

**EFFECT OF HUMIC ACID AND PUTRESCINE APPLICATION ON
GROWTH, YIELD, OIL QUALITY AND ECONOMICS IN *Sesamum indicum***

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2026

DECLARATION

I, hereby declare that the research work embodied in the Ph.D. thesis titled "**Effect of Humic Acid and Putrescine Application on Growth, Yield, Oil Quality and Economics in *Sesamum indicum***" in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Prasann Kumar (UID:21784)** working as **Associate Professor**, in the School of Agriculture (Agronomy) of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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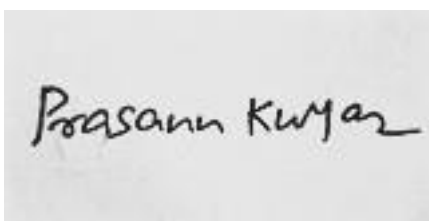
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CERTIFICATE-I

This is to certify that the work reported in the Ph. D. thesis entitled “**Effect of Humic Acid and Putrescine Application on Growth, Yield, Oil Quality and Economics in *Sesamum indicum***” submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in **Department of Agronomy, School of Agriculture**, is a research work carried out by **Amit Raj, 12215738**, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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ABSTRACT

Quality production is a key determinant of nutritional security and is critical to individual health and a country's economy. Sesame (*Sesamum indicum*) is one of the oldest and most important oilseed crops, with advantages including a high oil content, an excellent fatty acid profile, and antioxidant properties. The production of edible oil in India from sesame is substantial; however, sesame productivity is low due to poor soil fertility, nutrient imbalances, and vulnerability to abiotic stresses. As the population continues to rise and cultivable land steadily declines, the need to increase crop productivity has become a significant challenge. Limited nutrient availability and poor physiological performance during critical growth stages are among the limiting factors for the yield and quality of oil extracted from the sesame crop. Extensive use of chemical fertilizers often contributes to soil erosion and inefficient nutrient uptake, thereby affecting crop production and oil production. In this regard, biostimulants such as humic acid and polyamines, including putrescine, have become the focus of research as agents to enhance plant use efficiency, physiological processes, and metabolic activities. Humic acid improves soil properties, root development, and nutrient uptake, whereas putrescine, a naturally occurring polyamine, regulates plant development, stress resistance, and metabolism, including lipid synthesis. The experiment aimed to assess the effects of these agrochemicals on morpho-physiological variables, yield properties, and biochemical variables related to sesame oil quality, fatty acid profiles, and physicochemical properties of the oil. Specific attention was paid to evaluating the potential to improve productivity and oil quality under field conditions and to enhance profitability through the combined use of humic acid and putrescine. The purpose of the investigation was to find adequate dosages of humic acid and putrescine that would maximize the growth and yield of sesame, the quality of the oil, and the future of the economy. It is anticipated that the research results will be highly informative on sustainable sesame cropping methods. Biostimulants will serve as sustainable resources to enhance crop productivity, oil content, and farm revenue amid resource constraints. The present study investigated the effects of applying humic acid and putrescine on growth, yield, oil quality, and economics in sesame (*Sesamum indicum*). Consecutive two field experiments were conducted at the Department of Agronomy research farm

under the School of Agriculture at Lovely Professional University in Phagwara, Punjab, India during *kharif* seasons in 2023 and 2024 with one variety of sesame crop- Punjab Til No. 2. Different levels of humic acid (0, 500, 1000, and 1500 ppm), were applied in combination with putrescine (0, 50, 100, and 150 ppm) as a foliar application under factorial randomized block design to know their effect on growth, physiology, biochemical and yield attributing characters of the sesame crop. Various morphological, physiological, biochemical, and yield-attributing parameters of the sesame crop were studied through evidence-based observations at multiple time points in this experiment. Application of humic acid and putrescine enhances morpho-physiological and biochemical parameters, as well as quality parameters, of the sesame crop, thereby positively impacting crop production.

Keywords: Sesame, Humic acid, Putrescine, Yield, Oil Quality, Biochemical, Sustainability, Zero-hunger

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Place: LPU, Phagwara

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LIST OF ABBREVIATIONS

Symbol used	Abbreviation
;	Semicolon
%	Percent
:	Colon
⁰ C	Degree Celsius
Cm	Centimeter
Cm ²	Square Centimeter
Conc	Concentration
DAS	Days after sowing
DDW	Double distilled water
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic
FW	Fresh Weight
DW	Dry Weight
g	Gram
g cm ⁻³	Gram per cubic centimeter
HCl	Hydrochloric Acid
H ₂ SO ₄	Sulphuric Acid
H ₂ O ₂	Hydrogen Peroxide
q ha ⁻¹	Quintal per hectare

HA	Humic Acid
Put	Putrescine
mg	Milligram
mg kg-1	Milligram per kilogram
ml	Milliliter
N	Normal
NaOH	Sodium Hydroxide
Nm	Nano Meter
NO	Nitrous Oxide
POD	Peroxidase
ppm	Parts Per Million
R	Replication
rpm	Rotation Per Minute
RWC	Relative Water Content
T	Treatment
PAL	Phenylalanine ammonia lyase
w/v	weight by volume
MSI	Membrane Stability Index
MII	Membrane Injury Index
v/v	volume by volume
OP	Osmotic Potential

PV	Peroxide value
H.I.	Harvest index
pH	Soil reaction
OC	Organic matter
N	North
S	South
W	West
E	East
K	Potassium
P	Phosphorous
N	Nitrogen
CGR	Crop growth rate
RGR	Relative growth rate
NAR	Net assimilation rate
SPAD	Soil plant analysis development

INTRODUCTION

1. General Background

1.1 Global Importance of Agriculture in Ensuring Food and Nutritional Security

Human civilization is based on agriculture, a key component of global food and nutritional security. It ensures the production of food, feed, fiber, and biofuel, thereby supporting the existence and growth of societies worldwide. Agricultural systems are strained by rising demand to feed a growing global population, projected to reach approximately 10 billion by 2050, amid scarce natural resources such as land, water, and energy (Khatri et al., 2024). Food security is achieved through availability, stability, utilization, and accessibility. Agriculture also supplies staple foods, such as cereals, pulses, fruits, and vegetables, to meet caloric requirements while providing dietary diversity and health benefits. Agricultural diversity is also essential to nutritional security, which concerns the consumption of vital nutrients required for growth, disease resistance, and overall health. Legumes, oils, fruits, and other foods are sources of micronutrients, essential fatty acids, vitamins, minerals, and proteins (Dwivedi et al., 2026). Malnutrition, comprising undernutrition, micronutrient deficiency, and obesity, is a global issue. Diversifying agriculture with nutrient-dense crops, such as oilseeds, is a primary measure to address nutritional problems. Moreover, agriculture is closely linked to livelihoods and national economies. In most developing countries, more than 50% of the population depends on agriculture, directly or indirectly. The sector makes a substantial contribution to gross domestic product, rural employment, and agro-processing. Beyond its economic dimensions, agriculture affects environmental sustainability. Conservation tillage, integrated crop management, agroforestry, and crop rotation are environmentally friendly farming methods that support soil health, biodiversity conservation, and climate change resilience (Bhattacharya & Mondal, 2025). Nevertheless, the industry faces limitations, including climate change, land degradation, water scarcity, rising input costs, and post-harvest losses. Thus, the future of food and nutritional security worldwide depends on enhancing productivity, adopting

climate-sensitive agriculture, and adopting resource-saving technologies (Kabato et al., 2025).

1.2 Role of Oilseed Crops in Global and Indian Agriculture

Oilseed crops are essential to global agricultural systems because of their nutritional, industrial, and economic importance. They are cultivated across diverse agro-climatic zones and provide edible oils, proteins, and bioactive compounds essential for human health. Major oilseeds grown worldwide include soybean (*Glycine max*), rapeseed-mustard (*Brassica spp.*), groundnut (*Arachis hypogaea*), sunflower (*Helianthus annuus*), sesame (*Sesamum indicum*), safflower (*Carthamus tinctorius*), linseed (*Linum usitatissimum*), and oil palm (*Elaeis guineensis*). Recent FAO and USDA projections for 2024–2025 indicate that global oilseed production is expected to exceed 670 million tonnes, with soybean alone accounting for over 62% of total output. Brazil, the United States, Argentina, and China continue to dominate global soybean cultivation and processing, shaping international edible oil and protein meal markets (dos Reis et al., 2025). Rapeseed-mustard cultivation remains significant in Canada, the European Union, China, and India, while sunflower seed production remains concentrated in Russia, Ukraine, and Argentina (Swamikannu et al., 2024).

In India, oilseeds are the second-largest crop group by cultivated area, after cereals, and are central to the agricultural economy of dryland regions. According to the 2024–2025 agrarian estimates, India cultivates oilseeds on approximately 28–29 million hectares, producing around 40–42 million tonnes annually. Major oilseed crops grown in India include groundnut, soybean, rapeseed-mustard, sesame, sunflower, niger, and castor. The leading oilseed-producing states are Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Uttar Pradesh, Karnataka, Telangana, and Tamil Nadu (Mathur et al., 2023). In India, oilseeds used in the agricultural sector rank second in acreage, after food grains. India is also among the world's largest producers of groundnut, mustard, soybean, sesame, sunflower, and castor (Srivastava & Srivastava, 2023). These crops are grown in rainfed agricultural systems, especially in semi-arid and dryland areas. Oilseed crops play an essential role in the rural livelihoods of farmers, providing income and employment opportunities, particularly for small and marginal farmers.

Nutritionally, oilseeds provide essential polyunsaturated fatty acids that the human body cannot synthesize, including linoleic and linolenic acids. They are also rich in tocopherols (vitamin E), antioxidants, dietary fiber, and quality plant proteins (Sumara et al., 2023). Oilseed meals and cakes, produced after oil extraction, are also a significant source of protein in animal feed, particularly for poultry and livestock. Most oilseed crops in crop rotation improve soil health. Leguminous oilseeds such as groundnut and soybean enhance soil fertility through symbiosis with rhizobia to fix atmospheric nitrogen, enhance nutrient cycling, and improve soil sustainability (Rajput et al., 2024). Therefore, oilseed crops are of dual importance, as they enhance the nutritional quality of diets and maintain agricultural and economic sustainability at the global and Indian levels.

1.3 Contribution of Oilseeds to Edible Oil Production, Industrial Uses, and Economy

Edible oils are primarily derived from oilseeds and used in food processing, frying, and cooking. Soybean oil, mustard oil, groundnut oil, sunflower oil, and sesame oil are commonly consumed in household diets and the commercial food industry. As the world has become increasingly urbanized and lifestyles have changed alongside rising incomes, the consumption of edible oils has increased steadily. In other nations, such as India, the rate of edible oil consumption is growing faster than domestic production, resulting in increased reliance on imports, particularly palm oil. There are also non-edible and industrial uses of oilseeds (Jumrani & Meenakshi, 2023). Castor oil is used, e.g., in pharmaceuticals, lubricants, surfactants, cosmetics, and biodegradable polymers. Linseed oil is used in paints and varnishes, and sesame oil is valued in medical and traditional health care systems (Singh et al., 2023). Other valuable by-products of the oilseed processing industry include oilseed cakes, which serve as high-protein animal feed and as organic fertilizer to restore soil fertility. The oilseed industry contributes economically to the country through trade, agro-processing, and employment. Enhancing domestic oilseed production increases the food system's self-reliance, reduces import expenditures, and increases farmers' incomes and economic stability (Suganthi et al., 2025). The increased focus on renewable energy has also driven up the price of oilseeds used in biodiesel production, linking them to the new

bio-economy. Therefore, oilseeds are an essential part of agro-industrial development and the national economy.

1.4 Constraints in Oilseed Productivity and Quality under Current Production Systems

Although essential, oilseed crops are generally less productive than cereals. This disparity is driven by several factors: a high percentage of oilseed production occurs in areas that receive rainfall, exposing crops to drought, inconsistent rainfall, heat stress, and climate change. These conditions adversely affect flowering, seed development, and oil biosynthesis. Oilseed crops are also grown on poor soils with low organic matter and deficiencies of essential nutrients, including sulfur, boron, zinc, and molybdenum. Unbalanced fertilizer use reduces oil content and seed quality (Karmakar et al., 2024). Several biotic stresses affect oilseeds. Aphids, rust and mosaic virus (soybean), whitefly (sunflower), and leaf spot (groundnut) are among the pests and pathogens that destroy mustard crops, soybean, sunflower, and groundnut, respectively (Irshad et al., 2024). These pests and pathogens cause a significant reduction in oil yield and quality. The use of high-yielding, disease-resistant, and high-oil oleic varieties is limited. Seed production and distribution systems are often weak, limiting farmers' access to high-quality seed. Contamination, rancidity, and aflatoxin accumulation due to improper drying, storage, and processing affect market value and food safety. There is a need to enhance oilseed production and quality through integrated approaches that include genomics-based crop enhancement, CRISPR-based breeding, combined nutrient and pest control, increased irrigation efficiency, strengthened value chains, and the use of new storage and processing methods (Yadav & Langyan, 2025).

2. Sesame (*Sesamum indicum*) Crop Profile

2.1 Origin, Domestication, and Global Distribution

Sesame (*Sesamum indicum* L.) is the oldest known oilseed crop and is revered as the Queen of oilseeds. It has been hypothesized that it was domesticated archaeologically in the Indian subcontinent and in some areas of Mesopotamia more than 4,000 years ago. The Indian subcontinent is considered the main center of origin, and substantial

genetic variation persists in eastern and southern India. A secondary center of diversity is also identified in East Africa, particularly in Ethiopia and Sudan, where numerous landraces are adapted to diverse ecological niches. In ancient cultures, sesame was highly valued culturally and economically, particularly in the Babylonian, Egyptian, and Indus Valley societies, where it served as a source of edible oil, ritual offerings, and medicinal use (Angamuthu et al., 2025). Sesame spread through early trade routes between India and East Asia and Africa, and later to the Mediterranean and American territories. In the modern agricultural environment (2024-2025), sesame is grown in Asia, Africa, and parts of South America. Major producers include Sudan, Myanmar, India, Tanzania, Nigeria, China, and Ethiopia. Sudan and Myanmar are major exporters of sesame worldwide, owing to their large areas of commercial low-input farming. The increasing demand for natural antioxidants, cold-pressed oils, and nutraceutical ingredients in global markets has led to the expansion of sesame cultivation in emerging markets (Sharma et al., 2025).

2.2 Status of Sesame Cultivation in India

India is a major producer of sesame due to its high agro-ecological potential, experience, and consumer preference. In the 2024-2025 agricultural year, sesame was grown on approximately 1.6 to 1.8 million hectares, and national production was estimated at 0.7 to 0.9 million tonnes. Average output ranges from 450 to 600 kg per hectare, which is relatively low compared with the potential of improved varieties and hybrid lines (<https://upag.gov.in>). Sesame is primarily grown during the kharif (monsoon) season in most states, but in some states, such as West Bengal, Tamil Nadu, and Andhra Pradesh, rabi and summer seasons are also used for cultivation. The top sesame-producing states are Rajasthan, Uttar Pradesh, Madhya Pradesh, Gujarat, West Bengal, Tamil Nadu, Telangana, Odisha, and Maharashtra. Rajasthan accounts for a large share of the total cultivated area, and Gujarat and Madhya Pradesh also contribute substantially to the marketable surplus of cotton due to improved agronomic practices and seed replacement programs. The crop provides livelihood support to small and marginal farmers in rainfed agroecosystems, particularly in tribal districts across central, southern, and eastern India. Sesame is often cultivated on low-input, marginal soils with little irrigation or mechanization. Despite production stability issues, sesame

remains economically viable due to its high value in domestic and export markets, particularly given the growth in demand for cold-pressed and organic sesame oil (Haque et al., 2025).

2.3 Morphology, Growth Physiology, and Reproductive Biology

Sesame is an herbaceous annual that varies widely in growth habit, ranging from short, bush-like to tall, branching. Depending on genotype and environmental conditions, the plant generally grows to a height of 60-150 cm. The root system comprises a deep taproot and lateral roots, enabling the plant to adapt to water-deficient environments. Its stem is quadrangular and may be covered with fine hairs or a waxy coating, which helps it adapt to arid environments (Bisht et al., 2025). The arrangement of leaves is either opposite or alternate, or both; they are broad and straightforward in the lower canopy and narrow and lanceolate in the upper canopy, an adaptation for photosynthetic efficiency. Sesame is a zygomorphic flower, mostly solitary, and is carried in leaf axils. They are typically white or light pink, with possible variation in color among landraces. The reproductive biology of sesame is characterized by self-pollination, which is the most common, but cross-pollination may also occur via insect vectors such as bees. The flowering period is also extended, occurring gradually along the stem. The crop duration ranges from 75 to 120 days, depending on cultivar and environmental conditions. The fruit is a capsule, typically with four locules and numerous tiny seeds (Dawar et al., 2025). Capsule dehiscence is a significant cause of seed loss at harvest, affecting harvest management and yield security. Sesame is physiologically known to be drought-tolerant due to optimal stomatal control, osmotic regulation, and storage of stress-related metabolites, such as proline and flavonoids. It is, however, sensitive to water excess, particularly during seedling establishment and flowering (Jeyaraj & Beevy, 2024).

2.4 Nutritional and Medicinal Importance of Sesame Seeds and Oil

Sesame seeds are known for their high-quality oil, typically 45-55 percent oil and 18-25 percent protein. The oil contains a high proportion of unsaturated fatty acids, including oleic (Omega-9) and linoleic (Omega-6), which are nutritionally beneficial for cardiovascular health and lipid metabolism (Subrahmaniyan et al., 2024). Among

the factors that make sesame oil stand out is its content of natural antioxidants, such as sesamin, sesamol, and tocopherols, which make the oil more stable and longer-lasting on shelves by preventing oxidative rancidity. These lignans possess anti-inflammatory, anti-hyperlipidemic, and hepatoprotective properties; consequently, sesame oil has been used as a food ingredient in various nutraceutical preparations (Li et al., 2024). Sesame seeds also contain minerals such as calcium, iron, zinc, phosphorus, and magnesium, which further enhance the nutritional profile of this food. According to traditional and Ayurvedic medicine, sesame oil is referred to as teela taila and is widely used for the digestive system, lubricating joints, healing wounds, and carrying herbal preparations. Modern pharmacological research continues to investigate its applications in the treatment of oxidative stress, metabolic diseases, and degenerative diseases in older adults (Gupta & Kumar, 2025).

2.5 Major Production Constraints

Although economically and nutritionally significant, sesame production is constrained by several challenges, leading to unreliable yields and low productivity. Most varieties grown are landraces that have received limited genetic enhancement. The crop is exposed to weather fluctuations, particularly during flowering or capsule development. Sesame is also grown on marginal soils, and low amounts of fertilizer are used. Low nutrient uptake is due to limited exchange between roots and soil and inefficient uptake mechanisms (Dawar et al., 2025). High temperatures, drought, and excessive rainfall during establishment significantly affect crop performance. Waterlogging leads to root damage and reduced yield. The diseases and pests affecting the crop include phyllody, powdery mildew, bacterial leaf spot, leaf rollers, whiteflies, and capsule borers. Capsule shattering during maturity causes substantial seed loss. Post-harvest inefficiency is further exacerbated by the lack of mechanization and standardized harvest methods (Banik, 2023).

3. Importance of Oil Quality and Economics in Sesame

3.1 Chemical composition of sesame oil (fatty acids, antioxidants, and lignans such as sesamol, sesamin, and sesamol)

Sesame oil contains mostly unsaturated fatty acids, accounting for about 82-86 percent of the total oil content. Linoleic acid (C18:2) is the major fatty acid, ranging from 41-48 percent, and oleic acid (C18:1) ranges from 37-45 percent, depending on genotype and environmental factors. There are smaller amounts of saturated fatty acids, such as stearic acid (4-6%) and palmitic acid (8-10%). The oleic-to-linoleic acid ratio is considered a key parameter of oil stability and nutritional value, with higher ratios associated with greater oxidative stability and shelf life (Sumara et al., 2023). Recent gene research indicates that differences in expression of the FAD2 (Fatty Acid Desaturase 2) and SAD (Stearoyl-ACP Desaturase) genes are the primary determinants of the oleic-to-linoleic acid ratio, and this study offers breeders essential opportunities to improve quality through these means (Gangurde et al., 2025). In addition to fatty acids, sesame oil contains several bioactive antioxidants, including tocopherols, phenolic compounds, and lignans, which are found exclusively in the product and contribute to its stability. The most characteristic of these are sesamin, sesamol, and sesamol. Sesamin and sesamol occur naturally in unroasted or un-oil-extracted sesame seeds and undergo thermal conversion during oil extraction or roasting to produce sesamol, a high-potency antioxidant with vigorous free-radical scavenging activity. Sesamol is essential for inhibiting the auto-oxidation of unsaturated fatty acids, thereby preventing rancidity (Yang et al., 2025). More recent studies also indicate that these lignans regulate the cellular antioxidant enzyme pathways, including Nrf2-Keap1, superoxide dismutase, and catalase, and improve physiological antioxidant defense in humans (Luo et al., 2023). Phytosterols such as β -sitosterol, campesterol, and stigmasterol, which are present in it, contribute to its anti-inflammatory and cholesterol-lowering effects, making it even more beneficial for cardiovascular health (Zio et al., 2024).

3.2 Oil stability, oxidative resistance, and health benefits

An important parameter affecting storage, processing, and market value is oil stability. Unsaturated fatty acids in most edible oils are susceptible to oxidation, leading to off-flavors, nutrient loss, and reduced shelf life. Nevertheless, the oxidative stability of sesame oil is exceptionally high, ranking among the highest of vegetable oils. This is because its lignan profile is unique and thus stable. Sesamol is an effective radical

quencher; sesamin and sesamolin exhibit synergistic effects on the stability of tocopherols and inhibit their oxidation even under intense processing, such as deep frying. Experimental studies have shown that sesame oil retains oxidative stability 3-5 times longer than sunflower and soybean oils under accelerated storage conditions (Al Amin et al., 2023). Health-wise, the high levels of polyunsaturated fatty acids make sesame oil beneficial for cardiovascular health, as it lowers LDL cholesterol and increases HDL cholesterol (Oboulbige et al., 2023). Sesamin has been shown to affect lipid metabolism by regulating the activity of D5- and D6-desaturases, thereby inhibiting triglyceride synthesis in the liver (Bai et al., 2024). Sesame lignans also have antihypertensive, anti-inflammatory, anticarcinogenic, and hepatoprotective properties. The bioactivity of these substances increases when oil is consumed in the cold-pressed state, in which thermal degradation is less pronounced. Sesame oil (daily intake) can be used to reduce biomarkers of oxidative stress in humans, which is why it can be used in functional food products.

3.3 Market demand and consumer preference for oil quality traits

The demand for sesame oil varies by region, influenced by cultural dietary practices, processing methods, and the health benefits it provides. Roasted sesame oil is used in East Asian cultures such as Japan, Korea, and China, primarily as a cooking flavoring oil because of the characteristic aroma it develops during thermal transformation of lignans and other volatile compounds. Cold-pressed sesame oil is used in cooking, religious practices, and Ayurvedic medicine in India and the Middle East. Current market trends indicate increased demand for unrefined, cold-pressed, organic sesame oil, driven by consumer awareness of natural antioxidants and clean-label food products. According to the World Platform, the color of sesame seeds, oil content, percentage of free fatty acids, lignan composition, and the absence of contamination play significant roles in grading and pricing the product. White-seeded strains are favored for high-end export, whereas brown- or black-seeded strains are used in nutraceutical products because of their higher antioxidant content. Indian sesame export values have shown an upward trend, with a shift from the traditional trading of raw commodities to the marketing of value-added products such as hulled seeds, cold-pressed oil, and roasted oil. Given the projected growth in the global sesame market

through 2030, improvements in the quality characteristics of sesame oil products offer significant potential to increase foreign exchange revenues (Tang et al., 2024; Latino et al., 2022; Hamam et al., 2022).

3.4 Economic importance of sesame cultivation for smallholder farmers

Small and marginal farmers are the major sesame growers in rainfed areas of India, Africa, Myanmar, and Pakistan. It is an appropriate livelihood crop in areas where other oilseeds and cereals fail because of its low input requirements, drought resistance, and tolerance of marginal soils. Sesame is suitable for crop rotation and intercropping systems and improves farm sustainability and income stability. In India, sesame is cultivated in the Kharif, Rabi, and summer seasons to enable farmers to utilize residual soil moisture and align agricultural activities with market demand. Sesame is also a high-value export commodity, providing farmers with an opportunity to access foreign markets where high-quality seeds fetch premium prices. It is a cash crop in drylands and other regions, where it is cultivated by tribes and sold immediately after harvest to meet household needs. Women's contributions to planting, harvesting, and post-harvest processing further strengthen rural socioeconomic structures. Nevertheless, smallholders' income potential is often constrained by price volatility, quality rejections, and the absence of post-harvest infrastructure. Government interventions, such as Minimum Support Prices (MSPs) and cluster-based production programs, are aimed at enhancing profitability (Dossa et al., 2023; Lukurugu et al., 2023; Bedasa & Tekalign, 2024).

3.5 Yield quality economic trade-offs in sesame production

The central issue in sesame production is the balance between oil quality and seed yield. Varieties with high yield are sometimes low in lignan content or have altered fatty acid profiles, whereas traditional landraces with good oil quality are often low-yielding. Drought and heat are among the environmental pressures that can increase oil concentration at the expense of total biomass and seed production. The physiological basis of this trade-off is linked to carbon allocation pathways: environmental stress shifts plant metabolism toward secondary metabolite synthesis (including lignans), whereas favorable conditions favor carbohydrate accumulation and increased seed

mass (Salam et al., 2023). To address this trade-off, contemporary breeding programs focus on genotype x environment x management (GxExM) interactions. Increasing the content of high-oleic acid in sesame is achieved through marker-assisted selection of FAD2 gene variants, with no effect on yield. CRISPR-based technologies and genomic selection are likewise under investigation to enhance agronomic performance by improving lignan biosynthesis pathways. To optimize the trade-off, agronomic strategies, including balanced fertilization, moisture conservation, timely sowing, and the use of biofertilizers, are employed to enhance oil yield and quality. Sesame oil contains 45-55% oil, with a high content of unsaturated fatty acids, predominantly linoleic acid (40-48%) and oleic acid (35-45%). The saturated fraction consists of palmitic (8-10%) and stearic (4-6%) acids (Abdiani et al., 2024). The peculiarity of sesame oil is that it contains natural antioxidants, in particular tocopherols (vitamin E) and phenolic substances that contribute to improved nutritional value. The oil is especially valued for its lignans, such as sesamin, sesamolin, and sesamol. These lignans also confer antioxidant, anti-inflammatory, and lipid-regulating effects on sesame oil, making it superior to most other edible oils in terms of nutritional benefits and shelf life (Wei et al., 2022).

4. Humic Acid in Crop Production

Humic substances are becoming a significant component of sustainable agriculture in the modern world because of their capacity to improve soil quality, stimulate plant growth, and enhance nutrient efficiency. Humic materials are the most persistent component of soil organic matter, produced through the natural decomposition of plant and animal remains, and are well known for their benefits to soil and crops. Humic acid (HA) is the most agronomically important among them and offers a range of advantages that can be achieved chemically, physically, and biologically. Interest in the use of humic acid has been revived in recent years by growing concern over soil degradation, declining nutrient-use efficiency, and the need for environmentally friendly methods of crop production.

4.1 Nature, Origin, and Composition of Humic Substances

Humic compounds are high-molecular-weight organic materials of complex structure that develop over extended periods through the humification of vegetation and microbial remains. These are separated into three significant fractions, humic acid, fulvic acid, and humin, based on solubility and molecular weight. Humic acid is soluble in alkaline solutions and precipitates at acidic pH. Fulvic acid is soluble at all pH, and humin is insoluble in both acidic and alkaline conditions. Humic acids are heterogeneous macromolecules that include aromatic rings, aliphatic chains, carboxyl (-COOH), phenolic (-OH), quinone, and methoxyl groups, which define a high cation exchange capacity, redox activity, and the ability to form stable complexes with nutrients (Nabi et al., 2025). Current structural characterization of humic acid by NMR spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and liquid chromatography-mass spectrometry (LC-MS) shows that the structure is organized in a supramolecular structure due to hydrophobic interactions and hydrogen bonding. Humic acid is adsorbed within this structure and can stimulate microbial activity, regulate soil-plant biochemical processes, and adsorb nutrients. Humic acids are typically derived from lignite, leonardite, peat, compost, and vermicompost. Modern extraction methods, such as alkaline extraction and membrane filtration, are used to obtain purified humic acid products for agricultural applications (Anielak et al., 2025). Humic acids are chemically diverse due to parent materials, environments, and extraction procedures, which affect their functional properties in soil and plant systems.

4.2 Impact of Humic Acid on Physical, Chemical, and Biological Properties of Soil

Humic acid is central to improving soil physical structure through increased aggregate stability, reduced bulk density, and improved soil aeration. Humic acid enhances soil water infiltration and retention by binding soil particles into aggregates, a process that is particularly beneficial in sandy and degraded soils. Enhanced water retention capacity enables crops to withstand short-term drought stress and promotes improved root development. The application of humic acid at rates of 10-20 kg ha⁻¹ had a dramatic effect on the formation of macroaggregates in dryland soils, resulting in improved soil tilth and reduced soil erosion. Humic acid also increases the cation exchange capacity (CEC) of soil, enabling it to store vital nutrients such as potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), and ammonium (NH₄⁺) (Yang et al.,

2024). Humic acid forms chelated complexes with micronutrients such as iron, zinc, copper, and manganese, thereby increasing their solubility and availability to plants. Humic acid contains functional groups such as carboxyl and phenolic groups that help buffer soil pH, limit nutrient leaching, and improve fertilizer efficiency. Recent studies highlight the capacity of humic acid to inhibit phosphorus fixation in alkaline soils by interacting with calcium ions, thereby enhancing P availability to crops. Biologically, humic acid enhances the functioning of soil microbes, including those that support nutrient cycles, decompose organic matter, and promote plant growth (Piccolo et al., 2025). Humics are carbon sources for soil microbes and serve as substrates for enzymes such as dehydrogenase, urease, and phosphatase. Humic acid enhances the population of beneficial microbes such as *Azotobacter*, *Bacillus*, *Pseudomonas*, and mycorrhizal fungi, which leads to better soil health and improved interactions between plants and microbes (Hilles et al., 2025).

4.3 Physiological and Biochemical Functions of Plants

Humic acid has pronounced physiological effects on plants, including increased root growth, enhanced nutrient uptake, and stimulation of hormones and enzymes. Stimulation of root architecture is among the most widely reported effects of humic acid. Humic acid enhances lateral root emergence, growth, and root hair development, thereby increasing root surface area for nutrient uptake. Many of these effects are attributable to the hormone-like properties of humic acid, particularly its interactions with auxin signaling. Humic acid can regulate the expression of auxin-responsive genes, including *Aux/IAA* and *ARF*, leading to plasticity in the root system. Humic acid also improves nutrient uptake by increasing membrane permeability and H^+ -ATPase activity in roots. This enzyme protonates the rhizosphere, increasing nutrient solubility and promoting active ion uptake. Humic acid controls the expression of transporter genes, including *NRT1.1* (initiating nitrate assimilation), *PHO1* (phosphate assimilation), and *IRT1* (iron assimilation), to enhance nutrient uptake. Humic substances also enhance chlorophyll production, photosynthesis, and antioxidant enzyme activities, including superoxide dismutase, catalase, and peroxidase, and assist plants in overcoming oxidative stress. Humic acid enhances the production of primary metabolites, such as sugars, amino acids, and organic acids, which are beneficial for

plant growth and stress tolerance. It also promotes secondary metabolism, including flavonoids, phenolics, and lignin, which contribute to structural strength and defense mechanisms. The net physiological effect of humic acid is to increase plant vigor, stress tolerance, and nutrient-use efficiency (Piccolo et al., 2025; Decsi et al., 2024; Wang et al., 2024).

4.4 Reported Effects on Growth, Yield, and Quality of Oilseed and Field Crops

Research conducted globally over the past ten years shows positive effects of humic acid on the growth and productivity of a wide range of crops, including oilseeds, cereals, pulses, vegetables, and fruit crops. Humic acid application has been reported to improve plant height, root biomass, branch count, flowering, and pod or capsule development in oilseed crops such as sesame, groundnut, sunflower, soybean, and mustard. According to research conducted in India (ICAR-IISR, 2023-2024), foliar- or soil-applied humic acid at 0.1-0.3% increased sesame seed yield and oil content by 12-18% and 2-4%, respectively. Additionally, improvements in oil quality, oleic acid content, and antioxidant activity were reported. Humic acid in soybean stimulates nodulation, nitrogen fixation, and seed protein content. Groundnut and sunflower show positive associations with higher shelling percentage, seed filling, and oil recovery. Field trials in China and Turkey (2022-2024) demonstrated that yield gains (10-25%) in sunflower and rapeseed are attributable to increased nutrient uptake efficiency and expanded photosynthetic potential. Humic acid increases grain weight, chlorophyll, and water-use efficiency in some cereals, such as maize and wheat. The net positive effect on crop quality is associated with improved nutrient balance, stress resistance, and physiological functionality. Humic acid also alleviates the adverse effects of abiotic stresses such as drought, salinity, and temperature extremes by regulating osmolyte levels, enhancing membrane stability, and strengthening antioxidant enzyme activities. Incorporating humic acid into fertilizer programs reduces the use of chemical fertilizers by 15-25% while maintaining crop yields (Alizadeh et al., 2022; Mekdad et al., 2022; Osvalde et al., 2024; Morsy et al., 2024).

4.5 Humic Acid Application in Sustainable Agriculture Relevance

Humic acid has great potential in sustainable agricultural systems because it can increase soil fertility, enhance nutrient-use efficiency, and support crop development while reducing reliance on chemical fertilizers. As the world's agricultural systems face challenges such as soil degradation, declining organic matter, structural stresses imposed by climatic factors, and escalating fertilizer costs, humic acid is a viable alternative: a cheaper, more sustainable, and eco-friendlier option that will not hamper sustainable development objectives. Humic acid supports long-term soil health by enhancing soil structure and microbial activity, thereby increasing carbon sequestration and reducing greenhouse gas emissions. Its efficiency in improving fertilizer use reduces losses from leaching and volatilization, making nutrient management more effective and safer for the environment. Compost or vermicompost is applied to the soil to provide humic acid, which serves as a natural growth stimulant and soil conditioner in organic farming systems. Humic acid is an essential component of regenerative agriculture, conservation agriculture, and integrated nutrient management systems. It contributes to soil resilience, climate-sensitive agriculture, and resource-efficient crop production and is therefore relevant to modern frameworks for sustainable farming. As extraction technology improves and molecular knowledge of the interaction between humic acid and plants advances, humic acid will likely become a significant input to agricultural systems in the future (Lumactud et al., 2022; Datta et al., 2022; Maffia et al., 2025).

5. Putrescine and Polyamines in Plant Physiology

5.1 Polyamines: Putrescine, Spermidine, Spermine

Polyamines are small polycationic biomolecules that play critical roles in plant physiology because they interact with nucleic acids, membranes, proteins, and reactive oxygen species (ROS). The primary polyamines in plants are putrescine (Put), spermidine (Spd), and spermine (Spm). They are synthesized by decarboxylation of ornithine to putrescine (ODC) or of arginine to spermidine (ADC), and then serve as precursors to higher polyamines through the activities of SPDS (spermidine synthase) and SPMS (spermine synthase). Recent work has highlighted that polyamine biosynthesis is tightly regulated by transcription factors, including MYB, WRKY, and

NAC, which modulate ADC/ODC activity in response to developmental and stress stimuli. Polyamines are also implicated in cellular redox homeostasis because they can bind negatively charged molecules, stabilize chromatin, and affect gene expression. Increased attention over the past two years has brought polyamines to the forefront as metabolic centers that interact with ethylene, ABA, NO, and GABA signaling, indicating that they not only act as metabolites but also as signaling molecules. Their ubiquitous presence in organelles, including the cytosol, chloroplasts, mitochondria, and the apoplast, is yet another reason they have a systemic effect on plant growth, development, and stress adaptation (Mohajeri et al., 2024; Kolesnikov et al., 2024; Chamoto et al., 2024; Alayunt, 2025).

5.2 Putrescine plays a role in cell division, differentiation, and growth regulation

Putrescine is the most common and dynamically regulated polyamine in plant development and serves as a key regulator of cell division, cell elongation, and tissue differentiation. Putrescine increases DNA replication by stabilizing DNA polymerase complexes and mediating chromatin accessibility, thereby facilitating rapid progression through the G1/S cell cycle phase. Recent mechanistic studies (2022-2025) indicate that putrescine interacts with cyclin-dependent kinases (CDKs) and increases expression of CYCD3 and CYCA2, key genes involved in meristem activity. Putrescine promotes growth of the root apical meristem, leading to the formation of the elongation zone and increased vascular differentiation, which, in turn, improves nutrient uptake and root system architecture. Putrescine regulates cell wall plasticity in shoots and leaves by modulating pectin methylesterase activity, resulting in increased leaf expansion. It also controls turnover of auxin efflux carriers (PIN proteins), thereby regulating the auxin gradients necessary for organogenesis. Recent studies have found that exogenous putrescine application improves shoot regeneration efficiency in tissue culture systems by modulating the WUSCHEL-CLAVATA stem cell circuit, indicating broader developmental contributions. Therefore, putrescine is a biochemical mediator of plant morphogenesis that links metabolic state, hormone dynamics, and cell-cycle activity (Li et al., 2024; Huang et al., 2023; Alkan et al., 2024; Joshi et al., 2024).

5.3 Mechanisms of Action in Stress Tolerance (Drought, Salinity, Oxidative Stress)

Polyamines, especially putrescine, have become key players in the plant stress response, which operates at biochemical, molecular, and physiological levels. Putrescine promotes thylakoid membrane stabilization, preserves chlorophyll integrity, and reduces stomatal conductance under drought stress through the ABA-polyamine crosstalk pathway, which increases NCED3-dependent ABA synthesis, resulting in improved water-use efficiency. Putrescine mitigates Na⁺ toxicity under salinity stress by activating the ion transporters SOS1 and NHX1, thereby enhancing ion homeostasis and cytosolic K⁺/Na⁺ balance. Polyamines further regulate the key antioxidant enzymes SOD, CAT, APX, and GR, thereby significantly decreasing ROS concentrations during oxidative stress. A 2024 study emphasized the role of polyamine oxidase (PAO)-mediated putrescine catabolism in producing controlled bursts of hydrogen peroxide, a secondary messenger that activates stress-responsive genes, such as DREB2A, HSP70, and MYB42. Another emerging mechanism is polyamine-induced nitric oxide (NO) signaling, which promotes osmotic adjustment by increasing proline and glycine betaine levels. Moreover, polyamines enhance mitochondrial function by stabilizing ATP synthase complexes and inhibiting lipid peroxidation, thereby maintaining metabolic activity during stress. These results reveal that putrescine is a protective osmolyte and a signaling molecule that coordinates complex stress-relaxation mechanisms (Mabudi Bilasvar et al., 2022; Ma et al., 2022; Farooq et al., 2025).

5.4 Effect on Flowering, Reproductive Success, and Seed Development.

Polyamines strongly influence plant reproductive biology, affecting flower induction, pollen viability, fertilization efficiency, embryo development, and seed filling. During floral transition, putrescine levels rise significantly. In this context, putrescine regulates floral integrator genes, including FT, SOC1, and LFY, and promotes the timing of phase change. Spermidine and spermine are present at high levels in plant reproductive organs, pollen grains, and anthers, where they maintain membrane fluidity, stabilize nucleic acids, and protect against oxidative damage, all of which are essential prerequisites for pollen germination and pollen tube elongation. Recent results from 2023-2025 show that polyamines regulate Ca²⁺ fluxes at the tip of the pollen tube, thereby enabling the organization of actin filaments and vesicle trafficking that drive

tube growth. During fertilization and subsequent embryo growth, putrescine increases the activity of enzymes involved in carbohydrate mobilization and protein biosynthesis, thereby enhancing embryo survival and seed vitality. Polyamines also regulate the expression of genes related to storage protein deposition, lipid biosynthesis, and hormonal homeostasis, particularly ABA and GA, thereby influencing seed size, density, and nutrient content during the seed-filling stages. In addition, polyamine-deficient mutants tend to have low seed set, reduced grain weight, and disrupted embryogenesis, underscoring the necessity of putrescine and its derivatives for reproductive success. Polyamines positively affect floret fertility and seed quality and can be promising targets for crop enhancement and yield stability (Noveyri et al., 2022; González-Hernández et al., 2022; Mahmoud & Abdelhameed, 2022; Najafi et al., 2025).

5.5. Polyamine use to enhance crop productivity and quality

Exogenous polyamine application has become popular over the past 20 years, particularly in research aimed at increasing the growth and productivity of plant species and improving quality characteristics in cereals, legumes, oilseeds, fruits, and vegetables. Putrescine applied to foliar surfaces or soil has been shown to stimulate biomass growth, chlorophyll accumulation, photosynthetic rate, and nutrient-use efficiency, primarily by promoting root development and increasing nitrogen uptake. Putrescine treatments have resulted in substantial increases in grain yield in cereals such as wheat and maize, driven by enhanced grain filling and spikelet fertility, both linked to heightened activity of sucrose synthase and starch phosphorylase. Polyamines enhance fruit firmness, sugar content, pigmentation, and shelf life of horticultural products by modulating enzymes that loosen cell walls and by enhancing ROS-scavenging mechanisms. Oilseed crops such as sesame, rapeseed, and sunflower become oilier and have improved fatty acid profiles after putrescine application, as genes that regulate lipid synthesis are upregulated (i.e., ACC, FAD2, LACS, and DGAT). Research also indicates that polyamines mitigate yield penalties caused by abiotic stress by maintaining reproductive processes and defending metabolic pathways. Recent developments in nanoformulations have led to the encapsulation of polyamines to achieve sustained release and uptake, resulting in improved effects at

reduced dosages. Overall, research findings indicate that polyamines, including putrescine, are effective bio-stimulants that can enhance both yield and quality when used at optimal levels (Yang et al., 2024; Puente-Moreno et al., 2025; Tyagi et al., 2023).

6. Research studies on Humic Acid, Putrescine, and Oilseed Crops

6.1 Studies on humic acid application in cereals, legumes, and oilseeds

Over the past several years, a body of research has accumulated showing that humic acid (HA) is a widely applicable biostimulant that enhances soil functionality and crop productivity, with average yield increases of 8-15%, significant increases in nitrogen uptake and nitrogen-use efficiency, particularly in medium-organic carbon soils with sufficient precipitation. Mechanistic research has elucidated HA's actions across a variety of scales: at the soil level, HA enhances aggregate stability, water-holding capacity, and cation exchange capacity through its high density of carboxyl and phenolic functional groups, thereby reducing nutrient leaching and improving root access to mobile nutrients; at the rhizosphere level, HA forms soluble chelates with micronutrients (Fe, Zn, Mn) and improves their bioavailability, and as a result interacts with plasma membrane H⁺-ATPases, increasing root length density and fine root growth in cereals (wheat and maize), resulting in increased early vigor and water uptake during intermittent drought. HA also affects legumes. It has been demonstrated that HA can enhance nodulation efficiency and symbiotic nitrogen fixation by rhizobia by increasing both microbial colonization and nod gene expression, thereby improving plant nitrogen status without proportional increases in synthetic N fertilizer. In oilseeds (sunflower, rapeseed, groundnut and sesame), HA treatments consistently report increased vegetative growth, greater pod/capsule set, and small but significant percentage increases in seed oil; mechanistic metabolomics suggest that HA increases primary carbon flux to fatty acid biosynthesis (raising ACC and FAS pathway intermediates) and activates antioxidant metabolism in developing seeds, which also leads to yield and quality increases. These agronomic and mechanistic effects are, however, context-dependent, with HA responding best in soils with low to moderate organic matter and moderate rainfall regimes, and responses are dampened in highly

alkaline soils or overly N-rich soils (Osvalde et al., 2024; Ampong et al., 2022; Bhatt & Singh, 2022).

6.2 Research on putrescine application in stress management and productivity improvement

The simplest polyamine, putrescine (Put), has become the focus of an increasingly rich body of literature demonstrating that it can alleviate abiotic stress or enhance crop productivity when used as a seed-priming agent, foliar spray, or root-zone treatment. Put is known to strengthen mechanical stress resilience in plants through membrane stabilization, osmotic regulation, and regulated redox signaling. Put has been shown to bind to membrane phospholipids and cell-wall pectin, preventing electrolyte leakage during dehydration; to induce the accumulation of compatible solutes, e.g., proline and soluble sugars, that maintain cellular turgor during drought; and to generate controlled bursts of hydrogen peroxide (H₂O₂), which are proposed to trigger ant There are recent studies demonstrating such mechanisms in other species: foliar or root-applied Put promoted photosynthetic performance, reduced ROS accumulation, and increased chlorophyll fluorescence (Fv/Fm) in ornamental and field crops. Put seed priming increased germination and seedling vigor of seeds planted dry or in saline water: cereal and oilseeds. Field tests demonstrate that Put treatments can reduce yield loss from drought or salinity by enhancing reproductive success (fewer flower/seed abortions) and maintaining assimilate partitioning to sinks. Interestingly, doses in the low millimolar (usually 0.5 to 2 mM) range (when foliar applied) or 50 to 200 ppm (when seed primed) induce an optimal balance between effectiveness and phytotoxicity risk in promoting biomass and yield components without growth penalties. These studies, as a whole, place putrescine as a convenient and mechanistically well-defined biostimulant for reducing the effects of stress in planting systems, but the best methods and times of application are specific to crops and environments (Noveyri et al., 2022; González-Hernández et al., 2022; Mahmoud & Abdelhameed, 2022; Najafi et al., 2025).

6.3 Reports on combined /synergistic application of growth-promoting biostimulants

A recent trend in agronomic research is the synergistic effect of humic acid with polyamines and other biostimulants (seaweed extracts, amino acid formulations, PGPR, micronutrients), yielding better results than those of individual products. Mechanistic studies suggest possible foundations for such synergy. HA can improve soil physical structure and root exploration, thereby enabling higher root uptake capacity. At the same time, exogenous Put or in situ polyamine induction primes plant cellular defenses and spurs cell division and reproductive resilience. In combination, HA can increase the translocation and root uptake of applied polyamines and micronutrients, and Put can increase HA-induced membrane and transporter activities at the root surface. Results for combinations that included HA + microbial inoculants significantly improved soil enzyme activity and nutrient cycling, and HA + foliar Put sprays provided additive benefits for chlorophyll retention, yield, and oil content in oilseed trials. Convergence points at the signaling level have also been identified: a combination of treatments activates MAPK cascades and parallel signaling pathways among ROS, NO, and phytohormone modules (ABA, auxin), resulting in coordinated transcriptional reprogramming that benefits both growth and stress tolerance. In many cases, when HA is used with beneficial microbes or polyamines, there is an opportunity to reduce the quantity of chemical fertilizer used without any changes to yields, reflecting gains in productivity and sustainability. The literature, however, emphasizes that formulation compatibility, sequence/timing of application, and local soil chemistry are paramount to realizing synergy; poorly formulated combinations may result in antagonistic interactions or no effect whatsoever (Noveyri et al., 2022; González-Hernández et al., 2022; Osvalde et al., 2024; Ampong et al., 2022).

6.4 Specific findings in sesame and related oilseed crops

The seeds of sesame and other oilseeds have been the focus of numerous applied research studies on HA and Put, given the crop's sensitivity to moisture stress and shattering and the importance of oil quality. In India, Africa, and parts of the Middle East, field experiments suggest that HA enhances sesame root growth, nutrient uptake (especially N, P, K, and micronutrients), capsule set, and seed yield by double-digit percentages in numerous trials. At the same time, HA treatments have been linked to only single-digit percentage increases in seed oil percentage and to a higher antioxidant

capacity of seed oil, which is probably due to HA-mediated activation of fatty-acid biosynthesis and increased phen. Sesame putrescine interventions show significant advantages for reproductive resilience: foliar or seed treatments result in fewer floral abortions and better pollen retention during terminal drought, leading to better capsule retention and seed weight. Molecular studies and germplasm analyses indicate that the application of Put can increase lipid metabolism (genes that regulate oleic/linoleic ratios, such as FAD2 family members) and lignan biosynthesis (precursors of sesamin and sesamolin), thereby having quantifiable effects on yield and oil quality. Combined HA in sesame has been reported to improve outcomes. HA improves root uptake and soil nutrient interactions, whereas Put improves reproductive performance and seed biochemical quality, leading to better economic performance in smallholder environments. Similar patterns are observed in comparative studies of other oilseeds (sunflower, rapeseed, groundnut): HA enhances growth and N-use efficiency, and Put enhances reproductive development under stress, leading to improved oil yield and quality. These crop-specific results indicate that integrated HA-Put-based biostimulant treatments are promising for improving the yield and marketability of sesame and related oilseeds as long as their doses and timing of application are optimized to suit the local environment (Mahmoud & Abdelhameed, 2022; Najafi et al., 2025; Yang et al., 2024; Puente-Moreno et al., 2025; Tyagi et al., 2023).

7. Knowledge Gaps and Research Rationale

7.1 Limitations in current understanding of sesame growth, yield, and oil quality modulation

Despite sesame (*Sesamum indicum* L.) being one of the oldest and most important oilseed crops cultivated in semi-arid and tropical regions, substantial knowledge gaps remain regarding the integrated regulation of its growth, productivity, and oil quality under changing environmental and nutritional conditions. Earlier studies have primarily emphasized morphological, agronomic, or phenological responses, with comparatively limited attention to the biochemical, physiological, and molecular mechanisms governing carbon partitioning, reproductive development, capsule formation, seed filling, and lipid biosynthesis. Furthermore, the influence of abiotic stresses such as

drought, salinity, heat stress, and nutrient limitations on hormonal signaling networks, source–sink dynamics, and plastidial acetyl-CoA metabolism has not been comprehensively elucidated in sesame compared with other major oilseed crops. Similarly, the regulation of key genes associated with oil biosynthesis and quality, including FAD2, FAD3, CYP81Q1, CYP92B14, and oleosin-related proteins, under diverse agronomic and nutrient management practices remains inadequately understood. Although environmental factors and soil fertility are known to influence fatty acid composition, lignan biosynthesis, antioxidant capacity, and overall oil quality, the mechanistic interactions through which organic amendments, biostimulants, and integrated nutrient management modify these processes are still insufficiently characterized. Therefore, a comprehensive synthesis linking agronomic practices with physiological, biochemical, and molecular responses is needed to bridge existing knowledge gaps and support the development of targeted strategies for improving sesame productivity, oil quality, and resilience under diverse agroecological conditions (Yadav et al., 2022; Rauf et al., 2024; Li et al., 2023).

7.2 Lack of mechanistic studies on humic acid and putrescine effects in sesame

Humic acid (HA) and putrescine (Put) are accepted biostimulants that have shown beneficial effects in several crops. However, there is minimal mechanistic understanding of these biostimulants in sesame physiology. Most available research uses HA and Put as empirical growth stimulators without elucidating their molecular signaling or effects on nutrient transporters, redox homeostasis, membrane integrity, or hormonal balance. For example, HA has been shown to induce H-ATPases, root morphogenesis, and NPK uptake by modulating transporter gene families. However, the effects of these processes on sesame remain unexplored. Similarly, putrescine is a diamine polyamine and a central figure in stabilizing nucleic acids, regulating ion channels, enhancing ROS scavenging activity, and regulating the arginine- and S-adenosylmethionine (SAM)-derived polyamine pathway. Nevertheless, few records of sesame-specific data on Put-mediated remodeling of osmoprotective metabolites, proline turnover, dehydrin expression, or chloroplast biogenesis under field conditions are available. The interactive effects of HA and Put on sesame root-shoot signaling, the photosynthetic apparatus, the antioxidant defense cascade, and lipid accretion have not

been examined at the transcript, metabolite, or enzyme activity levels. The exact physiological windows, dose-response thresholds, transport kinetics, and biochemical pathways that HA and Put target in sesame have not been investigated without the use of fine-scale mechanistic studies. This significant knowledge gap impedes the rational development of biostimulant-based agronomy for this crop (Wahid et al., 2025; Arora et al., 2024; Singhal et al., 2023).

7.3 Minimal combination of growth, yield, oil quality, and economic research

Most prior sesame research has treated growth parameters, yield traits, oil quality traits, and economic viability as independent variables, resulting in fragmented knowledge that does not support holistic agronomic decision-making. Studies focused on growth typically quantify plant height, leaf area, chlorophyll index, or dry-matter distribution without linking these measures to downstream outcomes such as capsule productivity or lipid biosynthesis in the seed. Similarly, yield-oriented studies seldom employ comprehensive oil profiling, which encompasses changes in fatty acids, lignan proportions, tocopherol content, oxidative stability indicators, and metabolic markers. Where available, economic analyses primarily focus on gross returns or cost-benefit ratios rather than on physiological drivers or eco-efficiency metrics. This prevents the integration of models, so fundamental relationships, such as the impact of HA-induced root enhancement on nutrient remobilization efficiency or the impact of Put-mediated stress tolerance on oil stability, are not studied extensively. Moreover, recent developments, including machine-learning-based yield and quality prediction, life-cycle costing, carbon footprint estimation, and input-use efficiency analyses, are not widely covered in the sesame agronomy literature. This lack of integration between biological responses and economic viability at the farm scale prevents stakeholders from implementing evidence-based interventions to maximize profitability and productivity. There is thus an urgent need for a multidimensional integration of agronomic, physiological, biochemical, and economic data (Noveyri et al., 2022; González-Hernández et al., 2022; Osvalde et al., 2024; Ampong et al., 2022).

7.4 Reason behind using a combination of humic acid and putrescine as agronomic interventions.

Humic acid and putrescine are used concurrently, and this approach is a promising strategy because of their complementary and potentially synergistic mechanisms of action. HA improves the physicochemical characteristics of soils, promotes the growth of microbial consortia, enhances nutrient chelation, and increases root absorptive capacity by activating aquaporins and ion transporters. Meanwhile, putrescine coordinates stress-reduction pathways by stabilizing thylakoid membranes, Ca^{2+} signaling, the ascorbate-glutathione cycle, and polyamine-sensitive transcription factors. Collectively, the compounds may exert a combined effect on critical determinants of sesame productivity, including increased nutrient uptake by HA, which could provide substrates for putrescine-mediated biosynthetic pathways, such as chlorophyll synthesis, amino acid metabolism, and lipid synthesis. Additionally, HA could enhance the transport of putrescine across tissues by regulating membrane permeability and proton gradients. Their interaction is also postulated to affect lipid metabolic regulators such as ACCase, GPAT, LACS, and desaturases, thereby influencing seed oil content and quality. Additionally, co-application would potentially decrease hormonal regulation, especially auxin, cytokinin, abscisic acid, and ethylene signaling, which would provide an ideal internal environment to promote reproductive success and retain capsules. This dual intervention has never been systematically tested in sesame. Hence, it is a new method with high potential to improve growth vigor, yield stability, and oil quality under changing environmental conditions (Noveyri et al., 2022; González-Hernández et al., 2022; Mahmoud & Abdelhameed, 2022; Najafi et al., 2025).

7.5 Expected contribution of the present study to science and practice

The current research will likely help fill crucial knowledge gaps in the field by providing the first comprehensive assessment of the effects of humic acid and putrescine on sesame growth, physiological performance, yield components, and oil quality under field conditions. Scientifically, it aims to provide new mechanistic evidence of nutrient-hormone-polyamine interactions, stress resilience and adaptation,

and lipid biosynthetic control, as influenced by biostimulants. Measuring agronomic and physiological indicators, in combination with biochemical profiling of oils, will provide the appropriate environment to address how HA and Put influence photosynthesis, antioxidant capacity, assimilate translocation, membrane function, and metabolic fluxes toward lignan and fatty acid production. The results could also increase knowledge of how extrinsic biostimulants regulate sesame genetic pathways, thereby guiding future genomic or transcriptomic investigations. In practice, the results will be applied to evidence-based agronomy among farmers to optimize sesame productivity with minimal chemical inputs and enhance the marketability of the oil. An economic evaluation will be integrated to ensure that actionable recommendations on dose optimization, input cost efficiency, and return on investment are provided to enhance the potential for adoption. In the end, the investigation will help develop sustainable production systems for sesame; an ecologically sustainable, mechanistically based intervention program will be found to enhance yield stability, food quality, and financial viability, particularly under partial and more challenging agroclimatic conditions (Li et al., 2024; Huang et al., 2023; Alkan et al., 2024; Joshi et al., 2024).

Objectives of the proposed work

1. To study the effect of humic acid and putrescine on the growth and yield of sesame
2. To study the effect of treatments on biochemical and physiological changes in the leaves of sesame
3. To study the effect of treatments on oil quality
4. To study the effect of treatments on economics

REVIEW OF LITERATURE

The Effect of Humic Acid and Putrescine on Growth and Yield of Sesame

Rajpar et al. (2011) conducted an agronomic study to examine how applying humic acid affected *Brassica compestris* L. growth, yield, and oil content in alkaline-calcareous, weakly fertile soils. At rates of 0, 3.1, 6.3, and 9.3 kg/acre, humic acid was given to the soil at the time of seeding. When humic acid was applied at 6.3 kg/acre, growth and yield characteristics were substantially higher than those of the other treatments.

Anaz et al. (2021) examined how different plant densities responded to humic acid application with respect to canola genotype growth and yield. The results showed that, compared with controls, the administration of humic acid resulted in significantly increased canola plant growth and yield.

Hatami (2017) conducted an agronomic study to examine how humic acid and zinc treatments affected sunflower production and yield components under stress. Compared with alternative therapies, the application of humic acid significantly increased sunflower development and yield.

Savita and Girish (2019) examine how humic chemicals affect nutrient absorption and soybean yield. The application to the foliage of humic substances separated from organic matter (0.2%) at 40 DAS and the application of humic compounds at a rate of 5 kg ha⁻¹ during sowing resulted in a significantly higher grain and haulm yield as compared to the control.

Kandil and Essam (2015) examine the impact of planting methods and humic acid on sesame (*Sesamum indicum* L.). When humic acid was applied at 3.6 kg/ha, the growth and yield parameters were substantially higher than those of the other treatments.

Vani et al. (2017) investigated how INM affected the characteristics and quality of summer sesamum yield on sandy loam soil in 2013. The results showed that 100% RDN

produced significantly more oil and seeds than 100% RDN after 1% humic acid was sprayed on the foliage.

Farzi Aminabad et al. (2021) investigate the effects of SA and putrescine on rapeseed plant performance under increasing water deficit. The results showed that, compared with other treatments, salicylic acid and putrescine treatments significantly increased rapeseed growth and production.

Wei et al. (2013) conducted an agronomic study to examine the physiological and morpho-anatomical responses of sesame to waterlogging. The results showed that, compared with the control treatment, an increase in putrescine levels significantly enhanced growth and yield.

Reddy et al. (2021) reported that applying 100% recommended nitrogen through inorganic fertilizer combined with a 1% foliar spray of humic acid at branching and flowering significantly improved sesame growth and yield. This treatment produced the greatest plant height (169.3 cm), branches (5.36 plant⁻¹), capsules (107.71 plant⁻¹), seeds (53.75 capsule⁻¹), and seed yield (1058 kg ha⁻¹; 12.07 g plant⁻¹), while also achieving the highest economic returns with a benefit–cost ratio of 2.33 and net realization of ₹36,664 ha⁻¹.

Hayati et al. (2022) two-year field study assessed the foliar treatments of HA (0, 0.25, 0.5 g L⁻¹) and nano-chelate iron (0, 1, 2 g L⁻¹) in combination with irrigation levels (50, 75, and 100% the water was available) were evaluated in a two-year field trial. Both inputs mitigated the effects of stress; however, drought significantly reduced nutrient uptake and oil properties. When 0.5 g L⁻¹ humic acid and 2 g L⁻¹ nano-chelate iron were applied combined, the most significant grain production (1036 kg ha⁻¹) was achieved, increasing output by 21–27% over the control.

During the 2021–2022 kharif season, **Gajanan (2022)** compared various combinations of RDF, drip application, and humic substances. Nitrate-N and ammonium-N levels were significantly increased by drip application of humic compounds, particularly HS-1 at 0.2%, which also enhanced nutrient uptake and dry-matter accumulation. The

100% RDF drip produced the maximum seed and stalk yields. Notably, using 75% RDF with HS-1 maintained nutrient availability, suggesting that humic compounds can increase nitrogen-use efficiency while reducing the need for fertiliser.

An experiment by **Noveyri et al. (2022)** at Birjand University in 2018 assessed the effects of calcium nitrate and putrescine on sesame under different moisture conditions. Foliar sprays of calcium nitrate (0, 5, 10 mM) and putrescine (0.5 mM) were applied in conjunction with irrigation at 100%, 75%, and 50% of the water requirement. The combination of moisture stress and both inputs considerably impacted grain yield, proline, capsule number, and chlorophyll characteristics. While foliar spray of 10 mM calcium nitrate with putrescine produced the best thousand-seed weight and grain yield, showing considerable physiological improvement under moisture limitation, severe drought (50%) drastically decreased production.

Salama (2023) investigated the effects of foliar applications of putrescine and benzyladenine on chia under water stress in an agronomic study conducted in 2020–2021 and 2022–2023. Compared with full irrigation, irrigation at 70% and 40% ET_c significantly reduced growth and yield parameters, seed oil percentage, and unsaturated fatty acids. Nevertheless, under both stress levels, the use of putrescine (25 ppm) and benzyladenine (50 ppm), either separately or in combination, markedly enhanced growth traits, yield parameters, water-use efficiency, and oil quality.

Desoky et al. (2023) demonstrated that foliar application of polyamines, particularly putrescine, effectively mitigated drought stress in sesame under 75% and 50% ET_c irrigation. Putrescine enhanced chlorophyll content, gas exchange, water status, nutrient uptake, antioxidant activity, osmoprotectant accumulation, vascular development, oil content, seed yield, and water productivity while reducing oxidative stress, highlighting its potential for improving sesame performance under arid conditions.

Gholipour (2020) conducted a field study on the effects of putrescine, calcium nitrate, or moisture levels on the amino acid content and biochemical characteristics of sesame during drought. Moisture stress significantly reduced grain yield and altered the concentrations of protein, soluble carbohydrates, proline, lysine, and methionine. The

combination of 10 mM calcium nitrate and 50% water, containing putrescine, produced the highest methionine level, suggesting significant stress-mitigating effects. Meaningful relationships among putrescine, calcium nitrate, and moisture level were demonstrated, showing how they act in concert to increase protein content and osmolyte levels. Overall, sesame's biochemical performance was enhanced, and the effects of moisture stress were successfully mitigated by foliar application of calcium nitrate and putrescine.

Shah et al. (2023) examined how three mustard cultivars' growth, physiology, nutrient uptake, and yield characteristics were affected by six plant growth regulators (IAA, EBL, GA₃, putrescine, salicylic acid, and triacontanol) in a field setting. Compared with the water control, foliar sprays applied at 50 and 70 DAS significantly improved all evaluated characteristics. The PGR that most strongly increased biomass, photosynthetic rate, stomatal characteristics, seed yield, and oil yield was 24-epibrassinolide, especially in the cultivar Nath Sona. Overall, the study showed that exogenous PGR treatment, particularly EBL, significantly improved mustard's morphological and yield performance.

Luiz et al. (2022) reported that humic acid, particularly when combined with the plant growth-promoting bacteria *Azospirillum brasilense* and *Bacillus subtilis*, enhanced white sesame germination and early seedling growth. The combined treatment significantly improved germination, root and shoot length, root biomass, and seedling vigour, demonstrating the potential of humic acid–microbial integration for better crop establishment.

In a split-plot factorial study at Shahed University in Tehran, **Ayoubizadeh et al. (2020)** assessed the impact of drought stress and spray application of the fulvic acid & iron nano-chelate on the production and quality of oil of two sesame varieties. While full irrigation and coupled foliar feeding produced the highest yields, drought conditions dramatically reduced grain yield and oil content. Halil demonstrated superior agronomic characteristics, including biological yield, seed weight, and capsule number. While full irrigation improved palmitic and stearic fractions, irrigation cut-off increased

oleic and linoleic acids. Cultivar-specific responses and the beneficial effects of fulvic acid and nano-chelate under water stress were highlighted in the study.

El-Khayat (2024) investigated the impact of foliar applications of the humic acid & micronutrients on growth, chemical composition, and oil production of borage (*Borago officinalis* L.) throughout two seasons at Benha University. In addition to producing the highest levels of nitrogen, phosphate, potassium, and carbohydrates, humic acid treated at 4 kg/fed, when combined with Fe at 100 ppm, significantly improved vegetative attributes, such as branch number and herb biomass. Similarly, inflorescence biomass, seed production, and fixed oil % were all markedly enhanced by humic acid at 4 kg/ha with zinc at 100 ppm. GC-MS analysis revealed a substantial increase in oil components upon the application of humic acid and Fe relative to the control.

Javed et al. (2022) examined the effects of several humic acid treatment techniques on mung bean field performance at the University of Agriculture, Faisalabad. Various dosages of soil application, foliar spraying, and seed priming were among the treatments. In terms of pod-bearing branches, pod quantity, seed weight, plant height, yield, and protein content, adding 4 kg ha⁻¹ of humic acid to the soil outperformed all other treatments. In every aspect, untreated seeds exhibited the lowest values. The study found that the most effective method for increasing mung bean growth, yield, and quality was to apply 4 kg ha⁻¹ of humic acid directly to the soil.

The Effect of Humic Acid and Putrescine on Biochemical and Physiological Changes in Leaves of Sesame

Lotfi et al. (2015) investigate the physiological & biochemical reactions of mustard to HA during stress. The results demonstrated that mustard plants treated with 0.006 g/L HA had the highest ascorbate peroxidase activity. The soluble protein content of plants treated with HA was 42, 8.5, and 15% higher than that of control plants under well-watered, mild water stress, & severe water stress, respectively.

Nouriyani (2019) performed an agronomic experiment to examine the local cultivar of Dezful. Humic acid 1.5% was found to be the most efficient seed priming treatment for

enhancing germination, biochemical alterations, and seedling growth of sesame. Humic acid 1.5% seed priming performed noticeably better than the control.

Ghassemi-Golezani et al. (2019) conducted an agronomic study in which foliar sprays containing putrescine and salicylic acid exhibited significantly higher antioxidant enzyme activities, water content, membrane integrity, and chlorophyll content than other treatments.

Mizani et al. (2025) demonstrated that foliar application of fulvic acid and nano-potassium improved sesame yield, oil quality, and drought tolerance. Under drought stress, these treatments enhanced antioxidant activity, proline accumulation, lignan content, and fatty acid composition, while the combination of 1.5 ppt nano-potassium with chitosan maximized seed yield and 2.5 ppt nano-potassium with fulvic acid produced the highest oil content.

Forotaghe et al. (2021) reported that humic acid application enhanced the biochemical and antioxidant responses of plants under deficit irrigation (80–60% FC). Humic acid increased protein, proline, soluble carbohydrates, mineral uptake, and antioxidant enzyme activities (SOD and POD), thereby improving stress tolerance and supporting plant performance under water-limited conditions.

Shahdadi et al. (2025) assessed the effects of humic acid, chemical fertilisers, and biofertilisers, applied separately and in combination, on the growth, yield, and grain protein content of sesame under three irrigation regimes (40, 60, and 80% wetness). Nutrient treatments and watering levels had significant effects on plant height, number of sub-branches, number of leaves, number of capsules, weight per 1000 grains, biological production, grain production, and harvest index. By improving water-use efficiency and cell development, the combined treatments of chemical fertiliser, mycorrhizae, and humic acid increased growth, yield components, and grain protein. In contrast, reduced irrigation (80% moisture) significantly reduced all growth and yield traits compared with 40% moisture.

Experiments by **Nassiri-Mahallati & Jahan (2020)** assessed sesame (*Sesamum indicum* L.) performance in an arid environment under limited irrigation,

superabsorbent polymer (SAP), and foliar-applied humic acid (HA). A split-strip plot design was used to arrange the treatments, which included full and deficit irrigation, SAP (80 kg ha⁻¹), HA (6 kg ha⁻¹), and control. The results demonstrated that SAP effectively increased soil water content, canopy cover, biomass, seed yield, water-use efficiency, and harvest index under deficit irrigation. The benefits of SAP and HA as environmentally friendly inputs for improving sesame growth, yield, and water productivity under water-limited conditions were confirmed by AquaCrop model simulations, which closely matched observed values ($R^2 = 0.88$ and 0.92).

Weerasekara et al. (2021) studied the effects of priming soybean seeds with humic acid (HA) on germination, vigour, and seedling performance under controlled environmental conditions with soil moisture levels of 50 and 80 % of field capacity. Primed seeds that were stored for a year were treated with 0.2 g/L HA for 1-7 hours. Compared with non-primed seeds, five-hour priming improved germination traits, seedling emergence, and vigour index; decreased lipid peroxidation and electrical conductivity; and increased antioxidant enzyme activities (CAT and POD). The treatment improved the vigour and emergence of slightly aged soybean seeds and encouraged consistent field establishment and greater seedling growth, suggesting that HA priming successfully reduces storage-induced degradation.

A factorial RCBD with three replications was used in **Muhammadamin & Mahmood's (2023)** field study at Grdmala and Grdarasha, Erbil, in 2021 to evaluate the effects of two nipping practices on the development, production, and standard of sesame (Somar genotype) after five different fertiliser treatments (Control, suggested NPK, Nano-NPK, Humic acid, and Nano-NPK + Humic acid). Seed yields were highest (3.23 and 2.45 t ha⁻¹) in the Nano-NPK+Humic acid and Nano-NPK combinations, and lowest (2.41 and 1.43 t ha⁻¹) in the control plots. Yields were improved by nipping, and the highest seed yield per plant and oil content (57.71–59.49%) was obtained when Nano-NPK + Humic acid was combined with nipping. Across most metrics, Grdmala outperformed Grdarasha.

Salamati et al. (2024) examined the effects of foliar-applied amino acids and soil-applied HA on the morphophysiological traits, yield, and water productivity of sesame

(Darab1 and local cultivars) under drip irrigation in a field experiment conducted in Behbahan, Khuzestan, in 2021–2022. The findings showed that, compared with the control, biostimulant treatments significantly improved all examined features. The efficacy of biostimulants was demonstrated by regression analysis, which revealed that seeds per capsule and capsules per plant had the most positive effects on yield and water productivity.

A study by **Raj et al. (2025)** analysed the contributions of putrescine and HA to the management of sesame phyllody, a phytoplasma-caused phloem disease spread by plant hoppers. Evaluations of disease progression were conducted 10, 20, and 30 days following the third spraying, using different combinations of the two growth regulators. Humic acid (1000 ppm) and putrescine (150 ppm) yielded the most significant disease suppression, followed by humic acid (500 ppm) and putrescine (50 ppm). These treatments can significantly reduce disease incidence and improve sesame plant health.

The **Hussein et al. (2023)** study analysed the effect of putrescine priming or drought tension on wheat growth, seed yield, and physiological characteristics. Using 50% and 100% water irrigation, putrescine at concentrations of 0.25, 0.5, and 1 mM was used to prime wheat seeds. On the other hand, putrescine priming, particularly at one mM, improved yield components, growth, root and shoot biomass, carotenoids, soluble sugars, amino acids, phenols, and chlorophyll a and b under both normal and water-deficient conditions. According to these findings, putrescine enhances wheat performance and successfully reduces the effects of drought.

Ghareeb et al. (2024) found that foliar application of benzyladenine (BA), yeast extract (YE), and mixed amino acids (MAA) significantly enhanced sesame growth, yield, and seed quality. These treatments improved vegetative growth, flowering, capsule formation, photosynthetic pigments, nutrient accumulation, protein, sugars, amino acids, and oil content, with MAA (4 mL L⁻¹) and YE (100 mL L⁻¹) producing the greatest improvements in seed oil percentage and yield.

Farsaraei et al. (2021) evaluated how putrescine seed priming affected the medicinal pumpkin's tolerance to salinity. The seeds were exposed to 0, 30, 60, and 90 mM NaCl after priming with 0-, 0.5-, or 1-mM putrescine. Reduced germination percentage,

seedling vigour index, fresh and dry weights of radicles and plumules, tissue water content, photosynthetic pigments, and α -amylase activity were observed in plants subjected to salinity stress. In contrast, increased mean germination time, proline, and malondialdehyde content were observed. Priming with putrescine, particularly at one mM, significantly improved salinity-related seedling growth and germination. Correlations between the seedling vigour index and proline, malondialdehyde, and mean germination time were negative, while correlations between the index and germination percentage were positive.

Mahmoud & Abdelhameed (2022) investigated the impact of heat stress on sesame in El-Wadi El-Gedeed using modified titanium organic frameworks (MIL-125-NH₂) that contained polyamines such as spermidine (Spd), spermine (Spr), and putrescine (Put)—applying chemicals to plant parts by spraying reduced lower oil content and heat-induced losses in seed production. Seed weights rose 1.39, 1.92, and 2.16 times compared to the control group after receiving Put@MIL-125-NH₂, Spr@MIL-125-NH₂, and Spd@MIL-125-NH₂ treatments, respectively, which led to significant improvements in yield, growth, and biochemical composition. The results demonstrated that these compounds had the potential to enhance sesame yields under hot weather.

Kochi et al. (2025) investigated how drought stress affected sesame plants that were treated with foliar sprays of salicylic acid (SA) and jasmonic acid (JA). The irrigation schedules included depleting the soil moisture by 25%, 50%, and 75%; the PGR treatments included SA (0.5, 1, 1.5 mM), JA (50, 100, 150 μ M), and a control. Antioxidant enzyme activity and oxidative stress markers were both elevated during periods of severe drought, which also reduced the relative water content. Using 1.5 mM SA as a foliar spray improved seed yield, chlorophyll, carotenoids, and CAT activity, while 100 μ M JA increased proline content and decreased ion leakage. Both PGRs significantly reduced drought-induced oxidative damage, thereby increasing sesame's drought resistance.

Aghajanzadeh et al. (2025) evaluated the effect of putrescine (Put) on mitigating zinc (Zn) toxicity in canola (*Brassica napus* L.). Both with and without 0.2 mM Put, plants

were subjected to 10 μ M Zn. Toxic levels of zinc lowered chlorophyll a and b contents and decreased shoot biomass by 70% and root biomass by 63%, respectively. In addition to nitrate and total nitrogen concentration, essential genes involved in nitrogen metabolism (NR, GS2, NRT2.1, and NRT1.5) were silenced. By reducing Zn accumulation, increasing pigments, protein biosynthesis, and nitrogen uptake, and upregulating genes involved in nitrogen metabolism, exogenous Put alleviated Zn stress and improved plant development under Zn toxicity.

Movahedi et al. (2025) aimed to determine the effects of inoculating lentil (*Lens culinaris* Medik.) seeds with *Pseudomonas fluorescens* and applying foliar spermidine at 0, 0.5, and 1 mM on lentil development and stress tolerance in dryland settings. The most significant improvements in plant height, leaf area index, chlorophyll content, proline, CAT, POD, SOD, and grain yield were observed when *P. fluorescens* was inoculated alone. The results show that using *P. fluorescens* is a long-term, effective strategy to enhance lentil drought resistance and yield.

Ghasemi et al. (2023) assessed the effects of varying irrigation levels and mycorrhizal inoculation on Yekta and Naz, two sesame cultivars. While mycorrhizal colonisation, oil content, and seed output were negatively affected by water deficiency, antioxidant activity, total phenols, and flavonoids were positively affected. Oleic, palmitic, and stearic acids were all elevated by water stress, whereas oleic and linoleic acids were enhanced by inoculation. Even under dry conditions, mycorrhizal treatment can increase sesame yield and the levels of beneficial compounds.

The Effect of Humic Acid and Putrescine on Oil Quality of Sesame

A field experiment conducted by Dehsheikh et al. (2020) evaluated the effects of humic acid and biofertilizers on the quality, quantity, and microbial activity of Thai basil essential oils. The findings indicated that the application of biofertilizers, soil organic matter, essential oil content, yield, and nitrogen element all had positive effects.

Vani et al. (2017) performed agronomic research in 2013 to determine how integrated nitrogen management affected the yield characteristics and quality of summer sesamum grown on sandy loam soil. It was shown that using 100% RDN resulted in a

substantially increased seed and oil output, comparable to using 100% RDN plus a 1% foliar spray of humic acid.

Choudhary et al. (2017) researched how organic matter and mineral fertilisers affected the quality and economics of sesame. By incorporating organic manure FYM @ 5 t/hac + vermicompost @ 2.5 t/hac or mineral nutrients S via 20 kg + Fe via 10 kg + Zn via 5 kg/hac, the findings showed a significant improvement in oil content, protein content, and oil output compared to other treatments.

Seervi et al. (2018) performed an agronomic study that examined the impact of foliar sprays of micronutrients on sesame crop (*Sesamum indicum L.*) yield, yield characteristics, and oil content. T9, which contained a combination of micronutrients, outperformed the other treatments in both yield and oil content. Similarly, T9 (a combination of all the micronutrients) had substantially greater yield metrics and oil content than the control, which had significantly lower values.

Farzi Aminabad et al. (2021) conducted an agronomic experiment on the effects of water deficiency and growth regulators on safflower grain and oil yields. Foliar application of putrescine, which dramatically increased yield and oil content, was the most effective treatment for increasing safflower production under both normal and stressful conditions.

Alizadeh et al. (2022) reported that foliar application of humic acid (1.5 g L⁻¹) significantly improved rapeseed yield, oil content, and fatty acid composition under both timely and delayed planting. Humic acid enhanced oleic and linoleic acid levels while reducing erucic acid and glucosinolates, demonstrating its effectiveness in improving yield and oil quality, particularly under optimal planting conditions.

In their two-year field study in Egypt, **Nassar et al. (2020)** investigated how potassium humate (KH) affected sesame growth, seed production, and seed quality in alluvial soils, either alone or in conjunction with iron, zinc, and manganese. The most significant changes in photosynthetic pigments, yield attributes, and biochemical quality were observed after three foliar spray applications, although two or one were

also effective. A quadruple mixture of KH, Fe, Zn, and Mn was the most effective treatment, followed by triple and dual combinations; KH alone had the lowest results. To maximise sesame performance, it was best to spray the leaves three times with KH, then apply all three micronutrients simultaneously.

In a split-factorial field experiment, **Kazemi and Eskandari (2024)** studied the effect of HA and Si on sesame plants in response to water stoppage during blooming. Water stress increased seed protein and decreased capsule number, seed set, plant height, weight per 1000 grains, and oil content. Compared with untreated plants, plants treated with 6 kg ha⁻¹ of foliar-applied humic acid showed significant increases in seed yield, plant height, biological yield, oil percentage, and protein content. Similarly, critical yield and quality attributes were improved with two mM silicon. Under stressful conditions, a combination of 6 kg ha⁻¹ of humic acid and two mM silicon significantly enhanced water productivity while simultaneously increasing oil and protein yields.

In a two-factor experiment conducted in Iran, **Nargesi et al. (2022)** studied the impact of foliar sprays of organic and amino acids on the biochemical characteristics of three different olive varieties. Combinations of arginine, glutamine, humic acid, and fulvic acid were used as treatments. 'Arbequina' had the highest oil content, while 'Zard' had the best Brix value, protein, carotenoids, anthocyanins, and phenols, according to the results. Humic acid alone maximised phenols and anthocyanins, whereas chlorophyll and protein were both improved by the addition of arginine. The maximum oil content was produced by the combination of "Arbequina × glutamine," according to the interaction effects. When it came to increasing oil yield in Arbequina olives, foliar glutamine spray was the clear winner.

In a two-year factorial split-plot study conducted in Karaj, Iran, **Amiri et al. (2020)** examined the effects of humic acid (0.3% concentration) and plant density on the qualitative characteristics of six different rapeseed varieties. Seed production, oil output, canopy temperature, fatty acid composition, glucosinolate content, and humic acid application were significantly affected by interactions between plant density and cultivar. Jerry, when grown at a density of 40 plants per square metre and treated with humic acid, yielded the most oil and seeds, and had the highest concentrations of oleic,

linoleic, and palmitic acid. Jerry was determined to be the best cultivar for the conditions studied due to its low levels of erucic acid and glucosinolates.

Khuong et al. (2022) reported that foliar application of B at concentrations of 0, 50, 100, 150, and 200 mg L⁻¹ significantly improved growth, yield components, and oil accumulation in black sesame. Plant height, leaf number, chlorophyll content, and pod development were all highest at 150 mg L⁻¹, with 46.2 pods plant⁻¹, compared with 27.2 in the control. The seed oil content improved by 5.3%, and the grain production increased to 1.10-1.32 t ha⁻¹ when compared to the control treatment. Based on the findings, the optimal B concentration to increase sesame growth, fruit set, yield, and oil content is 150 mg L⁻¹.

Jalilian et al. (2022) examined the impact of organic fertilisation on sesame. They found that soil micronutrient status and crop yield were significantly improved when green manures were mixed with animal manure at rates of 0, 10, and 20 t ha⁻¹. Fenugreek green manure had the most significant impact on improving micronutrient availability, whereas applying 20 t ha⁻¹ of manure increased soil Cu, Zn, Mn, and Fe by 27, 22, 7.5, and 6% relative to the control, respectively. Use of 20 t ha⁻¹ manure and fenugreek together resulted in the best dry matter, grain yield (1929.4 kg ha⁻¹), and oil production compared to unfertilised plots, demonstrating a 44-52% increase. This further supports the idea that organic amendments play a favourable role in sesame farming.

Mabudi Bilasvar et al. (2022) conducted field experiments in 2017 and 2018. They found that rapeseed's biochemical and physiological responses were markedly enhanced when exposed to exogenous SA (1 mM) and Put (1 mM) under different watering regimes. Osmolytes such as glycine betaine and proline, as well as soluble sugars, were found to increase in response to water stress. The introduction of SA, along with Put compounds, further enhanced osmolyte accumulation while decreasing proline levels. The duration of seed filling, dry matter, and yield were all improved by both treatments, particularly under drought. By extending seed filling and increasing the

unsaturation index, SA and Put increased oil content, even when water stress reduced oil accumulation and the levels of unsaturated fatty acids. When tested under stressful conditions, SA spray significantly increased seed and oil yield.

The study by **Alsajri et al. (2024)** compared potassium chloride with potassium sulphate and found that plants treated with potassium sulphate were taller, heavier, more branched, with more capsules, heavier seeds, higher yields, and higher oil content. Increasing potassium levels, particularly to 300 kg ha⁻¹, resulted in improved growth and yield. Higher soil gypsum content (15.87%) also improved several agricultural characteristics relative to soils with lower gypsum levels. The interactions among potassium supply, potassium level, and gypsum content were significant for all the factors examined. The results show that higher doses of potassium sulphate can improve sesame growth in soils containing gypsum.

Ninama et al. (2025) examined the effects of clipping, plant growth regulators, and weed management strategies on the performance of summer sesame in medium-black calcareous soils. Clipping at 45 DAS, together with foliar application of GA₃ (200 ppm at 50 DAS), significantly improved plant height, biomass accumulation, and branching. Additionally, the application of pendimethalin (50 g ha⁻¹ PE) followed by quizalofop-p-ethyl (0.05 kg ha⁻¹ PoE) was more effective in reducing weed populations, thereby enhancing crop growth and yield. The results show that combining timely trimming, GA₃ spraying, and effective weed management can significantly improve the development and yield of summer-grown sesame.

Edalateyan Kharazi and Asgharzadeh (2023) examined the effects of topical applications of SA, putrescine, and spermidine to *Nigella sativa* leaves on plant development, production, and oil-related characteristics under drought stress. Full irrigation, which uses 100% of the field capacity, always yields the highest values, whereas drought gradually lowers viable capsules, seed number, biomass, and yield. When foliar salicylic acid was applied at one mM, drought-induced damage was effectively reduced, and plant height, dry weight, oil content, and seed yield were significantly improved. In the presence of 1 mM salicylic acid, the essential oil content

rose in response to extreme stress. The best therapy for increasing growth and productivity was found to be complete irrigation with salicylic acid.

The Effect of Humic Acid and Putrescine on the Economics of Sesame

Anaz et al., (2021) investigated the response of different canola (*Brassica napus* L.) genotypes to foliar application of humic acid under varying plant population densities. The study demonstrated that humic acid significantly enhanced vegetative growth, yield components, and seed yield across different planting densities. In addition, treated plants recorded improved resource-use efficiency and superior economic returns compared with the untreated control, highlighting the potential of humic acid as an effective biostimulant for sustainable canola production.

Hatami (2017) evaluated the combined effects of humic acid (HA) and zinc (Zn) application on sunflower growth and productivity under drought-stress conditions. The findings revealed that humic acid markedly alleviated the adverse effects of water deficit by improving plant growth, nutrient uptake, and yield attributes. Consequently, plants receiving humic acid produced significantly higher seed yield and better economic returns than the control and other treatment combinations, demonstrating the beneficial role of humic acid in enhancing sunflower productivity under limited water availability.

Choudhary et al. (2017) investigated the effects of organic compost and mineral nutrients on sesame quality characteristics and economic outcomes during the kharif season. The results indicated significantly elevated protein content, oil production, and net income (₹ 45531 M3) or (₹ 47646 T3) when organic manures were applied, specifically FYM at 5 t/hac combined with vermicompost at 2.5 t/hac, alongside mineral nutrients S at 20 kg, Fe at 10 kg, and Zn at 5 kg/hac.

Farzi Aminabad et al. (2021) conducted a field experiment to assess the effects of exogenous SA and Put on rapeseed performance under varying levels of water deficit. Compared with other treatments, salicylic acid and putrescine had a substantially greater impact on rapeseed yield and economics.

Wei et al. (2013) conducted an agronomic study to investigate the morpho-anatomical and physiological responses of sesame (*Sesamum indicum* L.) to waterlogging stress. Waterlogging markedly affected plant growth, root development, photosynthetic efficiency, and overall plant performance. However, sesame plants exhibited an increase in endogenous putrescine levels as part of their adaptive response to flooding stress. Elevated putrescine accumulation helped maintain cellular integrity, improved antioxidant defense mechanisms, and enhanced physiological tolerance to waterlogging. These beneficial effects contributed to better plant growth, improved yield performance, and greater economic returns compared with untreated plants, indicating the important role of putrescine in mitigating waterlogging-induced damage in sesame.

In a two-year field study conducted in Egypt, **Ewais et al. (2025)** investigated the profitability and efficiency of land use by applying humic acid and various intercropping patterns involving peanuts and sesame. Higher doses of humic acid (8 g L⁻¹) applied to the leaves significantly increased the land equivalent ratio (LER) in comparison to smaller levels. When the maximum humic acid rate was used, the intercropping system consisting of 100% peanuts and 100% sesame yielded the highest economic returns and LER. Net profitability and land-use values (up to 1.90) were significantly higher with this treatment than with peanuts grown alone. Overall system productivity and intercropping performance are both improved by humic acid, as indicated by the results.

The long-term experiment conducted by **Sangeetha et al. (2025)** from 2020 to 2023 examined the organic cultivation of sesame stalk compost. The results showed that the compost significantly improved soil health and crop performance. Fertilised composts contain more nutrients and humic substances than unfertilised composts. Compost derived from sesame stalks, treated at a rate of 5 t ha⁻¹, enhanced soil organic carbon, reduced bulk density, significantly reduced penetration resistance, and adjusted pH. More than only higher grain protein, mineral content, and oil output, the yield achieved with compost (about 780 kg ha⁻¹) was on par with that of artificial fertilisers. An eco-

friendly option that maintains high productivity and soil quality, sesame stalk compost was singled out in the study.

Kishk (2024) examined the effects of biostimulants, harvest timings, and storage durations on the sesame cv—Shandawil 3 presentation. Compared with the impact of spraying either biostimulants, alone or vinasse, the mixture of spirulina and chlorella had a much greater effect on plant growth, branching, capsule quantity, seed yield, and seed quality. Compared with earlier harvests, growth and yield parameters were higher when the crop was harvested at 120 days. Optimal germination and seed quality were maintained for storage durations of 0-6 months, whereas vigour was diminished beyond 18 months. Highlighting their potential for sesame enhancement in reclaimed areas, the interaction of Spirulina + Chlorella with the optimal harvest date resulted in the highest biological and seed productivity.

Harshitha et al. (2021) examined the effects of hormone therapies on mustard yields in sandy loam soils in Rajendranagar, Hyderabad. The treatments that yielded the best growth, yield, and economic returns combined GA_i at 45 ppm with 1.5% humic acid applied at two-day intervals during both stages. Then, the treatment that used the acid only during flowering ranked second. In Southern Telangana, the economic advantage of the flowering-only treatment was evidenced by its higher benefit-cost ratio, attributable to lower cultivation expenses. However, the combined-stage application produced the highest yield.

Aly & Abdelsalam (2025) studied the effects of priming seeds with selenium selenate and humic acid on the germination and behaviour of Brassica juncea seedlings. Salt accumulation increased, whereas germination, chlorophyll content, and hydration status were significantly reduced as NaCl concentration increased from 0 to 200 mM. Sericite priming of seeds at concentrations ranging from 0.5 to 1 mM significantly improved germination characteristics, seedling biomass, chlorophyll pigments, and K⁺/Na⁺ balance. Additionally, humic acid mitigated salt-induced ion imbalance and increased germination percentage, vitality, and root-to-shoot growth, particularly at a

5% concentration. In salty environments, early seedling performance was improved by both Se and HA treatments.

Chowardhara et al. (2022) examined how putrescine, ethanol, and acetic acid could reduce the harmful effects of cadmium on mustard seedlings. Increased oxidative stress and diminished antioxidant defence were observed following Cd (0.5 mM) exposure, which drastically reduced growth, biomass, and chlorophyll content. Improving seedling development, reducing lipid peroxidation, and the AsA-GSH cycle were all achieved through supplementation with putrescine (0.5 mM), ethanol (0.3-0.6%), and acetic acid (250 μ M). Although ethanol and acetic acid also considerably reduced Cd stress and enhanced tolerance mechanisms, the results showed that putrescine was the most effective treatment.

Sharma et al. (2023) examined how putrescine alleviates high-temperature stress in four mustard species. Electrolyte leakage, lipid peroxidation, and antioxidant enzyme activities were elevated in heat-exposed seedlings; nevertheless, relative moisture content, chlorophyll content, and developmental rate were significantly reduced. Tolerance was higher in RH-1566 and RH-1999-42, whereas sensitivity was higher in RH-1707 and RH-1708. By boosting growth features, improving hydration status, and strengthening antioxidant defence while minimising oxidative damage, the use of exogenous putrescine (1 mM) significantly reduced heat injury. The research demonstrated that putrescine is an effective stress reliever and highlighted that different varieties respond differently to heat.

A study by **Shah et al. (2023)** investigated the impact of six regulators of plant growth on the growth, physiological features, and yield of three *Brassica juncea* cultivars. Plants treated with IAA, 24-epibrassinolide, GA_i, putrescine, salicylic acid, and triacontanol at 50 and 70 DAS showed a considerable improvement in morphological characteristics, photosynthesis, nutrient uptake, and ultimate yield when compared to plants treated with water. Nath Sona was the cultivar that showed the most promise. In terms of dry matter accumulation, photosynthetic rate, stomatal characteristics, seed and oil output, and overall effectiveness, 24-epibrassinolide stood out among all treatments.

Kibar & Kibar (2025) investigated how salicylic acid and putrescine could protect cowpea from harm caused by salt. Exogenous administration of putrescine and salicylic acid considerably mitigated the effects of 100 mM NaCl on seed germination and growth. Seedling weight, height, & chlorophyll content were enhanced in procedures with 0.5 mM putrescine relative to salt-stressed controls, while procedures with 1.0 mM putrescine augmented plumule length & germination. The most effective treatments were 0.5- and 1.0-mM putrescine under salinity. These results provide further evidence that putrescine and salicylic acid can help cowpeas establish better in soils with high salinity by mitigating salinity stress.

Li et al. (2021) evaluate a multi-year field trial to assess the effect of humic acid on peanuts under continuous cropping. The rates of humic acid application at 50, 100, and 150 kg ha⁻¹ consistently increased yield and stress tolerance relative to untreated controls. The treatments increased chlorophyll content, nutrient uptake, and the levels of essential metabolic enzymes, with 150 kg ha⁻¹ demonstrating the most significant effects in many years. The activities of nitrate reductase, sucrose phosphate synthase, and antioxidant enzymes were elevated, whereas MDA levels decreased in both leaves and roots. Humic acid modified endogenous hormone concentrations. Humic acid significantly mitigated the effects of continuous cropping and enhanced peanut productivity.

Mahdavi et al. (2021) conducted a two-year study in Guilan province to examine the effects of changed irrigation levels and HA doses on peanut yield and its attributes. Irrigation treatments comprised rainfed conditions and the provision of 60%, 80%, and 100% of the crop's water requirements, administered at rates of 0, 3, 6, and 9 L ha⁻¹ in conjunction with humic acid. Rainfed circumstances without humic acid produced the lowest output (9750 kg ha⁻¹), in contrast to plants that were irrigated at 80% with 9 L ha⁻¹ of humic acid, which produced the highest biological yield (9750 kg ha⁻¹)—adding 9 litres of humic acid per hectare greatly improved seed yields. We found that the area's peanut productivity was highest when we applied 9 L ha⁻¹ HA and irrigated it at 80%.

Chaitra et al. (2024) conducted a field trial with thirteen different treatments. It was found that the combination of a 0.2% foliar spray applied 30 days after planting and 20 kg ha⁻¹ of humic acid from vermicompost produced the highest levels of plant length, leaf area index (LAI), and chlorophyll content. In terms of pod output, oil content, crude protein, and number of pods per plant, this treatment was the most productive. The research showed that groundnut growth, yield, and quality were significantly enhanced when humic acid from vermicompost was added, compared with conventional methods.

MATERIALS AND METHODS

An experiment titled "**Effect of Humic Acid and Putrescine Application on Growth, Yield, Oil Quality and Economics in *Sesamum indicum***" was carried out at Lovely Professional University field, Jalandhar, Punjab, during the *Kharif* season of 2023 and 2024. The following section of this chapter details the investigation's materials and methods. This chapter provides a concise overview of all materials and procedures, including the experimental site, soil characteristics, weather, treatments, and all agronomic operations. The findings of the OPSTAT statistical analysis on the experimental field data are shown both in table and graph form.

3.1 Experimental Site

3.1.1 Location of the Experiments

This experiment was carried out at the agricultural research farm of Lovely Professional University in Phagwara, Punjab, during the *Kharif* seasons of 2023 and 2024. Latitude 31°23'1.8" North, longitude 75°23'03.02" East, and an elevation of 252 meters above sea level are the coordinates of the research field (Figure 3.1.1).

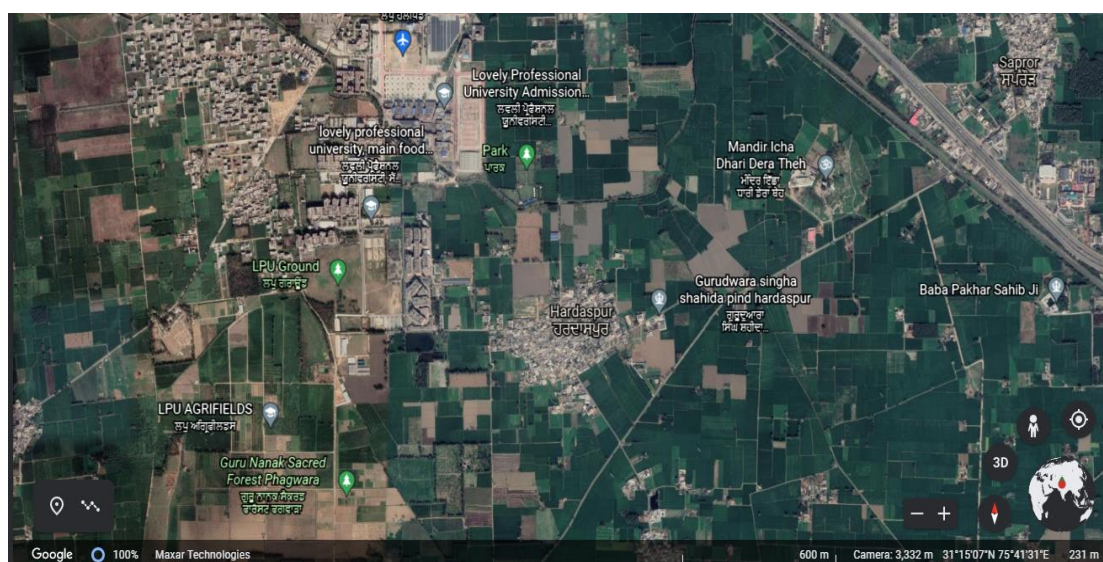


Figure 3.1.1 Source: Google Map

3.2 Experiment Design and Treatments Details

3.2.1 Experiment Design: The experiment was conducted using the Factorial Randomised Block Design. The total area needed for the experiment was approximately 1100m². The experiment involved 16 treatments and 3 replications, resulting in a total of 48 plots. All treatments were organised with randomisation to ensure distribution across the plots. The dimensions of each plot were 5.3m×4.3m, resulting in a total area of 22.7m². Separate irrigation channels, each measuring 1 metre in width, were established to facilitate individual irrigation for each plot.

3.2.2 Weather and Climate: The experiment's trial location observed varying conditions of hot and cold climates. The typical temperature reaches approximately 45°C. The weather parameters, including average maximum and minimum temperatures, rainfall, relative humidity, and evaporation rate, were sourced from the website throughout the season, from sowing to harvesting.

3.2.3 Properties of Soil: The various standard procedures assess soil properties. The determination of soil pH, soil electrical conductivity, and macronutrients was conducted using the following procedures.

Soil Ph: A pH meter is used to assess the soil's pH level. Soil samples were extracted from various depths in the field and stored in polyethylene bags. Measure 12.5g of soil into a 150ml beaker. The soil must be devoid of clods. Incorporate 50 ml of distilled water into the same cup. Agitate the soil solution for 30 minutes. Activate the pH meter and calibrate it using buffer pH solutions. Submerge the pH meter's probe in the soil solution and record the measurements (Sawarkar, 2012).

Soil EC (electrical conductivity): The electrical conductivity of the soil was measured using an EC meter. Use of 10g of air-dry soil were placed in a bottle, to which 50ml of distilled water were added, followed by shaking on a mechanical shaker for one hour to effectively dissolve soluble salts. The EC meter was calibrated using a 0.01M KCl solution prior to introducing the sample to the electrode. An electric conductivity cell was subsequently placed into the soil solution without disturbing the soil sample, and measurements were recorded (Sawarkar, 2012).

Soil organic carbon(%): The organic carbon content in the soil was quantified using the Walkley and Black Wet Oxidation technique (Jackson, 1973) and reported as a percentage.

Available Nitrogen Content: The nitrogen content of soil samples was assessed. The technique comprised three phases: digestion, distillation, and titration. In digestion-1 mg of the soil sample was placed in the digestion tube, followed by the addition of 10ml of concentrated sulphuric acid and 5g of catalyst combination, after which digestion commenced. The beginning temperature was 100°C, and efficient digestion commenced at 360°C. Upon completion of the digestive process, the hue transitioned to light green, ultimately resulting in a colourless solution. Subsequent distillation was conducted following the cooling of the digested material. One side hose should be immersed in 20ml of 4% boric acid solution, and 40 ml of NaOH was introduced into the distillation unit. Heat can transfer with the flow, and ammonia gas is absorbed by boric acid. The hue of the sample transitioned from pinkish to green. A blank sample was executed concurrently without the dirt. The final stage was conducting titration with 0.02N sulphuric acid. At the conclusion, the colour transitioned from greenish to pinkish once more. The volume of sulphuric acid utilised for titration was recorded (Upadhyay & Sahu, 2012).

Available Phosphorus: Olsen's approach is the most appropriate for estimating the available phosphorus in the soil sample. Introduce 1g of soil sample into a 150ml conical flask and incorporate a small amount of charcoal into the same flask. Subsequently, introduce 0.5N NaOHCO₃ into the same flask and agitate it using an electric shaker for thirty minutes. Subsequently, strain the material using Whatman's number 1 filter paper. From the filtered content, Transfer 5 ml of the solution into a 25ml volumetric flask. Conduct a parallel analysis of the blank solution. Subsequently, incorporate 0.5ml of 5N H₂SO₄ and agitate thoroughly until CO₂ evaluations are eliminated. Subsequently, introduce 4 ml of ascorbic acid and distilled water to the designated mark on the volumetric flask. Subsequently, combine the substances and assess the strength of the blue colour using a colorimeter at a wavelength of 760 µm, recording the measurements (Thakur, 2012).

Available Potassium: A flame photometer was utilised to measure the exchangeable potassium in the experimental soil. Measure 5g of soil sample and transfer it to a 50ml conical flask. Subsequently, introduce 25ml of ammonium acetate solution into the same conical flask and agitate thoroughly for five minutes. Pass the solution through Whatman's number 1 filter paper. Withdraw 5ml of the filtered content and transfer it to a 25ml volumetric flask. Prepare a standard potassium (K) solution with varying concentrations of 0, 2, 4, 6, 8, and 10 ppm. Introduce the prepared solution into a flame photometer and record the measurement (Baghel, 2012).

Table 3.1: Soil data of sesame during kharif season 2023-2024 & 2024-2025

Sr. No	Particulars	2023	2024
1.	pH	7.86	7.84
2.	EC (dS/m)	0.445	0.448
3.	Soil organic carbon (%)	0.34	0.37
4.	Available nitrogen (kg/ha)	241.54	242.14
5.	Available phosphorus (kg/ha)	23.4	23.7
6.	Available potassium (kg/ha)	148.2	148.9

3.3 Experimental Site and Details of the Experiment

3.3.1 Experimental Site: The field experiment was carried out at the Agronomy Research Farm, Department of Agronomy, Lovely Professional University, during the kharif seasons of 2023 and 2024. The experimental plots possess healthy soil with regular topography and consistent textural composition, and they are connected to the primary irrigation channel, which links to the tube well for efficient and timely irrigation. It possesses an effective drainage system for expelling excess water during experimentation.

3.3.2 Details of the Experiment: The field experiment was conducted in the kharif seasons of 2023 and 2024 using a Factorial Randomised Block Design (FRBD) with three replications and sixteen treatments. The following outlines the experimental details: Title: Effect of Humic Acid and Putrescine Application on Growth, Yield, Oil Quality and Economics in *Sesamum indicum*, Experimental Design: Factorial randomized block design (FRBD), Replications- 3, Treatment- 16.

3.3.3 Treatment Details: The chosen concentrations of humic acids (0, 500, 1000, and 1500 ppm) and putrescine (0, 50, 100, and 150 ppm) were guided by published reports which stated their efficacy in regulating crop growth, nutrient uptake, stress tolerance and yield in sesame and other oilseed crops. These dose levels present a gradient from control to high concentration to investigate dose-dependent responses and identification of the optimum combination under field conditions. Under the agro-climatic situation of the place of experiment, the experiment was conducted to observe the performance of various treatments under the actual practice of cultivation.

Table 3.2: Details of Treatments

Treatment	Details
Treatments (T ₁)	Humic acid (0 ppm) + Putrescine (0 ppm)
Treatments (T ₂)	Humic acid (0 ppm) + Putrescine (50 ppm)
Treatments (T ₃)	Humic acid (0 ppm) + Putrescine (100 ppm)
Treatments (T ₄)	Humic acid (0 ppm) + Putrescine (150 ppm)
Treatments (T ₅)	Humic acid (500 ppm) + Putrescine (0 ppm)
Treatments (T ₆)	Humic acid (500 ppm) + Putrescine (50 ppm)
Treatments (T ₇)	Humic acid (500 ppm) + Putrescine (100 ppm)
Treatments (T ₈)	Humic acid (500 ppm) + Putrescine (150 ppm)
Treatments (T ₉)	Humic acid (1000 ppm) + Putrescine (0 ppm)
Treatments (T ₁₀)	Humic acid (1000 ppm) + Putrescine (50 ppm)
Treatments (T ₁₁)	Humic acid (1000 ppm) + Putrescine (100 ppm)
Treatments (T ₁₂)	Humic acid (1000 ppm) + Putrescine (150 ppm)
Treatments (T ₁₃)	Humic acid (1500 ppm) + Putrescine (0 ppm)
Treatments (T ₁₄)	Humic acid (1500 ppm) + Putrescine (50 ppm)
Treatments (T ₁₅)	Humic acid (1500 ppm) + Putrescine (100 ppm)
Treatments (T ₁₆)	Humic acid (1500 ppm) + Putrescine (150 ppm)

T: Treatments

3.3.4 Layout:

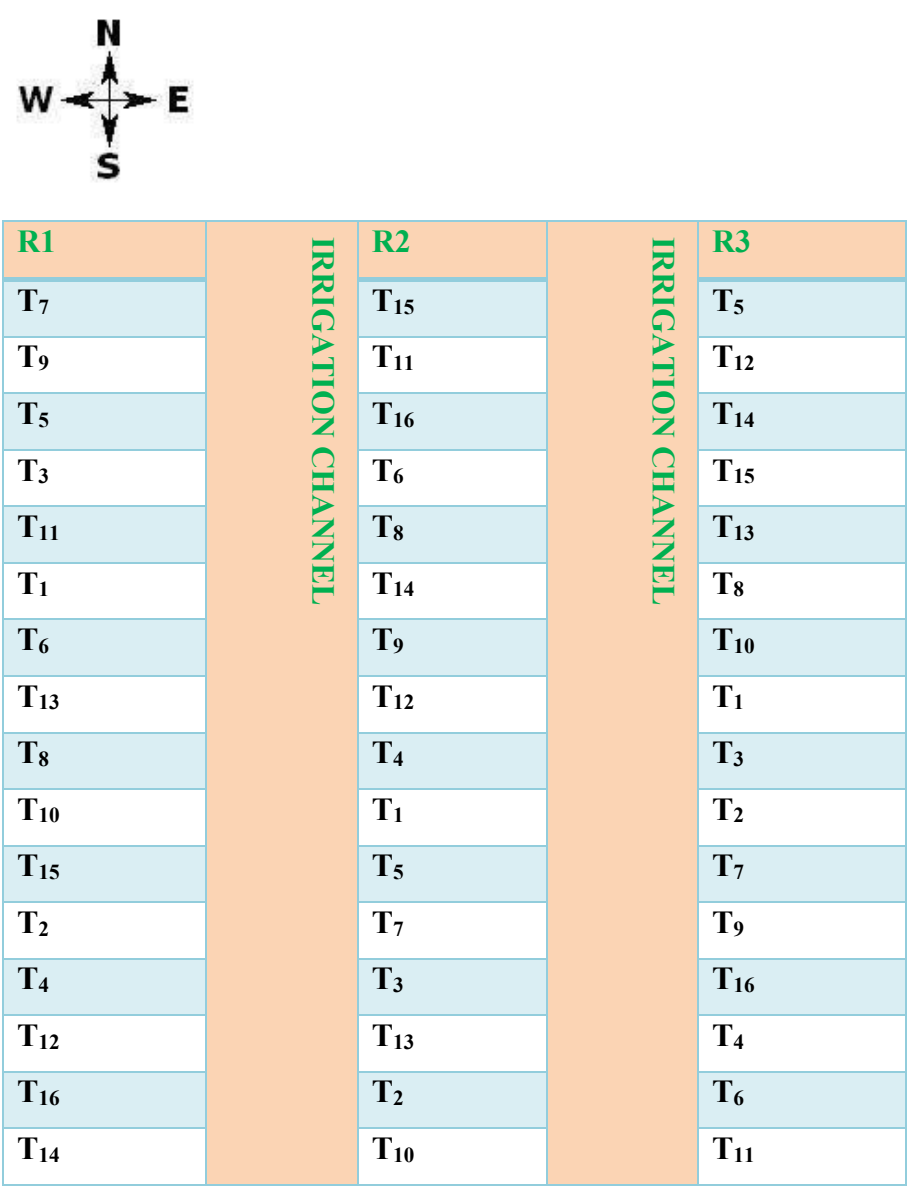


Figure 3.1.2

3.3.5 Details of Layout:

Table 3.3: Details of Layout

Sl. No	Particulars	Remark
1	Design	Factorial Randomized Block Design
2	No. of treatments	16
3	No. of replications	3
4	Total no. of plots	16x3= 48
5	Gross plot size	1100m ²
6	Net plot size	4.2x2.7m ²
7	Sowing method	Line sowing
8	Replication border	1.0m
9	Plot border	0.5m
10	Row-to-row spacing	30cm
11	Plant-to-plant spacing	15cm
12	No. of rows per plot	13
13	No. of plants per row	32
14	Variety	Punjab Till No. 2
15	Date of first sowing	25/07/2023
16	Date of first harvest	28/10/2023
17	Date of second sowing	20/07/2024
18	Date of second harvest	22/10/2024

3.3.6 Cultivation Details

Table 3.4: Cultivation Details 1st Trail

S. No	Operations	Date
1	Preparatory tillage	
	(a) Ploughing with tractor-drawn disc plough	19/07/2023
	(b) Followed by disc harrow and rotavator	19/07/2023
	(c) Planking	22/07/2023
2.	Layout	22/07/2023
3.	Fertilizer application	25/07/2023
4.	Sowing	25/07/2023
	Thinning	10/08/2023
5.	Intercultural operations	25/08/2023
6.	Irrigation	
	First	15/08/2023
	Second	10/09/2023
7.	Treatment Spray (First Trail)	
	(a) 15 DAS	09/08/2023
	(b) 45 DAS	09/09/2023
8.	Observation Taken (First Trail)	
	(a) 30 DAS	24/08/2023
	(b) 60 DAS	25/09/2023
	(c) At Harvest (90 DAS)	25/10/2023
9.	Harvesting	28/10/2023

Table 3.5: Cultivation Details 2nd Trail

S. No	Operations	Date
1	Preparatory tillage	
	(a) Ploughing with tractor-drawn disc plough	15/07/2024
	(b) Followed by disc harrow and rotavator	15/07/2024
	(c) Planking	18/07/2024
2.	Layout	18/07/2024
3.	Fertilizer application	20/07/2024
4.	Sowing	20/07/2024
	Thinning	08/08/2024
5.	Intercultural operations	18/08/2024
6.	Irrigation	
	First	20/08/2024
	Second	15/09/2024
7.	Treatment Spray (First Trail)	
	(a) 15 DAS	05/08/2024
	(b) 45 DAS	04/09/2024
8.	Observation Taken (First Trail)	
	(a) 30 DAS	19/08/2024
	(b) 60 DAS	20/09/2024
	(c) At Harvest (90 DAS)	20/10/2024
9.	Harvesting	22/10/2024

3.4 Weather Details of 2023 (July-October) and 2024 (July-October)

Table 3.6 Weather Details of 2023 (July-October) and 2024 (July-October)

	Temperature (°C)				Relative humidity(%)				Rainfall(mm)		Evaporation(mm)	
Date	Max 2023	Min 2023	Max 2024	Min 2024	Max 2023	Min 2023	Max 2024	Min 2024	2023	2024	2023	2024
1(Jul)-7(Jul)	34	25	33	26	85	62	88	70	12	0.5	2.9	4
8(Jul)-14(Jul)	32	25	36	27	88	69	90	62	10	0	3	6
15(Jul)-21(Jul)	35	27	37	28	86	66	88	64	3	0	3	5
22(Jul)-28(Jul)	33	26	35	27	91	74	89	72	12	0	2	5
29 (Jul)-4(Aug)	35	27	35	28	90	68	93	76	6	1.5	2	4
5(Aug)-11(Aug)	34	26	33	27	90	73	90	73	2	2.2	2	4
12(Aug)-18(Aug)	34	27	32	26	90	72	93	75	0	4.8	4	2
19(Aug)-25(Aug)	35	27	34	26	91	70	94	73	0	4	2	4.7
26(Aug)-1(Sept)	33	26	33	25	92	62	94	72	2	0.5	3	4.8
2(Sept)-8(Sept)	35	25	33	26	92	61	95	74	0	1.6	5	4.2
9(Sept)-15(Sept)	34	26	34	25	91	70	93	69	0	0.3	3	4.2
16(Sept)-22(Sept)	32	24	34	24	92	70	94	64	3	0	2	4.5
23(Sept)-29(Sept)	33	21	33	24	93	63	94	69	0	0.2	3	4.7
30(Sept)-6(Oct)	33	18	35	21	92	49	94	61	0	1.4	1	3.6
7(Oct)-13(Oct)	32	18	33	18	91	52	93	51	0.9	0	2	3.6
14(Oct)-20(Oct)	28	16	34	17	91	54	93	46	0	0	3	3.3

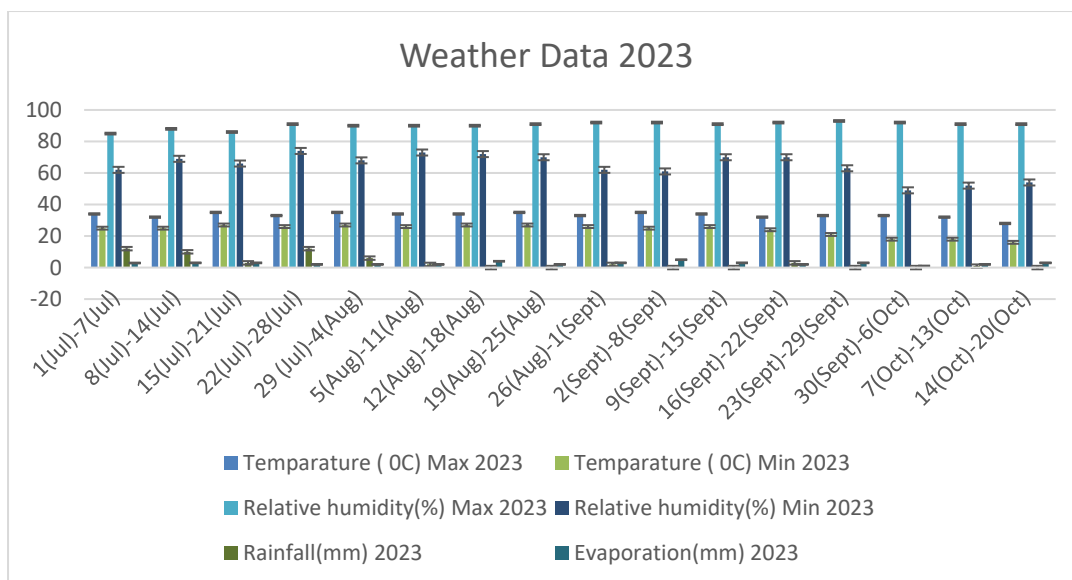


Figure 3.1.3: Weekly meteorological data during the crop growth (1July 2023 to 20 October 2023)

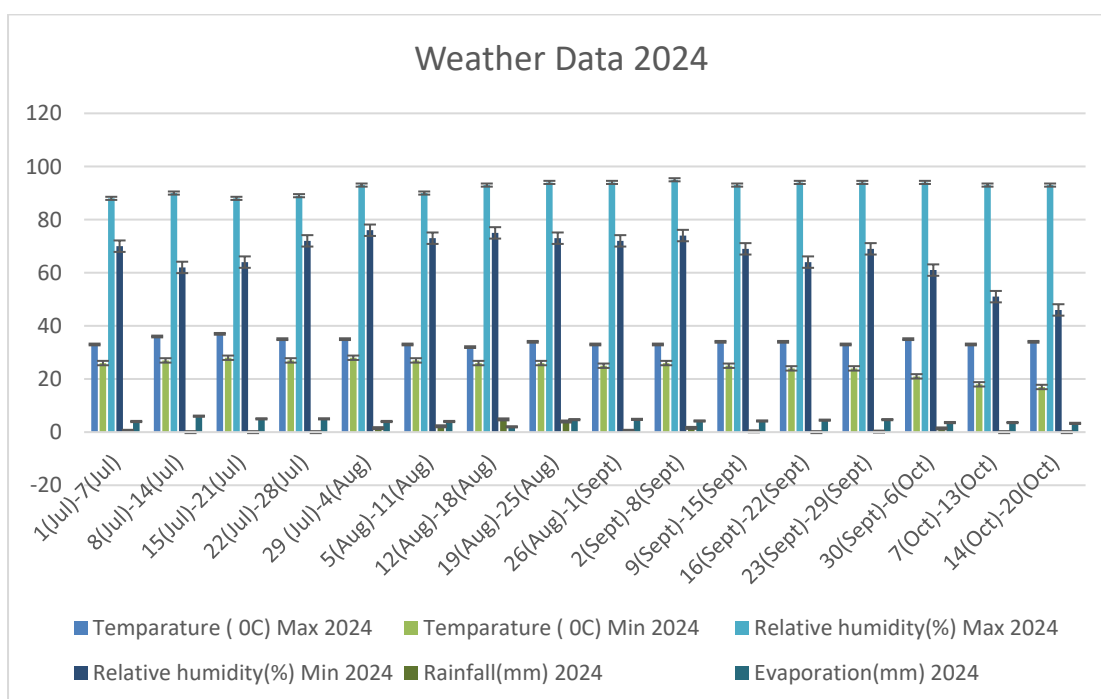


Figure 3.1.4: Weekly meteorological data during the crop growth (1July 2024 to 20 October 2024)

3.5 Cultivation Details: The experimental cultivation procedures are described in detail below.

3.5.1 Selection and preparation of field: The plot's level terrain and consistent fertility were key factors in its selection for the experiment. The ground was prepared for subdividing into plots according to the pattern and requirements after two rounds of deep ploughing, two rounds of cross harrowing with a tractor-drawn disc harrow, and a levelling operation.

3.5.2 Layout preparation: Following the specifications and the design, labourers use a disc plough to evenly divide the plots and prepare the trial layout.

3.5.3 Planting material: Sesame variety Punjab till no. 2 was selected for conducting this research. An authorised certified seed producer, "Punjab Agriculture University, Ludhiana, Punjab," was the source of the seed. No damaged or healthy seeds are sown.

3.5.4 Sowing: Each line of seeds was sown at a depth of 4-5 cm, with a spacing of 30x15 cm.

3.5.5 Description of variety: The plant is highly branched and produces dense capsules. Long, hairless capsules stand in a straight line across from one another. The white, assertive seeds contain 49% oil. Seeds are more palatable, have less crude fibre, and are tender. Tolerance to phyllody and cercospora leaf blight is present in this cultivar. On the other hand, it takes 90 days to reach maturity. The yield per acre for this cultivar is 2.8 quintals.

3.5.6 Application of fertilizers: Before planting, drill an area of 21 kg N (45 kg urea) per acre. Steer clear of over-manuring since it promotes high vegetative growth.

3.5.7 Treatment application: A knap-sack sprayer was used to apply the treatments as a foliar spray every 30 days. At 15 and 45 days after the planting, treatments were applied, and observations were taken at 30, 60, and 90 days after the crop was sown.

3.6 Cultural Operations after Sowing

3.6.1 Weeding: Three weeks after seeding, provide one hoeing. Within two days of seeding, a pre-emergence treatment of Lasso 50 EC (Alachlor) @ 3000 ml/ha dissolved in 500 litres of water was made.

3.6.2 Irrigation: The sesame crop needs very little irrigation. For the duration of the crop's life, one irrigation was given. Depending on the situation, because the monsoon season usually brings enough rainfall for the crop. Rainwater can negatively impact development; thus, it should never be left in the field for an extended period. Instead, it should be drained away.

3.6.3 Plant protection: Throughout the crop's existence, perfect care was taken to protect the plants. Some serious diseases and insect pests were noted throughout the crop season. When insect pests were observed in the experimental field, the sesame crop was given the proper attention at various intervals.

3.6.4 Harvesting: Plants should be harvested when they begin to turn pale. To prevent shattering, harvesting must be done promptly. To stack the plants, knot them into little bundles after harvesting. The entire produce can be collected with two shakes of the bundles.

3.6.5 Sampling technique: In each plot, five plants were chosen at random for data gathering, and they were tagged to facilitate identification so that intervalic observations could be made. 15 and 45 DAS were used for the treatments. Following the initial observation, which was made at 30 DAS, observations were made every 30 days, or 60 and 90 DAS. The final observation was statistically examined using OPSTAT software, and the average value of the recorded data was computed.

3.7 Observations Recorded

3.7.1 Morphological Parameters

3.7.1.1 Plant height (cm): Five plants were first chosen at random from each plot, and they were tagged to record the observations. Using a metre scale, the primary shoot length from ground level to the tip of the growth point was measured at 30, 60, 90 DAS, and harvest.

3.7.1.2 Dry matter accumulation per plant (g): After fresh weight, dry weight is obtained by air drying it in the sun for three to four days, and then it is placed in a hot air oven at 50⁰C for thirty-six hours to remove moisture. The average dry matter accumulation at various crop stages was calculated using the average of five plants. Weighing and observations were made after drying in a hot air oven.

3.7.1.3 Stem girth (mm): The Vernier calliper was used to measure the stem girth at intervals of 30, 60, and 90 DAS from the base of the plant to the tip of the stem. Each replicate's tagged plant's mean stem girth was computed and reported in centimetres.

3.7.1.4 Number of primary branches: To make observations, five plants were first chosen at random from each plot and tagged. Afterwards, at 30, 60, 90 DAS, and harvest time, the number of primary branches per plant was recorded.

3.7.1.5 Internodal length (cm): Five plants were first chosen at random from each plot, finding two nodes (the places where a leaf attaches to the stem) in a row on the main stem is the first step in measuring the internodal length of a plant. Next, measure the distance between the two nodes using a meter scale at 30, 60, 90 DAS, and harvest.

3.7.1.6 Number of leaves per plant: To make observations, five plants were first chosen at random from each plot and tagged. Next, at 30, 60, and 90 DAS as well as at harvest time, the number of leaves on each plant was counted.

3.7.1.7 Leaf area (cm²): Using a leaf area meter, the amount of a plant's canopy covered by its leaves was measured in the lab following leaf collection at 30, 60, and 90 DAS.

3.7.1.8 Days to 50% flowering: The "days to 50% flowering" is the number of days you recorded, which tracks the growth of the plants from the day of planting (or emergence) until half of the plants in your field or plot have at least one open flower.

3.7.2 Physiological Parameters

3.7.2.1 Leaf area index: Williams (1946) introduced the concept of Leaf Area Index (LAI). It is the proportion of crop foliage to the terrestrial area for a specified duration. The Leaf Area Index (LAI) should be optimal at the maximum ground cover area, where the crop canopy gets the greatest solar radiation.

$$LAI = \frac{\text{Total Leaf Area of a Plant}}{\text{Ground Area occupied by the Plant}}$$

3.7.2.2 Crop growth rate(CGR): The method was proposed by Watson (1956). The CGR indicates the dry matter collected per unit area per unit time (g m⁻² day⁻¹).

$$CGR = \frac{(W2 - W1)}{T2 - T1}$$

Where W2 & W1 are plant dry weight at time T2 & T1.

3.7.2.3 Relative growth rate(RGR): The term was introduced by Williams in 1946. Relative Growth Rate (RGR) quantifies the increase in total plant dry weight over a specified time interval relative to the initial weight, defined as the dry matter increases per unit biomass per unit time, or grammes of dry weight increase per gramme of dry weight, expressed as unit dry weight/unit dry weight/unit time ($\text{g g}^{-1} \text{ day}^{-1}$).

$$RGR = \frac{(\log_e W2 - \log_e W1)}{(T2 - T1)}$$

1. Where, W1 and W2 are whole plant dry weight
2. T1 and T2 respectively T1 and T2 are time interval in days

3.7.2.4 Net assimilation rate (NAR): Williams (1946) used the term NAR. NAR is defined as the increase of dry matter per unit of leaf area or per unit of leaf dry weight over a specified time. The NAR quantifies the average photosynthetic efficiency of leaves within a crop community.

$$NAR = \frac{(W2 - W1)}{T2 - T1} \times \frac{(LNA2 - LNA1)}{A2 - A1}$$

1. Where W₁ and W₂ are the dry weight of the whole plant at time T₁ and T₂, respectively,
2. A₁ and A₂ are leaf weights of leaf area at t₁ and t₂, NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time ($\text{g m}^{-2} \text{ day}^{-1}$).

3.7.2.5 Days to physiological maturity: As the capsule began to mature, the number of plants/plots was recorded every other day. Upon the maturation of the capsule, the number of plants was recorded in relation to the total plant populations of each plot within the designated area, and the total days were determined by counting the interval from sown to maturity. The days to maturity were calculated based on the matured capsules for each individual plot.

3.7.3 Biochemical Parameters

3.7.3.1 Chlorophyll Index (SPAD): Chlorophyll was quantified with a SPAD meter at 30, 60, and 90 days after sowing (DAS). The concentration of chlorophyll can be assessed by measuring the same leaf three times using a SPAD meter and calculating the average of the results (Arregui, 2006).

3.7.3.2 Total soluble sugar [Sadasuvam and Manickam (1992)]: Using a technique suggested by Sadasuvam and Manickam (1992), we were able to measure the total soluble sugar content of the plant sample. To quickly and easily determine the total soluble sugar in the plant sample, one can use the anthrone reaction as a foundation. Furfural is formed by dehydrating carbohydrates with concentrated sulfuric acid. When furfural and anthrone combine, a blue-green complex is formed, which may be detected calorimetrically at 630 nm.

Reagents:

1. Ethanol (80%)
2. Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% sulphuric acid. Prepare fresh before use.
3. Standard glucose: Stock-dissolve 100mg of glucose in 100ml water, working standard-10 ml of the stock diluted to 100ml with distilled water.

Procedure: 100 mg of leaf sample was homogenised with 10 ml of ethanol till all the leaf tissues were fully digested. Then, the extract of the sample was centrifuged at 5000 rpm for 15 min. The volume of the extract was 100 ml, adding distilled water. One ml of the extract was taken in a test tube and 6 ml of the anthrone reagent were added to each test tube. The tube was then placed in a boiling water bath for 10 min, after which they were allowed to cool in running water. A blank was prepared similarly but without a leaf sample. After some time, a blue colour developed in the test tubes, and the intensity of the blue colour was measured at 620 nm by a spectrophotometer. The standard curve calculated the amount of sugar in the leaf sample.

Preparation of The Standard Curve for Estimation of Total Soluble Sugar: 10 mg of the glucose was dissolved in 100 ml of distilled water, or a working standard was prepared by diluting 10 ml of standard glucose stock with 100 ml of distilled water. Different concentrations of the sugar solution were prepared from this stock solution by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the stock solution in a separate test tube. The

final volume of these test tubes was made to 3 ml by adding distilled water, and after that, 6 ml of the Anthrone reagent was added to each test tube. They were boiled in a water bath, as described above. The solution was cooled and the intensity of the blue colour was read at 620 nm. The standard curve was prepared by plotting the absorbance value on the y-axis against the sugar concentration in the solution on the x-axis.

3.7.3.3 Total soluble protein [Bradford, MM. (1976)]: Bradford (1976) suggested a method for estimating the total soluble protein content in plant samples, and that is exactly what was done. The assay operates on the principle that protein binding causes a change in the absorbance maxima of an acidic Coomassie Brilliant Blue G-250 solution from 465 nm to 595 nm. When the anionic form of the dye is stabilized by ionic and hydrophobic interactions, it changes color visibly. An experiment like this is useful since the extinction coefficient of a dye-albumin complex solution remains constant across a concentration range of 10 times.

Reagents:

1. Sodium phosphate buffer (pH 7.4)
2. **Solution A:** To prepare the Sodium phosphate buffer, 13.9 g of 0.1 M sodium dihydrogen phosphate (NaH_2PO_4) was dissolved in distilled water, and the volume was up to 1000 ml.
3. **Solution B:** To prepare the Sodium phosphate buffer, 26.82 g of 0.1 M disodium hydrogen phosphate (Na_2HPO_4) was dissolved in distilled water, and the volume was up to 1000 ml.
4. Solution A and solution B were mixed in a ratio of 19:81, and the final pH (7.4) was adjusted with the help of a pH meter.
5. **Dye concentration:** Dissolve 100mg of Coomassie brilliant blue G 250 in 50 ml of 95% ethanol. Add 100 ml of concentrated orthophosphoric acid. Add distilled water to a final volume of 200 ml. Store in an amber bottle in the refrigerator, the solution is stable for at least six months. Mixed concentrated dye solution with distilled water at the ratio of 1:4. Filter with Whatman No. 1 paper if any precipitate occurs.

Procedure: 100 mg of plant sample was taken and transferred into a mortar. The 10 ml of cold extraction was added. The mortar was put into the ice bucket and cursed with a pestle's help till a fine slurry was made. The homogenates were centrifuged at 15,000

rpm for 15 minutes. The supernatant was collected and used as crude protein extract. Took 5ml diluted dye, 0.2 ml of leaf crude protein extract, and 0.8 ml of distilled water; mix well and allow the colour to develop for at least five minutes but not longer than 30 minutes. The red dye turns blue when it binds to proteins, Read the absorbance at 595 nm in the spectrophotometer.

Preparation of the standard curve for estimating total soluble protein: The standard curve was prepared using 0.1-1.0 ml BSA (Bovine Serum Albumin). The standard curve was prepared by plotting the absorbance value on the y-axis against the sugar concentration in the solution on the x-axis. The amount of total soluble protein expressed in mg/g of sample.

3.7.3.4: Membrane stability index [Premchandra et al (1990)]: Premachandran et al. (1990) provided the formula that was used to compute the MSI. Solute leakage, also known as electrolyte leakage, is one indirect way to assess membrane degradation by monitoring cells and the MSI. One possible explanation for the stimulatory effect of stress on Electro Leyte leakage is damage to the plasma membrane.

Reagent: Double Distilled Water

Procedure: Leaves were taken from the youngest fully-grown leaf. The membrane stability index (MSI) and Membrane Injury Index were estimated by placing 200 mg of leaves in 10 ml double-distilled water in two sets. One set was heated at 40°C for 30 minutes in a water bath and the electrical conductivity (C1) was measured. The second set was boiled at 100°C in a boiling water bath for 10 min and the conductivity (C2) was measured; both conductivities were measured using a conductivity meter (ME977-C, Max Electronics, India).

1. The MSI and MII were calculated using the formula described below:

$$MII = \frac{EC1}{EC2} \times 100$$

$$MSI = 100 - MII$$

3.7.3.5 Total Phenol [Mahadevan and Sridhar (1982)]: Mahadevan and Sridhar (1982) provided the protocol for measuring the amount of total phenol. A blue complex, molybdenum blue, is formed when phenol combines with the oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent in an alkaline environment; this complex can be detected at 650 nm using a spectrophotometer.

Reagents:

1. 80% Ethanol
2. Folin-Ciocalteu reagent (FCR)
3. 20% Na₂CO₃
4. Stock Standard (100mg catechol in 100ml of water). Working standard is prepared by dilution of Stock Standard 10 times.

Procedure: Five hundred mg leaf samples were crushed in 3 ml 80% ethanol and centrifuged at 10,000 rpm for 20 min. Residue and supernatant were separated. The supernatant was saved. The residue was rewashed with 2 ml of 80% ethanol, and the supernatant was saved. Finally, the supernatants were mixed, and the final volume was 5 ml with 80% ethanol. 1 ml of the above supernatant (extract) was taken, and to it, 1 ml of Folin Ciocalteu reagent and 2 ml of sodium carbonate were added. The mixture was heated for 1 minute, and absorbance was recorded at 650 nm. A calibration curve of the known dilution of pyrocatechol was made following the same procedure as that of the sample, and the number of phenols in the sample was expressed as mg g⁻¹ fresh weight (FW).

Preparation of the standard curve for estimation of total phenol: Catechol (100 mg) was taken and dissolved in 100 ml distilled water, and then the stock solution was diluted to 1:10. The 0.2, 0.4, 0.6, 0.8-, and 1.0-ml aliquots were taken into different test tubes, and the volume was raised to 1ml by adding distilled water. The pink colour was developed the same way as for the sample, and absorbance was determined with the help of a spectrophotometer.

3.7.3.6 Total soluble proline [Bates *et al.* (1973)]: The method described by Bates *et al.* (1973) was used to determine the free proline content in the leaves. Sulphosalicylic acid is used to extract proteins from tissues, which then precipitate as a compound with other substances. In an acidic environment, the red color is produced when the extracted proline combines with ninhydrin.

Reagents

1. Acidic ninhydrin reagent. Dissolved 1.25 g of ninhydrin in a mixture of warm 30 ml of glacial acetic acid and 20 ml of 6M phosphoric acid (pH 1.0) with agitation until it is dissolved. Stored at 4°C and used within 24 hours
2. 3% Aqueous sulphosalicylic acid

3. Glacial Acetic Acid
4. Toluene
5. Standard proline solution.

Procedure: A leaf sample of 100 mg was homogenised in 10 ml of sulphosalicylic acid (3%) using mortar and pestle. It was centrifuged at 6000 rpm for 10 min, and the supernatant was collected. The 2.0 ml of the extract was taken in the test tube with 2 ml each of glacial acetic acid and ninhydrin reagent. The reaction mixture was boiled in a water bath at 100°C for 30 min till brick red colour developed. After cooling the reaction mixture, 5 ml of toluene was added and transferred to a separating funnel. The absorbance was read at 520 nm using a spectrophotometer against toluene as blank.

Preparation of the standard curve for estimating proline: Proline (10 mg) was dissolved in 3% aqueous sulphosalicylic acid and then diluted to 100 ml. The 0.2, 0.4, 0.6, 0.8-, and 1.0-ml aliquots were taken into different test tubes, and the volume was raised to 2 ml by adding 3% aqueous sulphosalicylic acid solution. Colour was developed the same way as for the sample, and absorbance was determined with the help of a spectrophotometer.

3.7.3.7 PAL [Subba Rao *et al.* (1970)]: The activity of the L-Phenylalanine Ammonia Lyase (PAL) enzyme was measured according to the protocol given by Subba Rao *et al.* (1970).

Principle: The enzyme may be assayed by measuring the appearance of trans-cinnamic acid from phenylalanine spectrophotometrically at 650 nm.

Reagents

1. 0.2M Sodium Borate Buffer, pH 8.7
2. 0.01M L-Phenylalanine (pH 8.7): Prepared in sodium borate buffer
3. 0.05M Tris-HCl buffer, pH 8.8
4. 1N HCl
5. Peroxide-free ether
6. 0.05N NaOH: prepared fresh from 1N NaOH
7. Standard Cinnamic Acid: Prepared in borate buffer
8. Mercaptoethanol (0.8ml/litre)

Procedure: 3.0 grams of fresh leaf sample was ground in 2.6 ml sodium borate buffer containing 2-mercaptoethanol (0.8ml/litre). This was centrifuged at 7000g for 10 min

at 2-4°C. The supernatant was saved. The pH of the supernatant was adjusted to 5.5 with the help of acetic acid (1M). Incubate 1 ml of 0.05M Tris-HCl buffer, 0.5 ml of 0.01M L-phenylalanine, and 0.4 ml of water at 30°C for 5 min. The reaction was initiated by adding 0.1 ml of the enzyme, which was incubated for 60 min at 30°C. A blank sample was prepared without phenylalanine. The reaction was stopped by the addition of 0.5 ml of 1N HCl. The mixture was extracted with the help of 3.5 ml of ether twice. The ether phase was removed, and the residue pooled and dried under air. The residue was dissolved in 3 ml of 0.05N NaOH. This was kept at room temperature overnight. The mixture was centrifuged at 2000g for 15 min, and the supernatant was saved. 1 ml of the above supernatant (extract) was taken, and 1 ml of Folin Ciocalteu reagent was added. The mixture was heated for 1 min, and absorbance was recorded at 650 nm. A calibration curve of known dilution of cinnamic acid was made following the same procedure as that for the sample. The amount of PAL in the sample was expressed as μ moles of cinnamic acid produced /min/mg protein.

Preparation of the standard curve for estimation of pal activity: Cinnamic acid (100 mg) was taken and dissolved in 100 ml borate buffer, and then the stock solution was diluted to 1:10. The 0.2, 0.4, 0.6, 0.8, and 1.0 ml of working standard solution were taken into different test tubes, and the volume was raised to 1ml by adding borate buffer. Absorbance was determined with the help of a spectrophotometer at 650 nm.

3.7.3.8 Flavonols [Desoky et al., 2001]: The flavanol content was estimated by the protocol given by Akkol *et al.*, 2008. The basic principle of the Aluminium chloride colourimetry method is that Aluminium chloride forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of Flavonols and flavonoids.

Reagents

1. Methanol 80%
2. Sodium Acetate
3. Aluminium Chloride.

Procedure: To estimate Flavonols, a 0.05g plant sample was extracted by boiling 80% methanol for 3 hours. One ml of methanol extract, 3 ml of sodium acetate and 1 ml of aluminium chloride solution were mixed. The absorbance was recorded at 445nm after 2.5hrs.

3.7.3.9 Flavonoids [Desoky et al., 2001]: The acid-stable compounds can be formed with aluminum chloride, flavones, flavanols, and either the C-3 or C-5 hydroxyl groups on the C-4 keto group. There are acid labile complexes that aluminum chloride forms with the ortho-dihydroxyl groups found on the A- or B-ring of flavonoids. The calibration curve is constructed using quercetin as a reference material. A standard calibration curve was constructed using standard quercetin solutions of varying concentrations.

Reagents

1. Quercetin
2. Methanol
3. Aluminium chloride
4. Potassium acetate.

Procedure: The aluminium chloride colourimetric method was used to determine the total flavonoid content of the sample. Quercetin was used to make the standard calibration curve for total flavonoid determination. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol, and then the standard solutions of quercetin were prepared by serial dilutions using methanol (5-200 $\mu\text{g/mL}$). An amount of 0.6 mL diluted standard quercetin solutions or extracts were separately mixed with 0.6 mL of 2% aluminium chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against a blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian). The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were carried out in triplicate.

3.7.4 Yield Parameters

3.7.4.1 Number of capsules per plant: At harvest time, the number of capsules per plant was manually counted, and the information was entered into a notebook.

3.7.4.2 Capsule length: A 15 cm ruler scale can be used to measure the length of a capsule, and the results were entered into a notebook for future use.

3.7.4.3 Number of seeds per capsule: The number of seeds in each capsule was manually counted by splitting the capsule in half. After counting the seeds, the information was entered into a notebook for later use.

3.7.4.4 Weight of 1000 seeds (g): After threshing and winnowing, 100 randomly selected seed samples were extracted from each treatment's net harvested plot in three replications. To determine the 1000 seed weight in gram, their weight was measured and then multiplied by ten.

3.7.4.5: Biological yield: Excluding root biomass, the complete yield of the net plot stays in the field post-harvest for sun drying and is subsequently weighed. The total biological yield per hectare was determined by multiplying the biological yield obtained from the net plot area by an appropriate conversion factor.

3.7.4.6 Seed yield ($q\ ha^{-1}$): Following threshing and winnowing, the seeds were weighed to determine the yield per plot. It was ultimately expressed in $q\ ha^{-1}$.

3.7.4.7 Stalk yield ($q\ ha^{-1}$): Total stalk yield was determined by subtracting seed yield from biological yield. Stalk yield was measured in $q\ ha^{-1}$ from each net plot area.

3.7.4.8 Harvest index: The harvest index was determined as the ratio of economic yield to biological yield using the subsequent formula. It was given by Fisher in 1962.

$$Harvest\ Index = \frac{Economic\ Yield}{Biological\ Yield} \times 100$$

3.7.4.9 Oil content (100 g of harvested seeds): The oil content from sesame seeds can be extracted with an oil expeller equipment. It must be immaculate prior to utilisation. The sesame seeds are placed in the upper bowl of the machine. The oil is automatically taken from the tube connected to the machine's base. The oil cake and oil are expelled independently from distinct apertures. The oilcake is expelled from the front of the tube located in the chamber of the oil expeller. The oil is gathered in a beaker and transferred to a measuring cylinder for quantification. The oil cake is gathered in a basin and subsequently weighed with a balance to determine the mass of the extracted oil cake.

3.7.4.10 Oil cake yield (100 g of harvested seeds): The weight of the oil cake can be estimated by measuring the oil cake produced from 100g of sesame seeds during the oil extraction process. Utilise a weighing balance to measure the oil cake.

3.7.4.11 Crude protein of oil cake: According to Jackson (1967), a modified approach was used to determine the nitrogen content in sesame oil cake. The nitrogen content was determined using the wet digestion method. Wet digestion was performed on oven-dried samples (0.5 g) from each treatment using H₂SO₄, potassium sulphate, copper sulphate, and selenium powder. After the material had been digested, it was transferred to a 100 ml volumetric flask and filled up to 100 ml with distilled water. After adding 5 ml of the distilled sample to a distillation tube, 10 ml of NaOH was added. Finally, water was added to the mixture. The 5 ml boric acid flask was held under the condenser until a purple color was observed. The distilled sample was subsequently titrated with concentrated H₂SO₄ until a pinkish hue was observed. In order to get the final figure, the volume was finally used.

$$\text{Nitrogen content \%} = \frac{R (\text{sample} - \text{blank}) \times \text{Normality of acid} \times \text{Atomic weight of N} \times 100}{\text{Weight of Sample (g)} \times 1000}$$

For determination of protein content, the following formula was used:

$$\text{Protein content (\%)} = \text{micro - Kjeldahl nitrogen content (\%)} \times 6.25$$

3.7.5 Quality Parameters of Oil

3.7.5.1 Peroxide value [standard of ISO 3960-2007]: The Peroxide values were predicted using the ISO 3960-2007 standard. 5g of the oil sample was dissolved in glacial acetic acid: chloroform (3:2, v/v, 30 ml) mixture, followed by 1ml of saturated potassium iodide solution. Further on, the desired amount of distilled water was added and then titrated gradually against sodium thiosulphate solution (0.01ml), where the starch solution (1%) was used as an indicator (Sharma et al., 2006).

$$\text{POV (meq per 1000g)} = \frac{(V_s - V_b) \times F \times N \times 1000}{W} = \frac{(V_s - V_b) \times F \times 1 \times 1000}{W \times 1000} = \frac{(V_s - V_b) \times F \times 10}{W}$$

Where, V_s = Titration volume of sample (ml), V_b = Titration volume of blank (ml), F = Factor of 0.01N Na₂S₂O₃ solution, W = Weight of oil, N = Normality of Na₂S₂O₃ solution

3.7.5.2 Anisidine value [ISO 6885:2006 standards]: The ISO 6885:2006 standards were followed to predict Anisidine values. In acidic conditions, the resultant sample has undergone a reaction with p-anisidine, and the values have been collected (WHO, 1983).

Procedure: Take 0.3g oil sample in 10ml flask. Dissolve it in iso-octane in 10ml flask. Measure O.D. of 2.5ml sample at 350nm against blank (O.D. of iso-octane). 0.5ml of p-anisidine reagent added to cuvette. Place in incubator for 10 min. Measure its O.D. at 350nm.

$$\text{Formula} = \frac{10\text{ml} \times (1.2 \times (AS2 - AB2) - (AS1 - AB1))}{W \text{ sample}}$$

3.7.5.3 Totox value (TV): The Totox value of oil is measured using the formula, (de Abreu et al., 2010),

Formula: $Tv = (2 * Pv) + pAv$, where Pv = Peroxide value, p-Av = para Anisidine value.

3.7.5.4 Acid value [AOCS [Cd 38-63] (Alimentarius, 1999)]: The acid value of the cooking oil is measured using the titration method by AOCS [Cd 38-63] (Alimentarius, 1999).

Reagents

1. **Phenolphthalein Indicator:** Add 1g phenolphthalein in 100ml ethanol
2. **Sodium hydroxide titrant:** Add 4g of sod. Hydroxide in 1000ml distilled water.
3. **Ethanol ether sol.:** Prepare mixture of ethanol and diethyl ether (1:1 v/v). Neutralize with sod. Hydroxide titrant and add 1ml phenolphthalein indicator until pink colour observed

Procedure: Take a known amount of oil sample. Place it in 250ml conical flask. Add 50ml ethanol ether solution and shake well. Titrate against sodium hydroxide titrant until solution turns pink for 30s.

$$\text{Formula} = \frac{\text{Titre value} \times 0.1 \times 56.1}{10}$$

3.7.5.5 Iodine value [Crowe and White, (2001)]: About 5g of the sample were treated with an excess of Iodobromine (IBr) in glacial acetic acid. The reaction between Iodobromine and Potassium iodide gives the amount of iodine from the given sample. The determination of iodine was calculated using the formula, (Crowe and White, 2001).

Reagents

1. Iodine monochloride Reagent
2. Potassium Iodide
3. 0.1N Sod. thiosulphate

Procedure: Take 10ml of oil sample. Add 20ml Iodine monochloride reagent in flask and mix it. Incubate for ½ an hr. in the dark. Make a blank by adding 10ml chloroform to the flask. Add 10ml of potassium iodide solution. Rinse the sides of the flask using 10ml distilled water. Titrate against sod. Thiosulphate solution until pale straw colour. Add 1 ml starch indicator (purple colour observed). Titrate until the solution turns colourless. Follow the same for blank and observe colour

Calculation: Vol. of Sod. Thiosulphate used= (Blank-Test) ml.

$$\text{Formula: } \frac{(B - S) \times N \times 126.9}{W}$$

3.7.5.6 Refractive Index [digital refractometer]: The refractive index was measured by a digital refractometer or a hand operator refractometer. The values were recorded and should be write on a notebook to keep it safe.

Procedure

1. Take a drop of oil sample with the help of dropper
2. Place it over stage of refractometer
3. Reading will show on the digital box

3.7.5.7 Density [Zahir et al., 2017]: The density of the various oil mixtures has been calculated using a Relative Density (R. D) bottle with a capacity of 25 mL (Zahir *et al.*, 2017).

3.7.5.8 Viscosity [By using Cannon – Fenske]: Using Cannon-Fenske (Fisher Scientific, Pitts-burgh, PA) glass capillary kinematic viscometers in a steady-temperature bath. A programmable water bath (Model F25-HE, Julabo USA Inc. Allentown, PA) was utilized to confirm the exact and concurrent results. The trials have been made with ASTM D445 for viscosity determination. The difference in viscosity as a function of temperature was calculated using this formula (Noureddini et al., 1992),
 $V = c \times t,$

where c = Viscometer Constant (mm^2/s^2), t = Time, $\mu = v \times p$, where, v = Viscosity in mm^2/s^2 , p = Density of the oil.

3.7.5.9 Fatty Acid content:

Purpose: Convert triglyceride fatty acids to methyl esters (FAMES) and analyse by GC (GC-FID or GC-MS).

Reagents & standards:

1. Methanol (HPLC grade), toluene or hexane (solvent), KOH in methanol (0.5—1 M) or 1% sodium methoxide, or 3F₃- methanol reagent for methylation.
2. Internal standard: methyl nonadecanoate (C19:0) or methyl heptadecanoate (C17:0)
3. FAME reference mix for ID and calibration (37 FAME mix or equivalent).
PMC+1

Sample prep (one commonly used protocol Ichihara et al. style):

1. Weigh =50-100 mg oil into a glass vial.
2. Add known amount of internal standard (e.g., 100 pg methyl nonadecanoate).
3. Add 1 mL toluene (to dissolve oil) + 2 mL methanolic KOH (0.5 M) and vortex 30 s (saponification/transesterification). Alternatively use 14% BF₃ in methanol and heat gently (60 °C, 10—20 min) when required for complete esterification.
4. After reaction, add 1-2 mL hexane and 5 mL saturated NaCl (or water) — mix and allow phases to separate. Collect the organic (hexane) layer.
5. Dry over anhydrous Na₂SO₄ if needed, filter, concentrate to N 1 mL and transfer to GC vial.

GC conditions (example starting method)

1. Column: 100 m x 0.25 mm x 0.20 μm polar capillary column for FAMES (e.g., SP-2560) or long polar GC column per EN141 03 application notes. Use a GCMS compatible column. +1
2. Carrier gas: Helium, constant flow 1.0 mL•min⁻¹.
3. Injector: Split (e.g., 50: 1) for concentrated samples; use splitless for trace analysis.

4. Oven program (example): 100⁰ C hold 2 min → ramp 4 ⁰ C•min⁻¹ to 240⁰ C -+ hold 15 min. (Adapt to column and sample).
5. MS: EI 70 ev, scan 50-550 m/z (or use selected ion monitoring for quantitation).
Use GC-FID if quant with external/internal standard is required

Identification & quantification

1. Identify peaks by retention times vs FAME standard mix and by mass spectra (NIST library).
2. Quantify using internal standard: calculate response factor of each FAME or apply certified response factors (or use calibration curves for major FAMES). Apply response-factor correction for saturated vs unsaturated esters if high accuracy needed.
3. Blank (solvent), reagent blank (methylation reagents), replicate injections, spiked recovery (known oil spiked with standard)

3.7.6 Economics

3.7.6.1 Cost of cultivation (₹ ha⁻¹): In the economic analysis, various variable cost components were evaluated by treatment, encompassing expenses related to land preparation, seeds, fertilisers, pest control techniques, and irrigation. The labour necessary for seeding, irrigation, weeding, top dressing, spraying, harvesting, and threshing was assessed according to current market pricing.

3.7.6.2 Gross returns (₹ ha⁻¹): Gross returns were determined by multiplying the seed and stalk production of sesame (q ha⁻¹) by the current market prices, expressed in rupees per hectare.

3.7.6.3 Net returns (₹ ha⁻¹): Net returns were determined by subtracting cost of cultivation from gross yields and reported in rupees per hectare.

3.7.6.4 B:C ratio: Benefit-cost ratio was obtained by dividing gross returns (₹ ha⁻¹) with cost of cultivation.

$$\text{Benefit cost ratio} = \frac{\text{Gross returns (₹ ha} - 1\text{)}}{\text{Cost of cultivation (₹ ha} - 1\text{)}}$$

3.1.5 Photographs of Field work and Lab work

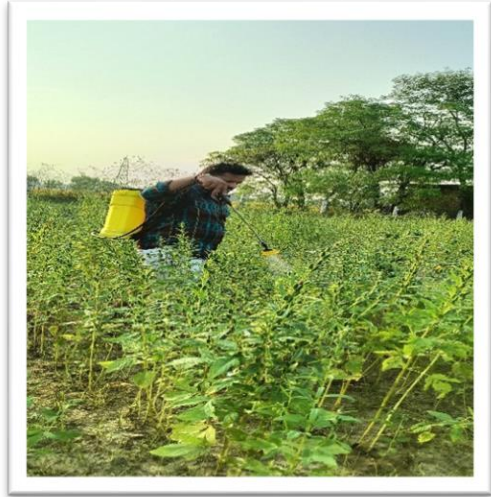
Field preparation and layout



Germination and data collection



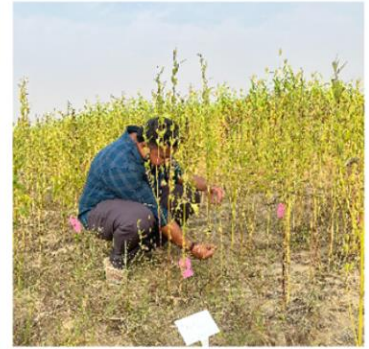
Spraying



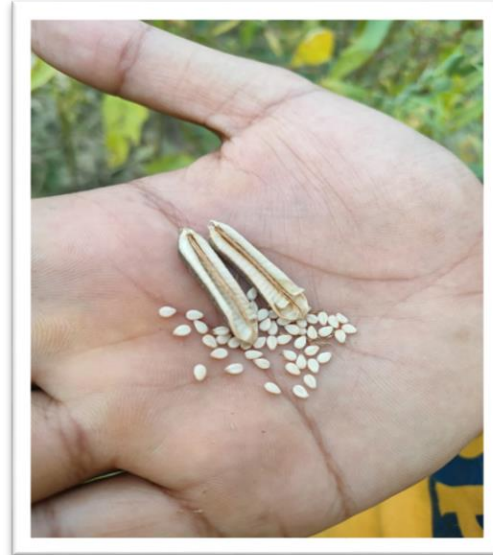
Flowering



Data Collection



Harvesting



Yield



Lab Work









RESULTS AND DISCUSSION

The field experiment for this research, titled "**Effect of Humic Acid and Putrescine Application on Growth, Yield, Oil Quality, and Economics in *Sesamum indicum*,**" took place in the Department of Agronomy at Lovely Professional University, Punjab, in the kharif seasons of 2023 and 2024, respectively. In this chapter, results are presented and discussed.

This study aimed to evaluate the impact of Humic Acid and Putrescine on the growth, physiology, yield, oil quality, and economic viability of the sesame variety Punjab Till No. 2 at 30, 60, and 90 days after sowing (DAS). The seed was sourced from an authorized certified seed provider, "Punjab Agriculture University, Ludhiana, Punjab." The chosen plot size for the experiment is $5.3 \times 4.3 \text{ m}^2$, for a total area of 22.7 m^2 . The seeds have been successfully sown in the research fields. The experiment was organized according to the statistical design FRBD, with treatments administered at 15 and 45 days after sowing (DAS). The treatments administered were sourced from the local market in Phagwara. Humic acid and putrescine were used after identifying the optimal concentrations in previous investigations. Observations were conducted at 30, 60, and 90 DAS across all treatments. The detailed plan of treatments are; T₁- Humic acid (0 ppm) + Putrescine (0 ppm), T₂- Humic acid (0 ppm) + Putrescine (50 ppm), T₃- Humic acid (0 ppm) + Putrescine (100 ppm), T₄- Humic acid (0 ppm) + Putrescine (150 ppm), T₅- Humic acid (500 ppm) + Putrescine (0 ppm), T₆- Humic acid (500 ppm) + Putrescine (50 ppm), T₇- Humic acid (500 ppm) + Putrescine (100 ppm), T₈- Humic acid (500 ppm) + Putrescine (150 ppm), T₉- Humic acid (1000 ppm) + Putrescine (0 ppm), T₁₀- Humic acid (1000 ppm) + Putrescine (50 ppm), T₁₁- Humic acid (1000 ppm) + Putrescine (100 ppm), T₁₂- Humic acid (1000 ppm) + Putrescine (150 ppm), T₁₃- Humic acid (1500 ppm) + Putrescine (0 ppm), T₁₄- Humic acid (1500 ppm) + Putrescine (50 ppm), T₁₅- Humic acid (1500 ppm) + Putrescine (100 ppm), T₁₆- Humic acid (1500 ppm) + Putrescine (150 ppm). Essential nutrients were applied at sowing, and two irrigations were administered to achieve optimal growth and production. This chapter presents the results obtained from the experiment conducted in 2023 and 2024. This

encompasses all observations related to the crop's morphological characteristics, biochemical properties, yield attributes, oil quality, and economic factors. The experiment comprised three primary components. The initial phase of the investigation aimed to assess the morpho-physiological parameters of the sesame crop across all treatments at 30, 60, and 90 days after sowing (DAS). The second part details the biochemical responses of sesame plants subjected to different treatment regimens. The experimental procedure is detailed in Chapter 3. This chapter presents and analyzes the recorded data. The results of the *Kharif* season research experiments are organized under the subsequent headings:

4.1 Effects of humic acid and putrescine on growth parameters of sesame cultivation

4.1.1 Plant Height (cm): The study effect of different treatments on plant height (cm) of sesame across two growing seasons (2023 and 2024), with measurements taken at 30, 60, and 90 (at harvest) days after sowing (DAS) (Table 4.1.1 and Figure 4.1.1) In the 2023 season, plant height measurements for the HA1 treatment (0 ppm) were 32.85 cm, 103.9 cm, and 107.0 cm at 30, 60, and 90 DAS, respectively. The HA2 treatment (500 ppm) showed a slight improvement, with heights of 33.38 cm, 116.0 cm, and 120.9 cm at the corresponding growth stages. Further increases were observed under the HA3 treatment (1000 ppm), which recorded plant heights of 34.82 cm, 121.7 cm, and 126.3 cm. The highest values were observed in the HA4 treatment (1500 ppm), with plant heights of 38.03 cm, 129.1 cm, and 134.4 cm at 30, 60, and 90 DAS, respectively. In the 2023 season, plant height under the P1 treatment (0 ppm) measured 32.53 cm, 112.6 cm, and 116.0 cm at 30, 60, and 90 DAS, respectively. A slight increase was observed with the P2 treatment (50 ppm), which recorded heights of 36.47 cm, 118.4 cm, and 122.6 cm at the same growth stages. The P3 treatment (100 ppm) resulted in further improvement, with plant heights of 35.07 cm, 119.0 cm, and 124.0 cm. The maximum plant height values were obtained under the P4 treatment (150 ppm), reaching 35.00 cm, 121.0 cm, and 126.0 cm at 30, 60, and 90 DAS, respectively. In the 2024 season, plant height measurements for the HA1 treatment (0 ppm) were 33.56 cm, 93.3 cm, and 106.4 cm at 30, 60, and 90 DAS, respectively. The HA2 treatment (500 ppm) showed a slight improvement, with heights of 34.23 cm, 98.5 cm, and 118.0 cm at the corresponding growth stages. Further increases were observed under the HA3 treatment (1000 ppm), which recorded plant heights of 34.89 cm, 97.7 cm, and 125.4 cm. The highest values were observed in the HA4 treatment (1500 ppm), with plant heights of 35.51 cm, 103.6 cm, and 128.0 cm at 30, 60, and 90 DAS, respectively. In the 2024 season, plant height under the P1 treatment (0 ppm) measured 33.58 cm, 93.4 cm, and 114.7 cm at 30, 60, and 90 DAS, respectively. A slight increase was observed with the P2 treatment (50 ppm), which recorded heights of 35.36 cm, 97.8 cm, and 118.9 cm at the same growth stages. The P3 treatment (100 ppm) resulted in further improvement, with plant heights of 34.96 cm, 100.8 cm, and 121.8 cm. The maximum plant height

values were obtained under the P4 treatment (150 ppm), reaching 34.58 cm, 101.2 cm, and 122.5 cm at 30, 60, and 90 DAS, respectively.

4.1.1a Interaction between different treatments on plant height (cm) of sesame at 30 DAS

The interactive effect of different treatments on sesame plant height (cm) at 30 DAS was significant in 2023 and 2024 (Table 4.1.1a). At 30 DAS in 2023, the combination HA4+P2 (41.05 cm) recorded the highest plant height, followed by HA4+P3 (38.91 cm), HA4+P1 (36.99 cm), and HA2+P4 (36.92 cm). The lowest plant height was observed in HA2+P1 (27.62 cm). At 30 DAS in 2024, the treatment HA4+P2 (38.84 cm) again recorded the highest plant height, followed by HA3+P4 (37.11 cm), HA4+P3 (36.04 cm), and HA2+P2 (35.52 cm). The lowest plant height in 2024 occurred in HA1+P4 (32.82 cm).

4.1.1b Interaction between different treatments on plant height (cm) of sesame at 60 DAS

The interactive effect of different treatments on sesame plant height (cm) at 60 DAS was significant in 2023 and 2024 (Table 4.1.1b). At 60 DAS in 2023, the treatment HA4+P4 (134.4 cm) recorded the highest plant height, followed by HA4+P3 (130.0 cm), HA4+P1 (126.7 cm), and HA4+P2 (125.5 cm). The lowest plant height was recorded in HA1+P1 (93.6 cm). At 60 DAS in 2024, the treatment HA4+P4 (107.1 cm) again recorded the highest plant height, followed by HA4+P3 (106.7 cm), HA4+P2 (102.7 cm), and HA3+P4 (102.6 cm). The lowest plant height in 2024 was observed in HA1+P1 (87.0 cm).

The current study demonstrates that the use of humic acid (HA) and putrescine (P) markedly improved vegetative growth of sesame, particularly plant height, across both growing seasons. The steady increase in height with increasing amounts of both HA and P indicates a strong dose-response effect. This means that they can change the physiological processes that control stem elongation, nutrient uptake, and hormone levels in the open field. Humic acid has a positive effect on the height of sesame plants because it does a lot of different things, like making nutrients more available, stimulating the growth of the root system, and speeding up metabolic processes that

help the shoots grow. Humic substances function as natural growth regulators by enhancing membrane permeability, promoting nutrient uptake, and activating enzymes involved in nitrogen assimilation and carbon metabolism. HA also acts as an auxin, promoting cell growth and division in apical meristems, thereby increasing plant height. The use of humic acid has also been linked to increased chlorophyll biosynthesis and photosynthetic efficiency, which may help explain why plants grow better during critical stages of crop development. The application of putrescine also significantly increased sesame plant height, particularly at higher concentrations. Putrescine is an important polyamine that regulates cell division, maintains cellular structure, and accelerates the activity of enzymes that degrade cell walls, which are necessary for cell elongation. Polyamines are also known to modulate hormone signaling, thereby enhancing meristematic activity and internode elongation. Enhanced antioxidant defense in the presence of putrescine diminishes oxidative stress, enabling plants to reallocate metabolic resources toward vegetative growth rather than stress alleviation. Putrescine's ability to maintain turgor and regulate osmotic balance supports continued elongation growth, particularly when plants are growing rapidly. The joint analysis of HA and P treatments indicates that both biostimulants promote growth independently, but they may do so through different pathways. HA primarily helps cells obtain nutrients and use them more efficiently, whereas putrescine supports cell growth and accelerates division and elongation. This complementary action is consistent with earlier research on oilseed crops, which has shown that combining organic biostimulants and polyamines improves shoot development and overall plant health. The sustained height increases observed throughout the study were likely due to improved root growth from HA treatment and increased cellular expansion from P. The fact that the effects of the treatments remained consistent across seasons suggests that these biostimulants are sufficiently compelling to function in the field, even when environmental conditions change. The significant increase in plant height during the middle and late stages of growth indicates that both HA and P applications enhance plant resilience and improve resource use efficiency. Such improvements are essential for agriculture because taller sesame plants often have greater photosynthetic area, improved reproductive potential, and enhanced yield components. The results indicate that humic acid and putrescine may be effective growth-promoting agents for sesame.

Their capacity to regulate nutrient absorption, hormonal homeostasis, and stress physiology offers a promising strategy for sustainable sesame cultivation, particularly in contexts where soil fertility or climatic conditions may constrain crop yield. Ongoing research that integrates molecular, physiological, and agronomic perspectives will help us better understand how these treatments improve sesame growth and yield (Rathor et al., 2023; Rajpar et al., 2011; Kandil & Essam, 2015).

Table 4.1.1 Effect of different treatments on plant height (cm) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	32.85	103.9	107.0	33.56	93.3	106.4
HA 2 (500 ppm)	33.38	116.0	120.9	34.23	98.5	118.0
HA 3 (1000 ppm)	34.82	121.7	126.3	34.89	97.7	125.4
HA 4 (1500 ppm)	38.03	129.1	134.4	35.51	103.6	128.0
SEm $\pm$	0.58	0.92	0.75	0.55	0.15	0.76
C.D. (p=0.05)	1.69	2.69	2.20	1.62	0.44	2.21
Factor (Putrescine)						
P 1 (0 ppm)	32.53	112.3	115.9	33.58	93.4	114.7
P 2 (50 ppm)	36.47	118.4	122.6	35.36	97.8	118.9
P 3 (100 ppm)	35.07	119.0	124.0	34.96	100.8	121.8
P 4 (150 ppm)	35.00	121.0	126.0	34.58	101.2	122.5
SEm $\pm$	0.58	0.92	0.75	0.55	0.15	0.76
C.D. (p=0.05)	1.96	2.69	2.20	1.62	0.44	2.21
Interaction	3.39	5.38	NS	3.23	0.88	NS

HA=Humic Acid, P=Putrescine,SEm $\pm$ =Standard mean of error, C.D=Critical difference, DAS=Days after sowing, Cm=Centimeter

Table 4.1.1a Interaction effect of humic acid and putrescine application on plant height (cm) at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	33.01	33.57	32.11	32.72	32.85	32.99	33.28	35.16	32.82	33.56
HA2	27.62	35.52	33.48	36.92	33.38	34.14	35.52	33.01	34.24	34.23
HA3	32.52	35.76	35.79	35.20	34.82	34.08	33.81	34.57	37.11	34.89
HA4	36.99	41.05	38.91	35.19	38.03	33.01	38.84	36.04	34.14	35.51
Mean	32.53	36.47	35.07	35.00		33.55	35.36	34.69	34.58	
SEm±	1.16					1.11				
C.D (P=0.05)	3.39					3.23				

HA=Humic Acid, P=Putrescine, SEm± =Standard mean of error, C.D=Critical difference, DAS=Days after sowing, Cm=Centimeter

Table 4.1.1b Interaction effect of humic acid and putrescine application on plant height (cm) at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	93.6	107.8	106.6	107.5	103.9	87.0	94.5	95.2	96.4	93.3
HA2	111.0	117.8	117.1	118.1	116.0	98.6	97.7	99.0	98.9	98.5
HA3	118.1	122.3	122.3	124.2	121.7	89.9	96.2	102.2	102.6	97.7
HA4	126.7	125.5	130.0	134.4	129.1	98.1	102.7	106.7	107.1	103.6
Mean	112.3	118.4	119.0	121.0		93.4	97.8	100.8	101.2	
SEm±	1.85					0.30				
C.D (P=0.05)	5.38					0.88				

HA=Humic Acid, P=Putrescine, SEm± =Standard mean of error, C.D=Critical difference, DAS=Days after sowing, Cm=Centimeter

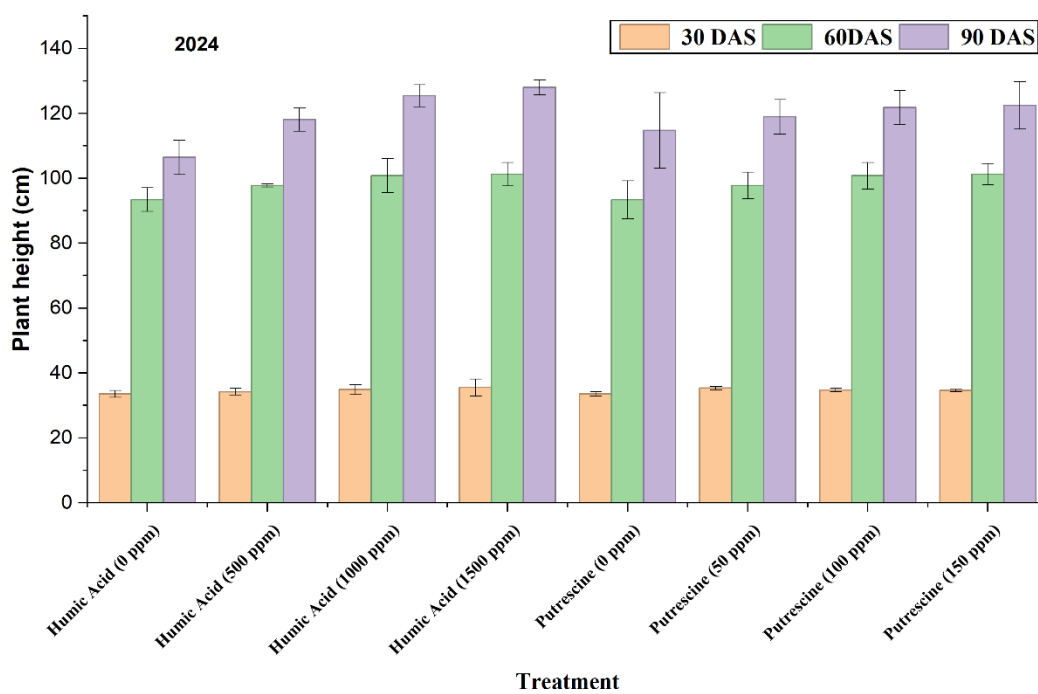
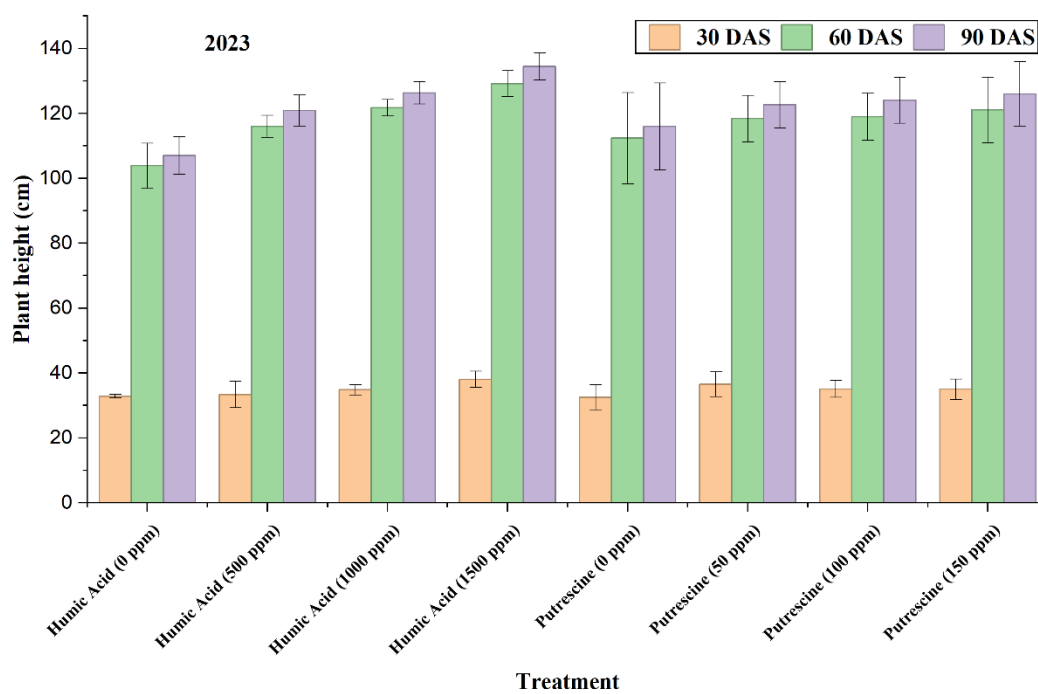


Figure 4.1.1 Effect of different treatments on plant height (cm) of sesame during kharif 2023 & 2024

4.1.2 Dry Matter Accumulation per Plant (g): The effect of different treatments on dry matter accumulation per plant (g) of sesame across two growing seasons (2023 and 2024), with measurements taken at 30, 60, and 90 (at harvest) days after sowing (DAS) (Table 4.1.2 and Figure 4.1.2). In the 2023 season, dry matter accumulation exhibited a clear positive response to increasing levels of humic acid. Under the HA1 treatment (0 ppm), dry matter values were 1.14, 7.73, and 9.51 g at 30, 60, and 90 DAS, respectively. A modest improvement was evident with the HA2 treatment (500 ppm), which recorded 1.19 g, 9.80 g, and 11.89 g at the same growth stages. Substantial enhancement occurred with the HA3 treatment (1000 ppm), where dry matter increased to 1.33 g, 13.29 g, and 15.58 g across the respective intervals. The highest biomass accumulation was observed under HA4 (1500 ppm), with 1.53 g at 30 DAS, 16.09 g at 60 DAS, and 18.05 g at 90 DAS, indicating a strong dose-dependent increase. Similarly, putrescine treatments also demonstrated progressive increases in dry matter production. The P1 control (0 ppm) recorded 1.20 g, 9.50 g, and 10.98 g at 30, 60, and 90 DAS, respectively. A noticeable rise was observed with P2 (50 ppm), which achieved 1.32 g, 11.75 g, and 13.74 g. Further enhancement occurred under P3 (100 ppm), with values of 1.33 g, 12.42 g, and 14.78 g. The maximum dry matter accumulation in the putrescine treatments was observed at P4 (150 ppm), reaching 1.35, 13.23, and 15.25 g at 30, 60, and 90 DAS, respectively. These results collectively indicate a consistent, concentration-dependent improvement in biomass production under both humic acid and putrescine applications. In the 2024 season, dry matter accumulation exhibited a clear positive response to increasing levels of humic acid. Under the HA1 treatment (0 ppm), dry matter values were 1.50 g, 7.58 g, and 8.6 g at 30, 60, and 90 DAS, respectively. A modest improvement was evident with the HA2 treatment (500 ppm), which recorded 1.55 g, 9.49 g, and 11.52 g at the same growth stages. Substantial enhancement occurred with the HA3 treatment (1000 ppm), where dry matter increased to 1.48 g, 12.67 g, and 14.13 g across the respective intervals. The highest biomass accumulation was observed under HA4 (1500 ppm), with 1.66 g at 30 DAS, 15.91 g at 60 DAS, and 17.37 g at 90 DAS, indicating a substantial dose-dependent increase. Similarly, putrescine treatments also demonstrated progressive increases in dry matter production. The P1 control (0 ppm) recorded 1.50 g, 9.10 g, and 9.93 g at 30, 60, and 90 DAS, respectively. A noticeable rise was observed with P2 (50 ppm), which achieved

1.62 g, 11.36 g, and 12.80 g. Further enhancement occurred under P3 (100 ppm), with values of 1.52 g, 12.24 g, and 14.18 g. The maximum dry matter accumulation in the putrescine treatments was observed at P4 (150 ppm), reaching 1.55, 12.94, and 14.71 g at 30, 60, and 90 DAS, respectively.

4.1.2a Interaction between different treatments on dry matter accumulation per plant (g) of sesame at 30 DAS

The interactive effect of different treatments on dry matter accumulation per plant (g) of sesame at 30 DAS was significant in 2023 and 2024 (Table 4.1.2a). At 30 DAS in 2023, the combination HA4+P4 (1.85 g) recorded the highest dry matter, followed by HA3+P2 (1.51 g), HA4+P3 (1.48 g), and HA3+P3 (1.45 g). The lowest dry matter was observed in HA1+P1 (1.08 g). At 30 DAS in 2024, the treatment HA4+P2 (1.87 g) again recorded the highest dry matter, followed by HA2+P1 (1.66 g), HA1+P4 (1.65 g), and HA4+P1 (1.63 g). The lowest dry matter in 2024 occurred in HA1+P1 (1.26 g).

These results collectively indicate a consistent, concentration-dependent improvement in biomass production under both humic acid and putrescine applications. The present study demonstrated that foliar application of humic acid and putrescine markedly enhanced dry matter accumulation in sesame during both growing seasons, confirming their effectiveness as growth-promoting biostimulants under field conditions. The progressive increase in biomass with increasing concentrations of both compounds indicates a dose-dependent response, suggesting improvements in physiological activity, nutrient assimilation, root growth, and source–sink relationships that collectively promoted vegetative development and reproductive productivity. Humic acid exerted a particularly pronounced influence on dry matter production, especially at the higher application rates. This observation is consistent with previous reports indicating that humic substances enhance carbon fixation, mineral nutrient uptake, enzyme activity, and cellular metabolism through hormone-like effects while simultaneously improving soil physicochemical characteristics. Greater root proliferation, enhanced microbial activity within the rhizosphere, and improved nutrient availability likely contributed to increased biomass accumulation. Similar responses have been reported in sesame and other oilseed crops, where humic acid supplementation increased chlorophyll concentration, photosynthetic efficiency, and

assimilate translocation, ultimately resulting in higher dry matter production. Putrescine also significantly increased dry matter accumulation, emphasizing the central role of polyamines in regulating plant growth and development. The improvement in biomass observed with increasing putrescine concentrations may be attributed to its ability to stabilize cellular membranes, regulate ionic homeostasis, stimulate antioxidant defense mechanisms, and maintain metabolic activity under field conditions. In addition, putrescine promotes cell division, delays leaf senescence, enhances nitrogen metabolism, and supports protein synthesis, thereby sustaining vegetative growth and increasing biomass production. Previous investigations in sesame and other oilseed species have likewise demonstrated that exogenous putrescine improves photosynthetic performance, osmotic adjustment, and stress tolerance, leading to greater dry matter accumulation. The comparable response pattern observed during both experimental seasons indicates that the beneficial effects of humic acid and putrescine remained consistent despite seasonal environmental variation. Such stability is particularly valuable for sesame cultivation because the crop is frequently exposed to abiotic constraints, including high temperature, nutrient deficiency, and irregular rainfall. By strengthening plant vigor, improving nutrient-use efficiency, and enhancing physiological resilience, these treatments likely minimized the adverse influence of environmental fluctuations on biomass production. These findings corroborate recent studies demonstrating that humic acid- and polyamine-based biostimulants effectively improve crop growth and productivity under open-field conditions. Overall, the enhanced dry matter accumulation observed in the present investigation suggests that humic acid and putrescine interact synergistically with plant physiological and metabolic processes to improve biomass production, resource utilization, and tolerance to environmental stress, thereby contributing to sustainable sesame production. Their integration into sesame production systems could therefore serve as an effective agronomic strategy for boosting biomass and subsequent yield, particularly in environments prone to nutrient limitations and climatic stress (Anaz et al., 2021; Hatami, 2017; Savita & Girijrsh, 2015).

Table 4.1.2 Effect of different treatments on dry matter accumulation per plant (g) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	1.14	7.73	9.51	1.50	7.58	8.60
HA 2 (500 ppm)	1.19	9.80	11.89	1.55	9.49	11.52
HA 3 (1000 ppm)	1.33	13.29	15.58	1.48	12.67	14.13
HA 4 (1500 ppm)	1.53	16.09	18.05	1.66	15.91	17.37
SEm ±	0.02	0.29	0.34	0.03	0.04	0.02
C.D. (p=0.05)	0.06	0.86	0.99	0.01	0.14	0.06
Factor (Putrescine)						
P 1 (0 ppm)	1.20	9.50	10.98	1.50	9.10	9.93
P 2 (50 ppm)	1.32	11.75	13.74	1.62	11.36	12.80
P 3 (100 ppm)	1.33	12.42	14.78	1.52	12.24	14.18
P 4 (150 ppm)	1.35	13.23	15.25	1.55	12.94	14.71
SEm ±	0.02	0.29	0.34	0.03	0.04	0.02
C.D. (p=0.05)	0.06	0.86	0.99	0.01	0.14	0.06
Interaction	0.12	NS	NS	0.06	NS	NS

Table 4.1.2a Interaction effect of humic acid and putrescine application on dry matter accumulation per plant (g)at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	1.08	1.18	1.16	1.15	1.14	1.26	1.49	1.59	1.65	1.50
HA2	1.15	1.20	1.24	1.19	1.19	1.66	1.60	1.43	1.52	1.55
HA3	1.11	1.51	1.45	1.28	1.33	1.45	1.52	1.53	1.41	1.48
HA4	1.39	1.42	1.48	1.85	1.53	1.63	1.87	1.52	1.60	1.66
Mean	1.20	1.32	1.33	1.35		1.50	1.62	1.52	1.55	
SEm±	0.04					0.02				
C.D (P=0.05)	0.12					0.06				

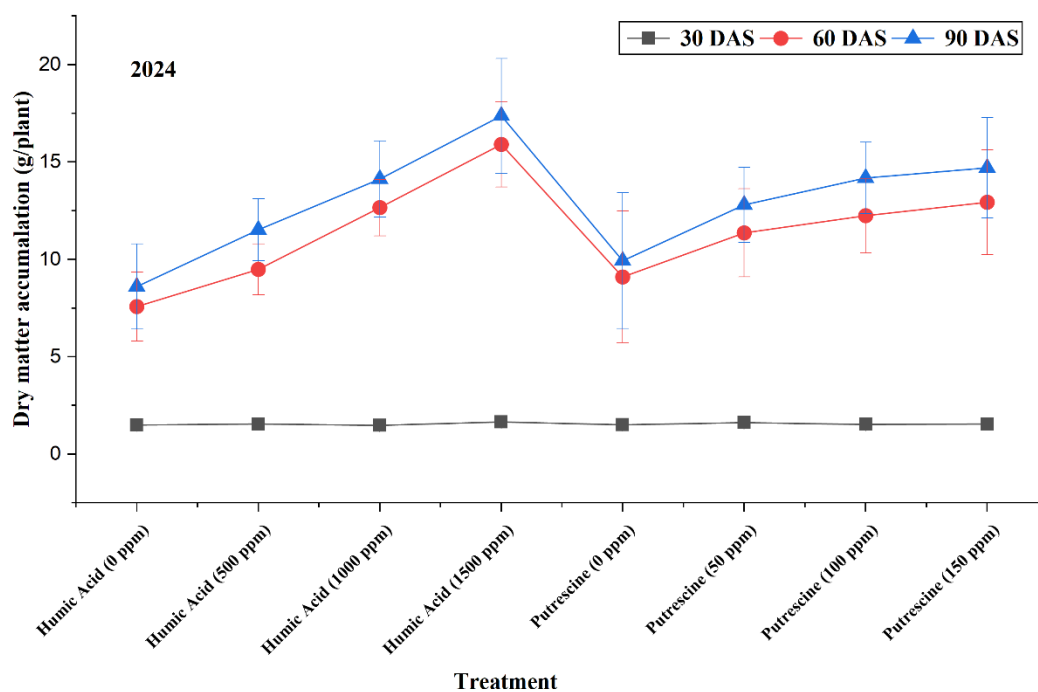
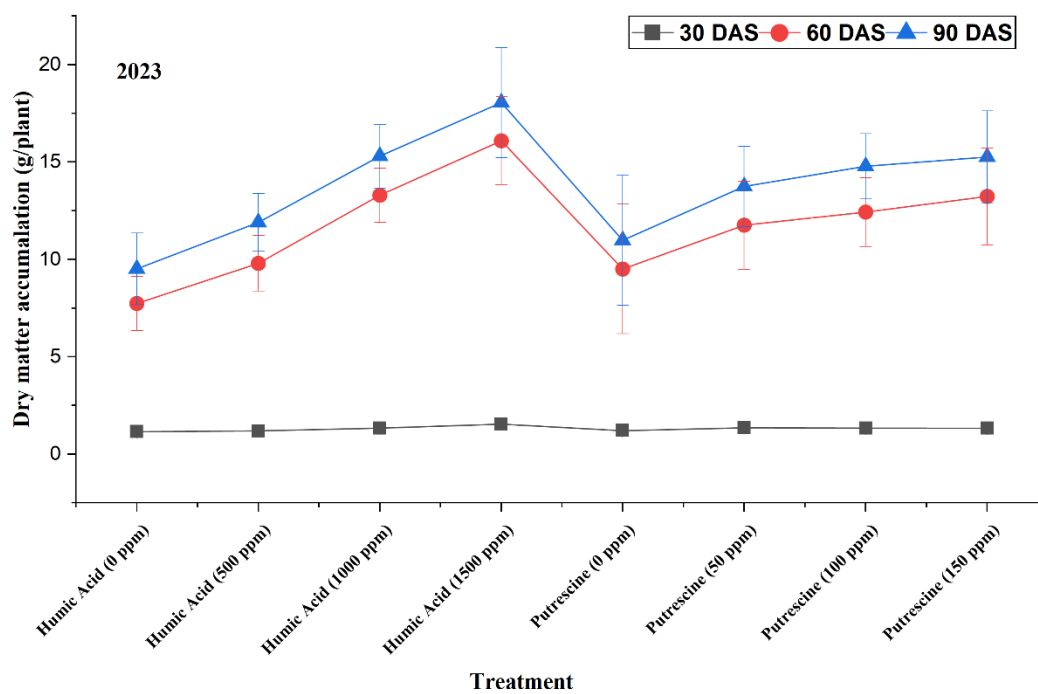


Figure 4.1.2 Effect of different treatments on dry matter accumulation per plant (g) of sesame during kharif 2023 & 2024

4.1.3 Stem Girth (mm): The effect of different treatments on stem girth (mm) of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.1.3 and Figure 4.1.3). In the 2023 season, humic acid application significantly enhanced stem girth relative to the untreated control. The HA1 control (0 ppm) recorded stem girth values of 2.67 mm, 3.94 mm, and 4.11 mm at 30, 60, and 90 DAS, respectively. Incremental increases were observed under HA2 (500 ppm), which achieved 2.89 mm, 4.61 mm, and 4.75 mm across the same intervals. A more pronounced improvement was observed with HA3 (1000 ppm), with measurements of 3.48 mm at 30 DAS, 4.13 mm at 60 DAS, and 4.20 mm at 90 DAS. The highest stem girth values were recorded under HA4 (1500 ppm), reaching 3.85 mm, 5.59 mm, and 5.60 mm at 30, 60, and 90 DAS, respectively, demonstrating the strong stimulatory effect of elevated humic acid concentration on stem thickening. A similar pattern was observed under putrescine treatments. The P1 control (0 ppm) showed stem girth values of 2.88 mm, 4.30 mm, and 4.58 mm at 30, 60, and 90 DAS. Application of 50 ppm putrescine (P2) enhanced girth to 3.25 mm, 4.45 mm, and 4.75 mm, while P3 (100 ppm) further increased values to 3.34 mm, 4.46 mm, and 4.67 mm. The highest response was observed at P4 (150 ppm), with stem girths of 3.41 mm, 4.55 mm, and 4.66 mm at the respective growth stages. In the 2024 season, the response pattern remained consistent, reinforcing the stability of treatment effects across years. The HA1 control (0 ppm) recorded stem girth values of 2.88 mm, 4.07 mm, and 4.14 mm at 30, 60, and 90 DAS. Improvement was evident under HA2 (500 ppm), with values of 3.34 mm, 4.66 mm, and 4.78 mm. The HA3 treatment (1000 ppm) further increased stem girth to 3.44 mm, 4.63 mm, and 4.86 mm. The maximum enhancement was observed under HA4 (1500 ppm), with stem diameters of 3.82 mm, 5.22 mm, and 5.52 mm, confirming the strong and consistent influence of higher humic acid concentrations on stem development. Putrescine treatments in 2024 also followed a progressive response pattern. The P1 control (0 ppm) showed stem girths of 3.13 mm, 4.38 mm, and 4.56 mm at 30, 60, and 90 DAS, respectively. Application of P2 (50 ppm) increased these values to 3.25 mm, 4.76 mm, and 4.95 mm. Slightly higher girth was observed under P3 (100 ppm), with girth measurements of 3.32 mm, 4.51 mm, and 4.68 mm. The most significant improvement

occurred in P4 (150 ppm), where stem girth reached 3.78 mm, 4.93 mm, and 5.10 mm at the respective stages.

4.1.3a Interaction between different treatments on stem girth (mm) of sesame at 30 DAS

The interactive effect of different treatments on stem girth (mm) of sesame at 30 DAS was significant in 2023 and 2024 (Table 4.1.3a). At 30 DAS in 2023, the combination HA4+P4 (4.50 mm) recorded the highest stem girth, followed by HA4+P3 (3.95 mm), HA3+P4 (3.78 mm), and HA4+P2 (3.66 mm). The lowest stem girth was observed in HA1+P1 (2.28 mm). At 30 DAS in 2024, the treatment HA4+P2 (4.25 mm) again recorded the highest dry matter, followed by HA4+P4 (3.94 mm), HA4+P3 (3.82 mm), and HA2+P4 (3.80 mm). The lowest stem girth in 2024 occurred in HA1+P1 (2.28 mm).

4.1.3b Interaction between different treatments on stem girth (mm) of sesame at 60 DAS

The interactive effect of different treatments on stem girth (mm) of sesame at 60 DAS was significant in 2023 and 2024 (Table 4.1.3b). At 60 DAS in 2023, the combination HA4+P4 (5.82 mm) recorded the highest stem girth, followed by HA4+P1 (5.43 mm), HA4+P3 (5.10 mm), and HA4+P2 (4.93 mm). The lowest stem girth was observed in HA1+P1 (3.19 mm). At 60 DAS in 2024, the treatment HA4+P2 (5.41 mm) again recorded the highest dry matter, followed by HA4+P4 (5.25 mm), HA4+P1 (5.14 mm), and HA4+P3 (5.10 mm). The lowest stem girth in 2024 occurred in HA1+P1 (3.19 mm).

4.1.3c Interaction between different treatments on stem girth (mm) of sesame at 90 DAS

The interactive effect of different treatments on stem girth (mm) of sesame at 90 DAS was significant in 2023 and 2024 (Table 4.1.3c). At 90 DAS in 2023, the combination HA4+P2 (6.18 mm) recorded the highest stem girth, followed by HA4+P1 (5.68 mm), HA4+P3 (5.37 mm), and HA4+P4 (5.16 mm). The lowest stem girth was observed in HA1+P1 (3.49 mm). At 90 DAS in 2024, the treatment HA4+P2 (5.73 mm) again recorded the highest dry matter, followed by HA4+P4 (5.49 mm), HA4+P3 (5.47 mm),

and HA4+P1 (5.37 mm). The lowest stem girth in 2024 occurred in HA1+P1 (3.26 mm).

Collectively, the results from both seasons demonstrate that humic acid, particularly at 1500 ppm, was more effective than putrescine in enhancing stem girth. The steady increase in stem thickness across treatments suggests improved cambial activity, enhanced vascular development, and greater structural integrity, all of which contribute to stronger plant architecture under field conditions. The present study demonstrated that both humic acid and putrescine significantly and progressively increased stem girth in sesame across two consecutive field seasons. The consistent improvement observed with increasing humic acid concentrations indicates a strong regulatory influence on structural growth processes, particularly those associated with secondary thickening. The superior performance of the highest humic acid treatment suggests that humic substances promote cambial cell activity, lignification, and vascular differentiation, ultimately resulting in thicker, more mechanically stable stems. Similar stimulatory effects of humic acid on stem development have been reported in soybean, sunflower, and sesame, supporting its role in modulating hormonal signaling, nutrient mobilization, and carbon assimilation pathways. Humic acid is known to interact with auxin-like mechanisms, stimulating plant growth via enhanced proton pump activity, improved mineral uptake, and activation of growth-related enzymes. These processes collectively strengthen stem architecture and increase biomass allocation to structural tissues. The positive, dose-dependent response observed in this study aligns with findings in other oilseed crops, in which humic acid application increased stem diameter, vascular tissue thickness, and shoot sturdiness under both optimal and stress conditions. Application of putrescine also produced marked improvements in stem girth, though its effect was comparatively moderate compared with humic acid. As a polyamine, putrescine contributes to cellular division, expansion, and cell wall stabilization, thereby enhancing stem rigidity and strength. The progressive increase in stem girth with rising putrescine concentrations suggests active involvement in regulating cambial dynamics and secondary metabolism. Similar reports in sesame, maize, and chickpea indicate that putrescine improves stem robustness by mitigating oxidative stress, enhancing antioxidant enzyme activity, and promoting lignin

biosynthesis. The consistency of treatment effects across seasons reinforces the reliability of both humic acid and putrescine as growth-promoting agents under open-field conditions. However, the greater magnitude of response under humic acid applications highlights its superior influence on structural development, likely due to its multifaceted role in nutrient acquisition, hormonal mimicry, and root–shoot communication. The combined evidence suggests that integrating humic acid and putrescine applications may confer significant agronomic benefits for sesame by improving stem strength, lodging resistance, and overall plant vigor (Anaz et al., 2021; Vani et al., 2017; Rajpar et al., 2011).

Table 4.1.3 Effect of different treatments on stem girth (mm) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	2.67	3.94	4.11	2.88	4.07	4.14
HA 2 (500 ppm)	2.89	4.61	4.75	3.34	4.66	4.78
HA 3 (1000 ppm)	3.48	4.13	4.20	3.44	4.63	4.86
HA 4 (1500 ppm)	3.84	5.59	5.60	3.82	5.22	5.52
SEm ±	0.06	0.03	0.04	0.05	0.03	0.03
C.D. (p=0.05)	0.19	0.09	0.12	0.15	0.09	0.09
Factor (Putrescine)						
P 1 (0 ppm)	2.88	4.30	4.58	3.13	4.38	4.56
P 2 (50 ppm)	3.25	4.45	4.75	3.25	4.76	4.95
P 3 (100 ppm)	3.34	4.46	4.67	3.32	4.51	4.68
P 4 (150 ppm)	3.41	4.55	4.66	3.78	4.93	5.10
SEm ±	0.06	0.03	0.04	0.05	0.03	0.03
C.D. (p=0.05)	0.19	0.09	0.12	0.15	0.09	0.09
Interaction	0.38	0.18	0.23	0.31	0.18	0.18

Table 4.1.3a Interaction effect of humic acid and putrescine application on stem girth (mm)at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	2.28	2.69	2.62	2.73	2.67	2.28	2.37	3.20	3.66	2.88
HA2	2.65	3.26	3.37	2.63	2.89	3.35	3.44	2.77	3.80	3.34
HA3	3.34	3.39	3.43	3.78	3.48	3.64	2.93	3.48	3.71	3.44
HA4	3.25	3.66	3.95	4.50	3.84	3.26	4.25	3.82	3.94	3.82
Mean	2.88	3.25	3.34	3.41		3.13	3.25	3.32	3.78	
SEm±	0.13					0.10				
C.D (P=0.05)	0.38					0.31				

Table 4.1.3b Interaction effect of humic acid and putrescine application on stem girth (mm)at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	3.19	4.30	4.29	3.84	3.94	3.19	3.84	4.30	4.93	4.07
HA2	4.75	4.45	4.24	4.37	4.61	4.73	4.70	4.24	4.95	4.66
HA3	3.84	4.07	4.14	4.17	4.13	4.45	5.08	4.39	4.60	4.63
HA4	5.43	4.93	5.10	5.82	5.59	5.14	5.41	5.10	5.25	5.22
Mean	4.30	4.45	4.46	4.55		4.38	4.76	4.51	4.93	
SEm±	0.06					0.06				
C.D (P=0.05)	0.18					0.18				

Table 4.1. Interaction effect of humic acid and putrescine application on stem girth (mm) at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	3.49	3.91	4.51	4.54	4.11	3.26	3.91	4.43	4.97	4.14
HA2	5.23	4.59	4.46	4.71	4.75	4.83	4.80	4.33	5.14	4.78
HA3	3.92	4.33	4.35	4.21	4.20	4.77	5.37	4.50	4.80	4.86
HA4	5.68	6.18	5.37	5.16	5.60	5.37	5.73	5.47	5.49	5.52
Mean	4.58	4.75	4.67	4.66		4.56	4.95	4.68	5.10	
SEm±	0.08					0.06				
C.D (P=0.05)	0.23					0.18				

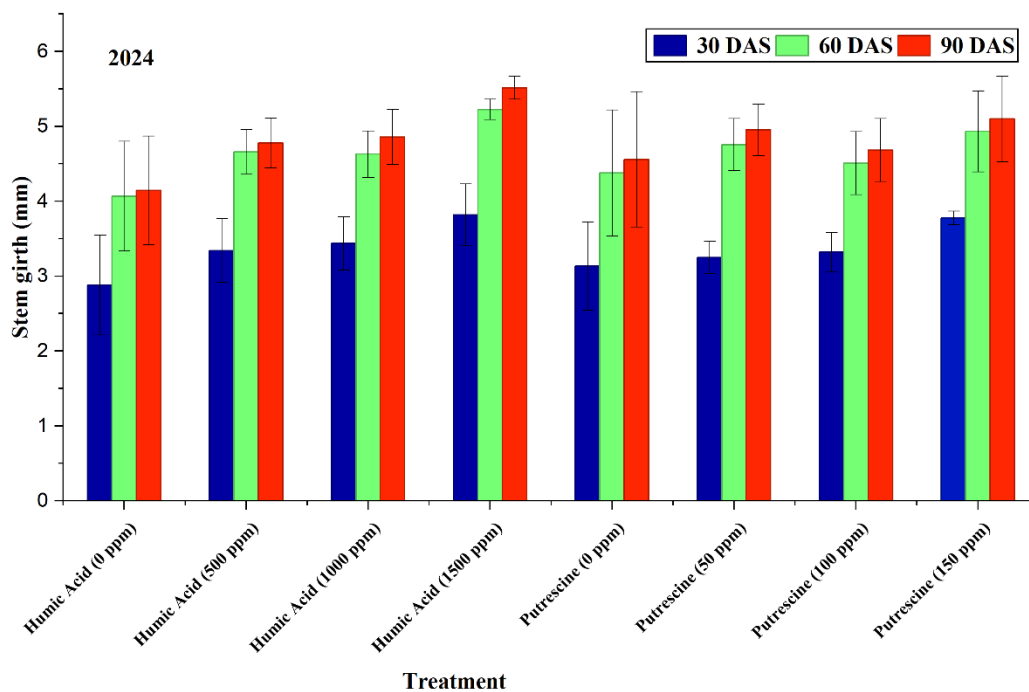
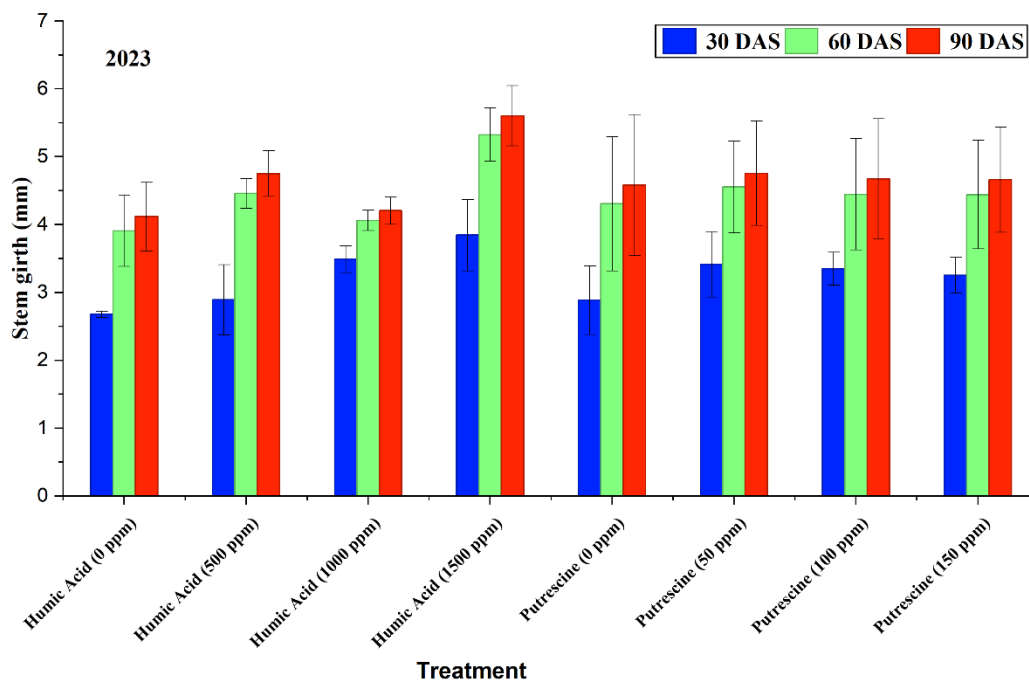


Figure 4.1.3 Effect of different treatments on stem girth (mm) of sesame during kharif 2023 & 2024

4.1.4 Number of Primary Branches: The effect of different treatments on the number of primary branches of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.1.4 and Figure 4.1.4). In the 2023 season, the application of humic acid exerted a marked positive influence on the number of primary branches compared with the untreated control. The HA1 control (0 ppm) produced only 2.06, and 2.06 branches at 60, and 90 DAS, respectively. A gradual enhancement was noted under HA2 (500 ppm), which recorded 2.33, and 2.33 branches at the corresponding stages. A more substantial improvement emerged with HA3 (1000 ppm), yielding 2.69, and 2.69 branches. The maximum stimulatory effect was observed with HA4 (1500 ppm), which produced 3.30, and 3.30 branches, indicating a strong dose-dependent response with increasing humic acid concentration. A parallel trend was evident under putrescine treatments. The P1 control (0 ppm) showed 2.40, and 2.40 branches at 60, and 90 DAS, respectively. Application of P2 (50 ppm) enhanced branching to 2.52, and 2.52, while P3 (100 ppm) promoted further increases, reaching 2.65, and 2.65. The highest branching response was observed under P4 (150 ppm), which produced 2.81, and 2.81 branches, indicating that higher putrescine concentrations synergistically enhance vegetative architecture. In the 2024 season, similar response patterns were recorded. The HA1 control (0 ppm) showed 2.52, and 2.52 branches, whereas HA2 (500 ppm) resulted in slight increments to 2.98, and 2.98 branches. A more pronounced improvement was found with HA3 (1000 ppm), registering 2.80, and 2.80 branches. The highest branching potential was again observed under HA4 (1500 ppm), with 3.02, and 3.02 branches at 60, and 90 DAS, respectively, confirming the consistent growth-promoting effect of elevated humic acid levels at these time points. Putrescine treatments showed a comparable enhancement trend in 2024. The P1 control (0 ppm) recorded 2.47, and 2.47 branches, while P2 (50 ppm) improved branching to 2.73, and 2.73. Further increases were documented under P3 (100 ppm), yielding 2.97, and 2.97 branches. The peak branching response occurred at P4 (150 ppm), producing 3.15, and 3.15 branches at 60, and 90 DAS, respectively, reaffirming the concentration-dependent enhancement of vegetative growth by putrescine.

4.1.4a Interaction between different treatments on the number of primary branches of sesame at 60 DAS

The interactive effect of humic acid and putrescine treatments on the number of primary branches at 60 DAS was significant in both 2023 and 2024 (Table 4.1.4a). In 2023, the treatment combination HA4+P4 (3.80) produced the highest number of primary branches, followed closely by HA4+P3 (3.50), HA4+P1 (3.06), and HA4+P2 (2.83). The lowest branching response was recorded under HA1+P1 (1.73), indicating minimal vegetative stimulation under the untreated conditions. A comparable trend was observed in 2024. The treatment HA4+P4 (3.47) again yielded the highest number of primary branches, followed by HA2+P4 (3.27), HA4+P3 (3.13), and HA1+P4 (3.07). As in the previous year, the lowest number of primary branches was documented under HA1+P1 (1.73), reaffirming the limited growth response in the absence of humic acid and putrescine supplementation.

4.1.4b Interaction between different treatments on the number of primary branches of sesame at 90 DAS

The interactive effect of different treatments on the number of primary branches of sesame at 90 DAS was significant in 2023 and 2024 (Table 4.1.4b). At 90 DAS in 2023, the combination HA4+P4 (3.80) recorded the number of primary branches, followed by HA4+P3 (3.50), HA4+P1 (3.06), and HA4+P2 (2.83). The lowest number of primary branches was observed in HA1+P1 (1.73). At 90 DAS in 2024, the treatment HA4+P4 (3.47) again recorded the highest number of primary branches, followed by HA2+P4 (3.27), HA4+P3 (3.13), and HA1+P4 (3.07). The lowest number of primary branches in 2024 occurred in HA1+P1 (1.73).

The present investigation revealed that both humic acid and putrescine exerted a pronounced, concentration-dependent enhancement in the number of primary branches of sesame during the 2023 and 2024 growing seasons. The consistent improvement observed with rising humic acid concentrations highlights its multifaceted role in stimulating vegetative development. Humic acid is known to mimic the activities of endogenous plant hormones, enhance nutrient uptake efficiency, and improve soil–root interactions, all of which jointly contribute to enhanced axillary bud activation and

increased shoot proliferation. These mechanisms explain the progressive increase in branching with higher humic acid levels in both seasons. Similar growth-promoting effects of humic substances have been reported in sesame, sunflower, and other oilseed crops, where humic acid applications improved canopy structure, shoot vigor, and branching intensity through improved nitrogen assimilation and regulation of auxin–cytokinin balance. Putrescine treatments showed a comparable pattern of enhancement, confirming the importance of polyamines in regulating vegetative architecture. Putrescine plays an essential role in cell division, maintenance of cellular integrity, and modulation of hormonal pathways that control apical dominance and lateral bud emergence. The observed concentration-dependent increase in branching aligns with previous studies demonstrating that exogenous putrescine enhances vegetative vigor, stimulates meristematic activity, and improves shoot initiation in various crops. Higher putrescine concentrations likely reduced ethylene-mediated growth inhibition while promoting cytokinin-mediated branching responses. The consistency of treatment effects across two seasons highlights the reliability and agronomic significance of humic acid and putrescine under open-field conditions. While both biostimulants improved branching, humic acid exhibited slightly stronger effects, particularly at advanced growth stages, suggesting broader physiological influence on nutrient dynamics and shoot development. Collectively, the findings underscore the potential of integrating humic acid and putrescine as effective bio-growth enhancers to improve vegetative performance and canopy architecture in sesame (Vani et al., 2017; Farzi Aminabad et al., 2021; Wei et al., 2013).

Table 4.1.4 Effect of different treatments on number of primary branches of sesame during kharif 2023 & 2024

Treatments	2023		2024	
	60 DAS	90 DAS	60 DAS	90 DAS
Factor (Humic Acid)				
HA 1 (0ppm)	2.06	2.06	2.52	2.52
HA 2 (500 ppm)	2.33	2.33	2.98	2.98
HA 3 (1000 ppm)	2.69	2.69	2.80	2.80
HA 4 (1500 ppm)	3.30	3.30	3.02	3.02
SEm ±	0.05	0.05	0.04	0.04
C.D. (p=0.05)	0.16	0.16	0.13	0.13
Factor (Putrescine)				
P 1 (0 ppm)	2.40	2.40	2.47	2.47
P 2 (50 ppm)	2.52	2.52	2.73	2.73
P 3 (100 ppm)	2.65	2.65	2.97	2.97
P 4 (150 ppm)	2.81	2.81	3.15	3.15
SEm ±	0.05	0.05	0.04	0.04
C.D. (p=0.05)	0.16	0.16	0.13	0.13
Interaction	0.33	0.33	0.27	0.27

Table 4.1.4a Interaction effect of humic acid and putrescine application on number of primary branches at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	1.73	2.20	2.10	2.21	2.06	1.73	2.40	2.87	3.07	2.52
HA2	2.26	2.33	2.30	2.43	2.33	2.74	3.07	2.87	3.27	2.98
HA3	2.53	2.73	2.70	2.80	2.69	2.60	2.80	3.00	2.80	2.80
HA4	3.06	2.83	3.50	3.80	3.30	2.80	2.67	3.13	3.47	3.02
Mean	2.40	2.52	2.65	2.81		2.47	2.73	2.97	3.15	
SEm±	0.11					0.09				
C.D (P=0.05)	0.33					0.24				

Table 4.1.4b Interaction effect of humic acid and putrescine application on number of primary branches at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	1.73	2.20	2.10	2.21	2.06	1.73	2.40	2.87	3.07	2.52
HA2	2.26	2.33	2.30	2.43	2.33	2.74	3.07	2.87	3.27	2.98
HA3	2.53	2.73	2.70	2.80	2.69	2.60	2.80	3.00	2.80	2.80
HA4	3.06	2.83	3.50	3.80	3.30	2.80	2.67	3.13	3.47	3.02
Mean	2.40	2.52	2.65	2.81		2.47	2.73	2.97	3.15	
SEm±	0.11					0.09				
C.D (P=0.05)	0.33					0.27				

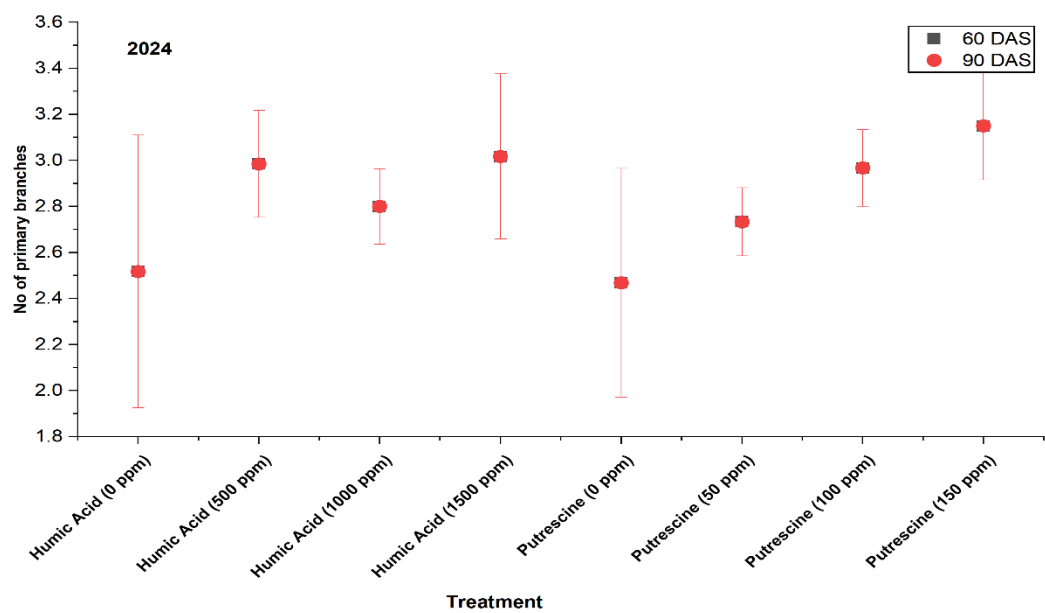
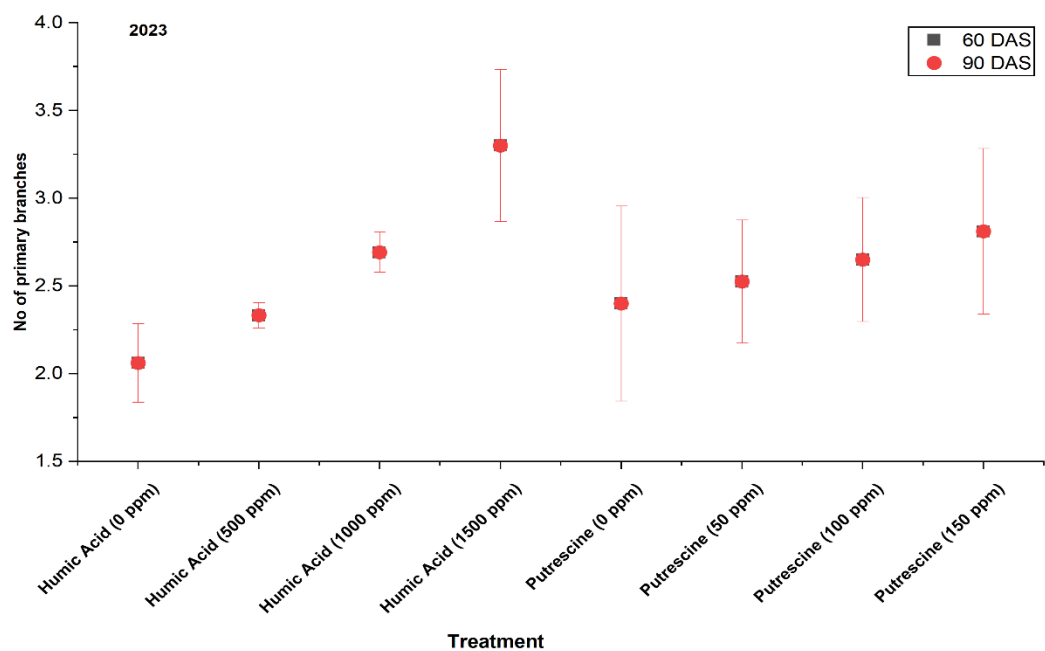


Figure 4.1.4 Effect of different treatments on number of primary branches of sesame during kharif 2023 & 2024

4.1.5 Internodal Length (cm): The effect of different treatments on internodal length (cm) of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.15 and Figure 4.1.5). In the 2023 season, the application of humic acid markedly improved internodal length relative to the untreated control. The HA1 control (0 ppm) exhibited the lowest internodal lengths, recording 6.28 cm, 10.76 cm, and 11.45 cm at 30, 60, and 90 DAS, respectively. A progressive increase was observed with HA2 (500 ppm), which registered 7.03 cm, 11.65 cm, and 11.84 cm across the same intervals. A more pronounced enhancement appeared under HA3 (1000 ppm), yielding 7.33 cm, 11.68 cm, and 11.69 cm. The maximum internodal elongation was observed with HA4 (1500 ppm), at 8.45 cm, 11.99 cm, and 12.42 cm at 30, 60, and 90 DAS, respectively, indicating a substantial dose-responsive increase with increasing humic acid concentration. A similar pattern was observed with putrescine treatments. The P1 control (0 ppm) exhibited internodal lengths of 6.40 cm, 10.94 cm, and 11.08 cm at 30, 60, and 90 DAS, respectively. Application of P2 (50 ppm) increased internodal length to 7.36 cm, 11.26 cm, and 11.46 cm, while P3 (100 ppm) induced further elongation, reaching 7.51 cm, 12.01 cm, and 12.33 cm. The highest response was observed under P4 (150 ppm), producing 7.82 cm, 12.10 cm, and 12.33 cm at 30, 60, and 90 DAS, respectively, indicating a robust concentration-dependent stimulatory effect of putrescine on vegetative growth and stem elongation. In the 2024 season, humic acid application continued to exert a pronounced positive effect on internodal length compared with the untreated control. The HA1 control (0 ppm) recorded the lowest internodal lengths, at 6.18 cm, 11.31 cm, and 11.42 cm at 30, 60, and 90 DAS, respectively. A steady improvement was observed under HA2 (500 ppm), with values of 6.87 cm, 11.49 cm, and 11.92 cm at the corresponding growth stages. A more notable enhancement emerged with HA3 (1000 ppm), yielding 7.88 cm, 11.10 cm, and 11.46 cm. The maximum stimulatory response was observed in HA4 (1500 ppm), which reached 8.56 cm, 11.49 cm, and 11.97 cm at 30, 60, and 90 DAS, respectively, indicating a substantial dose-dependent increase in internodal elongation with higher humic acid concentrations. A comparable pattern of improvement was observed under putrescine treatments. The P1 control (0 ppm) produced 7.05 cm, 10.95 cm, and 11.21 cm at 30, 60, and 90 DAS. Application of P2 (50 ppm) enhanced internodal length to

7.50 cm, 11.24 cm, and 11.61 cm, while P3 (100 ppm) resulted in further elongation, recording 7.38 cm, 11.45 cm, and 11.82 cm. The most pronounced effect was observed with P4 (150 ppm), which produced 7.55 cm, 11.76 cm, and 12.12 cm at 30, 60, and 90 DAS, respectively, highlighting the synergistic and concentration-dependent role of putrescine in promoting stem elongation and vegetative development.

4.1.5a Interaction between different treatments on internodal length (cm)s of sesame at 60 DAS

The interactive effect of humic acid and putrescine on internodal length at 60 DAS was significant in both 2023 and 2024 (Table 4.1.5a). In 2023, the combination HA4+P4 (13.17 cm) recorded the maximum internodal length, followed by HA4+P3 (12.62 cm), HA3+P4 (12.25 cm), and HA2+P4 (12.08 cm). The lowest internodal length was observed under HA1+P1 (9.90 cm), indicating minimal vegetative enhancement under untreated conditions and highlighting the strong dependence of stem elongation on combined humic acid and putrescine application. A similar trend persisted in 2024. The treatment HA4+P4 (12.31 cm) again exhibited the highest internodal length, followed by HA1+P3 (12.25 cm), HA2+P4 (11.96 cm), and HA4+P1 (11.55 cm). Consistent with the previous season, the lowest internodal length was recorded under HA1+P1 (9.90 cm), reaffirming the limited elongation potential in the absence of both bio-stimulants.

4.1.5b Interaction between different treatments on internodal length (cm)s of sesame at 90 DAS

The interactive effect of humic acid and putrescine on internodal length at 90 DAS was significant in both 2023 and 2024 (Table 4.1.5b). In 2023, the combination HA4+P4 (13.54 cm) exhibited the maximum internodal length, followed by HA4+P3 (12.75 cm), HA2+P4 (12.45 cm), and HA3+P4 (12.36 cm). The lowest internodal length was recorded under HA1+P1 (10.12 cm), reflecting minimal vegetative stimulation in the absence of humic acid and putrescine inputs. A similar pattern was observed in 2024. The treatment HA4+P4 (12.80 cm) again produced the highest internodal length, followed by HA2+P3 (12.18 cm), HA3+P4 (12.10 cm), and HA4+P2 (11.54 cm). Consistent with the previous year, the lowest internodal length was observed under

HA1+P1 (10.31 cm), reaffirming the limited elongation response in the absence of bio-stimulant supplementation.

The present study demonstrated that humic acid and putrescine significantly increased internodal length in sesame in a concentration-dependent manner during two successive growing seasons, confirming their effectiveness as plant growth-promoting biostimulants under field conditions. The consistent enhancement in internodal elongation with increasing humic acid concentrations indicates its strong influence on stem growth and structural development. Humic acid is well known for its hormone-like properties, particularly its ability to regulate auxin- and gibberellin-mediated pathways that stimulate cell elongation, vascular differentiation, and stem expansion. These physiological effects explain the sustained increase in internodal length observed in the present study and agree with previous reports showing that humic substances promote shoot elongation, stem thickening, and structural reinforcement in sesame and other oilseed crops under diverse environmental conditions. Likewise, foliar application of putrescine produced a marked increase in internodal length, highlighting the essential role of polyamines in vegetative growth regulation. Putrescine functions as an important regulator of cell division, membrane stability, nucleic acid metabolism, and protein synthesis, while also interacting with endogenous plant hormones responsible for stem elongation. Its application likely enhanced cellular expansion, maintained meristematic activity, and alleviated oxidative damage, thereby promoting longer internodes. These findings are consistent with previous studies demonstrating that exogenous polyamines improve stem elongation, assimilate partitioning, biomass accumulation, and vegetative vigor in sesame, soybean, maize, and other field crops. Although both treatments significantly enhanced internodal elongation, humic acid generally produced a greater response than putrescine, suggesting that its broader effects on nutrient availability, root development, root–shoot signaling, photosynthetic efficiency, and overall metabolic activity contributed more effectively to stem elongation and plant structural development. Collectively, these findings underscore the potential of integrating humic acid and putrescine into sesame cultivation strategies to enhance vegetative architecture and support improved crop productivity (Reddy et al., 2021; Wei et al., 2013; Hayati et al., 2022).

Table 4.1.5 Effect of different treatments on internodal length (cm) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	6.28	10.76	11.45	6.18	11.31	11.42
HA 2 (500 ppm)	7.03	11.65	11.84	6.87	11.49	11.92
HA 3 (1000 ppm)	7.33	11.68	11.69	7.88	11.10	11.46
HA 4 (1500 ppm)	8.45	11.99	12.42	8.56	11.49	11.97
SEm $\pm$	0.21	0.04	0.08	0.03	0.04	0.03
C.D. (p=0.05)	0.61	0.14	0.24	0.10	0.14	0.10
Factor (Putrescine)						
P 1 (0 ppm)	6.40	10.94	11.08	7.05	10.95	11.21
P 2 (50 ppm)	7.36	11.26	11.46	7.50	11.24	11.61
P 3 (100 ppm)	7.51	12.01	12.33	7.38	11.45	11.82
P 4 (150 ppm)	7.82	12.10	12.53	7.55	11.76	12.12
SEm $\pm$	0.21	0.04	0.08	0.03	0.04	0.03
C.D. (p=0.05)	0.61	0.14	0.24	0.10	0.14	0.10
Interaction	NS	0.27	0.49	NS	0.29	0.21

Table 4.1.5a Interaction effect of humic acid and putrescine application on internodal length (cm) at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	9.90	10.54	11.71	10.89	10.76	9.90	11.55	12.25	11.54	11.31
HA2	11.15	11.41	11.96	12.08	11.65	11.16	11.41	11.43	11.96	11.49
HA3	11.17	11.54	11.75	12.25	11.68	11.17	10.55	11.46	11.22	11.10
HA4	10.62	11.55	12.62	13.17	11.99	11.55	11.44	10.66	12.31	11.49
Mean	10.94	11.26	12.01	12.10		10.95	11.24	11.45	11.76	
SEm±	0.09					0.09				
C.D (P=0.05)	0.27					0.29				

Table 4.1.5b Interaction effect of humic acid and putrescine application on internodal length (cm) at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	10.12	10.97	12.35	11.77	11.45	10.31	11.77	11.73	11.87	11.42
HA2	11.23	11.57	12.10	12.45	11.84	11.55	11.84	12.18	12.10	11.92
HA3	11.30	11.57	12.13	12.36	11.69	11.43	10.96	11.71	11.72	11.46
HA4	11.67	11.74	12.75	13.54	12.42	11.54	11.87	11.66	12.80	11.97
Mean	11.08	11.46	12.33	12.53		11.21	11.61	11.82	12.12	
SEm±	0.16					0.07				
C.D (P=0.05)	0.49					0.20				

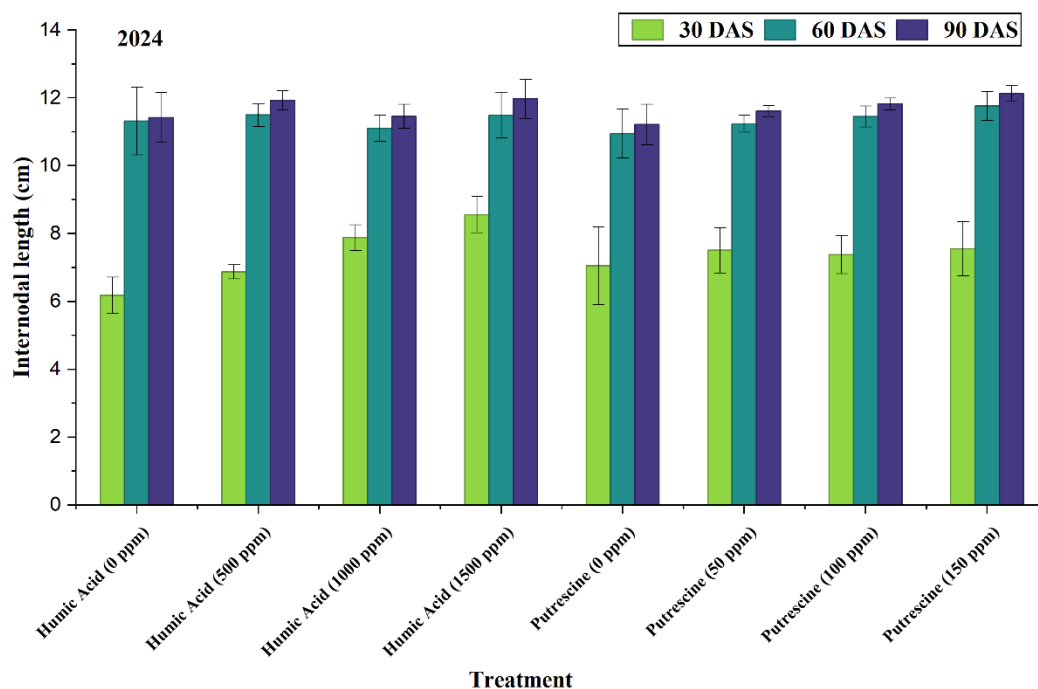
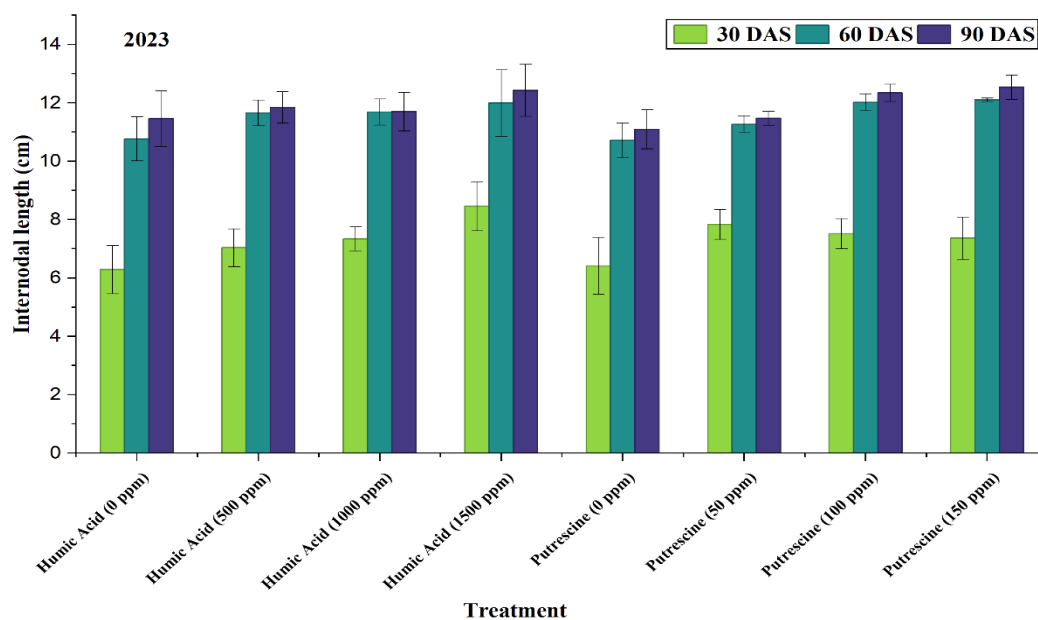


Figure 4.1.5 Effect of different treatments on internodal length (cm) of sesame during kharif 2023 & 2024

4.1.6 Number of Leaves per Plant: The effect of different treatments on the number of leaves per plant of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.1.6 and Figure 4.1.6). In the 2023 season, the application of humic acid exhibited a pronounced enhancement in the number of leaves per plant compared with the untreated control. The HA1 control (0 ppm) produced only 10.39, 51.88, and 10.86 leaves per plant at 30, 60, and 90 DAS, respectively. A progressive improvement was observed under HA2 (500 ppm), which recorded 11.00, 56.35, and 15.45 leaves per plant at the corresponding stages. A further increment occurred with HA3 (1000 ppm), yielding 11.55, 59.38, and 17.98 leaves per plant. The most substantial stimulatory effect was observed with HA4 (1500 ppm), yielding 12.71, 63.53, and 20.56 leaves per plant at 30, 60, and 90 DAS, respectively, indicating a substantial dose-responsive increase with increasing humic acid concentration. A comparable pattern was noted under putrescine treatments. The P1 control (0 ppm) recorded 10.86, 55.58, and 15.09 leaves per plant at 30, 60, and 90 DAS. Application of P2 (50 ppm) improved leaf production to 11.47, 57.68, and 16.20, while P3 (100 ppm) elicited further gains, reaching 11.58, 58.18, and 16.50 leaves per plant. The highest leaf proliferation was observed under P4 (150 ppm), with 11.75, 59.70, and 17.07 leaves per plant at 30, 60, and 90 DAS, respectively, highlighting the cumulative and growth-promoting influence of elevated putrescine concentrations on vegetative development. In the 2024 season, the application of humic acid resulted in a pronounced increase in the number of leaves per plant compared with the untreated control. The HA1 control (0 ppm) produced only 10.92, 57.40, and 10.12 leaves per plant at 30, 60, and 90 DAS, respectively. A progressive improvement was observed under HA2 (500 ppm), which recorded 10.65, 59.50, and 14.71 leaves per plant at the corresponding stages. A further increment occurred with HA3 (1000 ppm), yielding 10.38, 59.90, and 17.11 leaves per plant. The most substantial stimulatory effect was observed with HA4 (1500 ppm), yielding 11.70, 62.80, and 19.08 leaves per plant at 30, 60, and 90 DAS, respectively, indicating a substantial dose-responsive increase with increasing humic acid concentration. A comparable pattern was noted under putrescine treatments. The P1 control (0 ppm) recorded 10.30, 57.10, and 14.05 leaves per plant at 30, 60, and 90 DAS. Application of P2 (50 ppm) improved leaf production to 10.77, 59.80, and 14.73, while P3 (100

ppm) elicited further gains, reaching 10.88, 61.1, and 15.59 leaves per plant. The highest leaf proliferation was observed under P4 (150 ppm), with 11.70, 61.10, and 16.65 leaves per plant at 30, 60, and 90 DAS, respectively, highlighting the cumulative and growth-promoting influence of elevated putrescine concentrations on vegetative development.

4.1.6a Interaction between different treatments on the number of leaves per plant of sesame at 30 DAS

The interactive effect of humic acid and putrescine on the number of leaves per plant at 30 DAS was found to be significant in both 2023 and 2024 (Table 4.1.6a). In 2023, the combination HA4+P4 (13.53) exhibited the maximum number of leaves per plant, followed by HA4+P3 (12.86), HA4+P2 (12.40), and HA4+P1 (12.06). The lowest number of leaves per plant was recorded under HA1+P1 (10.53), reflecting minimal vegetative stimulation in the absence of humic acid and putrescine inputs. A similar pattern was observed in 2024. The treatment HA4+P4 (13.07) again produced the highest number of leaves per plant, followed by HA4+P2 (11.67), HA2+P4 (11.53), and HA1+P4 (11.33). Consistent with the previous year, the lowest number of leaves per plant was observed under HA1+P1 (10.13), reaffirming the limited elongation response in the absence of bio-stimulant supplementation.

The present findings clearly demonstrate that humic acid and putrescine substantially stimulate vegetative growth in sesame, with both inputs producing a consistent, concentration-dependent increase in the number of leaves per plant across seasons. The marked improvements observed at higher humic acid levels are consistent with recent evidence indicating that humic substances enhance leaf formation by improving carbon assimilation efficiency, accelerating nutrient uptake kinetics, and modulating hormone-like activities that promote meristematic activity. Enhanced root system architecture induced by humic acid—particularly increased lateral root density and surface area—likely contributed to improved mineral acquisition and sustained canopy development, a mechanism supported by the auxin-mimicking behaviour of humic fractions. Such physiological enhancements are crucial during early and mid-season growth when sesame establishes its photosynthetic and biomass-forming capacity. Application of

putrescine also exerted a significant positive effect on leaf proliferation, reflecting the well-established role of polyamines in regulating cell division, stabilizing thylakoid membranes, and enhancing leaf expansion via modulation of reactive oxygen species homeostasis. Elevated putrescine concentrations are known to promote ribosomal activity and accelerate protein synthesis, processes essential for rapid vegetative growth. Moreover, polyamines support stomatal conductance and chlorophyll retention under field conditions, which may have contributed to the sustained leaf production observed in the higher treatment levels. The synergistic improvements across seasons highlight the robustness of these biochemical inputs under open-field variability, suggesting that humic acid and putrescine act through complementary mechanisms: humic acid enhances nutrient-driven growth processes, whereas putrescine strengthens cellular division and structural stability. These results are consistent with emerging studies advocating integrated use of biostimulants to optimize growth, resilience, and yield potential in oilseed crops. Overall, the findings reaffirm the potential of humic acid and putrescine as sustainable growth-enhancement tools for sesame cultivation (Gajanan, 2022; Hayati et al., 2022; Noveyri et al., 2022).

Table 4.1.6 Effect of different treatments on number of leaves per plant of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	10.39	51.88	10.86	10.92	57.4	10.12
HA 2 (500 ppm)	11.00	56.35	15.45	10.65	59.5	14.71
HA 3 (1000 ppm)	11.55	59.38	17.98	10.38	59.9	17.11
HA 4 (1500 ppm)	12.71	63.53	20.56	11.70	62.8	19.08
SEm ±	0.12	0.55	0.26	0.19	0.44	0.07
C.D. (p=0.05)	0.34	1.61	0.781	0.56	1.28	0.20
Factor (Putrescine)						
P 1 (0 ppm)	10.86	55.58	15.09	10.30	57.1	14.05
P 2 (50 ppm)	11.47	57.68	16.20	10.77	59.8	14.73
P 3 (100 ppm)	11.58	58.18	16.50	10.88	61.1	15.59
P 4 (150 ppm)	11.75	59.70	17.07	11.70	61.6	16.65
SEm ±	0.120	0.555	0.26	0.19	1.28	0.07
C.D. (p=0.05)	0.34	1.61	0.78	0.56	0.44	0.20
Interaction	0.69	NS	NS	1.11	NS	NS

Table 4.1.6a Interaction effect of humic acid and putrescine application on number of leaves per plant at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	10.53	10.58	10.20	10.26	10.39	10.13	10.60	11.60	11.33	10.92
HA2	10.66	10.96	11.26	11.13	11.00	10.13	10.53	10.40	11.53	10.65
HA3	10.20	11.93	12.00	12.06	11.55	9.87	10.27	10.53	10.87	10.38
HA4	12.06	12.40	12.86	13.53	12.71	11.07	11.67	11.00	13.07	11.70
Mean	10.86	11.47	11.58	11.75		10.30	10.77	10.88	11.70	
SEm±	0.24					0.39				
C.D (P=0.05)	0.69					1.11				

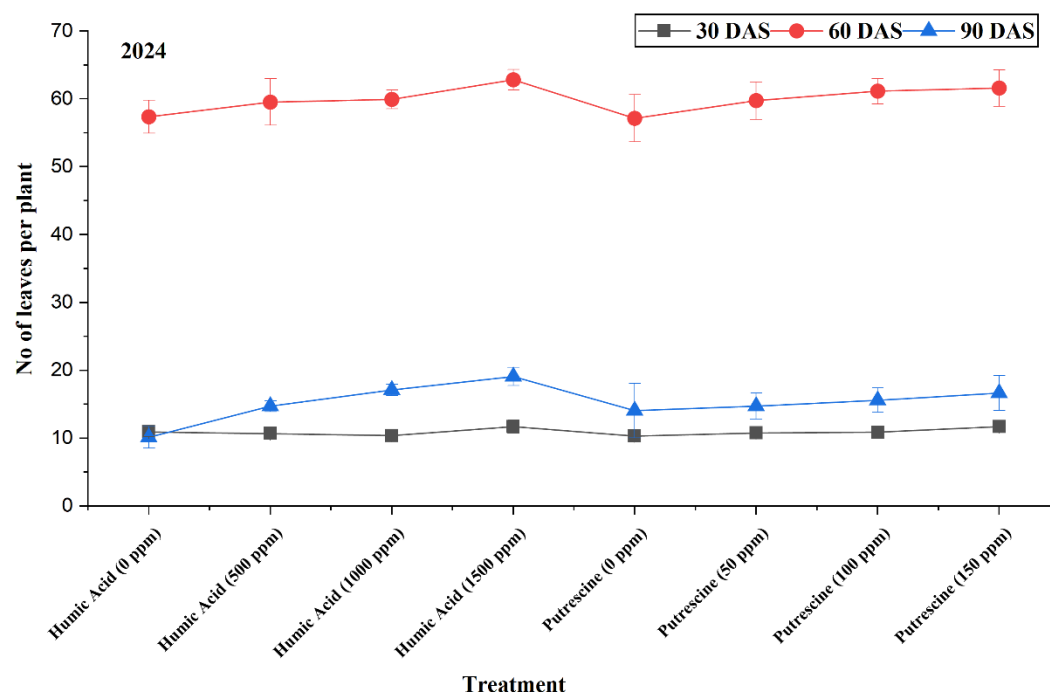
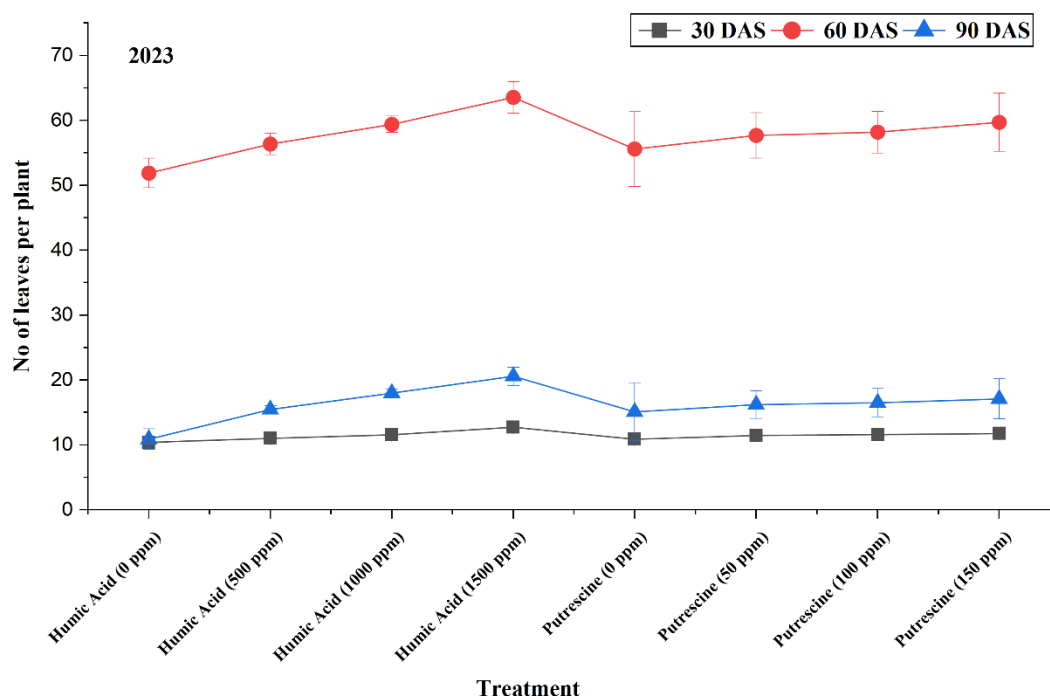


Figure 4.1.6 Effect of different treatments on number of leaves per plant of sesame during kharif 2023 & 2024

4.1.7 Leaf Area per Plant (cm²): The effect of different treatments on the number of leaf area per plant of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.1.7 and Figure 4.1.7). In the 2023 season, the application of humic acid resulted in a substantial enhancement in leaf area per plant compared with the untreated control. The HA1 control (0 ppm) produced leaf areas of 141.7 cm², 989 cm², and 230.4 cm² per plant at 30, 60, and 90 DAS, respectively. A steady improvement was evident under HA2 (500 ppm), with values of 158.0 cm², 1226 cm², and 349.76 cm² at the corresponding stages. This trend intensified with HA3 (1000 ppm), yielding leaf areas of 194.2 cm², 1433 cm², and 447.7 cm². The greatest stimulatory effect was observed with HA4 (1500 ppm), achieving 244.4 cm², 1669 cm², and 560.2 cm² at 30, 60, and 90 DAS, respectively, clearly demonstrating a robust dose-dependent enhancement as humic acid concentration increased. A similar response pattern was observed under putrescine treatments. The P1 control (0 ppm) produced 165.1 cm², 1199 cm², and 346.86 cm² of leaf area per plant at 30, 60, and 90 DAS, respectively. Application of P2 (50 ppm) enhanced leaf expansion to 178.4 cm², 1277 cm², and 375.5 cm², while P3 (100 ppm) promoted additional gains, reaching 195.6 cm², 1419 cm², and 431.3 cm². The maximum leaf area was recorded under P4 (150 ppm), with values of 199.3 cm², 1422 cm², and 434.2 cm² at 30, 60, and 90 DAS, respectively, underscoring the synergistic and growth-promoting effects of higher putrescine levels on canopy development. In the 2024 season, the application of humic acid resulted in a substantial enhancement in leaf area per plant compared with the untreated control. The HA1 control (0 ppm) produced leaf areas of 151.0 cm², 851 cm², and 200.4 cm² per plant at 30, 60, and 90 DAS, respectively. A steady improvement was evident under HA2 (500 ppm), with values of 156.5 cm², 944 cm², and 319.5 cm² at the corresponding stages. This trend intensified with HA3 (1000 ppm), yielding leaf areas of 172.3 cm², 1068 cm², and 396.0 cm². The greatest stimulatory effect was observed with HA4 (1500 ppm), achieving 221.2 cm², 1286 cm², and 484.9 cm² at 30, 60, and 90 DAS, respectively, clearly demonstrating a robust dose-dependent enhancement as humic acid concentration increased. A similar response pattern was observed under putrescine treatments. The P1 control (0 ppm) produced 155.2 cm², 937 cm², and 308.0 cm² of leaf area per plant at 30, 60, and 90 DAS, respectively. Application of P2 (50 ppm) enhanced

leaf expansion to 162.0 cm², 972 cm², and 334.3 cm², while P3 (100 ppm) promoted additional gains, reaching 182.9 cm², 1097 cm², and 361.3 cm². The maximum leaf area was recorded under P4 (150 ppm), with values of 200.7 cm², 1143 cm², and 397.3 cm² at 30, 60, and 90 DAS, respectively, underscoring the synergistic and growth-promoting effects of higher putrescine levels on canopy development.

4.1.7a Interaction between different treatments on leaf area per plant (cm²) of sesame at 30 DAS

The interactive effect of humic acid and putrescine on leaf area per plant at 30 DAS was significant in both 2023 and 2024 (Table 4.1.7a). During 2023, the treatment combination HA4+P4 (273.6 cm²) resulted in the best leaf area per plant, followed by HA4+P3 (258.1 cm²), HA4+P2 (239.1 cm²), and HA4+P1 (206.8 cm²). The minimum leaf area was observed under HA1+P1 (127.2 cm²), indicating a weak vegetative response in the absence of both humic acid and putrescine. A comparable pattern emerged in 2024. The HA4+P4 (274.1 cm²) treatment again produced the highest leaf area, followed by HA4+P3 (218.2 cm²), HA4+P2 (206.1 cm²), and HA4+P1 (186.2 cm²). Consistent with the previous year, the lowest leaf area per plant was recorded in HA1+P1 (122.1 cm²), reaffirming the limited canopy expansion when no biostimulant supplementation was provided.

4.1.7b Interaction between different treatments on leaf area per plant (cm²) of sesame at 60 DAS

The interactive effect of humic acid and putrescine on leaf area per plant at 60 DAS was significant in both 2023 and 2024 (Table 4.1.7b). In 2023, the treatment combination HA4+P4 (1844 cm²) produced the highest leaf area per plant, followed by HA4+P3 (1810 cm²), HA4+P1 (1525 cm²), and HA4+P2 (1498 cm²). The minimum leaf area was recorded under HA1+P1 (825 cm²), indicating a markedly reduced canopy development in the absence of humic acid and putrescine. A comparable trend was observed in 2024. The HA4+P4 treatment (1493 cm²) again resulted in the maximum leaf area per plant, followed by HA4+P3 (1315 cm²), HA4+P2 (1214 cm²), and HA3+P3 (1146 cm²). As in the previous year, the lowest leaf area was observed under

HA1+P1 (687 cm²), reaffirming the limited expansion of foliage when no biostimulant supplementation was provided.

4.1.7c Interaction between different treatments on leaf area per plant (cm²) of sesame at 90 DAS

The interactive effect of humic acid and putrescine on leaf area per plant at 90 DAS was significant in both 2023 and 2024 (Table 4.1.7c). In 2023, the treatment combination HA4+P4 (628.6 cm²) recorded the best leaf area per plant, followed by HA4+P3 (610.1 cm²), HA4+P2 (520.4 cm²), and HA4+P1 (481.7 cm²). The smallest leaf area was observed under HA1+P1 (182.1 cm²), reflecting markedly suppressed foliage expansion in the absence of humic acid and putrescine supplementation. A comparable trend emerged in 2024, where HA4+P4 (560.1 cm²) again produced the maximum leaf area per plant, followed by HA4+P3 (489.4 cm²), HA4+P2 (466.6 cm²), and HA3+P1 (423.6 cm²). Consistent with the previous season, the lowest leaf area was documented under HA1+P1 (147.2 cm²), reaffirming the restricted vegetative growth under untreated conditions.

The consistent increase in leaf area observed under applications of humic acid and putrescine across both growing seasons highlights the strong biostimulatory potential of these compounds in sesame cultivated under open-field conditions. The progressive increase in leaf expansion with rising humic acid concentrations is congruent with recent findings demonstrating that humic substances stimulate morphological and physiological attributes by improving nutrient uptake efficiency, activating plasma-membrane H⁺-ATPases, and promoting hormonal crosstalk associated with leaf morphogenesis. Humic acid is known to enhance auxin-like signaling, increase photosynthetic pigment biosynthesis, and improve carbon assimilation pathways, all of which directly contribute to greater leaf lamina development and canopy enlargement. Additionally, enhanced root hydraulic conductivity and rhizosphere microbial activation triggered by humic fractions may have further supported leaf area expansion through increased water absorption and nutrient mobilization. Application of putrescine likewise produced a marked increase in leaf area, reflecting the central regulatory role of polyamines in cellular expansion, membrane stabilization, and the maintenance of

chloroplast integrity. Putrescine enhances cell elongation by modulating expansin activity, supporting translational machinery, and buffering oxidative stress through ROS-scavenging pathways. The improvements in leaf area with higher putrescine doses are also consistent with findings that polyamines enhance stomatal conductance, stimulate carotenoid and chlorophyll synthesis, and maintain mesophyll cell turgor, thereby facilitating sustained photosynthetic performance under fluctuating field conditions. The season-to-season consistency in responses to both humic acid and putrescine suggests that these biostimulants confer robust physiological advantages regardless of environmental variation. Their complementary mechanisms—humic acid driving nutrient-linked biochemical enhancement and putrescine reinforcing cellular and structural stability—highlight their potential as integrated growth-promotion tools in sesame cultivation. Recent literature increasingly supports the combined use of biostimulants to optimize canopy architecture, radiation interception, and overall biomass production in oilseed crops (Salama, 2023; Gajanan, 2022; Noveyri et al., 2022).

Table 4.1.7 Effect of different treatments on leaf area per plant (cm²) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	141.7	989	230.4	151.0	851	200.4
HA 2 (500 ppm)	158.0	1226	349.8	156.5	944	319.5
HA 3 (1000 ppm)	194.2	1433	447.7	172.3	1068	396.0
HA 4 (1500 ppm)	244.4	1669	560.2	221.2	1286	484.9
SEm ±	1.39	1.65	1.37	1.01	1.39	1.53
C.D. (p=0.05)	4.06	4.80	3.98	2.94	4.04	4.44
Factor (Putrescine)						
P 1 (0 ppm)	165.1	1199	346.9	155.2	937	308.0
P 2 (50 ppm)	178.4	1277	375.5	162.0	972	334.3
P 3 (100 ppm)	195.6	1419	431.4	182.9	1097	361.3
P 4 (150 ppm)	199.3	1422	434.2	200.7	1143	397.3
SEm ±	1.39	1.65	1.37	1.01	1.39	1.53
C.D. (p=0.05)	4.06	4.80	3.98	2.94	4.04	4.44
Interaction	8.12	9.61	7.97	5.89	8.08	8.89

Table 4.1. Interaction effect of humic acid and putrescine application on leaf area per plant (cm²) at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	127.2	134.2	151.2	154.2	141.7	122.1	134.3	173.9	173.2	151.0
HA2	148.8	152.4	165.5	165.2	158.0	151.8	149.6	154.2	170.1	156.5
HA3	177.7	187.8	207.4	204.0	194.2	160.7	157.9	185.2	185.4	172.3
HA4	206.8	239.1	258.1	273.6	244.4	186.2	206.1	218.2	274.1	221.2
Mean	165.1	178.4	195.6	199.3		155.2	162.0	182.9	200.7	
SEm±	2.79					2.03				
C.D (P=0.05)	8.12					5.89				

Table 4.1.7b Interaction effect of humic acid and putrescine application on leaf area per plant (cm²) at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	825	995	1064	1073	989	687	792	975	949	851
HA2	1112	1196	1298	1296	1226	889	921	951	1017	944
HA3	1333	1420	1504	1476	1433	1053	962	1146	1112	1068
HA4	1525	1498	1810	1844	1669	1121	1214	1315	1493	1286
Mean	1199	1277	1419	1422		937	972	1097	1143	
SEm±	3.31					2.78				
C.D (P=0.05)	9.61					8.08				

Table 4.1.7c Interaction effect of humic acid and putrescine application on leaf area per plant (cm²) at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	182.1	207.4	265.2	266.7	230.4	147.2	180.5	213.7	260.4	200.4
HA2	311.7	340.0	374.5	372.8	349.8	287.6	304.2	337.8	348.3	319.5
HA3	412.0	434.1	475.8	468.8	447.7	373.6	386.1	404.1	420.3	396.0
HA4	481.7	520.4	610.1	628.6	560.2	423.6	466.6	489.4	560.12	484.9
Mean	346.7	375.5	431.4	434.2		308.0	334.3	361.3	397.3	
SEm±	2.74					3.06				
C.D (P=0.05)	7.97					8.89				

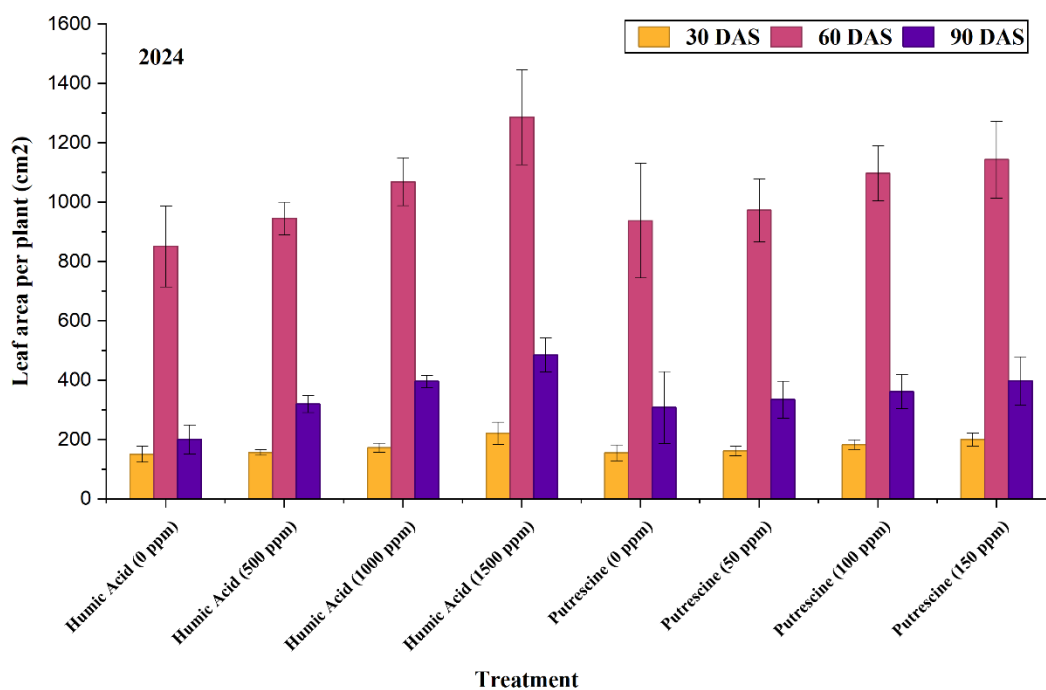
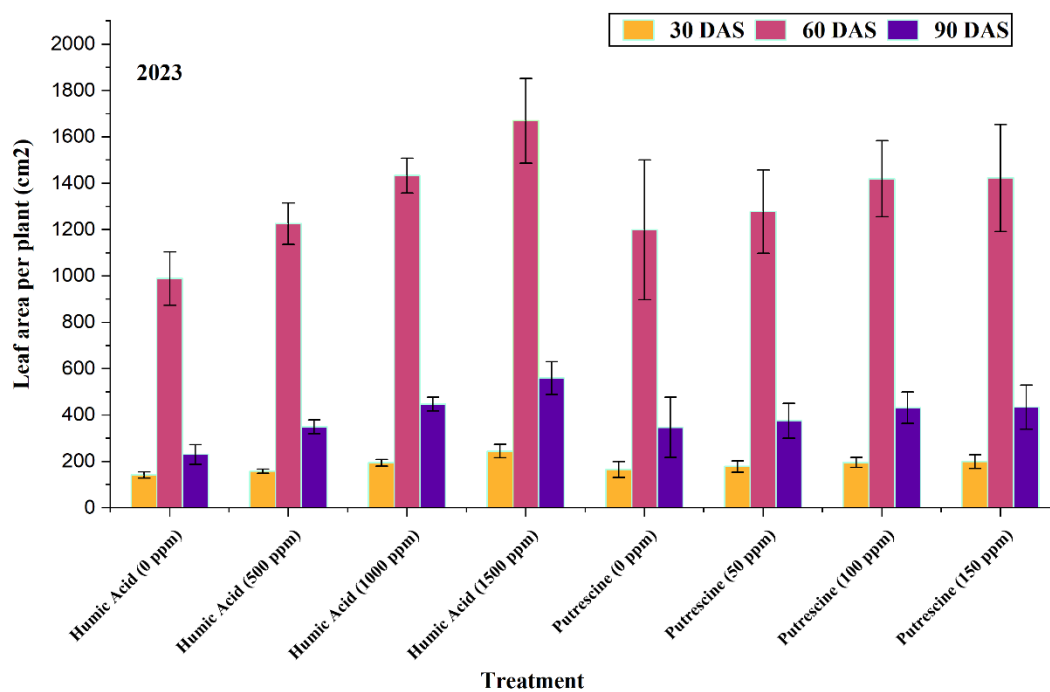


Figure 4.1.7 Effect of different treatments on leaf area per plant (cm²) of sesame during kharif 2023 & 2024

4.1.8 Days to 50% Flowering: The effect of different treatments on days to 50% flowering of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.1.8 and Figure 4.1.8). In the 2023 season, the number of days required to reach 50% flowering differed significantly across humic acid treatments. The earliest flowering occurred under HA4, which reached the 50% flowering stage in 46.25 days, followed by HA3 (49.50 days) and HA2 (49.51 days). The maximum duration was observed in the untreated control (HA1), which required 50.75 days to reach this developmental milestone. A similar trend was evident across putrescine treatments. The earliest flowering was recorded under P4, achieving 50% flowering in 48.58 days, followed by P2 (48.66 days) and P3 (49.25 days). The longest time to reach 50% flowering occurred in the untreated control (P1), which took 49.91 days, indicating delayed phenological progression in the absence of putrescine. In the 2024 season, the number of days required to reach 50% flowering differed clearly across humic acid treatments. The earliest flowering occurred under HA4, which reached the 50% flowering stage in 49.16 days, followed by HA3 (51.16 days) and HA2 (51.58 days). The maximum duration was observed in the untreated control (HA1), which required 52.41 days to reach this developmental milestone. A similar trend was evident across putrescine treatments. The earliest flowering was recorded under P4, achieving 50% flowering in 50.58 days, followed by P2 (50.83 days) and P3 (51.33 days). The longest time to reach 50% flowering occurred in the untreated control (P1), which took 51.58 days, indicating delayed phenological progression in the absence of putrescine.

4.1.8a Interaction between different treatments on 50 % flowering of sesame (2023)

The interactive effect of humic acid and putrescine on the number of days required to reach 50% flowering was significant in 2023 (Table 4.1.8a). In 2023, the treatment combination HA4+P4 recorded the earliest flowering, reaching the 50% flowering stage in 45.0 days, followed by HA4+P2 (45.3 days), HA4+P3 (45.7 days), and HA4+P1 (49.0 days). The most extended duration to reach 50% flowering was observed with the untreated combination HA1+P1, which required 51.6 days, indicating delayed phenological progression under minimal bio-stimulant input.

The present study demonstrated that humic acid and putrescine significantly influenced the time required to attain 50% flowering in sesame, indicating that both biostimulants effectively accelerated phenological development under open-field conditions. The progressive reduction in days to 50% flowering with increasing humic acid concentrations suggests that humic substances promote early vegetative growth and hasten the transition from vegetative to reproductive development. This finding is consistent with previous reports showing that humic acid improves hormonal balance, enhances carbon assimilation, stimulates root growth, and increases nutrient uptake, thereby accelerating physiological maturity and floral initiation. Putrescine contributes to floral transition by stabilizing cellular membranes, maintaining reactive oxygen species (ROS) homeostasis, regulating endogenous hormones, and activating metabolic pathways associated with flowering-time genes. Its influence on cell division, floral meristem differentiation, and protein synthesis likely contributed to the earlier attainment of 50% flowering observed at higher application levels. Comparable responses have been reported in sesame and other oilseed crops, where exogenous polyamines accelerated flowering through improved photosynthetic efficiency, enhanced assimilate partitioning, and better source–sink coordination. The similar treatment responses recorded during both growing seasons demonstrate the consistency and reliability of humic acid and putrescine under variable field environments, including fluctuations in temperature and soil moisture. The earlier flowering achieved at higher concentrations of both treatments suggests that improved nutrient acquisition, enhanced hormonal regulation, greater metabolic efficiency, and strengthened physiological activity collectively promoted a more rapid transition from vegetative growth to reproductive development, thereby contributing to improved crop establishment and productivity. These findings reinforce the growing evidence supporting the role of biostimulants in optimizing crop phenology, improving stress resilience, and enhancing reproductive efficiency in sesame cultivation (Desoky et al., 2023; Salama, 2023; Gholipour, 2020).

Table 4.1.8 Effect of different treatments on days to 50% flowering of sesame during kharif 2023 & 2024

Treatments	2023	2024
	Days to 50% flowering	Days to 50% flowering
Factor (Humic Acid)		
HA 1 (0ppm)	50.75	52.41
HA 2 (500 ppm)	49.91	51.58
HA 3 (1000 ppm)	49.50	51.16
HA 4 (1500 ppm)	46.25	49.16
SEm ±	0.27	0.30
C.D. (p=0.05)	0.79	0.87
Factor (Putrescine)		
P 1 (0 ppm)	49.91	51.58
P 2 (50 ppm)	48.66	50.83
P 3 (100 ppm)	49.25	51.33
P 4 (150 ppm)	48.58	50.58
SEm ±	0.27	0.30
C.D. (p=0.05)	0.79	NS
Interaction	1.58	NS

Table 4.1.8a Interaction effect of humic acid and putrescine application on 50% flowering (2023)

P HA	2023				
	P1	P2	P3	P4	Mean
HA1	51.6	50.0	51.0	50.3	50.7
HA2	50.0	50.3	50.3	49.0	49.9
HA3	49.0	49.0	50.0	50.0	49.5
HA4	49.0	45.3	45.7	45.0	46.2
Mean	49.9	48.6	49.5	48.5	
SEm±	0.54				
C.D (P=0.05)	1.58				

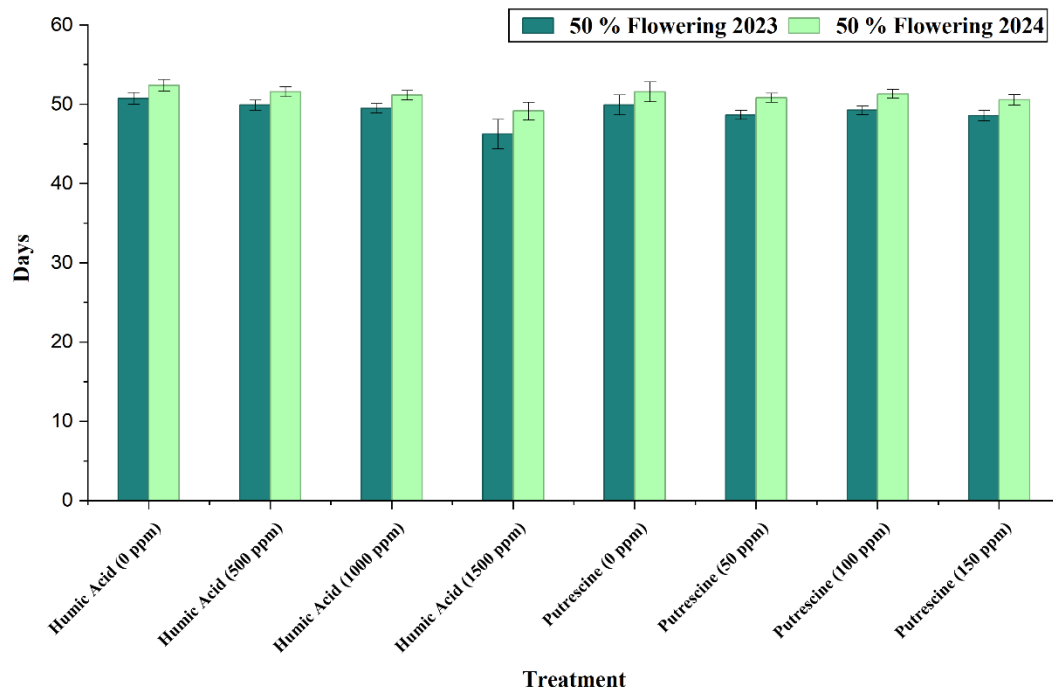


Figure 4.1.8 Effect of different treatments on days to 50% flowering of sesame during kharif 2023 & 2024

4.2 Effects of humic acid and putrescine on physiological parameters of sesame cultivation

4.2.1 Leaf Area Index: The influence of the various treatments on the leaf area index of sesame during the 2023 and 2024 seasons revealed a distinct, dose-dependent enhancement under both humic acid and putrescine applications (Table 4.1.2 and Figure 4.1.2). In the 2023 season, the application of humic acid markedly enhanced the leaf area index (LAI) compared with the untreated control. The HA1 control (0 ppm) recorded LAI values of 0.31, 2.20, and 0.51 at 30, 60, and 90 DAS, respectively. A consistent improvement was observed under HA2 (500 ppm), with values of 0.35, 2.72, and 0.77 at the corresponding growth stages. This enhancement was more pronounced with HA3 (1000 ppm), yielding LAI values of 0.43, 3.18, and 0.99. The highest stimulatory response was observed under HA4 (1500 ppm), achieving 0.54, 3.70, and 1.54 at 30, 60, and 90 DAS, respectively, indicating a substantial dose-responsive increase in canopy expansion with elevated humic acid concentration. A comparable physiological response was evident under putrescine treatments. The P1 control (0 ppm) produced LAI values of 0.36, 2.66, and 0.77 at 30, 60, and 90 DAS, respectively. Application of P2 (50 ppm) enhanced LAI to 0.39, 2.83, and 0.83, while P3 (100 ppm) generated further increases, reaching 0.43, 3.15, and 0.95. The maximum LAI was recorded under P4 (150 ppm), with values of 0.44, 3.16, and 0.96 at the respective stages, highlighting the cumulative and canopy-enhancing effects of higher putrescine concentrations on vegetative growth. In the 2024 season, the application of humic acid markedly enhanced the leaf area index (LAI) compared with the untreated control. The HA1 control (0 ppm) recorded LAI values of 0.33, 1.89, and 0.44 at 30, 60, and 90 DAS, respectively. A consistent improvement was observed under HA2 (500 ppm), with values of 0.34, 2.09, and 0.71 at the corresponding growth stages. This enhancement was more pronounced with HA3 (1000 ppm), yielding LAI values of 0.38, 2.37, and 0.88. The highest stimulatory response was observed under HA4 (1500 ppm), achieving 0.49, 2.85, and 1.07 at 30, 60, and 90 DAS, respectively, indicating a substantial dose-responsive increase in canopy expansion with elevated humic acid concentration. A comparable physiological response was evident under putrescine

treatments. The P1 control (0 ppm) produced LAI values of 0.34, 2.08, and 0.68 at 30, 60, and 90 DAS, respectively. Application of P2 (50 ppm) enhanced LAI to 0.36, 2.16, and 0.74, while P3 (100 ppm) generated further increases, reaching 0.40, 2.43, and 0.80. The maximum LAI was recorded under P4 (150 ppm), with values of 0.44, 2.53, and 0.88 at the respective stages, highlighting the cumulative and canopy-enhancing effects of higher putrescine concentrations on vegetative growth.

4.2.1a Interaction between different treatments on leaf area index of sesame at 30 DAS

The interactive effect of humic acid and putrescine on leaf area index (LAI) at 30 DAS was significant in both 2023 and 2024 (Table 4.2.1a). In 2023, the treatment combination HA4+P4 (0.61) produced the highest LAI, followed by HA4+P3 (0.57), HA4+P2 (0.53), and HA4+P1 (0.46). The lowest LAI was recorded under HA1+P1 (0.28), reflecting a poor vegetative response when neither humic acid nor putrescine was supplied. A similar trend was evident in the 2024 season. The HA4+P4 treatment (0.61) again yielded the highest LAI, followed by HA4+P3 (0.48), HA4+P2 (0.46), and HA4+P1 (0.41). As in the previous year, the minimum LAI was observed in HA1+P1 (0.21), reaffirming the restricted canopy development in the absence of biostimulant application.

4.2.1b Interaction between different treatments on leaf area index of sesame at 60 DAS

The interactive effect of humic acid and putrescine on leaf area index (LAI) at 60 DAS was significant in both 2023 and 2024 (Table 4.2.1b). In 2023, the treatment combination HA4+P4 (4.09) produced the highest LAI, followed by HA4+P3 (4.02), HA4+P1 (3.39), and HA4+P2 (3.33). The lowest LAI was recorded under HA1+P1 (1.83), reflecting a poor vegetative response when neither humic acid nor putrescine was supplied. A similar trend was evident in the 2024 season. The HA4+P4 treatment (3.31) again yielded the highest LAI, followed by HA4+P3 (2.92), HA4+P2 (2.69), and HA3+P3 (2.54). As in the previous year, the minimum LAI was observed in HA1+P1 (1.52), reaffirming the restricted canopy development in the absence of biostimulant application.

4.2.1c Interaction between different treatments on leaf area index of sesame at 90 DAS

The interactive effect of humic acid and putrescine on leaf area index (LAI) at 30 DAS was significant in both 2023 and 2024 (Table 4.2.1c). In 2023, the treatment combination HA4+P4 (1.39) produced the highest LAI, followed by HA4+P3 (1.35), HA4+P2 (1.15), and HA4+P1 (1.07). The lowest LAI was recorded under HA1+P1 (0.40), reflecting a poor vegetative response when neither humic acid nor putrescine was supplied. A similar trend was evident in the 2024 season. The HA4+P4 treatment (1.24) again yielded the highest LAI, followed by HA4+P3 (1.08), HA4+P2 (1.03), and HA4+P1 (0.41). As in the previous year, the minimum LAI was observed in HA1+P1 (0.32), reaffirming the restricted canopy development in the absence of biostimulant application.

The observed enhancement in leaf area index (LAI) under humic acid and putrescine treatments across both growing seasons demonstrates their significant role in regulating canopy development and overall physiological performance of sesame under open field conditions. The progressive increase in LAI with rising humic acid concentrations indicates a strong stimulatory influence on leaf expansion, photosynthetic surface development, and biomass accumulation. Humic substances are known to enhance enzymatic activity, stimulate root growth, and improve the assimilation of essential nutrients such as nitrogen and potassium, thereby driving greater leaf proliferation and surface-area development. Their auxin-like activity and ability to modulate cell wall loosening further contribute to increased leaf expansion, explaining the pronounced canopy growth observed at higher application levels. Putrescine treatments also produced a consistent increase in LAI, reaffirming the role of polyamines as key regulators of vegetative growth and stress-responsive physiological pathways. Putrescine enhances chloroplast development, stabilizes thylakoid membranes, and modulates stomatal function, thereby improving photosynthetic efficiency and sustaining leaf area retention. Its involvement in enhancing nitrogen metabolism and cell division contributes to more vigorous canopy formation, consistent with earlier

reports in sesame and other oilseed crops, in which polyamines promoted higher leaf area and chlorophyll stability under field and semi-arid conditions. The consistency of responses across seasons further suggests that both biostimulants provide resilience against environmental variability. The combined physiological effects of improved nutrient uptake, enhanced hormonal regulation, membrane stabilization, and efficient carbon assimilation likely explain the superior LAI observed at higher concentrations of humic acid and putrescine. These findings support their potential as effective agronomic interventions to maximize canopy development and improve productivity in sesame cultivation (Desoky et al., 2023; Shah et al., 2023; Gholipour, 2020).

Table 4.2.1 Effect of different treatments on leaf area index of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.31	2.20	0.51	0.33	1.89	0.44
HA 2 (500 ppm)	0.35	2.72	0.77	0.34	2.09	0.71
HA 3 (1000 ppm)	0.43	3.18	0.99	0.38	2.37	0.88
HA 4 (1500 ppm)	0.54	3.70	1.24	0.49	2.85	1.07
SEm ±	0.003	0.004	0.003	0.002	0.003	0.004
C.D. (p=0.05)	0.009	0.01	0.010	0.007	0.009	0.011
Factor (Putrescine)						
P 1 (0 ppm)	0.36	2.66	0.77	0.34	2.08	0.68
P 2 (50 ppm)	0.39	2.83	0.83	0.36	2.16	0.74
P 3 (100 ppm)	0.43	3.15	0.95	0.40	2.43	0.80
P 4 (150 ppm)	0.44	3.16	0.96	0.44	2.53	0.88
SEm ±	0.003	0.004	0.003	0.002	0.003	0.004
C.D. (p=0.05)	0.009	0.01	0.010	0.007	0.009	0.11
Interaction	0.01	0.02	0.02	0.01	0.01	0.22

Table 4.2.1a Interaction effect of humic acid and putrescine application on leaf area index at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.28	0.29	0.33	0.34	0.31	0.27	0.30	0.38	0.38	0.33
HA2	0.33	0.34	0.37	0.36	0.35	0.33	0.33	0.34	0.37	0.34
HA3	0.39	0.41	0.46	0.45	0.43	0.36	0.35	0.41	0.41	0.38
HA4	0.46	0.53	0.57	0.61	0.54	0.41	0.46	0.48	0.61	0.49
Mean	0.36	0.39	0.43	0.44		0.34	0.36	0.40	0.44	
SEm±	0.006					0.004				
C.D (P=0.05)	0.01					0.01				

Table 4.2.1b Interaction table of leaf area index at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	1.83	2.21	2.36	2.38	2.20	1.52	1.76	2.16	2.10	1.89
HA2	2.47	2.65	2.88	2.88	2.72	1.97	2.04	2.11	2.26	2.09
HA3	2.96	3.15	3.34	3.28	3.18	2.34	2.13	2.54	2.47	2.37
HA4	3.39	3.33	4.02	4.09	3.70	2.49	2.69	2.92	3.31	2.85
Mean	2.66	2.83	3.15	3.16		2.08	2.16	2.43	2.53	
SEm±	0.007					0.006				
C.D (P=0.05)	0.02					0.01				

Table 4.2.1c Interaction effect of humic acid and putrescine application on leaf area index at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.40	0.46	0.58	0.59	0.51	0.32	0.40	0.47	0.57	0.44
HA2	0.69	0.75	0.83	0.82	0.77	0.63	0.67	0.75	0.77	0.71
HA3	0.91	0.96	1.05	1.04	0.99	0.83	0.85	0.89	0.93	0.88
HA4	1.07	1.15	1.35	1.39	1.24	0.94	1.03	1.08	1.24	1.07
Mean	0.77	0.83	0.95	0.96		0.68	0.74	0.80	0.88	
SEm±	0.006					0.008				
C.D (P=0.05)	0.02					0.22				

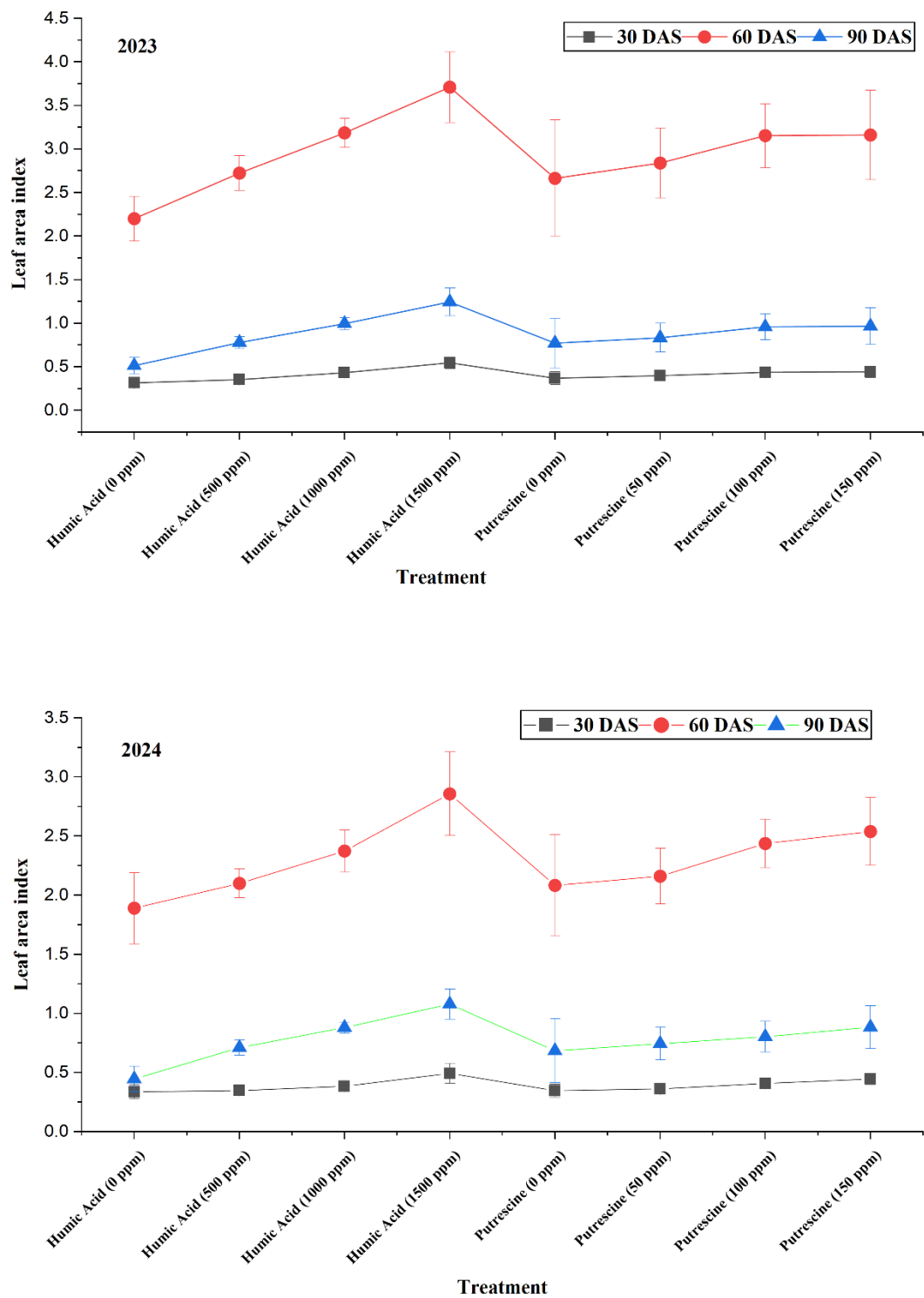


Figure 4.2.1 Effect of different treatments on leaf area index of sesame during kharif 2023 & 2024

4.2.2 Crop Growth Rate(CGR): The influence of the applied treatments on sesame crop growth rate (CGR), measured in $\text{g m}^{-2} \text{ day}^{-1}$, during the 2023 and 2024 seasons showed a pronounced, dose-responsive enhancement, with both humic acid and putrescine contributing to progressively higher growth rates as their concentrations increased (Table 4.2.2 and Figure 4.2.2). In the 2023 season, the crop growth rate (CGR) of sesame showed significant variation among the humic-acid treatments across the three growth intervals. During 0–30 DAS, CGR values were relatively low, with the untreated control (HA1) registering the minimum growth rate ($0.84 \text{ g m}^{-2} \text{ day}^{-1}$), while HA4 achieved the maximum ($1.13 \text{ g m}^{-2} \text{ day}^{-1}$). HA2 (0.88) and HA3 (0.99) recorded intermediate rates, indicating moderate biomass accumulation during early growth. A substantial rise in CGR was evident during 30–60 DAS, corresponding to the peak vegetative and early reproductive phase. HA4 again recorded the highest CGR (10.78), followed by HA3 (8.85) and HA2 (6.37), whereas HA1 (4.87) remained the lowest, reflecting the pronounced stimulatory effect of higher humic-acid concentrations on mid-season biomass production. During 60–90 DAS, CGR declined across all treatments as the crop approached physiological maturity. Nonetheless, HA4 (1.22) and HA2 (1.55) maintained comparatively higher CGR values, with HA3 (1.45) showing a moderate response and HA1 (1.31) continuing to exhibit the lowest rate. Overall, the CGR trend showed a characteristic rise from early to mid-growth stages followed by a late-season decline, with HA4 consistently outperforming all other treatments, confirming the beneficial role of higher humic-acid levels in promoting sustained biomass accumulation in sesame. A similar pattern was observed under putrescine treatments. During 0–30 DAS, CGR remained low across all treatments, with P1 recording the lowest value ($0.89 \text{ g m}^{-2} \text{ day}^{-1}$) and P4 the highest ($1.01 \text{ g m}^{-2} \text{ day}^{-1}$). P2 (0.98) and P3 (0.99) showed moderate early-season growth. During the 30–60 DAS interval, CGR increased sharply, indicating the phase of maximal vegetative expansion. P4 again recorded the highest CGR (8.80), followed by P3 (8.21) and P2 (7.70), whereas P1 (6.14) remained the lowest, indicating that elevated putrescine levels positively influence biomass production during this critical stage. During 60–90 DAS, CGR declined with crop maturity, whereas P4 (1.75) and P3 (1.74) maintained relatively higher CGR values, whereas P2 (1.47) showed a moderate response, and P1 (1.09) remained the lowest. Collectively, putrescine treatments exhibited the typical

CGR progression observed in sesame, with P4 consistently enhancing biomass accumulation and demonstrating the most substantial growth-promoting effect among the treatment levels. In the 2024 season, the crop growth rate (CGR) of sesame varied significantly among humic-acid treatments across the three growth intervals. During 0–30 DAS, CGR values were relatively low, with the untreated control (HA1) registering the minimum growth rate ($1.11 \text{ g m}^{-2} \text{ day}^{-1}$), while HA4 achieved the maximum ($1.22 \text{ g m}^{-2} \text{ day}^{-1}$). HA2 (1.15) and HA3 (1.09) recorded intermediate rates, indicating moderate biomass accumulation during early growth. A substantial rise in CGR was evident during 30–60 DAS, corresponding to the peak vegetative and early reproductive phase. HA4 again recorded the highest CGR (10.55), followed by HA3 (8.28) and HA2 (5.88), whereas HA1 (4.50) remained the lowest, reflecting the pronounced stimulatory effect of higher humic-acid concentrations on mid-season biomass production. During 60–90 DAS, CGR declined across all treatments as the crop approached physiological maturity. Nonetheless, HA2 (1.50) and HA4 (1.08) maintained comparatively higher CGR values, with HA3 (1.08) showing a moderate response and HA1 (0.75) continuing to exhibit the lowest rate. Overall, the CGR trend showed a characteristic rise from early to mid-growth stages followed by a late-season decline, with HA2 consistently outperforming all other treatments, confirming the beneficial role of higher humic-acid levels in promoting sustained biomass accumulation in sesame. A similar pattern was observed under putrescine treatments. During 0–30 DAS, CGR remained low across all treatments, with P3 recording the lowest value ($1.12 \text{ g m}^{-2} \text{ day}^{-1}$), whereas P2 exhibited the highest value ($1.20 \text{ g m}^{-2} \text{ day}^{-1}$). P4 (1.14) and P1 (1.13) showed moderate early-season growth. During the 30–60 DAS interval, CGR increased sharply, indicating the phase of maximal vegetative expansion. P4 again recorded the highest CGR (8.44), followed by P3 (7.94) and P2 (7.21), whereas P1 (5.64) remained the lowest, indicating that elevated putrescine levels positively influence biomass production during this critical stage. During 60–90 DAS, CGR declined with crop maturity, whereas P4 (1.43) and P3 (1.31) maintained relatively higher CGR values, whereas P2 (1.06) showed a moderate response, and P1 (0.61) remained the lowest. Collectively, putrescine treatments exhibited the typical CGR progression observed in sesame, with P4 consistently enhancing biomass accumulation and demonstrating the most substantial growth-promoting effect among the treatment levels.

The variation in crop growth rate (CGR) across humic-acid and putrescine treatments reflects their strong physiological influence on biomass accumulation and growth dynamics of sesame under open-field conditions. The gradual rise in CGR from early growth to the mid-season peak, followed by a decline toward maturity, aligns with the typical growth pattern of sesame; however, the magnitude of CGR enhancement under both biostimulants suggests significant improvements in photosynthetic efficiency, nutrient assimilation, and metabolic activity. Higher humic-acid levels consistently produced superior CGR values, particularly during the 30–60 DAS interval, which corresponds to the phase of rapid canopy expansion and reproductive initiation. This response can be attributed to the well-established role of humic substances in enhancing root architecture, facilitating nutrient uptake, and stimulating hormonal signaling pathways related to cell division and elongation. Humic acid has also been reported to improve nitrogen-use efficiency and carbon assimilation rates, thereby accelerating biomass productivity during critical growth phases. The sustained advantage, even during late-season growth, indicates prolonged metabolic activity and improved regulation of senescence. Putrescine treatments exhibited a similar CGR trajectory, with the higher concentrations generating stronger responses. Polyamines, such as putrescine, modulate physiological processes associated with photosynthesis, chlorophyll stability, and antioxidant defense systems. Their involvement in enhancing cell proliferation, reducing oxidative stress, and strengthening membrane integrity contributes to greater biomass formation, especially during peak vegetative stages. Putrescine's ability to delay senescence and sustain metabolic activity likely explains the comparatively higher CGR observed during the 60–90 DAS period. Overall, humic acid and putrescine effectively improved seasonal biomass accumulation by enhancing nutrient dynamics, photosynthetic performance, and stress-buffering capacity. These findings reinforce their potential as effective biostimulants for maximizing growth and productivity in sesame cultivation under open-field conditions (Shah et al., 2023; Luiz et al., 2022; Ayoubizadeh et al., 2020).

Table 4.2.2 Effect of different treatments on crop growth rate ($\text{g m}^{-2} \text{ day}^{-1}$) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	0-30 DAS	30-60 DAS	60-90 DAS	0-30 DAS	30-60 DAS	60-90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.84	4.87	1.31	1.11	4.50	0.75
HA 2 (500 ppm)	0.88	6.37	1.55	1.15	5.88	1.50
HA 3 (1000 ppm)	0.99	8.85	1.45	1.09	8.28	1.08
HA 4 (1500 ppm)	1.13	10.78	1.69	1.22	10.55	1.08
SEm $\pm$	0.01	0.21	0.38	0.008	0.03	0.03
C.D. (p=0.05)	0.04	0.63	0.14	0.02	0.11	0.11
Factor (Putrescine)						
P 1 (0 ppm)	0.89	6.14	1.09	1.13	5.62	0.61
P 2 (50 ppm)	0.98	7.70	1.47	1.20	7.21	1.06
P 3 (100 ppm)	0.99	8.21	1.74	1.12	7.94	1.31
P 4 (150 ppm)	1.01	8.80	1.75	1.14	8.44	1.43
SEm $\pm$	0.01	0.21	0.38	0.008	0.03	0.03
C.D. (p=0.05)	0.04	0.63	0.14	0.02	0.11	0.11
Interaction	NS	NS	NS	NS	NS	NS

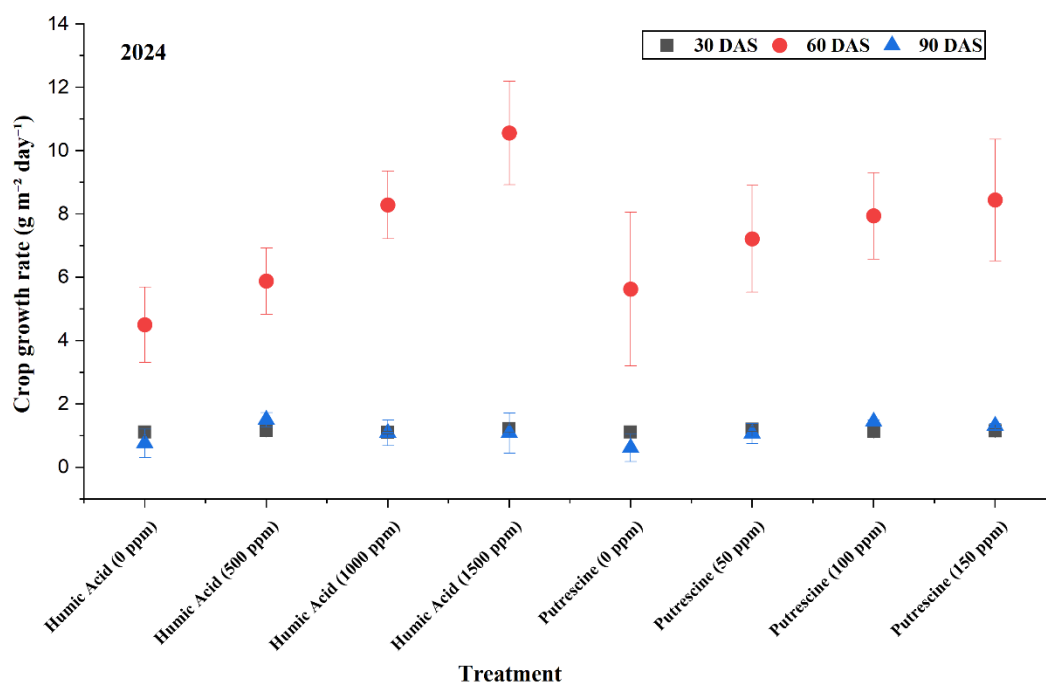
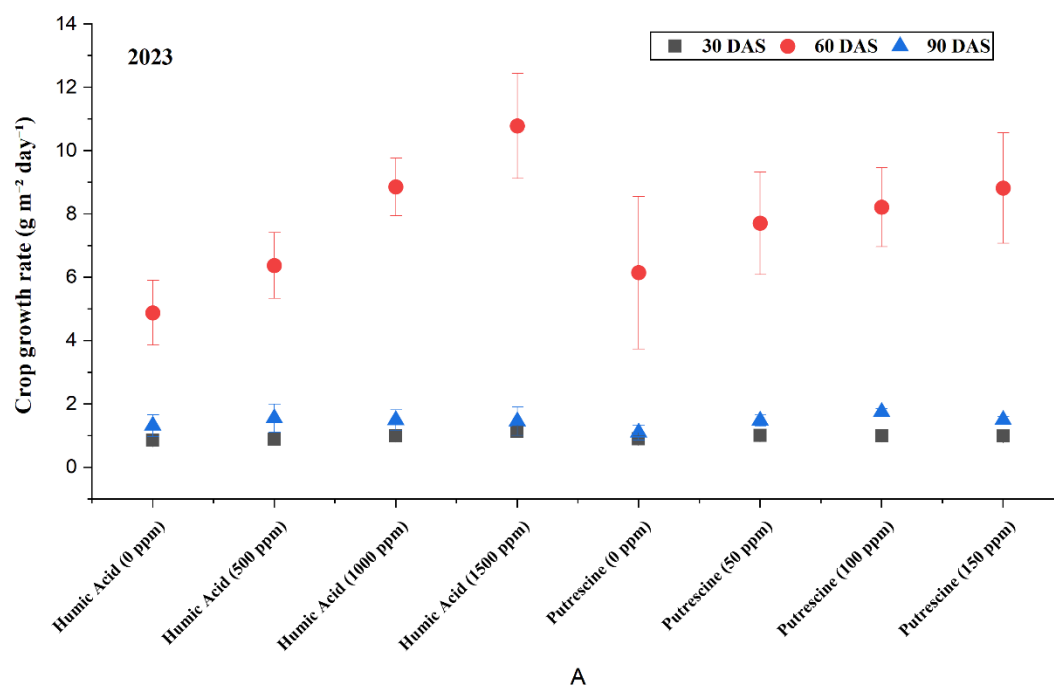


Figure 4.2.2 Effect of different treatments on crop growth rate ($\text{g m}^{-2} \text{ day}^{-1}$) of sesame during kharif 2023 & 2024

4.2.3 Relative Growth Rate (RGR): The influence of the applied treatments on sesame relative growth rate (RGR), measured in $\text{g g}^{-1} \text{ day}^{-1}$, during the 2023 and 2024 seasons showed a pronounced, dose-responsive enhancement, with both humic acid and putrescine contributing to progressively higher relative growth rates as their concentrations increased (Table 4.2.3). In the 2023 season, during the 0–30 DAS period, the relative growth rate (RGR) was generally higher across all treatments, attributable to rapid early vegetative expansion. The highest RGR was recorded under HA4 (0.014), followed by HA3 (0.009) and HA2 (0.006), whereas HA1 (0.005) showed the lowest value, indicating a clear dose-response pattern with increasing humic-acid concentration. During 30–60 DAS, RGR declined across all treatments as the crop transitioned to reproductive growth. However, HA4 (0.078) and HA3 (0.077) continued to exhibit distinctly higher RGR values, whereas HA2 (0.070) showed a moderate increase, and HA1 (0.063) remained the lowest. From 60–90 DAS, RGR further decreased as plants approached physiological maturity. A gradual reduction was observed across treatments, with HA2 (0.007) and HA1 (0.007) showing relatively higher RGR values compared with HA3 (0.005) and HA4 (0.004). This decline reflects the natural slowdown in biomass accumulation during late growth, although higher humic-acid levels still supported more sustained metabolic activity earlier in the season. A similar pattern was observed under putrescine treatments. During the 0–30 DAS period, the relative growth rate (RGR) was generally higher across all treatments, attributable to rapid early vegetative expansion. The highest RGR was recorded under P4 (0.009), followed by P3 (0.009) and P2 (0.009), whereas P1 (0.006) showed the lowest value, indicating a clear dose-response pattern with increasing putrescine concentration. During 30–60 DAS, RGR declined across all treatments as the crop transitioned to reproductive growth. However, P4 (0.076) and P3 (0.073) continued to exhibit distinctly higher RGR values, while P2 (0.072) showed moderate enhancement and P1 (0.067) remained the lowest. From 60–90 DAS, RGR further decreased as plants approached physiological maturity. A gradual reduction was observed across treatments, with P3 (0.006) and P2 (0.006) showing relatively higher RGR values compared with P4 (0.005) and P1 (0.005). This decline reflects the natural slowdown in biomass accumulation during late growth, although higher putrescine levels still supported more sustained metabolic activity earlier in the season. In the 2024 season,

during the 0–30 DAS period, the relative growth rate (RGR) was generally higher across all treatments, attributable to rapid early vegetative expansion. The highest RGR was recorded under HA4 (0.017), followed by HA2 (0.015) and HA3 (0.013), whereas HA1 (0.013) showed the lowest value, indicating a clear dose-response pattern with increasing humic-acid concentration. During 30–60 DAS, RGR declined across all treatments as the crop transitioned to reproductive growth. However, HA4 (0.075) and HA3 (0.071) continued to exhibit distinctly higher RGR values, whereas HA2 (0.060) showed a moderate increase, and HA1 (0.053) remained the lowest. From 60–90 DAS, RGR further decreased as plants approached physiological maturity. A gradual reduction was observed across treatments, with HA2 (0.006) and HA1 (0.004) showing relatively higher RGR values compared with HA3 (0.004) and HA4 (0.003). This decline reflects the natural slowdown in biomass accumulation during late growth, although higher humic-acid levels still supported more sustained metabolic activity earlier in the season. A similar pattern was observed under putrescine treatments. During the 0–30 DAS period, the relative growth rate (RGR) was generally higher across all treatments, attributable to rapid early vegetative expansion. The highest RGR was recorded under P2 (0.016), followed by P4 (0.014) and P3 (0.014), whereas P1 (0.013) showed the lowest value, indicating a clear dose-response pattern with increasing putrescine concentration. During 30–60 DAS, RGR declined across all treatments as the crop transitioned to reproductive growth. However, P4 (0.070) and P3 (0.069) continued to exhibit distinctly higher RGR values, while P2 (0.064) showed moderate enhancement and P1 (0.058) remained the lowest. From 60–90 DAS, RGR further decreased as plants approached physiological maturity. A gradual reduction was observed across treatments, with P3 (0.005) and P4 (0.004) showing relatively higher RGR values compared with P2 (0.004) and P1 (0.003). This decline reflects the natural slowdown in biomass accumulation during late growth, although higher putrescine levels still supported more sustained metabolic activity earlier in the season.

4.2.3a Interaction between different treatments on the relative growth rate of sesame at 0-30 DAS

The interactive effect of humic acid and putrescine on relative growth rate (RGR) at 0–30 DAS was significant in both 2023 and 2024 (Table 4.2.3a). In 2023, the treatment

combination HA4+P2 (0.020) exhibited the highest RGR, followed by HA3+P4 (0.014), HA4+P3 (0.013), and HA3+P3 (0.013). The lowest RGR occurred under HA1+P1 (0.005), indicating a markedly weak vegetative response in the absence of both humic acid and putrescine. A comparable pattern was observed in 2024. The HA4+P2 treatment (0.021) again achieved the maximum RGR, followed by HA2+P2 (0.017), HA4+P4 (0.016), and HA4+P1 (0.016). Consistent with the previous season, the minimum RGR was recorded in HA1+P1 (0.008), reaffirming the limited early biomass accumulation when no biostimulant supplementation was provided.

4.2.3b Interaction between different treatments on the relative growth rate of sesame at 30-60 DAS

The interactive effect of humic acid and putrescine on relative growth rate (RGR) at 30-60 DAS was significant in both 2023 and 2024 (Table 4.2.3b). In 2023, the treatment combination HA4+P4 (0.085) exhibited the highest RGR, followed by HA4+P3 (0.081), HA3+P1 (0.078), and HA3+P4 (0.076). The lowest RGR occurred under HA1+P1 (0.054), indicating a markedly weak vegetative response in the absence of both humic acid and putrescine. A comparable pattern was observed in 2024. The HA4+P4 treatment (0.081) again achieved the maximum RGR, followed by HA4+P3 (0.081), HA3+P4 (0.076), and HA3+P (0.073). Consistent with the previous season, the minimum RGR was recorded in HA1+P1 (0.047), reaffirming the limited early biomass accumulation when no biostimulant supplementation was provided.

The relative growth rate (RGR) patterns observed under humic-acid and putrescine applications reflect their profound influence on early vegetative vigor, mid-season biomass accumulation, and late-stage metabolic dynamics in sesame. RGR was highest during the initial growth phase (0–30 DAS), coinciding with rapid cell division, leaf expansion, and root establishment. The marked enhancement in RGR at higher humic-acid concentrations can be attributed to stimulation of root proliferation, increased nutrient uptake efficiency, and activation of enzymatic pathways involved in carbon assimilation and metabolic turnover. Humic substances also enhance ATP production and hormonal signaling, particularly auxin-like activity, thereby accelerating early vegetative development. As sesame transitioned into the 30–60 DAS period, the decline

in RGR across treatments reflected the shift from active vegetative growth to reproductive development. Nevertheless, the relatively higher RGR under elevated humic-acid treatments indicates sustained photosynthetic efficiency and improved nutrient retranslocation, which have been widely reported in crops receiving humic-based biostimulants. Putrescine treatments exhibited a parallel physiological response. Polyamines, such as putrescine, are integral regulators of cell division, membrane stability, and chloroplast ultrastructure, thereby enhancing metabolic activity during early growth phases. Their ability to stabilize proteins and nucleic acids helps maintain higher growth rates even as plants enter reproductive transition. During the late growth stage (60–90 DAS), a natural decline in RGR was observed across all treatments; however, putrescine continued to support relatively higher metabolic activity by delaying senescence and mitigating oxidative stress. Overall, both humic acid and putrescine substantially improved RGR during critical developmental windows, reinforcing their role as effective biostimulants for enhancing physiological efficiency and growth resilience in sesame cultivated under open-field conditions (Luiz et al., 2022; Ayoubizadeh et al., 2020; Shah et al., 2023).

Table 4.2.3 Effect of different treatememnts on relative growth rate ($\text{g g}^{-1} \text{ day}^{-1}$) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	0-30 DAS	30-60 DAS	60-90 DAS	0-30 DAS	30-60 DAS	60-90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.005	0.063	0.007	0.013	0.053	0.004
HA 2 (500 ppm)	0.006	0.070	0.007	0.015	0.06	0.006
HA 3 (1000 ppm)	0.009	0.077	0.005	0.013	0.071	0.004
HA 4 (1500 ppm)	0.014	0.078	0.004	0.017	0.075	0.003
SEm $\pm$	0.001	0.001	0.0004	0.0003	0.0003	0.0004
C.D. (p=0.05)	0.002	0.003	0.001	0.001	0.001	0.001
Factor (Putrescine)						
P 1 (0 ppm)	0.006	0.067	0.005	0.013	0.058	0.003
P 2 (50 ppm)	0.009	0.072	0.006	0.016	0.064	0.004
P 3 (100 ppm)	0.009	0.073	0.006	0.014	0.069	0.005
P 4 (150 ppm)	0.009	0.076	0.005	0.014	0.07	0.004
SEm $\pm$	0.001	0.001	0.0004	0.0003	0.0003	0.0004
C.D. (p=0.05)	0.002	0.003	0.001	0.001	0.001	0.001
Interaction	0.003	0.005	N.S	0.002	0.002	NS

Table 4.2.3a Interaction effect of humic acid and putrescine application on relative growth rate ($\text{g g}^{-1} \text{ day}^{-1}$) at 0-30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.005	0.003	0.005	0.006	0.005	0.008	0.013	0.015	0.017	0.013
HA2	0.005	0.006	0.007	0.006	0.006	0.017	0.016	0.012	0.014	0.015
HA3	0.004	0.008	0.013	0.014	0.009	0.012	0.014	0.014	0.011	0.013
HA4	0.011	0.020	0.013	0.012	0.014	0.016	0.021	0.014	0.016	0.017
Mean	0.006	0.009	0.009	0.009		0.013	0.016	0.014	0.014	
SEm±	0.002					0.0006				
C.D (P=0.05)	0.003					0.002				

Table 4.2.3b Interaction effect of humic acid and putrescine application on relative growth rate ($\text{g g}^{-1} \text{ day}^{-1}$) at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.054	0.066	0.067	0.067	0.063	0.047	0.053	0.056	0.058	0.053
HA2	0.063	0.071	0.071	0.074	0.070	0.051	0.059	0.066	0.064	0.060
HA3	0.078	0.078	0.075	0.076	0.077	0.066	0.070	0.073	0.076	0.071
HA4	0.074	0.072	0.081	0.085	0.078	0.069	0.072	0.081	0.081	0.075
Mean	0.067	0.072	0.073	0.076		0.058	0.064	0.069	0.07	
SEm±	0.002					0.0006				
C.D (P=0.05)	0.005					0.002				

4.2.4 Net Assimilation Rate (NAR): The influence of the applied treatments on the net assimilation rate (NAR) of sesame, expressed in $\text{g cm}^{-2} \text{ day}^{-1}$, during the 2023 and 2024 seasons showed a marked, dose-dependent improvement, with both humic acid and putrescine inducing progressively higher NAR values as their concentrations increased (Table 4.2.4). In the 2023 season, the net assimilation rate (NAR) of sesame varied markedly across growth intervals and humic-acid treatments. During 0–30 DAS, NAR was highest in HA1 (0.007), followed closely by HA2 (0.007) and HA3 (0.005), while the lowest value was recorded in HA4 (0.004). This pattern indicates that lower humic-acid concentrations were associated with a greater carbon assimilation efficiency during the early vegetative phase. In the 30–60 DAS interval, NAR declined in all treatments as leaf age increased and photosynthetic capacity gradually diminished. Nevertheless, the relative trend remained consistent, with HA1 (0.011) again exhibiting the highest NAR, followed by HA2 (0.010) and HA3 (0.008), while HA4 (0.006) continued to show the lowest value. Across both intervals, sesame plants receiving lower concentrations of humic acid (HA1 and HA2) consistently displayed superior NAR performance, suggesting enhanced leaf metabolic activity and more efficient biomass conversion compared with higher-dose applications. A similar pattern was observed under putrescine treatments. During 0–30 DAS, NAR was highest in P3 (0.006), followed closely by P2 (0.006) and P4 (0.005), while the lowest value was recorded in P1 (0.005). This pattern suggests that increasing putrescine concentration contributed to improved carbon assimilation efficiency during the early vegetative stage. In the 30–60 DAS interval, NAR declined in all treatments as leaf age advanced and photosynthetic activity gradually weakened. However, the relative trend was similar, with P3 (0.010) again showing the highest NAR, followed by P2 (0.009) and P4 (0.008), whereas P1 (0.008) remained the lowest. Across both intervals, sesame plants receiving higher putrescine concentrations (P2, P3, and P4) consistently exhibited better NAR performance, indicating enhanced leaf metabolic activity and more efficient biomass assimilation compared with the lower-dose treatment (P1). In the 2024 season, the net assimilation rate (NAR) of sesame varied distinctly across the growth intervals and humic-acid treatments. During 0–30 DAS, NAR was highest in HA1 (0.002), followed closely by HA2 (0.002) and HA3 (0.001), whereas HA4 (0.001) recorded the lowest value. This trend indicates that lower humic-acid concentrations

were associated with higher carbon assimilation efficiency during the early vegetative phase. In the 30–60 DAS interval, NAR declined across all treatments as leaf age increased and photosynthetic capacity progressively decreased. However, the relative pattern remained consistent, with HA1 (0.001) maintaining the highest NAR, followed by HA2 (0.001) and HA3 (0.001), while HA4 (0.001) again exhibited the lowest assimilation rate. Across both intervals, sesame plants supplied with lower humic-acid concentrations (HA1 and HA2) consistently demonstrated superior NAR, reflecting enhanced leaf physiological activity and improved efficiency of biomass conversion compared with the higher concentrations. A similar response was observed under putrescine treatments. During 0–30 DAS, NAR was highest in P2 (0.002), followed by P1 (0.001) and P4 (0.001), whereas P3 (0.001) had the lowest value. This suggests that moderate levels of putrescine were more effective for carbon assimilation during early growth. In the 30–60 DAS interval, NAR declined across all treatments as photosynthetic tissues aged; however, the relative trend persisted, with P3 (0.001) showing the highest NAR, followed by P2 (0.001) and P4 (0.001), whereas P1 (0.001) remained the lowest. Over both intervals, sesame plants receiving higher putrescine concentrations (P2, P3, and P4) consistently exhibited improved NAR performance, indicating enhanced metabolic efficiency and more effective biomass assimilation relative to the lower-dose treatment (P1).

Variation in the net assimilation rate (NAR) of sesame across growth stages and biostimulant treatments revealed distinct physiological responses to humic acid and putrescine under field conditions. NAR declined with crop age owing to leaf senescence, reduced chlorophyll concentration, and lower photosynthetic efficiency, a common pattern in oilseed crops. Nevertheless, treatment differences demonstrated that lower humic acid concentrations consistently enhanced NAR by improving nutrient uptake, carbon fixation, and biomass conversion. Optimal humic acid levels stimulate plasma membrane H^+ -ATPase activity, increase nutrient transport, and enhance mesophyll conductance, thereby promoting photosynthetic efficiency. Excessive concentrations may reduce assimilation because of metabolic imbalance, indicating a hormetic response. In contrast, moderate to higher putrescine concentrations maintained greater NAR throughout crop development. Putrescine preserves

chloroplast integrity, delays senescence, enhances Rubisco activity, stabilizes thylakoid membranes, and strengthens antioxidant defenses, thereby sustaining photosynthesis and reducing oxidative damage. These physiological improvements enhanced biomass production and metabolic efficiency. Overall, optimized humic acid and putrescine applications complemented each other in maintaining photosynthetic performance and supporting efficient plant growth throughout sesame development. These findings reaffirm the potential of biostimulant-based strategies to improve physiological resilience and carbon-use efficiency in sesame cultivated in open fields (Ayoubizadeh et al., 2020; El-Khayat, 2024; Javed et al., 2022).

Table 4.2.4 Effect of different treatememnts on net assimilation rate ($\text{g cm}^{-2} \text{ day}^{-1}$) of sesame during kharif 2023 & 2024

Treatments	2023		2024	
	0-30 DAS	30-60 DAS	0-30 DAS	30-60 DAS
Factor (Humic Acid)				
HA 1 (0ppm)	0.007	0.011	0.002	0
HA 2 (500 ppm)	0.007	0.010	0.002	0.001
HA 3 (1000 ppm)	0.005	0.008	0.001	0.001
HA 4 (1500 ppm)	0.004	0.006	0.001	0.001
SEm $\pm$	0.0003	0.0004	0.0003	0.0003
C.D. (p=0.05)	0.0009	0.001	0.001	0.0009
Factor (Putrescine)				
P 1 (0 ppm)	0.005	0.008	0.002	0.001
P 2 (50 ppm)	0.006	0.009	0.002	0.001
P 3 (100 ppm)	0.006	0.010	0.001	0.001
P 4 (150 ppm)	0.005	0.008	0.001	0.001
SEm $\pm$	0.0003	0.0004	0.0003	0.003
C.D. (p=0.05)	0.0009	0.001	0.001	0.0009
Interaction	NS	NS	NS	NS

4.2.5 Days to physiological maturity: The effect of different treatments on days to physiological maturity of sesame across the 2023 and 2024 growing seasons demonstrated a clear, concentration-dependent enhancement under both humic acid and putrescine applications (Table 4.2.5 and Figure 4.2.3). In the 2023 season, days to maturity in sesame were significantly affected by both humic acid and putrescine applications, with a consistent pattern of accelerated physiological development at higher concentrations of each biostimulant. Among the humic-acid treatments, HA4 recorded the shortest maturity period (86.83 days), reflecting the most rapid progression toward physiological maturity, followed by HA3 (89.16 days) and HA2 (89.58 days). The most extended duration was observed under HA1 (90.41 days), indicating delayed crop maturation in the absence of humic acid input. A comparable pattern emerged across putrescine treatments. P4 showed the earliest maturity (88.41 days), followed by P2 (88.75 days) and P3 (89.25 days), whereas P1 required the most days to mature (89.58 days). Collectively, the results demonstrate that higher doses of humic acid and putrescine consistently shortened the maturity period by enhancing metabolic efficiency, promoting faster physiological transitions, and improving overall developmental rates in sesame. In the 2024 season, days to maturity in sesame were significantly affected by both humic acid and putrescine applications, with a consistent pattern of accelerated physiological development at higher concentrations of each biostimulant. Among the humic-acid treatments, HA4 recorded the shortest maturity period (88.33 days), reflecting the most rapid progression toward physiological maturity, followed by HA3 (90.83 days) and HA2 (91.25 days). The most extended duration was observed under HA1 (92.08 days), indicating delayed crop maturation in the absence of humic acid input. A comparable pattern emerged across putrescine treatments. P4 showed the earliest maturity (90.08 days), followed by P3 (90.58 days) and P2 (90.58 days), whereas P1 required the most days to mature (91.25 days). Collectively, the results demonstrate that higher doses of humic acid and putrescine consistently shortened the maturity period by enhancing metabolic efficiency, promoting faster physiological transitions, and improving overall developmental rates in sesame. The variation in days to maturity observed in sesame under humic-acid and putrescine applications reflects the capacity of these biostimulants to modulate developmental timing through their influence on physiological and metabolic

pathways. The consistent trend of earlier physiological maturity with increasing concentrations of both inputs suggests enhanced metabolic coordination, accelerated phenophase transition, and improved crop vigor under optimized biostimulant regimes. Humic acid, particularly at higher concentrations, has been widely recognized for its role in enhancing root activity, nutrient uptake efficiency, and hormonal signaling—factors collectively linked to more rapid progression toward reproductive maturity. Recent findings indicate that humic substances stimulate auxin- and cytokinin-like responses, thereby increasing sink–source interactions and promoting the faster accumulation of biomass required for maturation. Enhanced nutrient assimilation, particularly of nitrogen and potassium, further strengthens enzymatic activities associated with flowering and pod development, thereby shortening maturation cycles. The delayed maturity under untreated conditions in both seasons is consistent with previous reports showing that the absence of humic substances constrains metabolic turnover and slows physiological development. Application of putrescine also markedly accelerated maturity, reflecting the multifunctional regulatory role of polyamines in plant development. Putrescine enhances cellular division, stabilizes nucleic acids, and supports the biosynthesis of growth-related phytohormones, particularly gibberellins and cytokinins, which collectively shorten vegetative duration and expedite reproductive progression. Its involvement in mitigating oxidative stress via enhanced antioxidant activation further allows sesame plants to maintain stronger physiological performance during the critical flowering-to-maturity interval. Moreover, putrescine-mediated improvements in chloroplast stability and carbon assimilation lead to faster attainment of physiological maturity, as observed in many field-grown oilseed crops. These findings reaffirm the utility of biostimulant-based agronomic strategies for optimizing phenological behavior and improving crop scheduling in sesame production systems (El-Khayat, 2024; Javed et al., 2022; Luiz et al., 2022).

Table 4.2.5 Effect of different treatments on days to physiological maturity of sesame during kharif 2023 & 2024

Treatments	2023	2024
	Days to physiological maturity	
Factor (Humic Acid)		
HA 1 (0ppm)	90.41	92.08
HA 2 (500 ppm)	89.58	91.25
HA 3 (1000 ppm)	89.16	90.83
HA 4 (1500 ppm)	86.83	88.33
SEm ±	0.31	0.34
C.D. (p=0.05)	0.91	0.98
Factor (Putrescine)		
P 1 (0 ppm)	89.58	91.25
P 2 (50 ppm)	88.75	90.58
P 3 (100 ppm)	89.25	90.58
P 4 (150 ppm)	88.41	90.08
SEm ±	0.31	0.34
C.D. (p=0.05)	NS	NS
Interaction	NS	NS

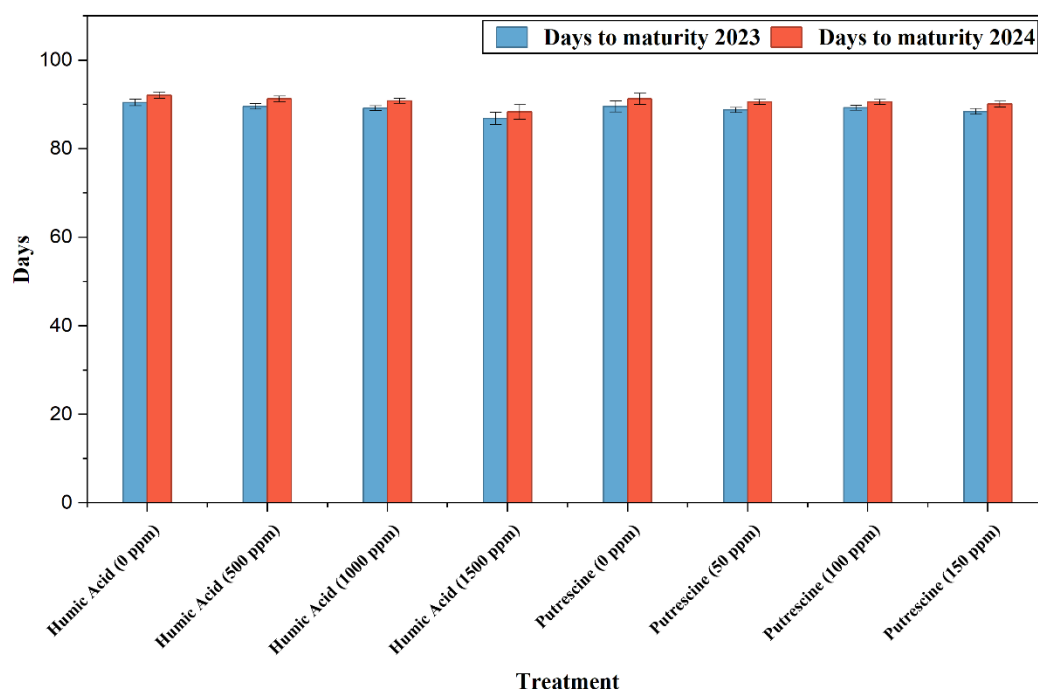


Figure 4.2.3 Effect of different treatments on days to physiological maturity of sesame during kharif 2023 & 2024

4.3 Effects of humic acid and putrescine on biochemical parameters of sesame cultivation

4.3.1 Chlorophyll index (SPAD): The influence of the applied treatments on sesame chlorophyll index measured in spad, during the 2023 and 2024 seasons, showed a pronounced, dose-responsive enhancement, with both humic acid and putrescine contributing to progressively higher chlorophyll index as their concentrations increased (Table 4.3.1 and Figure 4.3.1). In the 2023 season, the chlorophyll index of sesame varied significantly across growth stages and humic acid treatments. During the 0–30 DAS period, chlorophyll index values were generally higher in all treatments, reflecting rapid early vegetative growth. The maximum chlorophyll index was recorded under HA4 (40.43), followed by HA3 (35.04) and HA2 (33.19), whereas the lowest value was observed under the control treatment (HA1; 31.39). This trend indicates a clear dose-dependent enhancement in chlorophyll accumulation with increasing humic acid concentration. During the 30–60 DAS interval, chlorophyll index values increased further across all treatments as the crop progressed toward the reproductive phase. However, HA4 (43.06) and HA3 (40.40) maintained distinctly higher chlorophyll index values, while HA2 (41.55) showed moderate improvement and HA1 (37.40) continued to record the lowest values. From 60–90 DAS, chlorophyll index values declined across all treatments as plants approached physiological maturity. Nevertheless, HA4 (33.76) and HA3 (31.30) exhibited relatively higher chlorophyll index values than HA2 (30.81) and HA1 (27.71). This decline reflects the natural reduction in photosynthetic activity during late growth stages, although higher humic acid levels supported more prolonged chlorophyll retention earlier in the season. A comparable response pattern was observed under putrescine treatments. During 0–30 DAS, chlorophyll index values were higher across treatments, with P4 recording the maximum value (36.01), followed by P3 (35.10) and P2 (34.98). In contrast, the lowest value was observed under the control (P1; 33.97), indicating a positive dose-response effect of putrescine on chlorophyll development. During 30–60 DAS, the chlorophyll index increased across all treatments, with P4 (41.58) and P3 (41.29) exhibiting the highest values, P2 (40.95) showing a moderate increase, and P1 (38.59) remaining the lowest. From 60–90 DAS, chlorophyll index values declined as the crop approached maturity. However, P4

(31.52) and P2 (31.40) maintained relatively higher chlorophyll index values compared with P3 (30.69) and P1 (29.98). This reduction is consistent with normal senescence, although higher putrescine concentrations helped sustain chlorophyll levels during earlier growth stages. In the 2024 season, the chlorophyll index of sesame varied significantly across growth stages and humic acid treatments. During the 0–30 DAS period, chlorophyll index values were generally higher in all treatments, reflecting rapid early vegetative growth. The maximum chlorophyll index was recorded under HA4 (39.69), followed by HA3 (35.17) and HA2 (33.40), whereas the lowest value was observed under the control treatment (HA1; 31.07). This trend indicates a clear dose-dependent enhancement in chlorophyll accumulation with increasing humic acid concentration. During the 30–60 DAS interval, chlorophyll index values increased further across all treatments as the crop progressed toward the reproductive phase. However, HA4 (42.80) and HA3 (41.85) maintained distinctly higher chlorophyll index values, whereas HA2 (41.80) showed a moderate improvement, and HA1 (37.06) continued to record the lowest values. From 60–90 DAS, chlorophyll index values declined across all treatments as plants approached physiological maturity. Nevertheless, HA4 (33.79) and HA3 (31.55) exhibited relatively higher chlorophyll index values than HA2 (31.19) and HA1 (27.76). This decline reflects the natural reduction in photosynthetic activity during late growth stages, although higher humic acid levels supported more prolonged chlorophyll retention earlier in the season. A comparable response pattern was observed under putrescine treatments. During 0–30 DAS, chlorophyll index values were higher across treatments, with P4 recording the maximum value (36.22), followed by P3 (34.85) and P2 (34.21). In contrast, the lowest value was observed under the control (P1; 34.05), indicating a positive dose-response effect of putrescine on chlorophyll development. During 30–60 DAS, the chlorophyll index increased across all treatments, with P4 (41.95) and P3 (41.31) exhibiting the highest values, P2 (41.24) showing a moderate increase, and P1 (39.03) remaining the lowest. From 60–90 DAS, chlorophyll index values declined as the crop approached maturity. However, P4 (31.84) and P2 (31.73) maintained relatively higher chlorophyll index values compared with P3 (30.19) and P1 (35.53). This reduction is consistent with normal senescence, although higher putrescine concentrations helped sustain chlorophyll levels during earlier growth stages.

4.3.1a Interaction between different treatments on the chlorophyll index of sesame at 30 DAS

The interactive effect of humic acid and putrescine on chlorophyll index at 30 DAS was significant during both the 2023 and 2024 growing seasons (Table 4.3.1a). In 2023, the highest chlorophyll index was recorded under the combined treatment HA4+P4 (41.88), followed by HA4+P3 (40.25), HA4+P2 (39.90), and HA4+P1 (39.70). In contrast, the lowest chlorophyll index was observed under the control combination HA1+P1 (29.50), indicating a markedly reduced chlorophyll accumulation in the absence of both humic acid and putrescine. A similar response trend was observed in 2024. The HA4+P4 treatment again produced the maximum chlorophyll index (41.84), followed by HA4+P1 (39.38), HA4+P3 (38.62), and HA3+P2 (38.62). Consistent with the previous season, the minimum chlorophyll index was recorded under HA1+P1 (29.38), reaffirming limited photosynthetic pigment development in the absence of biostimulant supplementation.

4.3.1b Interaction between different treatments on the chlorophyll index of sesame at 60 DAS

The interactive effect of humic acid and putrescine on chlorophyll index at 60 DAS was significant during both the 2023 and 2024 growing seasons (Table 4.3.1b). In 2023, the highest chlorophyll index was recorded under the combined treatment HA4+P4 (44.75), followed by HA4+P3 (43.44), HA4+P2 (42.57), and HA4+P1 (41.47). In contrast, the lowest chlorophyll index was observed under the control combination HA1+P1 (32.78), indicating a markedly reduced chlorophyll accumulation in the absence of both humic acid and putrescine. A similar response trend was observed in 2024. The HA4+P4 treatment again produced the maximum chlorophyll index (44.30), followed by HA4+P2 (42.73), HA4+P1 (42.22), and HA3+P3 (41.96). Consistent with the previous season, the minimum chlorophyll index was recorded under HA1+P1 (32.46), reaffirming limited photosynthetic pigment development in the absence of biostimulant supplementation.

4.3.1c Interaction between different treatments on the chlorophyll index of sesame at 90 DAS

The interactive effect of humic acid and putrescine on chlorophyll index at 90 DAS was significant during both the 2023 and 2024 growing seasons (Table 4.3.1c). In 2023, the highest chlorophyll index was recorded under the combined treatment HA4+P4 (34.66), followed by HA4+P3 (34.14), HA4+P2 (33.78), and HA4+P1 (32.45). In contrast, the lowest chlorophyll index was observed under the control combination HA1+P1 (25.47), indicating a markedly reduced chlorophyll accumulation in the absence of both humic acid and putrescine. A similar response trend was observed in 2024. The HA4+P4 treatment again produced the maximum chlorophyll index (35.18), followed by HA4+P1 (3.55), HA4+P3 (3.25), and HA3+P2 (33.19). Consistent with the previous season, the minimum chlorophyll index was recorded under HA1+P1 (25.53), reaffirming limited photosynthetic pigment development in the absence of biostimulant supplementation.

Chlorophyll content, expressed as chlorophyll index (SPAD value), is a reliable indicator of leaf nitrogen status, photosynthetic capacity, and overall plant physiological performance. The consistent enhancement of chlorophyll index observed under humic acid and putrescine treatments across both seasons clearly indicates the positive influence of these biostimulants on the biochemical functioning of sesame plants. The dose-responsive improvement suggests that increasing concentrations of humic acid and putrescine effectively strengthened chlorophyll biosynthesis, stability, and retention throughout the crop growth period. Humic acid primarily enhances chlorophyll content through its beneficial effects on nutrient availability and uptake, particularly nitrogen, magnesium, and iron, which are essential components of the chlorophyll molecule and its biosynthetic enzymes. Humic substances improve soil physicochemical properties and stimulate root growth, thereby increasing the efficiency of macro- and micronutrient absorption. Enhanced nitrogen availability supports chlorophyll biosynthesis by increasing the synthesis of chlorophyll-binding proteins and photosynthetic enzymes. In contrast, improved iron availability activates key enzymes, such as protochlorophyllide reductase, involved in chlorophyll formation. These combined effects lead to higher chlorophyll accumulation, especially during

early and mid-vegetative growth stages. Additionally, humic acid influences hormonal signaling pathways, particularly auxin- and cytokinin-like activities, thereby delaying leaf senescence and maintaining chloroplast integrity. This hormonal modulation helps preserve chlorophyll content for a longer duration during reproductive growth, explaining the sustained chlorophyll index observed even at later stages. Improved membrane stability and reduced chlorophyll degradation further contribute to prolonged photosynthetic activity under humic acid application. Putrescine, a low-molecular-weight polyamine, enhances chlorophyll content through its multifaceted role in cellular metabolism and stress regulation. Polyamines are positively charged molecules that bind to negatively charged thylakoid membranes, stabilizing chloroplast ultrastructure and protecting chlorophyll pigments from oxidative damage. Putrescine also enhances nitrogen assimilation by regulating enzymes such as nitrate reductase and glutamine synthetase, thereby increasing the availability of nitrogen for chlorophyll synthesis. Another important mechanism by which putrescine improves chlorophyll index is its ability to suppress chlorophyll degradation by inhibiting chlorophyllase activity and delaying senescence-related gene expression. By reducing reactive oxygen species (ROS) accumulation and enhancing antioxidant enzyme activity, putrescine protects chlorophyll pigments from photooxidative damage, especially under field conditions where plants are exposed to fluctuating environmental stresses. The observed decline in chlorophyll index at later growth stages across all treatments reflects natural leaf senescence and resource remobilization toward developing reproductive organs. However, the relatively higher chlorophyll retention under higher doses of humic acid and putrescine suggests delayed senescence and prolonged photosynthetic competence. This sustained chlorophyll availability likely contributed to increased assimilate production, thereby supporting enhanced growth, yield, and seed quality. Overall, the synergistic effects of humic acid and putrescine on chlorophyll biosynthesis, nutrient assimilation, hormonal balance, antioxidant defense, and chloroplast stability account for the consistent improvement in the chlorophyll index across seasons. These findings confirm that both biostimulants play a crucial role in strengthening the biochemical foundation of sesame plants, thereby improving photosynthetic efficiency and crop productivity under open-field condition (Javed et al., 2022; Lotfi et al., 2015; Nouriyani, 2019).

Table 4.3.1 Effect of different treatments on chlorophyll index (spad) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	31.39	37.40	27.71	31.07	37.06	27.76
HA 2 (500 ppm)	33.19	41.55	30.81	33.40	41.80	31.19
HA 3 (1000 ppm)	35.04	40.40	31.30	35.17	41.85	31.55
HA 4 (1500 ppm)	40.43	43.06	33.76	39.69	42.80	33.79
SEm ±	0.26	0.35	0.24	0.23	0.27	0.21
C.D. (p=0.05)	0.76	1.01	0.70	0.66	0.80	0.61
Factor (Putrescine)						
P 1 (0 ppm)	33.97	38.59	29.98	34.05	39.03	30.53
P 2 (50 ppm)	34.98	40.95	31.40	34.21	41.24	31.73
P 3 (100 ppm)	35.10	41.29	30.69	34.85	41.31	30.19
P 4 (150 ppm)	36.01	41.58	31.52	36.22	41.95	31.84
SEm ±	0.26	0.35	0.24	0.23	0.27	0.21
C.D. (p=0.05)	0.76	1.01	0.70	0.66	0.80	0.61
Interaction	1.52	2.03	1.39	1.33	1.61	1.23

Table 4.3.1a Interaction effect of humic acid and putrescine application on chlorophyll index at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	29.50	31.70	31.90	32.47	31.39	29.38	30.58	30.97	33.35	31.07
HA2	32.97	31.87	35.46	32.47	33.19	32.67	31.50	37.02	32.40	33.40
HA3	33.69	36.47	32.78	37.21	35.04	34.77	36.12	32.49	37.28	35.17
HA4	39.70	39.90	40.25	41.88	40.43	39.38	38.62	38.92	41.84	39.69
Mean	33.97	34.98	35.10	36.01		34.05	34.21	34.85	36.22	
SEm±	0.52					0.46				
C.D (P=0.05)	1.52					1.33				

Table 4.3.1b Interaction effect of humic acid and putrescine application on chlorophyll index at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	32.78	37.21	39.70	39.90	37.40	32.46	37.06	38.19	40.54	37.06
HA2	40.25	41.88	42.54	41.54	41.55	39.92	41.00	43.88	42.41	41.80
HA3	39.87	42.14	39.47	40.14	40.40	41.51	44.17	41.21	40.53	41.85
HA4	41.47	42.57	43.44	44.75	43.06	42.22	42.73	41.96	44.30	42.80
Mean	38.59	40.95	41.29	41.58		39.03	41.24	41.31	41.95	
SEm±	0.70					0.54				
C.D (P=0.05)	2.03					1.61				

Table 4.3.1c Interaction effect of humic acid and putrescine application on chlorophyll index at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	25.47	28.47	27.41	29.48	27.71	25.53	28.59	27.26	29.67	27.76
HA2	30.14	30.89	31.74	30.47	30.81	30.05	31.91	31.46	31.32	31.19
HA3	31.84	32.44	29.47	31.46	31.30	33.01	33.18	28.82	31.19	31.55
HA4	32.45	33.78	34.14	34.66	33.76	33.55	33.25	33.19	35.18	33.79
Mean	29.98	31.40	30.69	31.52		30.53	31.73	30.19	31.84	
SEm±	0.48					0.42				
C.D (P=0.05)	1.33					1.23				

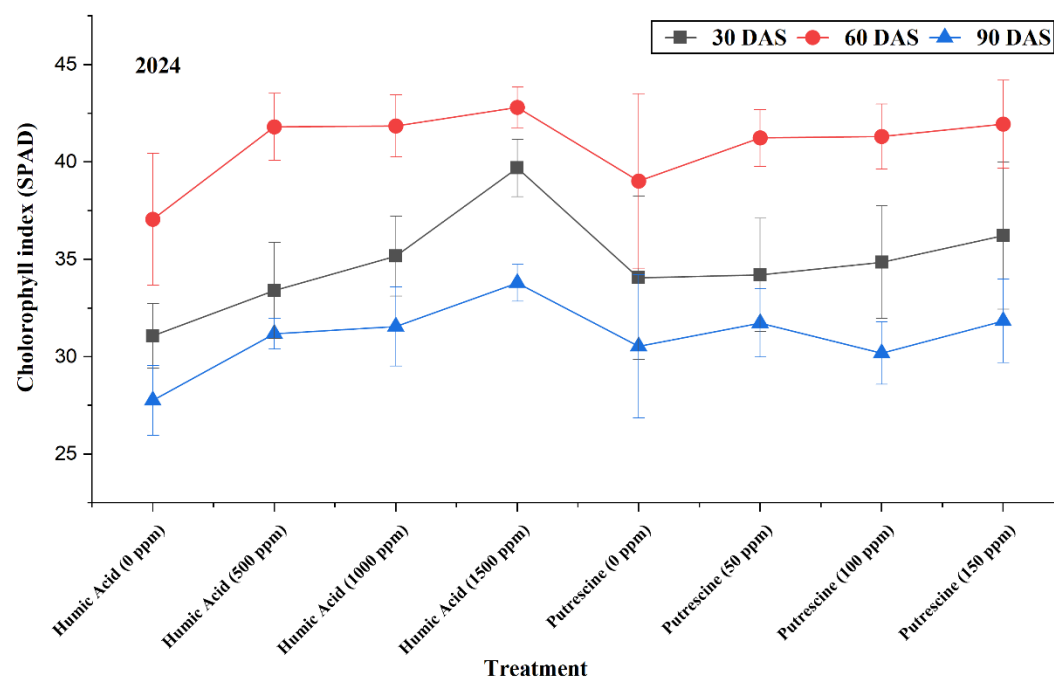
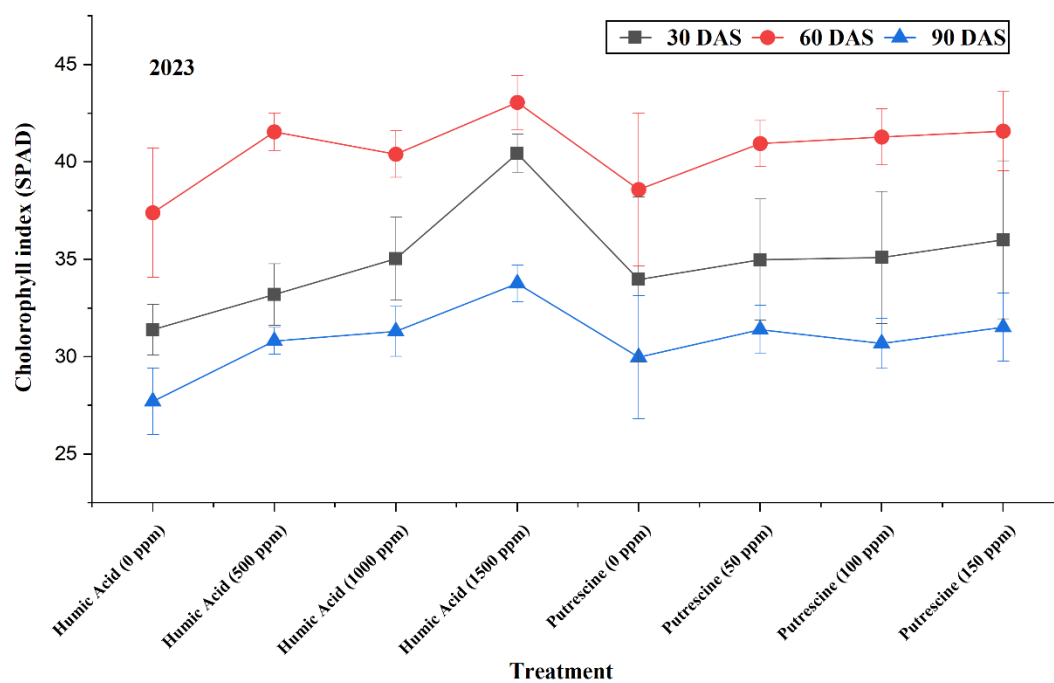


Figure 4.3.1 Effect of different treatments on chlorophyll index (spad) of sesame during kharif 2023 & 2024

4.3.2 Total Soluble Sugar (mg/g Fresh Weight): The applied treatments exerted a pronounced and concentration-dependent influence on total soluble sugar content in sesame during both the 2023 and 2024 growing seasons. Increasing levels of humic acid and putrescine consistently enhanced total soluble sugar accumulation across growth stages (Table 4.3.2 and Figure 4.3.2). In 2023, total soluble sugar content varied significantly across growth stages under different humic acid treatments. At 30 DAS, higher total soluble sugar levels were observed across treatments, reflecting active early vegetative growth. The maximum total soluble sugar content was recorded under HA2 (1.26), followed by HA4 (0.83) and HA3 (0.73), while the control treatment, HA1 (0.62), recorded the lowest value, indicating a clear treatment-induced improvement. At 60 DAS, total soluble sugar content increased further as the crop advanced toward the reproductive phase. Treatments HA2 (1.83) and HA4 (1.25) maintained significantly higher total soluble sugar levels, whereas HA3 (1.12) showed a moderate response, and HA1 (0.76) continued to record the minimum value. By 90 DAS, total soluble sugar content declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA2 (1.62) and HA4 (1.19) maintained relatively higher total soluble sugar levels than HA3 (0.97) and HA1 (0.60). This decline reflects the natural reduction in photosynthetic activity during later growth stages, although higher humic acid concentrations supported prolonged sugar retention earlier in the season. A comparable response pattern was observed under putrescine treatments. At 30 DAS, total soluble sugar content was higher across treatments, with P4 recording the maximum value (1.18), followed by P1 (0.88) and P3 (0.74). At the same time, P2 (0.63) exhibited the lowest value, indicating that putrescine positively influenced early carbohydrate accumulation. During 60 DAS, total soluble sugar content increased across all putrescine treatments. The highest values were recorded under P4 (1.57) and P1 (1.27), followed by P3 (1.20), whereas P2 (0.93) remained the lowest. At 90 DAS, total soluble sugar content declined as the crop approached maturity. Nevertheless, P4 (1.39) and P1 (1.14) maintained relatively higher total soluble sugar levels compared with P3 (1.05) and P2 (0.80). This reduction is consistent with normal senescence, although higher putrescine concentrations contributed to sustained carbohydrate levels during earlier growth phases. In 2024, the total soluble sugar content of sesame differed significantly among growth stages and humic acid

treatments. At 30 DAS, relatively higher total soluble sugar levels were recorded across treatments, reflecting vigorous early vegetative growth. The maximum total soluble sugar content was observed under HA2 (1.26), followed by HA4 (0.85) and HA3 (0.72), whereas the control treatment, HA1 (0.59), recorded the lowest value, demonstrating a clear treatment-induced enhancement. At 60 DAS, total soluble sugar content increased further as the crop progressed toward the reproductive phase. Treatments HA2 (1.84) and HA4 (1.23) maintained significantly higher sugar levels, while HA3 (1.09) exhibited a moderate response, and HA1 (0.76) continued to record the minimum value. By 90 DAS, total soluble sugar content declined across all treatments as plants approached physiological maturity. Despite this general reduction, HA2 (1.62) and HA4 (1.19) sustained comparatively higher sugar levels than HA3 (0.98) and HA1 (0.59). This decline reflects the natural reduction in photosynthetic activity during later growth stages, although higher humic acid concentrations facilitated the retention of carbohydrates during earlier phases of crop development. A similar response trend was observed under putrescine treatments. At 30 DAS, total soluble sugar content was elevated across treatments, with P4 recording the highest value (1.19), followed by P1 (0.89) and P3 (0.71). At the same time, P2 (0.63) registered the lowest value, indicating a positive role of putrescine in early carbohydrate accumulation. During 60 DAS, total soluble sugar content increased across all putrescine treatments. The highest values were recorded under P4 (1.57) and P1 (1.24), followed by P3 (1.17), whereas P2 (0.94) remained the lowest. At 90 DAS, total soluble sugar content declined as the crop approached maturity. Nevertheless, P4 (1.39) and P1 (1.13) maintained relatively higher sugar levels compared with P3 (1.05) and P2 (0.80). This reduction is consistent with normal senescence, although higher putrescine concentrations contributed to sustained carbohydrate availability during the early growth stages.

4.3.2a Interaction between different treatments on Total soluble Sugar of sesame at 30 DAS

The interaction between humic acid and putrescine exerted a significant influence on total soluble sugar content at 30 DAS during both the 2023 and 2024 growing seasons (Table 4.3.2a). In 2023, the highest total soluble sugar content was recorded under the combined treatment HA2+P4 (2.55), followed by HA4+P1 (1.54), HA3+P4 (1.11), and

HA2+P1 (1.08). In contrast, the lowest total soluble sugar content was observed under the control combination HA1+P1 (0.38), indicating a pronounced reduction in carbohydrate accumulation in the absence of both humic acid and putrescine. A comparable trend was evident in 2024. The HA2+P4 treatment again produced the maximum total soluble sugar content (2.58), followed by HA4+P1 (1.60), HA3+P4 (1.11), and HA2+P1 (1.09). Consistent with the previous season, the lowest total soluble sugar content was observed under HA1+P1 (0.35), reaffirming limited carbohydrate synthesis and assimilate accumulation when no biostimulant supplementation was provided.

4.3.2b Interaction between different treatments on Total soluble Sugar of sesame at 60 DAS

The interaction between humic acid and putrescine exerted a significant influence on total soluble sugar content at 60 DAS during both the 2023 and 2024 growing seasons (Table 4.3.2a). In 2023, the highest total soluble sugar content was recorded under the combined treatment HA2+P4 (3.02), followed by HA2+P3 (2.16), HA3+P1 (1.69), and HA4+P1 (1.58). In contrast, the lowest total soluble sugar content was observed under the control combination HA1+P3 (0.58), indicating a pronounced reduction in carbohydrate accumulation in the absence of both humic acid and putrescine. A comparable trend was evident in 2024. The HA2+P4 treatment again produced the maximum total soluble sugar content (3.03), followed by HA2+P3 (2.13), HA3+P1 (1.65), and HA4+P1 (1.53). Consistent with the previous season, the lowest total soluble sugar content was observed under HA1+P3 (0.55), reaffirming limited carbohydrate synthesis and assimilate accumulation when no biostimulant supplementation was provided.

4.3.2c Interaction between different treatments on Total soluble Sugar of sesame at 90 DAS

The interaction between humic acid and putrescine exerted a significant influence on total soluble sugar content at 90 DAS during both the 2023 and 2024 growing seasons (Table 4.3.2a). In 2023, the highest total soluble sugar content was recorded under the combined treatment HA2+P4 (2.70), followed by HA2+P3 (2.11), HA3+P1 (1.51), and

HA4+P1 (1.48). In contrast, the lowest total soluble sugar content was observed under the control combination HA1+P1 (0.55), indicating a pronounced reduction in carbohydrate accumulation in the absence of both humic acid and putrescine. A comparable trend was evident in 2024. The HA2+P4 treatment again produced the maximum total soluble sugar content (2.67), followed by HA2+P3 (2.13), HA3+P1 (1.50), and HA4+P1 (1.44). Consistent with the previous season, the lowest total soluble sugar content was observed under HA1+P1 (0.53), reaffirming limited carbohydrate synthesis and assimilate accumulation when no biostimulant supplementation was provided.

Total soluble sugars (TSS) constitute a major metabolic reserve in plants, functioning as structural metabolites and signaling compounds that regulate growth, development, and adaptation to environmental stress. The consistent enhancement of total soluble sugar content in sesame following humic acid and putrescine application during both growing seasons demonstrates the capacity of these biostimulants to improve carbon assimilation, carbohydrate metabolism, and source–sink relationships. Humic acid significantly increased soluble sugar accumulation by enhancing photosynthetic efficiency and nutrient acquisition, particularly nitrogen, potassium, and essential micronutrients required for carbohydrate synthesis and translocation. Increased nitrogen availability promotes chlorophyll biosynthesis and Rubisco activity, thereby improving photosynthetic carbon fixation and sugar formation. Potassium, whose uptake is stimulated by humic acid, facilitates phloem loading and efficient transport of photoassimilates, leading to greater soluble sugar accumulation during active vegetative growth. Furthermore, humic acid stimulates carbohydrate-metabolizing enzymes, including sucrose phosphate synthase, invertases, and amylases, promoting efficient conversion of assimilates into soluble sugars. The hormone-like properties of humic substances, resembling auxins and cytokinins, strengthen sink activity and delay leaf senescence, thereby sustaining carbohydrate production during vegetative and reproductive development. Although soluble sugar levels naturally declined during later growth stages because carbohydrates were increasingly mobilized toward developing seeds, humic acid-treated plants maintained comparatively higher sugar concentrations through prolonged photosynthetic activity and improved metabolic

performance. Putrescine further enhanced total soluble sugar accumulation by regulating carbon metabolism and protecting photosynthetic processes against oxidative stress. As an important polyamine, putrescine stabilizes thylakoid membranes, preserves chloroplast integrity, and maintains photosynthetic efficiency under field conditions. It also regulates the expression and activity of carbohydrate-metabolizing enzymes, including sucrose synthase and hexokinase, thereby promoting sugar biosynthesis and accumulation. Moreover, putrescine improves source–sink relationships by facilitating phloem loading and assimilate transport while maintaining cellular osmotic balance during changing environmental conditions. Its interaction with endogenous hormones, particularly abscisic acid and cytokinins, further regulates carbohydrate partitioning and sugar signaling throughout plant development. The reduction in soluble sugar concentration during later growth stages across treatments reflects the normal redistribution of carbohydrates toward reproductive sinks during seed filling. Nevertheless, higher putrescine concentrations maintained greater sugar availability during early developmental stages by delaying senescence, strengthening antioxidant defenses, preserving membrane stability, and reducing oxidative injury to photosynthetic tissues. Consequently, sustained photosynthetic activity allowed prolonged carbohydrate synthesis and storage. Overall, the integrated effects of humic acid and putrescine on photosynthesis, nutrient acquisition, enzyme regulation, osmotic adjustment, hormonal balance, and stress protection explain the consistent increase in total soluble sugar content observed in sesame. Enhanced carbohydrate metabolism and assimilate availability likely contributed directly to improved vegetative growth, reproductive development, seed formation, and higher productivity at elevated biostimulant concentrations. The results confirm that humic acid and putrescine function as effective biochemical enhancers, optimizing carbon assimilation and metabolic efficiency in sesame cultivated under open-field conditions (Ghassemi-Golezani et al., 2019; Lotfi et al., 2015; Mizani et al., 2025).

Table 4.3.2 Effect of different treatments on total soluble sugar of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.62	0.76	0.60	0.59	0.76	0.59
HA 2 (500 ppm)	1.26	1.83	1.62	1.26	1.84	1.62
HA 3 (1000 ppm)	0.73	1.12	0.97	0.72	1.09	0.98
HA 4 (1500 ppm)	0.83	1.25	1.19	0.85	1.23	1.19
SEm ±	0.01	0.01	0.01	0.01	0.01	0.01
C.D. (p=0.05)	0.03	0.04	0.03	0.03	0.04	0.04
Factor (Putrescine)						
P 1 (0 ppm)	0.88	1.27	1.14	0.89	1.24	1.13
P 2 (50 ppm)	0.63	0.93	0.80	0.63	0.94	0.80
P 3 (100 ppm)	0.74	1.20	1.05	0.71	1.17	1.05
P 4 (150 ppm)	1.18	1.57	1.39	1.19	1.57	1.39
SEm ±	0.01	0.01	0.01	0.01	0.01	0.01
C.D. (p=0.05)	0.03	0.04	0.03	0.03	0.04	0.04
Interaction	0.07	0.08	0.06	0.07	0.09	0.08

Table 4.3.2a Interaction effect of humic acid and putrescine application on total soluble sugar at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.38	1.05	0.45	0.59	0.62	0.35	1.07	0.38	0.57	0.59
HA2	1.08	0.58	0.81	2.55	1.26	1.09	0.57	0.81	2.58	1.26
HA3	0.52	0.36	0.94	1.11	0.73	0.54	0.35	0.88	1.11	0.72
HA4	1.54	0.53	0.76	0.47	0.83	1.60	0.54	0.77	0.49	0.85
Mean	0.88	0.63	0.74	1.18		0.89	0.63	0.71	1.19	
SEm±	0.02					0.02				
C.D (P=0.05)	0.07					0.07				

Table 4.3.2b Interaction effect of humic acid and putrescine application on total soluble sugar at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.60	0.99	0.58	0.88	0.76	0.61	1.00	0.55	0.88	0.76
HA2	1.22	0.94	2.16	3.02	1.83	1.17	1.02	2.13	3.03	1.84
HA3	1.69	0.55	0.99	1.25	1.12	1.65	0.50	0.93	1.27	1.09
HA4	1.58	1.26	1.05	1.12	1.25	1.53	1.24	1.06	1.11	1.23
Mean	1.27	0.93	1.20	1.57		1.24	0.94	1.17	1.57	
SEm±	0.02					0.02				
C.D (P=0.05)	0.08					0.09				

Table 4.3.2c Interaction effect of humic acid and putrescine application on total soluble sugar at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	0.55	0.60	0.56	0.68	0.60	0.53	0.62	0.53	0.68	0.59
HA2	1.02	0.64	2.11	2.70	1.62	1.06	0.63	2.13	2.67	1.62
HA3	1.51	0.60	0.64	1.13	0.97	1.50	0.62	0.68	1.11	0.98
HA4	1.48	1.35	0.88	1.05	1.19	1.44	1.34	0.88	1.11	1.19
Mean	1.14	0.80	1.05	1.39		1.13	0.80	1.05	1.39	
SEm±	0.02					0.02				
C.D (P=0.05)	0.06					0.08				

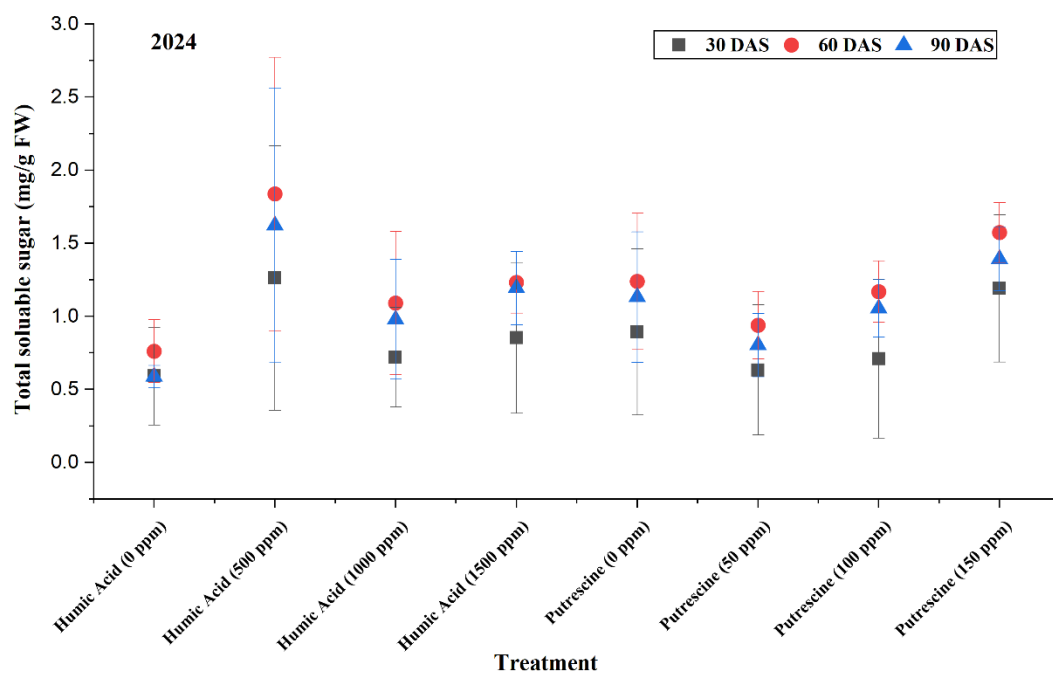
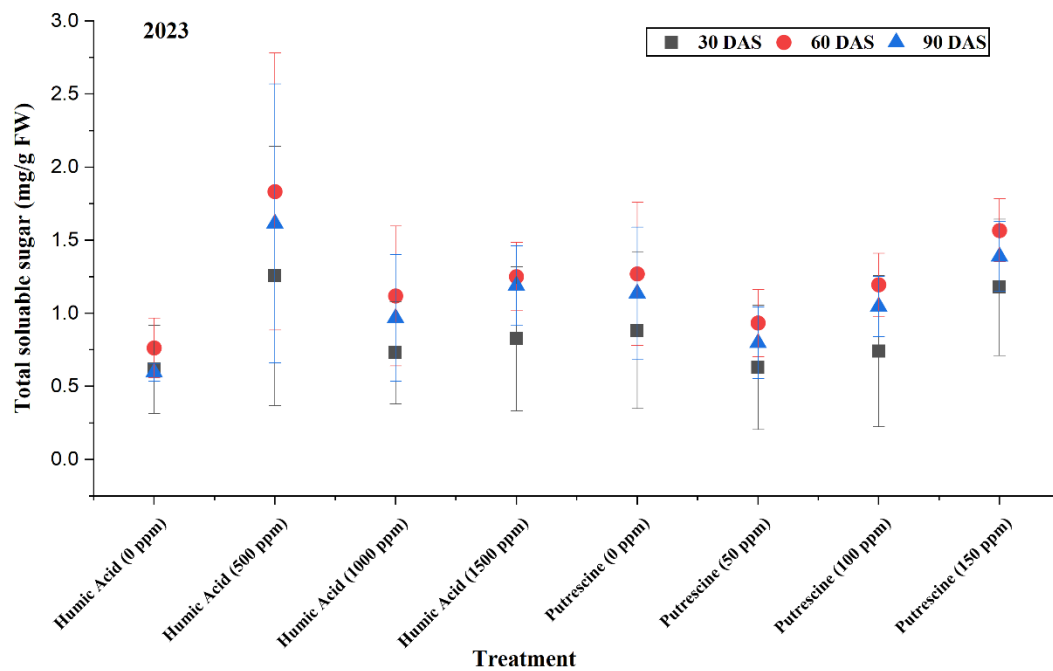


Figure 4.3.2 Effect of different treatments on total soluble sugar of sesame during kharif 2023 & 2024

4.3.3 Total Soluble Protein (mg g⁻¹ Fresh Weight): The applied treatments exerted a pronounced and concentration-dependent influence on the total soluble protein content of sesame during both the 2023 and 2024 growing seasons. Increasing concentrations of humic acid and putrescine consistently enhanced total soluble protein accumulation across different growth stages (Table 4.3.3 and Figure 4.3.3). In 2023, total soluble protein content varied significantly with growth stage under different humic acid treatments. At 30 DAS, higher protein levels were observed across treatments, reflecting vigorous early vegetative growth. The maximum total soluble protein content was recorded under HA3 (0.54), followed closely by HA4 (0.53) and HA2 (0.33), whereas the untreated control, HA1 (0.20), registered the lowest value, indicating a clear treatment-induced enhancement. At 60 DAS, total soluble protein content increased further as the crop progressed toward the reproductive phase. Treatments HA4 (1.08) and HA3 (1.01) maintained significantly higher protein levels, while HA2 (0.87) showed a moderate response, and HA1 (0.60) continued to record the lowest value. By 90 DAS, total soluble protein content declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA4 (1.64) and HA3 (1.49) exhibited higher protein levels than HA2 (1.40) and HA1 (1.00), suggesting greater metabolic activity at higher humic acid concentrations. A similar response pattern was observed under putrescine treatments. At 30 DAS, total soluble protein content was higher across treatments, with P4 recording the maximum value (0.46), followed by P2 (0.42) and P3 (0.37). At the same time, P1 (0.35) exhibited the lowest value, indicating that putrescine positively influenced early protein accumulation. At 60 DAS, total soluble protein content increased across all putrescine levels, with the highest values recorded under P4 (1.00) and P3 (0.90), followed by P1 (0.84), whereas P2 (0.81) remained the lowest. At 90 DAS, total soluble protein content declined as the crop approached maturity; however, P4 (1.54) and P3 (1.42) maintained relatively higher protein levels compared with P2 (1.24) and P1 (1.33). This decline is consistent with normal senescence, although higher putrescine concentrations contributed to sustained protein accumulation during earlier growth stages. In 2024, total soluble protein content varied significantly with growth stage under different humic acid treatments. At 30 DAS, higher protein levels were observed across treatments, reflecting vigorous early vegetative growth. The maximum total soluble protein content

was recorded under HA3 (0.55), followed closely by HA4 (0.54) and HA2 (0.34), whereas the untreated control, HA1 (0.19), registered the lowest value, indicating a clear treatment-induced enhancement. At 60 DAS, total soluble protein content increased further as the crop progressed toward the reproductive phase. Treatments HA4 (1.07) and HA3 (0.98) maintained significantly higher protein levels, while HA2 (0.85) showed a moderate response, and HA1 (0.61) continued to record the lowest value. By 90 DAS, total soluble protein content declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA4 (1.67) and HA3 (1.52) exhibited higher protein levels than HA2 (1.41) and HA1 (1.00), suggesting greater metabolic activity at higher humic acid concentrations. A similar response pattern was observed under putrescine treatments. At 30 DAS, total soluble protein content was higher across treatments, with P4 recording the maximum value (0.47), followed by P2 (0.43) and P3 (0.37). At the same time, P1 (0.35) exhibited the lowest value, indicating that putrescine positively influenced early protein accumulation. At 60 DAS, total soluble protein content increased across all putrescine levels, with the highest values recorded under P4 (1.03) and P3 (0.85), followed by P2 (0.82), whereas P1 (0.82) remained the lowest. At 90 DAS, total soluble protein content declined as the crop approached maturity; however, P4 (1.56) and P3 (1.43) maintained relatively higher protein levels compared with P1 (1.35) and P2 (1.25). This decline is consistent with normal senescence, although higher putrescine concentrations contributed to sustained protein accumulation during earlier growth stages.

Total soluble protein content is a key biochemical indicator reflecting nitrogen assimilation efficiency, enzymatic activity, and overall metabolic vigor of plants. The consistent increase in total soluble protein in sesame under humic acid and putrescine applications across both growing seasons clearly demonstrates the role of these biostimulants in enhancing nitrogen metabolism, protein biosynthesis, and physiological stability throughout crop development. Humic acid application markedly enhanced soluble protein accumulation by improving soil nutrient availability and root nutrient uptake, particularly nitrogen and micronutrients such as iron, magnesium, and zinc that are essential for amino acid synthesis and protein assembly. Improved nitrogen uptake promotes higher activity of nitrate reductase and glutamine synthetase, leading

to increased amino acid synthesis and the synthesis of structural and enzymatic proteins. Humic substances also stimulate root growth and membrane permeability, thereby facilitating efficient nutrient absorption and translocation to aerial tissues, supporting higher protein accumulation during active growth stages. Additionally, humic acid exhibits hormone-like activity, particularly auxin- and cytokinin-like effects, which stimulate cell division, protein synthesis, and delayed leaf senescence. Cytokinin-mediated delay in senescence helps maintain ribosomal integrity and enzymatic machinery, allowing sustained protein synthesis even during later growth stages. Putrescine, a low-molecular-weight polyamine, further increased total soluble protein content by regulating nitrogen metabolism and stress protection. Polyamines directly stabilize ribosomes, nucleic acids, and proteins, thereby enhancing translational efficiency and protein stability. Putrescine also upregulates key enzymes of nitrogen assimilation, such as nitrate reductase and aminotransferases, thereby increasing amino acid availability and subsequent protein synthesis. Moreover, putrescine plays a crucial role in mitigating oxidative stress by enhancing antioxidant defense systems, including superoxide dismutase and catalase activities. Reduced oxidative damage prevents protein degradation and preserves cellular structures involved in protein synthesis. Polyamines also interact with phytohormones, such as abscisic acid and cytokinins, influencing source–sink relationships and promoting efficient assimilate allocation to protein synthesis, particularly during early vegetative and flowering stages. The gradual decline in total soluble protein content toward physiological maturity observed across all treatments is consistent with normal developmental senescence and the remobilization of nitrogenous compounds from vegetative tissues to developing seeds. Overall, the synergistic effects of humic acid and putrescine on nutrient uptake, nitrogen assimilation, enzyme regulation, hormonal balance, and oxidative stress protection account for the consistent increase in total soluble protein content in sesame. These biochemical improvements directly contribute to improved growth performance, seed quality, and yield attributes. The findings confirm that humic acid and putrescine function as effective biostimulants, optimizing protein metabolism and physiological efficiency in sesame cultivated under open-field conditions (Mizani et al., 2025; Forotaghe et al., 2021; Ghassemi-Golezani et al., 2019).

Table 4.3.3 Effect of different treatments on total soluble protein of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.20	0.60	1.00	0.19	0.61	1.00
HA 2 (500 ppm)	0.33	0.87	1.40	0.34	0.85	1.41
HA 3 (1000 ppm)	0.54	1.01	1.49	0.55	0.98	1.52
HA 4 (1500 ppm)	0.53	1.08	1.64	0.54	1.07	1.67
SEm ±	0.01	0.01	0.01	0.009	0.01	0.01
C.D. (p=0.05)	0.03	0.04	0.05	0.02	0.04	0.05
Factor (Putrescine)						
P 1 (0 ppm)	0.35	0.84	1.33	0.35	0.82	1.35
P 2 (50 ppm)	0.42	0.83	1.24	0.43	0.82	1.25
P 3 (100 ppm)	0.37	0.90	1.42	0.37	0.85	1.43
P 4 (150 ppm)	0.46	1.00	1.54	0.47	1.03	1.56
SEm ±	0.01	0.01	0.01	0.009	0.01	0.01
C.D. (p=0.05)	0.03	0.04	0.05	0.02	0.04	0.05
Interaction	NS	NS	NS	NS	NS	NS

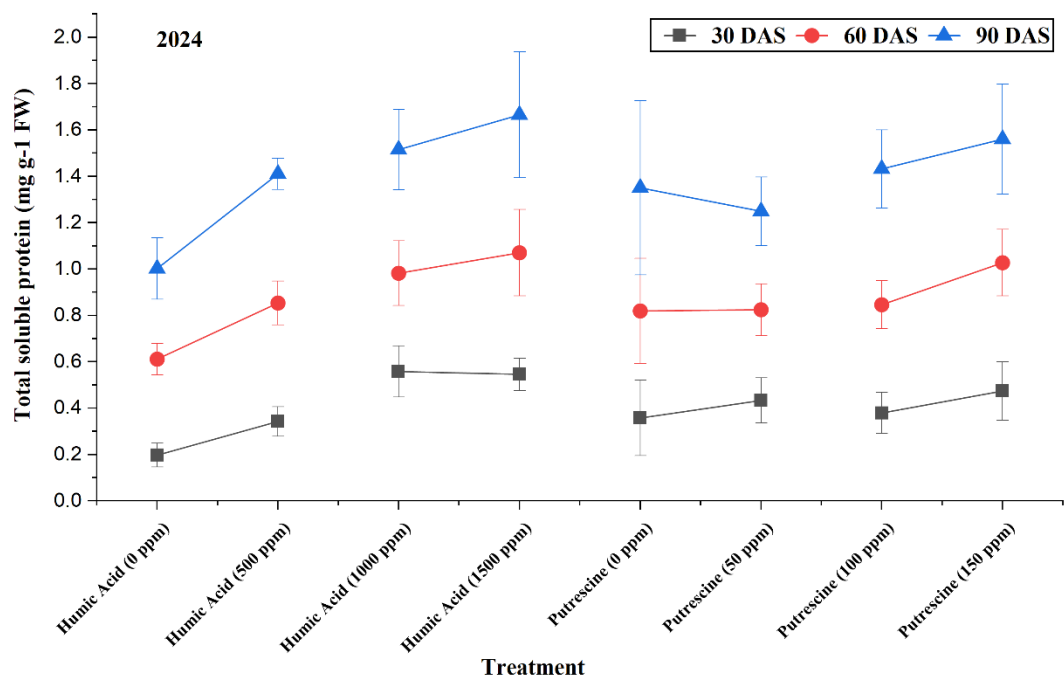
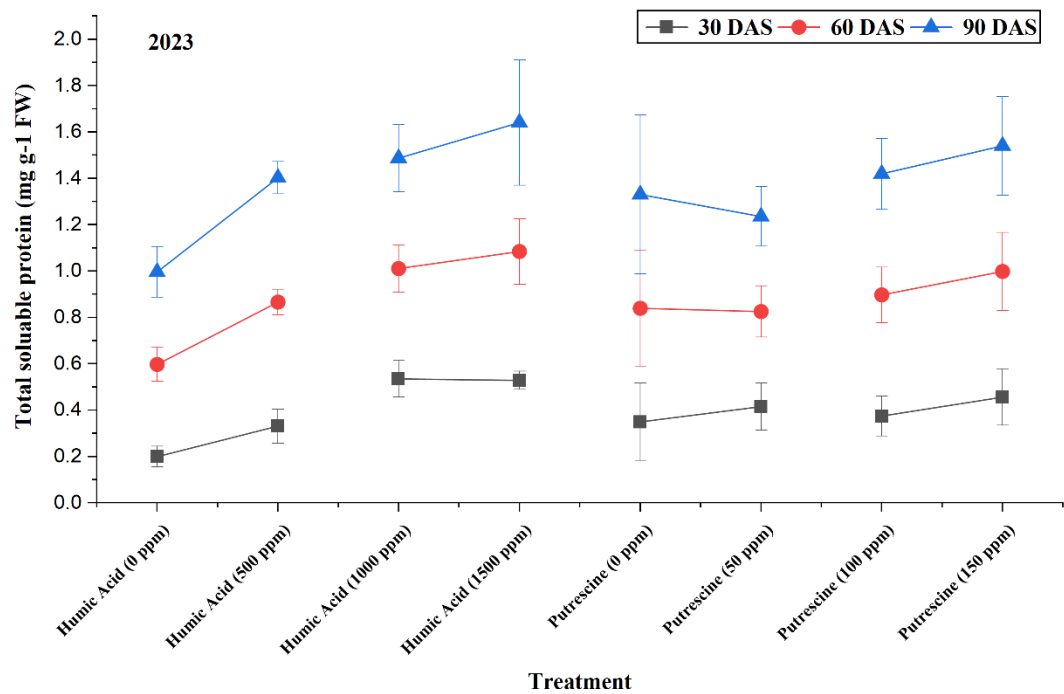


Figure 4.3.3 Effect of different treatments on total soluble protein of sesame during kharif 2023 & 2024

4.3.4 Membrane stability index: The applied treatments exerted a significant influence on the membrane stability index (MSI, %) of sesame at different growth stages (30, 60, and 90 DAS) during the 2023 and 2024 seasons (Table 4.3.4 and Figure 4.3.4). In 2023, the membrane stability index varied significantly across growth stages under different humic acid treatments. At 30 DAS, MSI values were relatively higher across treatments, indicating greater membrane integrity during early vegetative growth. The highest MSI was observed in the untreated control (HA1, 42.09%), followed by HA2 (37.49%) and HA3 (32.55%), whereas HA4 (32.79%) showed comparatively lower values. At 60 DAS, MSI values generally increased as the crop advanced toward the reproductive phase. Treatments HA1 (36.21%) and HA2 (34.93%) maintained significantly higher MSI, whereas HA3 (28.77%) showed a moderate response, and HA4 (29.45%) recorded the lowest value. By 90 DAS, MSI declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA1 (29.13%) and HA2 (20.54%) sustained comparatively higher MSI values than HA3 (19.96%) and HA4 (18.06%), indicating a gradual loss of membrane integrity during senescence. A comparable trend was observed under putrescine treatments. At 30 DAS, MSI values were higher across treatments, with P1 recording the highest value (39.56%), followed by P2 (37.55%) and P3 (35.25%), whereas P4 (35.26%) had the lowest value. At 60 DAS, MSI increased across all putrescine levels, with the highest values recorded under P1 (36.24%) and P2 (33.95%), followed by P3 (30.55%), whereas P4 (32.56%) remained relatively lower. At 90 DAS, MSI declined as the crop approached maturity; however, P1 (25.31%) and P2 (19.10%) maintained relatively higher MSI compared with P4 (22.95%) and P3 (20.32%). This decline corresponds to the normal senescence process, reflecting progressive membrane degradation at later growth stages. In 2024, the membrane stability index varied significantly across growth stages under different humic acid treatments. At 30 DAS, MSI values were relatively higher across treatments, indicating greater membrane integrity during early vegetative growth. The highest MSI was observed in the untreated control (HA1, 42.05%), followed by HA2 (38.89%) and HA4 (33.52%), whereas HA3 (31.78%) showed the lowest value. At 60 DAS, MSI values generally increased as the crop advanced toward the reproductive phase. Treatments HA1 (37.98%) and HA2 (32.52%) maintained significantly higher MSI, whereas HA3 (30.36%) showed a

moderate response, and HA4 (28.73%) recorded the lowest value. By 90 DAS, MSI declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA1 (27.68%) and HA3 (20.98%) sustained comparatively higher MSI values than HA2 (19.57%) and HA4 (17.18%), indicating a gradual loss of membrane integrity during senescence. A comparable trend was observed under putrescine treatments. At 30 DAS, MSI values were higher across treatments, with P1 recording the maximum value (39.09%), followed by P2 (35.64%) and P3 (29.47%), while P4 (27.50%) exhibited the lowest value. At 60 DAS, MSI increased across all putrescine levels, with the highest values recorded under P1 (36.97%) and P2 (35.64%), followed by P3 (29.47%), whereas P4 (27.50%) remained relatively lower. At 90 DAS, MSI declined as the crop approached maturity; however, P1 (25.42%) and P4 (21.89%) maintained relatively higher MSI compared with P3 (19.55%) and P2 (18.57%). This decline corresponds to the normal senescence process, reflecting progressive membrane degradation at later growth stages.

4.3.4a Interaction between different treatments on the membrane stability index of sesame at 30 DAS

The interaction between humic acid and putrescine significantly influenced the membrane stability index (MSI) at 30 DAS during both the 2023 and 2024 growing seasons (Table 4.3.4a). In 2023, the highest MSI was recorded under the control treatment HA1+P1 (50.66%), followed by HA2+P2 (43.48%), HA1+P4 (41.24%), and HA3+P2 (40.83%). In contrast, the lowest MSI was observed with the HA4+P2 combination (25.37%), indicating reduced membrane integrity under this treatment. A similar response pattern was observed in 2024. The HA1+P1 (control) treatment again recorded the maximum MSI (50.86%), followed by HA2+P3 (43.96%), HA2+P2 (43.63%), and HA1+P4 (41.61%). Consistent with the previous season, the minimum MSI was observed under HA4+P2 (21.65%), indicating a pronounced decline in membrane stability at higher humic acid levels in combination with putrescine during early growth stages.

Membrane stability index (MSI) is a critical physiological parameter that reflects the integrity and functional efficiency of cellular membranes under varying growth and

environmental conditions. The observed variation in MSI across growth stages and treatment levels during both seasons highlights the dynamic nature of membrane integrity in sesame and its modulation by humic acid and putrescine applications. Higher MSI values during early vegetative growth indicate intact membrane structures, optimal lipid composition, and efficient functioning of membrane-bound proteins. As crop development progressed toward the reproductive and maturity stages, a gradual decline in MSI was evident across treatments, consistent with natural senescence-associated membrane degradation. Senescence is characterized by increased lipid peroxidation, loss of membrane fluidity, and enhanced electrolyte leakage due to accumulation of reactive oxygen species (ROS). The comparatively higher MSI observed under lower or untreated humic acid and putrescine levels suggests that membrane integrity was primarily maintained by inherent cellular mechanisms during non-stressful growth phases. However, higher concentrations of humic acid and putrescine resulted in relatively lower MSI values, which may reflect enhanced metabolic activity rather than membrane damage per se. Increased metabolic turnover, elevated respiration, and accelerated growth rates under higher biostimulant doses can increase membrane permeability due to rapid membrane remodeling and active solute transport. Humic acid influences membrane properties indirectly through its effects on nutrient uptake, ion transport, and hormonal balance. By enhancing root growth and nutrient availability, humic substances stimulate metabolic processes that increase membrane-associated enzyme activities. This can temporarily alter membrane permeability, particularly during active growth and reproductive phases. Moreover, humic acid can modify membrane lipid composition by influencing fatty acid desaturation and sterol content, affecting membrane fluidity and stability across developmental stages. Putrescine, as a polyamine, plays a dual role in membrane dynamics. At optimal concentrations, polyamines stabilize membranes by binding to negatively charged phospholipids and proteins, thereby protecting membranes against oxidative damage. However, at higher concentrations, putrescine can enhance membrane permeability by increasing ion fluxes and modulating membrane transporters, which may result in lower MSI values despite improved physiological performance. This phenomenon reflects increased membrane activity rather than structural breakdown. Additionally, polyamines are closely associated with ROS

signaling pathways. While putrescine enhances antioxidant enzyme activity and scavenges free radicals, it also participates in the controlled generation of ROS, which act as signaling molecules for growth and development. Such ROS-mediated signaling can transiently increase membrane permeability, particularly during the flowering and seed-filling stages, thereby contributing to variations in MSI. The decline in MSI at later growth stages across all treatments corresponds with senescence-driven degradation of membrane lipids and proteins, increased lipoxygenase activity, and reduced antioxidant defense. Although humic acid and putrescine delayed senescence-related physiological decline by improving nutrient status and metabolic efficiency, complete prevention of membrane degradation at maturity is unlikely given programmed cellular aging. Overall, the modulation of MSI by humic acid and putrescine reflects a balance between membrane stabilization and enhanced metabolic activity. While early growth stages exhibited inherently higher membrane integrity, biostimulant-induced physiological acceleration influenced membrane dynamics during later stages. These findings underscore that changes in MSI under biostimulant application should be interpreted in the context of growth stimulation, metabolic intensity, and developmental stage rather than membrane damage alone (Forotaghe et al., 2021; Mizani et al., 2025; Shahdadi et al., 2025).

Table 4.3.4 Effect of different treatments on the membrane stability index of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	42.09	36.21	29.13	42.05	37.98	27.68
HA 2 (500 ppm)	37.49	34.93	20.54	38.89	32.52	19.57
HA 3 (1000 ppm)	32.55	28.77	19.96	31.78	30.36	20.98
HA 4 (1500 ppm)	32.79	29.45	18.06	33.52	28.73	17.18
SEm ±	0.26	0.55	0.71	0.86	1.84	1.29
C.D. (p=0.05)	0.75	1.59	2.08	2.50	5.35	3.76
Factor (Putrescine)						
P 1 (0 ppm)	39.56	36.24	25.31	39.09	36.97	25.42
P 2 (50 ppm)	37.55	33.95	19.10	36.30	35.64	18.57
P 3 (100 ppm)	35.25	30.55	20.32	36.84	29.47	19.55
P 4 (150 ppm)	32.56	28.61	22.95	34.00	27.50	21.89
SEm ±	0.26	0.55	0.71	0.86	1.84	1.29
C.D. (p=0.05)	0.75	1.59	2.08	2.50	5.35	3.76
Interaction	1.51	NS	NS	5.01	NS	NS

Table 4.3.4a Interaction effect of humic acid and putrescine application on membrane stability index at 30 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	50.66	40.52	35.95	41.24	42.09	50.86	38.69	37.05	41.61	42.05
HA2	35.48	43.48	39.02	31.97	37.49	34.84	43.63	43.96	33.13	38.89
HA3	34.21	40.83	30.56	24.61	32.55	32.16	41.26	29.29	24.41	31.78
HA4	37.89	25.37	35.48	32.43	32.79	38.52	21.65	37.07	36.86	33.52
Mean	39.56	37.55	35.25	32.56		39.09	36.30	36.84	34.00	
SEm±	0.52					1.72				
C.D (P=0.05)	1.51					5.01				

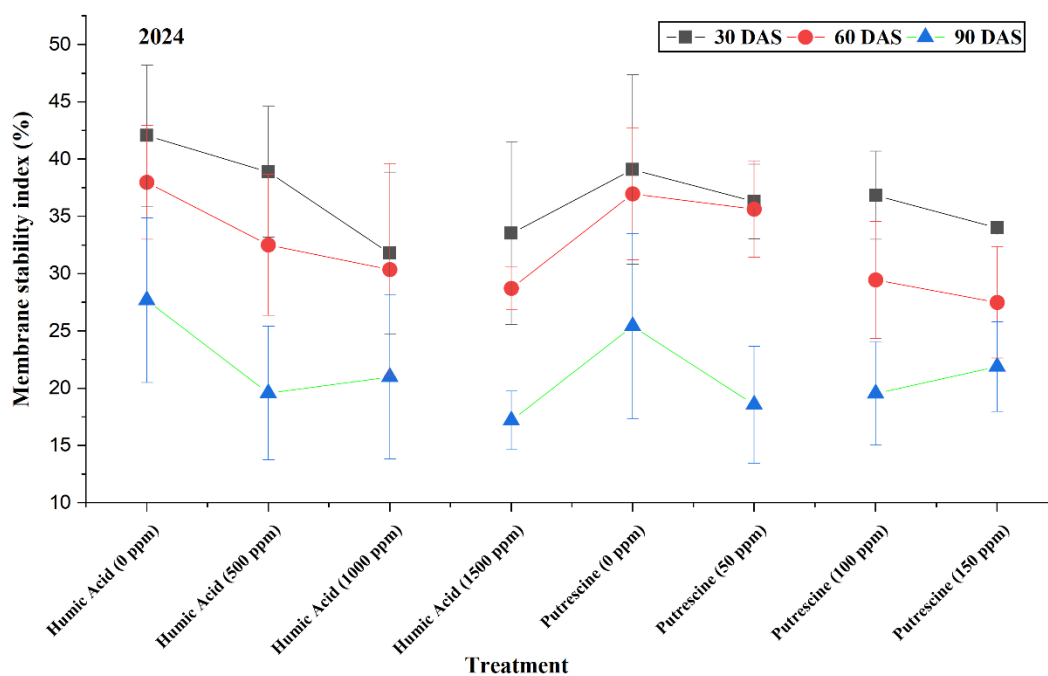
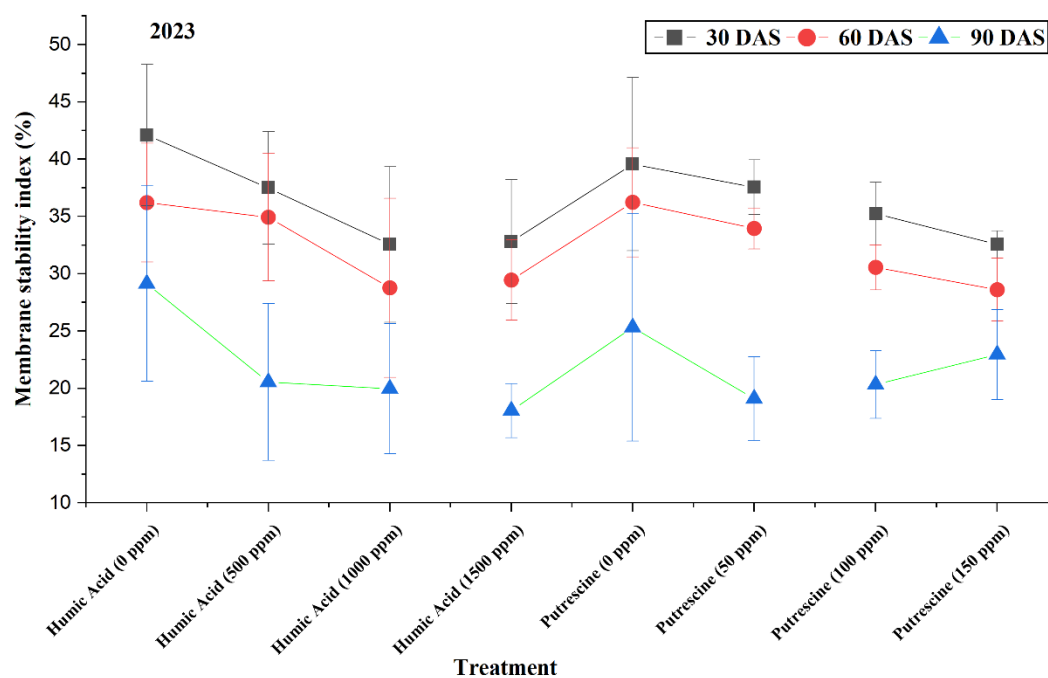


Figure 4.3.4 Effect of different treatments on the membrane stability index of sesame during kharif 2023 & 2024

4.3.5 Total Phenol (mg/g Fresh Weight): The applied treatments exerted a significant influence on the total phenol of sesame at different growth stages (30, 60, and 90 DAS) during the 2023 and 2024 seasons (Table 4.3.5 and Figure 4.3.5). In 2023, total phenol content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, relatively higher phenol levels were observed across treatments, reflecting active early vegetative growth. The highest total phenol content was recorded for HA4 (4.97), followed by HA2 (4.89) and HA3 (4.75), whereas the untreated control, HA1 (3.92), had the lowest value, indicating a treatment-induced increase. At 60 DAS, total phenol content increased further as the crop progressed toward the reproductive phase. Treatments HA3 (5.21) and HA4 (4.97) maintained significantly higher phenol levels, whereas HA2 (4.66) showed a moderate response, and HA1 (3.87) continued to record the minimum value. By 90 DAS, total phenol content declined across all treatments as plants approached physiological maturity. Despite this general reduction, HA2 (5.47) and HA4 (5.42) exhibited higher phenol levels than HA3 (4.98) and HA1 (3.87), indicating improved antioxidant status with humic acid application. A comparable response pattern was observed under putrescine treatments. At 30 DAS, total phenol content was higher across treatments, with P4 recording the maximum value (5.16), followed by P3 (4.63) and P1 (4.61). At the same time, P2 (4.12) exhibited the lowest value, suggesting a positive effect of putrescine on early phenolic metabolism. During 60 DAS, phenol content increased in all treatments, with the highest values recorded under P4 (5.31) and P3 (4.85), followed by P1 (4.44), whereas P2 (3.80) remained the lowest. At 90 DAS, total phenol content increased further as the crop approached maturity. Nevertheless, P4 (6.03) and P3 (5.15) maintained relatively higher phenol levels compared with P1 (4.71) and P2 (3.85). These trends indicate that higher putrescine concentrations sustained phenolic compound accumulation, thereby enhancing antioxidant potential during later growth stages. In 2024, total phenol content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, relatively higher phenol levels were observed across treatments, reflecting active early vegetative growth. The highest total phenol content was recorded for HA3 (5.61), followed by HA4 (5.59) and HA2 (5.16), whereas the untreated control, HA1 (4.24), had the lowest value, indicating a clear treatment-induced enhancement. At 60 DAS, total phenol content increased further as the crop progressed toward the

reproductive phase. Treatments HA4 (6.95) and HA3 (6.35) maintained significantly higher phenol levels, whereas HA2 (5.52) showed a moderate response, and HA1 (4.73) continued to record the minimum value. By 90 DAS, total phenol content declined across all treatments as plants approached physiological maturity. Despite this general reduction, HA4 (6.11) and HA3 (5.58) exhibited higher phenol levels than HA2 (4.65) and HA1 (3.24), indicating improved antioxidant status with humic acid application. A comparable response pattern was observed under putrescine treatments. At 30 DAS, total phenol content was higher across treatments, with P4 recording the maximum value (5.35), followed by P3 (5.18) and P2 (5.06). At the same time, P1 (5.01) exhibited the lowest value, suggesting a positive effect of putrescine on early phenolic metabolism. During 60 DAS, phenol content increased in all treatments, with the highest values recorded under P4 (7.00) and P3 (6.15), followed by P2 (5.46), whereas P21 (4.94) remained the lowest. At 90 DAS, total phenol content decreased further as the crop approached maturity. Nevertheless, P4 (5.40) and P2 (4.90) maintained relatively higher phenol levels compared with P3 (4.90) and P1 (4.34). These trends indicate that higher putrescine concentrations sustained phenolic compound accumulation, thereby enhancing antioxidant potential during later growth stages.

4.3.5a Interaction between different treatments on Total Phenol of sesame at 90 DAS

The interaction between humic acid and putrescine significantly influenced total phenol content at 90 DAS during both the 2023 and 2024 growing seasons (Table 4.3.5a). In 2023, the highest total phenol content was recorded under the combined treatment HA2+P4 (6.82), followed by HA3+P4 (6.66), HA4+P4 (6.17), and HA4+P3 (5.76). In contrast, the lowest total phenol content was observed under the control combination HA1+P1 (2.67), indicating markedly reduced phenolic accumulation in the absence of both humic acid and putrescine. A comparable trend was observed in 2024. The treatment HA4+P2 produced the maximum total phenol content (6.51), followed by HA4+P4 (6.31), HA3+P2 (5.91), and HA4+P3 (5.81). Consistent with the previous season, the minimum total phenol content was again recorded under HA1+P1 (2.05), reaffirming the limited phenolic biosynthesis and antioxidant potential when no biostimulant supplementation was provided.

Phenolic compounds are vital secondary metabolites in plants, playing a central role in antioxidant defense, stress tolerance, and regulation of growth and development. The observed enhancement of total phenol content in sesame under humic acid and putrescine treatments across growth stages and seasons indicates a strong biostimulant-mediated modulation of phenolic metabolism. Higher phenol accumulation during early vegetative stages reflects active metabolic flux through the phenylpropanoid pathway, which is closely associated with rapid cell division and tissue differentiation. Humic acid application is known to stimulate key enzymes involved in secondary metabolism, particularly phenylalanine ammonia-lyase (PAL), the primary regulatory enzyme of phenolic biosynthesis. By improving nutrient availability, especially nitrogen and micronutrients such as iron and zinc, humic substances enhance enzymatic activity and carbon skeleton availability required for phenolic compound synthesis. The sustained increase in phenolic content during the reproductive phase suggests that humic acid contributes to improved redox homeostasis and metabolic efficiency. Enhanced photosynthetic performance under humic acid application provides a greater supply of assimilates, which are diverted toward secondary metabolite production. Additionally, humic substances exert hormone-like effects, particularly auxin- and cytokinin-like activities, which indirectly stimulate phenolic biosynthesis through gene-level regulation of antioxidant pathways. Putrescine, a low-molecular-weight polyamine, plays a pivotal role in regulating phenolic metabolism through its involvement in cellular signaling and stress response pathways. Polyamines are closely linked with the shikimate and phenylpropanoid pathways, serving as modulators of PAL and polyphenol oxidase activities. Increased phenolic accumulation under putrescine application reflects its ability to induce mild oxidative signaling, which activates antioxidant defense mechanisms without causing cellular damage. Furthermore, putrescine contributes to membrane stabilization and ROS scavenging, reducing oxidative degradation of phenolic compounds during later growth stages. This protective role enables sustained phenolic retention even as plants approach maturity, when natural senescence processes typically reduce antioxidant capacity. Polyamines also enhance the expression of genes involved in flavonoid and phenolic acid biosynthesis, strengthening the non-enzymatic antioxidant system of plants. The decline in phenolic content toward physiological maturity across treatments is

consistent with developmental regulation of secondary metabolism. As assimilates are increasingly diverted toward seed filling and storage compound synthesis, the allocation to secondary metabolites diminishes. However, the comparatively higher phenol levels under humic acid and putrescine treatments indicate a delayed senescence effect, which preserves antioxidant activity for a longer duration. The combined influence of humic acid and putrescine thus reflects a synergistic enhancement of antioxidant metabolism. Humic acid primarily improves substrate availability and enzymatic efficiency, while putrescine acts as a metabolic regulator and signaling molecule. Together, they strengthen the antioxidant defense network, improving stress resilience and biochemical quality of sesame plants. Overall, the enhanced phenolic content under biostimulant application signifies improved oxidative balance, metabolic flexibility, and physiological robustness. These biochemical adjustments are critical for sustaining plant performance under varying environmental conditions and contribute to improved crop quality and stress tolerance in sesame cultivation (Shahdadi et al., 2025; Forotaghe et al., 2021; Nassiri-Mahallati & Jahan, 2020).

Table 4.3.5 Effect of different treatments on total phenol of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	3.92	3.57	3.87	4.24	4.739	3.24
HA 2 (500 ppm)	4.89	4.66	5.47	5.16	5.524	4.65
HA 3 (1000 ppm)	4.75	5.21	4.98	5.61	6.355	5.58
HA 4 (1500 ppm)	4.97	4.97	5.42	5.59	6.955	6.11
SEm ±	0.03	0.03	0.04	0.04	0.05	0.04
C.D. (p=0.05)	0.08	0.10	0.13	0.12	0.16	0.12
Factor (Putrescine)						
P 1 (0 ppm)	4.61	4.44	4.71	5.01	4.949	4.34
P 2 (50 ppm)	4.12	3.80	3.85	5.06	5.464	4.93
P 3 (100 ppm)	4.63	4.85	5.15	5.18	6.155	4.90
P 4 (150 ppm)	5.16	5.31	6.03	5.35	7.005	5.40
SEm ±	0.03	0.03	0.04	0.04	0.05	0.04
C.D. (p=0.05)	0.08	0.10	0.13	0.12	0.16	0.12
Interaction	NS	NS	0.27	NS	NS	0.26

Table 4.3.5a Interaction effect of humic acid and putrescine application on total phenol at 90 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	2.67	3.44	4.89	4.46	3.87	2.05	2.70	3.70	4.50	3.24
HA2	5.86	4.35	4.84	6.82	5.47	3.70	4.60	5.30	5.00	4.65
HA3	4.90	3.25	5.10	6.66	4.98	5.80	5.91	4.80	5.80	5.58
HA4	5.41	4.35	5.76	6.17	5.42	5.80	6.51	5.81	6.31	6.11
Mean	4.71	3.85	5.15	6.03		4.34	4.93	4.90	5.40	
SEm±	0.08					0.08				
C.D (P=0.05)	0.27					0.27				

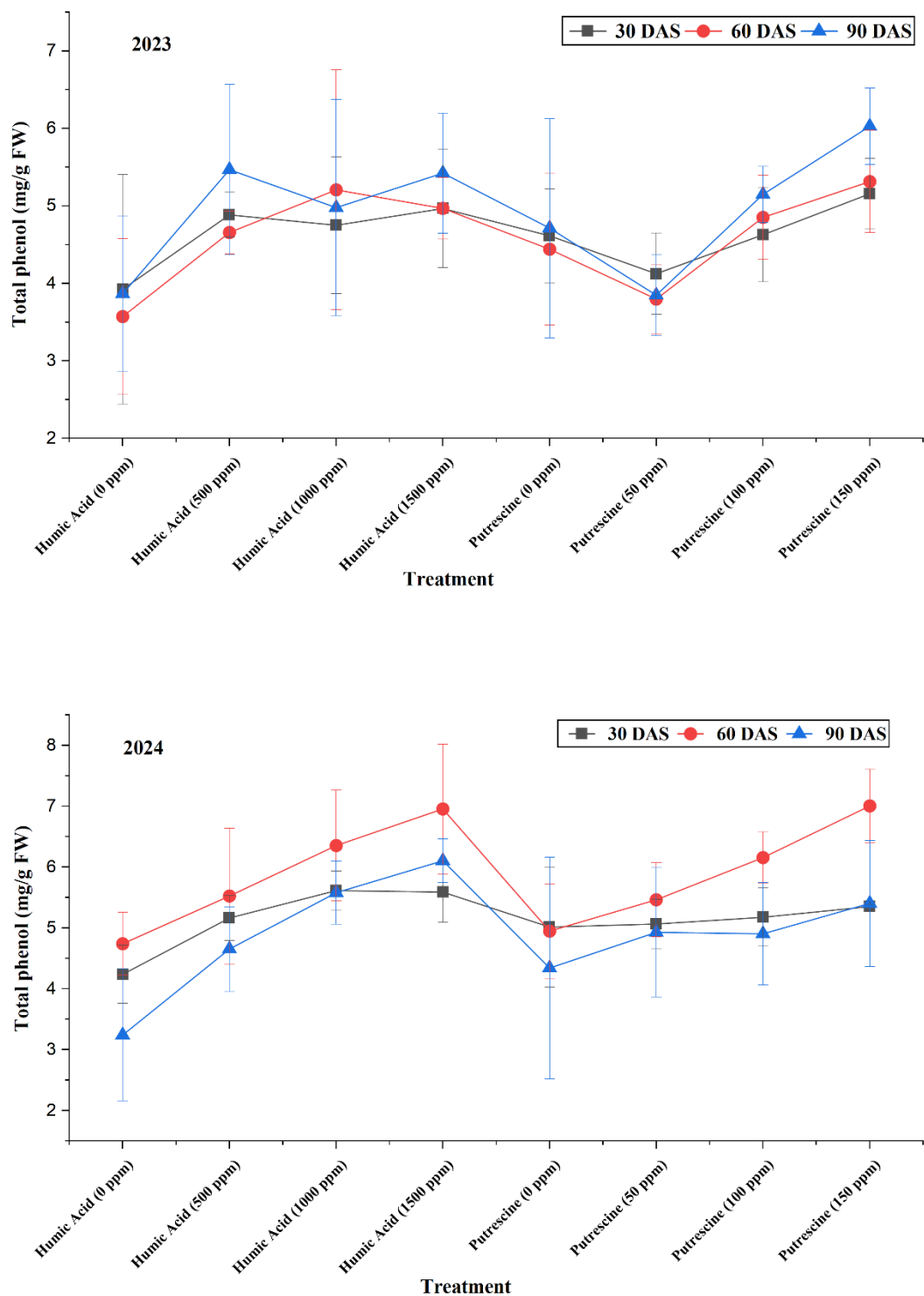


Figure 4.3.5 Effect of different treatments on total phenol of sesame during kharif 2023 & 2024

4.3.6 Total Soluble Proline: The applied treatments exerted a significant influence on the total free proline of sesame at different growth stages (30, 60, and 90 DAS) during the 2023 and 2024 seasons (Table 4.3.6 and Figure 4.3.6). In 2023, total free proline content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, relatively higher free proline levels were observed across treatments, reflecting active early vegetative growth. The highest free proline content was recorded under HA4 (0.680), followed by HA3 (0.461) and the control HA1 (0.328), whereas HA2 (0.212) recorded the lowest value, indicating a clear treatment-induced enhancement. At 60 DAS, free proline content increased further as the crop progressed toward the reproductive phase. Treatments HA4 (0.585) and HA3 (0.372) maintained significantly higher free proline levels, whereas HA1 (0.263) showed a moderate response, and HA2 (0.168) continued to exhibit the lowest value. By 90 DAS, free proline content declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA4 (0.532) and HA3 (0.327) sustained comparatively higher free proline levels than HA1 (0.216) and HA2 (0.134), suggesting improved stress tolerance and antioxidant status under humic acid application. A similar response pattern was observed under putrescine treatments. At 30 DAS, free proline content was higher across treatments, with P4 recording the maximum value (0.492), followed by P3 (0.476) and P1 (0.418). At the same time, P2 (0.295) exhibited the lowest value, indicating a positive effect of putrescine on early proline metabolism. During 60 DAS, free proline content increased in all treatments, with the highest values recorded under P4 (0.422) and P3 (0.397), followed by P1 (0.344), whereas P2 (0.224) remained the lowest. At 90 DAS, free proline content declined as the crop approached maturity; however, P4 (0.388) and P3 (0.345) maintained relatively higher levels compared with P1 (0.283) and P2 (0.193). These trends indicate that higher putrescine concentrations promote sustained proline accumulation, thereby enhancing osmotic adjustment and antioxidant potential during later growth stages. In 2024, total free proline content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, relatively higher free proline levels were observed across treatments, reflecting active early vegetative growth. The highest free proline content was recorded under HA4 (0.679), followed by HA3 (0.464) and the control HA1 (0.319), whereas HA2 (0.202) recorded the lowest value, indicating a clear

treatment-induced enhancement. At 60 DAS, free proline content increased further as the crop progressed toward the reproductive phase. Treatments HA4 (0.562) and HA3 (0.367) maintained significantly higher free proline levels, whereas HA1 (0.274) showed a moderate response, and HA2 (0.165) continued to exhibit the lowest value. By 90 DAS, free proline content declined across all treatments as plants approached physiological maturity. Despite this overall reduction, HA4 (0.544) and HA3 (0.332) sustained comparatively higher free proline levels than HA1 (0.215) and HA2 (0.136), suggesting improved stress tolerance and antioxidant status under humic acid application. A similar response pattern was observed under putrescine treatments. At 30 DAS, free proline content was higher across treatments, with P4 recording the maximum value (0.481), followed by P3 (0.471) and P1 (0.417). At the same time, P2 (0.296) exhibited the lowest value, indicating a positive effect of putrescine on early proline metabolism. During 60 DAS, free proline content increased in all treatments, with the highest values recorded under P4 (0.415) and P3 (0.393), followed by P1 (0.335), whereas P2 (0.225) remained the lowest. At 90 DAS, free proline content declined as the crop approached maturity; however, P4 (0.402) and P3 (0.347) maintained relatively higher levels compared with P1 (0.290) and P2 (0.187). These trends indicate that higher putrescine concentrations promote sustained proline accumulation, thereby enhancing osmotic adjustment and antioxidant potential during later growth stages.

Proline is a multifunctional amino acid that plays a pivotal role in plant stress physiology, serving as an osmoprotectant, antioxidant, molecular chaperone, and signaling molecule. The consistent increase in total free proline content in sesame under humic acid and putrescine treatments across growth stages and seasons indicates strong biostimulant-mediated regulation of stress-adaptive metabolism. Higher proline accumulation during early vegetative stages reflects active cellular metabolism and rapid tissue expansion, where proline supports osmotic balance and stabilizes proteins and membranes. Humic acid application has been shown to improve nutrient uptake efficiency, particularly nitrogen assimilation, which directly supplies substrates for amino acid biosynthesis, including proline. Enhanced nitrogen metabolism in the presence of humic substances stimulates the glutamate pathway, the primary route for

proline synthesis in plants. During the reproductive phase, elevated proline levels under higher humic acid concentrations suggest improved physiological resilience and metabolic plasticity. Humic substances are known to enhance root growth, water uptake, and enzymatic activity, thereby reducing physiological stress and triggering adaptive osmolyte accumulation. Additionally, humic acid induces mild oxidative signaling that activates stress-responsive genes, including those encoding Δ^1 -pyrroline-5-carboxylate synthetase (P5CS), the key regulatory enzyme in proline biosynthesis. The decline in proline content toward physiological maturity is consistent with developmental regulation, as assimilates are increasingly diverted toward seed filling and storage compounds. However, sustained proline levels under humic acid treatments indicate delayed senescence and prolonged stress-buffering capacity, supporting continued metabolic activity during later growth stages. Application of putrescine further amplified proline accumulation, underscoring the close metabolic and regulatory link between polyamines and proline metabolism. Polyamines share common precursors with amino acids and play a central role in modulating cellular redox status. Putrescine enhances proline accumulation by upregulating P5CS activity while simultaneously inhibiting proline dehydrogenase, thereby reducing proline degradation. Moreover, putrescine contributes to membrane stabilization and reactive oxygen species scavenging, thereby indirectly preserving proline pools by minimizing oxidative damage. The signaling role of polyamines in activating stress-responsive transcription factors also promotes coordinated regulation of osmolyte synthesis and antioxidant defense pathways. This interaction enables plants to maintain higher proline levels even as natural senescence progresses. The synergistic influence of humic acid and putrescine highlights a complementary mode of action: humic acid primarily enhances substrate availability, nutrient assimilation, and metabolic efficiency, whereas putrescine acts as a regulatory and protective molecule that modulates stress signaling and osmotic adjustment. Together, they strengthen cellular homeostasis, improve stress tolerance, and sustain biochemical integrity throughout crop development. These biochemical adjustments are crucial for maintaining physiological stability under fluctuating environmental conditions and for improving crop performance and adaptability (Nassiri-Mahallati & Jahan, 2020; Shahdadi et al., 2025; Weerasekara et al., 2021).

Table 4.3.6 Effect of different treatments on total free proline of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.328	0.263	0.216	0.319	0.274	0.215
HA 2 (500 ppm)	0.212	0.168	0.134	0.202	0.165	0.136
HA 3 (1000 ppm)	0.461	0.372	0.327	0.464	0.367	0.332
HA 4 (1500 ppm)	0.680	0.585	0.532	0.679	0.562	0.544
SEm ±	0.005	0.007	0.006	0.007	0.006	0.008
C.D. (p=0.05)	0.015	0.019	0.016	0.021	0.018	0.023
Factor (Putrescine)						
P 1 (0 ppm)	0.418	0.344	0.283	0.417	0.335	0.290
P 2 (50 ppm)	0.295	0.224	0.193	0.296	0.225	0.187
P 3 (100 ppm)	0.476	0.397	0.345	0.471	0.393	0.347
P 4 (150 ppm)	0.492	0.422	0.388	0.481	0.415	0.402
SEm ±	0.005	0.007	0.006	0.007	0.006	0.008
C.D. (p=0.05)	0.015	0.019	0.016	0.021	0.018	0.023
Interaction	NS	NS	NS	NS	NS	NS

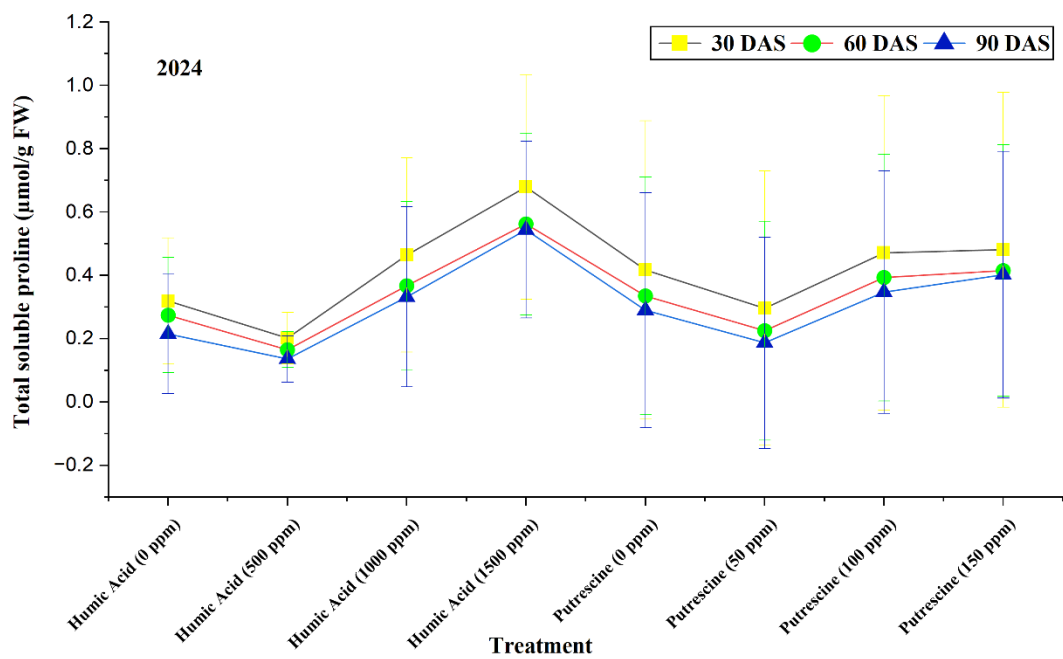
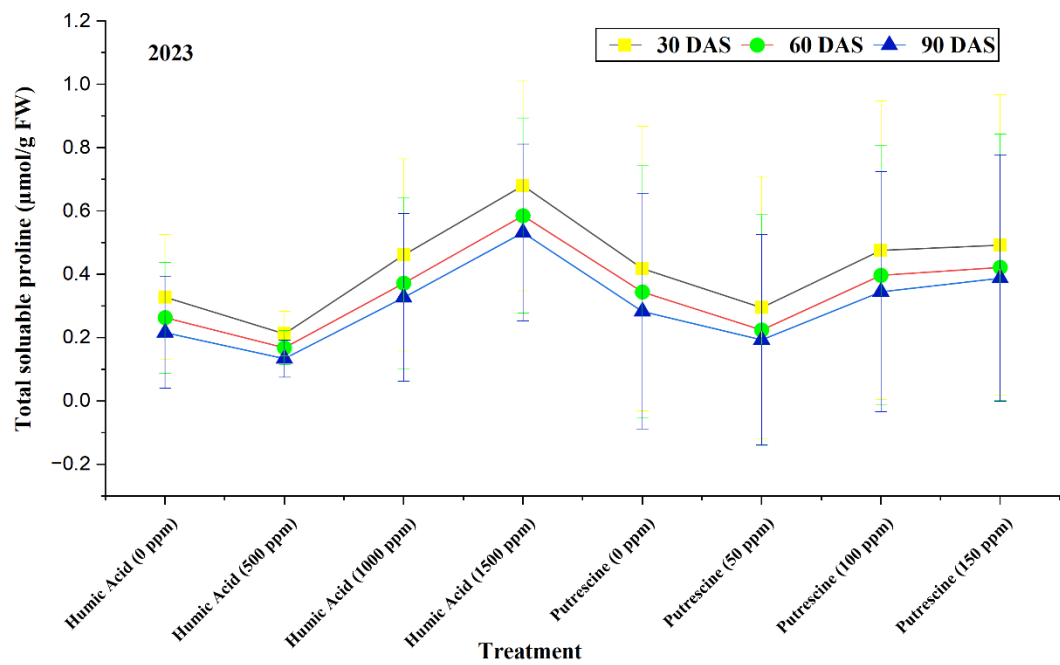


Figure 4.3.6 Effect of different treatments on total free proline of sesame during kharif 2023 & 2024

4.3.7 L-Phenylalanine Ammonia Lyase (PAL) (mg/g Fresh Weight): The applied treatments exerted a significant influence on the L-Phenylalanine Ammonia Lyase (PAL) of sesame at different growth stages (30, 60, and 90 DAS) during the 2023 and 2024 seasons (Table 4.3.7 and Figure 4.3.7). In 2023, phenylalanine ammonia-lyase (PAL) activity in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, relatively higher PAL activity was observed across treatments, reflecting active early vegetative growth. The highest PAL activity was recorded under HA4 (4.65), followed by HA3 (4.41) and HA2 (3.07), whereas the lowest value was observed under HA1 (1.98), indicating a clear treatment-induced enhancement. At 60 DAS, PAL activity increased further as the crop progressed toward the reproductive phase. Treatments HA4 (4.16) and HA3 (3.49) maintained significantly higher PAL activity, while HA2 (3.31) showed a moderate response, and HA1 (2.49) continued to record the minimum value. By 90 DAS, PAL activity increased across all treatments as plants approached physiological maturity. Despite this overall increase, HA3 (4.97) and HA2 (4.97) sustained comparatively higher PAL activity than HA4 (4.54) and HA1 (2.72), suggesting enhanced stress tolerance and activation of secondary metabolism under humic acid application. A similar response pattern was observed under putrescine treatments. At 30 DAS, PAL activity was higher across treatments, with P3 recording the maximum value (3.76), followed by P4 (3.74) and P2 (3.51), while P1 (3.11) exhibited the lowest value, indicating a positive influence of putrescine on early PAL activity. During 60 DAS, PAL activity increased in all treatments, with the highest values recorded under P4 (3.85) and P2 (3.57), followed by P1 (3.11), whereas P3 (2.94) remained the lowest. At 90 DAS, PAL activity declined slightly as the crop approached maturity; however, P4 (4.66) and P3 (4.38) maintained relatively higher PAL activity compared with P2 (4.09) and P1 (4.07). These trends indicate that higher putrescine concentrations promote sustained PAL activity, thereby enhancing antioxidant capacity and stress adaptation during later growth stages. In 2024, phenylalanine ammonia-lyase (PAL) activity in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, relatively higher PAL activity was observed across treatments, reflecting active early vegetative growth. The highest PAL activity was recorded under HA4 (4.71), followed by HA3 (4.58) and HA2 (2.98), whereas the lowest value was observed under HA1 (1.80),

indicating a clear treatment-induced enhancement. At 60 DAS, PAL activity increased further as the crop progressed toward the reproductive phase. Treatments HA4 (4.36) and HA3 (3.57) maintained significantly higher PAL activity, while HA2 (3.29) showed a moderate response, and HA1 (2.41) continued to record the minimum value. By 90 DAS, PAL activity increased across all treatments as plants approached physiological maturity. Despite this overall increase, HA2 (4.93) and HA3 (4.32) sustained comparatively higher PAL activity than HA4 (4.27) and HA1 (2.75), suggesting enhanced stress tolerance and activation of secondary metabolism under humic acid application. A similar response pattern was observed under putrescine treatments. At 30 DAS, PAL activity was higher across treatments, with P3 recording the maximum value (3.73), followed by P4 (3.55) and P2 (3.54). At the same time, P1 (3.25) exhibited the lowest value, indicating that putrescine positively influenced early PAL activity. During 60 DAS, PAL activity increased in all treatments, with the highest values recorded under P4 (3.98) and P2 (3.63), followed by P1 (3.22), whereas P3 (2.79) remained the lowest. At 90 DAS, PAL activity declined slightly as the crop approached maturity; however, P4 (4.42) and P3 (4.10) maintained relatively higher PAL activity compared with P2 (4.00) and P1 (3.75). These trends indicate that higher putrescine concentrations promote sustained PAL activity, thereby enhancing antioxidant capacity and stress adaptation during later growth stages.

4.3.7a Interaction between different treatments on phenylalanine ammonia-lyase (PAL) of sesame at 90 DAS

The interaction between humic acid and putrescine exerted a significant effect on phenylalanine ammonia-lyase (PAL) activity at 90 DAS during both the 2023 and 2024 growing seasons (Table 4.3.7a). In 2023, the highest PAL activity was observed under the combined treatment HA3+P4 (4.49), followed by HA4+P4 (4.30), HA4+P2 (4.29), and HA4+P3 (4.24). In contrast, the lowest PAL activity was recorded under the control combination HA1+P1 (1.97), indicating markedly reduced PAL induction in the absence of both humic acid and putrescine supplementation. A similar interaction pattern was evident in 2024. The treatment HA4+P2 recorded the maximum PAL activity (4.93), followed by HA3+P2 (4.63), HA4+P4 (4.56), and HA4+P3 (4.40). Consistent with the previous season, the minimum PAL activity was again observed

under HA1+P1 (1.70), reaffirming the limited activation of PAL-mediated phenylpropanoid metabolism and antioxidant potential when biostimulants were not applied.

Phenylalanine ammonia-lyase (PAL) is a key regulatory enzyme of the phenylpropanoid pathway, catalyzing the conversion of L-phenylalanine to trans-cinnamic acid and thereby initiating the biosynthesis of phenolics, flavonoids, lignin, and other secondary metabolites. The observed enhancement of PAL activity in sesame under humic acid and putrescine treatments across growth stages and seasons underscores the strong influence of these biostimulants on secondary metabolism and stress-defense pathways. Higher PAL activity during early vegetative growth reflects rapid metabolic turnover and the establishment of basal defense systems. Humic acid application significantly stimulates root growth, nutrient uptake, and carbon–nitrogen metabolism, which increases the availability of phenylalanine, the direct substrate for PAL. Improved nitrogen assimilation under humic substances enhances aromatic amino acid biosynthesis via the shikimate pathway, thereby supporting elevated PAL activity (Canellas & Olivares, 2023). Additionally, humic acid acts as a signaling molecule that elicits mild oxidative and hormonal responses, particularly auxin- and jasmonate-like responses, which are known to activate PAL gene expression. As sesame plants progress toward the reproductive stage, increased PAL activity under humic acid treatments indicates intensified secondary metabolite synthesis associated with structural development, reproductive success, and stress acclimation. Enhanced lignification and phenolic accumulation during this phase strengthen cell walls, improve vascular function, and protect reproductive tissues from oxidative and environmental stresses. Humic substances have been reported to upregulate defense-related enzymes by modulating redox homeostasis and activating transcription factors linked to phenylpropanoid metabolism. The sustained or further increase in PAL activity toward physiological maturity suggests prolonged activation of antioxidant and defense pathways. This response is critical during late growth stages when plants experience increased oxidative pressure due to senescence-related metabolic shifts. Elevated PAL activity supports the accumulation of phenolic antioxidants, which scavenge reactive oxygen species and delay cellular degradation. Application of putrescine also markedly

enhanced PAL activity, reflecting the close interaction between polyamine metabolism and phenylpropanoid biosynthesis. Polyamines function as signaling molecules that regulate stress-responsive gene expression, including PAL, through calcium signaling and mitogen-activated protein kinase (MAPK) cascades. Putrescine has been shown to induce PAL activity by stabilizing membranes, reducing oxidative damage, and enhancing phenolic compound synthesis under both normal and stress conditions. Moreover, putrescine contributes to maintaining cellular redox balance, a critical regulator of PAL activity. By limiting lipid peroxidation and preserving enzyme integrity, putrescine ensures sustained PAL function during later growth stages. The coordinated increase in PAL activity under putrescine treatments supports enhanced accumulation of phenolics, flavonoids, and related antioxidants, strengthening the plant's defensive capacity and metabolic resilience. The combined influence of humic acid and putrescine demonstrates a complementary mode of action: humic acid enhances metabolic capacity and substrate availability, while putrescine regulates signaling pathways and enzyme stability. Together, they promote sustained activation of the phenylpropanoid pathway, thereby enhancing antioxidant potential, stress tolerance, and biochemical robustness in sesame. Overall, increased PAL activity under these biostimulants reflects an adaptive metabolic reprogramming that enhances secondary metabolism, supports stress defense, and improves physiological efficiency throughout sesame growth and development (Weerasekara et al., 2021; Nassiri-Mahallati & Jahan, 2020; Muhammadamin & Mahmood's, 2023).

Table 4.3.7 Effect of different treatments on l-phenylalanine ammonia lyase (pal) of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	1.98	2.49	2.72	1.80	2.41	2.75
HA 2 (500 ppm)	3.07	3.31	4.97	2.98	3.29	4.93
HA 3 (1000 ppm)	4.41	3.49	4.97	4.58	3.57	4.32
HA 4 (1500 ppm)	4.65	4.16	4.54	4.71	4.36	4.27
SEm ±	0.08	0.09	0.15	0.10	0.12	0.10
C.D. (p=0.05)	0.25	0.28	0.45	0.30	0.35	0.30
Factor (Putrescine)						
P 1 (0 ppm)	3.11	3.11	4.07	3.25	3.22	3.75
P 2 (50 ppm)	3.51	3.57	4.09	3.54	3.63	4.00
P 3 (100 ppm)	3.76	2.94	4.38	3.73	2.79	4.10
P 4 (150 ppm)	3.74	3.85	4.66	3.55	3.98	4.42
SEm ±	0.08	0.09	0.15	0.10	0.12	0.10
C.D. (p=0.05)	0.25	0.28	0.45	0.30	0.35	0.30
Interaction	NS	0.56	NS	NS	0.70	NS

Table 4.3.7a Interaction table of L-Phenylalanine Ammonia Lyase (PAL) at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	1.97	2.82	2.20	2.98	2.49	1.70	2.98	1.83	3.14	2.41
HA2	2.92	3.27	3.45	3.62	3.31	3.35	3.05	3.47	3.29	3.29
HA3	3.71	3.90	1.86	4.49	3.49	4.00	3.88	1.46	4.93	3.57
HA4	3.83	4.29	4.24	4.30	4.16	3.84	4.63	4.40	4.56	4.36
Mean	3.11	3.57	2.94	3.85		3.22	3.63	2.79	3.98	
SEm±	0.18					0.24				
C.D (P=0.05)	0.56					0.70				

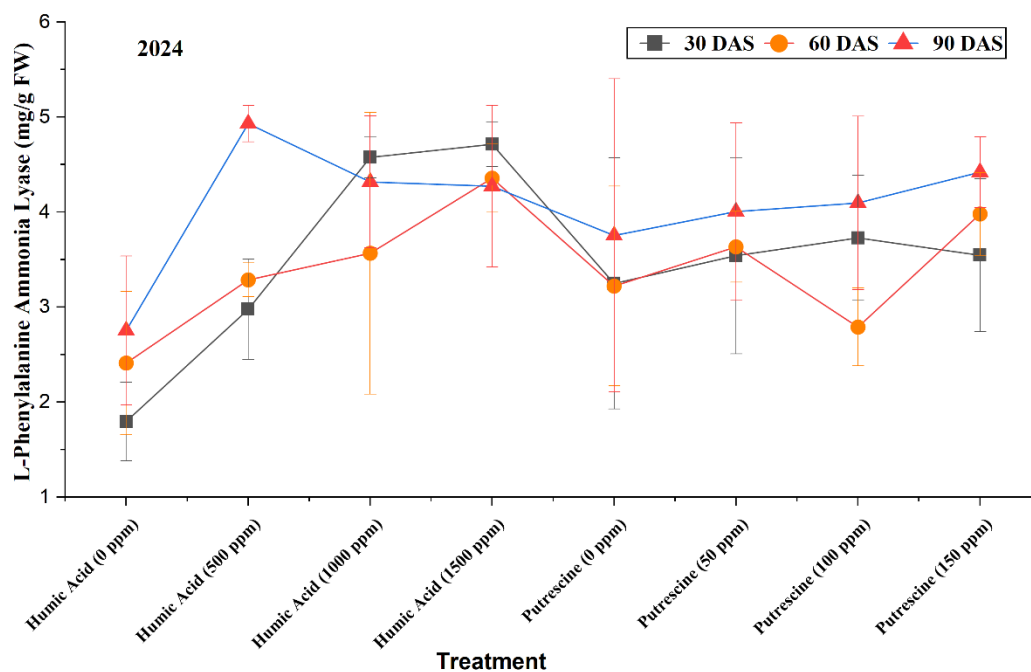
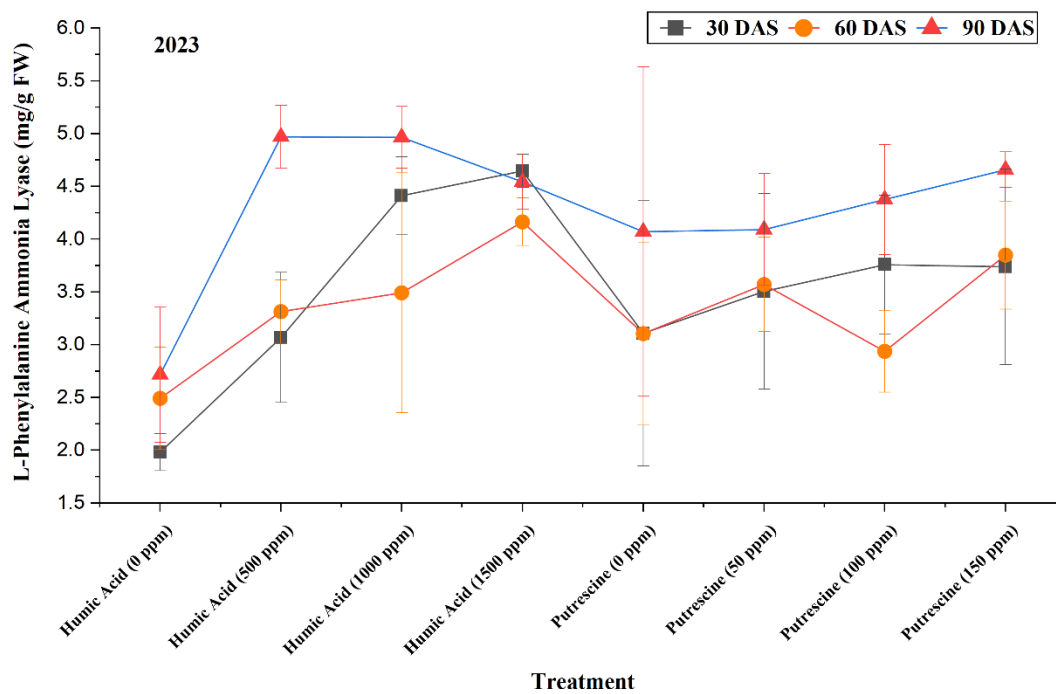


Figure 4.3.7 Effect of different treatments on l-phenylalanine ammonia lyase (pal) of sesame during kharif 2023 & 2024

4.3.8 Flavonols (mg/g Fresh Weight): The applied treatments exerted a significant influence on the flavonols of sesame at different growth stages (30, 60, and 90 DAS) during the 2023 and 2024 seasons (Table 4.3.8 and Figure 4.3.8). In 2023, flavonol content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, higher flavonol levels were observed across treatments, reflecting active early vegetative growth and early induction of secondary-metabolite synthesis. The highest flavonol content was recorded under HA2 (8.49), followed by HA4 (8.20) and HA1 (8.16), whereas HA3 (7.81) registered the lowest value, indicating a clear treatment-dependent response. At 60 DAS, flavonol content increased further as the crop advanced toward the reproductive phase. Treatments HA2 (9.44) and HA4 (9.01) maintained significantly higher flavonol levels, while HA1 (8.59) exhibited a moderate response, and HA3 (8.49) continued to record the minimum value. By 90 DAS, flavonol content increased across all treatments as plants approached physiological maturity. Despite this overall increase, HA2 (9.75) and HA4 (8.21) sustained comparatively higher flavonol levels than HA1 (8.48) and HA3 (7.78), suggesting enhanced stress tolerance and stimulation of secondary metabolism under humic acid application. A comparable response pattern was observed under putrescine treatments. At 30 DAS, flavonol content was higher across treatments, with P3 recording the maximum value (8.85), followed by P4 (8.14) and P1 (7.90). At the same time, P2 (7.78) exhibited the lowest value, indicating a positive role of putrescine in promoting early flavonol biosynthesis. At 60 DAS, flavonol content increased in all treatments, with the highest values observed under P3 (9.48) and P4 (8.97), followed by P1 (8.67), whereas P2 (8.57) remained the lowest. At 90 DAS, flavonol content declined slightly as the crop approached maturity; however, P3 (9.35) and P4 (8.97) maintained relatively higher flavonol levels compared with P1 (8.72) and P2 (8.57). These trends indicate that higher putrescine concentrations promote sustained accumulation of flavonols, thereby enhancing antioxidant capacity and stress adaptation during later growth stages. In 2024, flavonol content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, higher flavonol levels were observed across treatments, reflecting active early vegetative growth and early induction of secondary-metabolite synthesis. The highest flavonol content was recorded under HA2 (8.49), followed by HA4 (8.21) and HA1

(8.15), whereas HA3 (7.78) registered the lowest value, indicating a clear treatment-dependent response. At 60 DAS, flavonol content increased further as the crop advanced toward the reproductive phase. Treatments HA2 (9.64) and HA4 (9.00) maintained significantly higher flavonol levels, while HA1 (8.75) exhibited a moderate response, and HA3 (8.38) continued to record the minimum value. By 90 DAS, flavonol content increased across all treatments as plants approached physiological maturity. Despite this overall increase, HA (9.82) and HA2 (9.70) sustained comparatively higher flavonol levels than HA3 (8.49) and HA1 (8.37), suggesting enhanced stress tolerance and stimulation of secondary metabolism under humic acid application. A comparable response pattern was observed under putrescine treatments. At 30 DAS, flavonol content was higher across treatments, with P3 recording the maximum value (8.94), followed by P4 (8.00) and P1 (7.87). At the same time, P2 (7.82) exhibited the lowest value, indicating a positive role of putrescine in promoting early flavonol biosynthesis. At 60 DAS, flavonol content increased in all treatments, with the highest values observed under P3 (9.49) and P4 (9.08), followed by P1 (8.84), whereas P2 (8.37) remained the lowest. At 90 DAS, flavonol content declined slightly as the crop approached maturity; however, P3 (9.69) and P4 (9.29) maintained relatively higher flavonol levels compared with P2 (8.85) and P1 (8.56). These trends indicate that higher putrescine concentrations promote sustained flavonol accumulation, thereby enhancing antioxidant capacity and stress adaptation during later growth stages.

4.3.8a Interaction between different treatments on Flavonols of sesame at 90 DAS

The interaction between humic acid and putrescine had a significant effect on flavonol content at 90 DAS during both the 2023 and 2024 growing seasons (Table 4.3.8a). In 2023, the highest flavonol content was recorded under the combined treatment HA2+P3 (10.41), followed by HA4+P1 (9.86), HA2+P2 (9.81), and HA2+P1 (9.70). In contrast, the lowest flavonol content was observed under the control combination HA1+P1 (7.51), indicating markedly reduced flavonol induction in the absence of both humic acid and putrescine supplementation. A comparable interaction response was evident in 2024. The HA2+P3 treatment again produced the maximum flavonol content (10.46), followed by HA4+P3 (10.37), HA4+P2 (10.29), and HA4+P1 (10.03). Consistent with the previous season, the minimum flavonol content was recorded under HA1+P1

(7.18), reaffirming the limited activation of flavonol biosynthesis and antioxidant potential when biostimulant applications were omitted.

Flavonols are an essential class of flavonoids that are involved in plant defense, antioxidant protection, UV screening, and the regulation of growth and development. The observed enhancement of flavonol content in sesame in response to applications of humic acid and putrescine across growth stages and seasons indicates that both biostimulants play a decisive role in modulating secondary metabolism, particularly the phenylpropanoid–flavonoid pathway. Higher flavonol accumulation during early vegetative stages reflects early activation of secondary metabolism, associated with rapid cell division and the establishment of protective mechanisms. Humic acid is known to improve nutrient uptake efficiency, particularly nitrogen, phosphorus, iron, and micronutrients that are essential cofactors for enzymes involved in flavonoid biosynthesis. Improved mineral nutrition enhances carbon assimilation and shikimate pathway activity, thereby increasing the availability of phenylalanine, the precursor of flavonol synthesis. Moreover, humic substances function as signaling molecules that stimulate gene expression in phenylpropanoid metabolism, including key enzymes such as chalcone synthase and flavonol synthase, thereby promoting flavonol biosynthesis. As sesame plants advanced toward the reproductive phase, the continued increase in flavonol content under humic acid treatments suggests enhanced metabolic allocation toward protective secondary compounds. Flavonols play a critical role during flowering and reproductive development by protecting tissues from oxidative stress generated by high metabolic activity. Humic acid–induced improvements in photosynthetic efficiency and carbohydrate availability further support flavonol synthesis, as carbon skeletons derived from photosynthesis are diverted toward secondary metabolite production. The sustained accumulation of flavonols during later growth stages under humic acid application indicates delayed senescence and improved antioxidant capacity. Flavonols act as potent scavengers of reactive oxygen species (ROS), stabilizing membranes and protecting cellular macromolecules. Enhanced flavonol levels, therefore, contribute to improved stress tolerance under environmental fluctuations commonly experienced during crop maturation. Application of putrescine also significantly enhanced flavonol accumulation across growth stages, highlighting

the close interaction between polyamine metabolism and flavonoid biosynthesis. Polyamines such as putrescine function as growth regulators and signaling molecules that modulate transcriptional and enzymatic activities associated with secondary metabolism. Putrescine has been shown to upregulate PAL activity and downstream flavonoid biosynthetic enzymes through calcium-dependent signaling and MAP kinase cascades, thereby promoting flavonol synthesis. Additionally, putrescine stabilizes cellular membranes and reduces oxidative damage by regulating ROS homeostasis. This redox-regulatory function indirectly promotes flavonol accumulation, as mild oxidative signaling has been shown to stimulate flavonoid biosynthesis. The maintenance of higher flavonol levels during later growth stages under putrescine treatments suggests improved antioxidant buffering capacity and enhanced adaptation to physiological stress associated with senescence. The complementary roles of humic acid and putrescine indicate a coordinated mechanism: humic acid enhances nutrient availability, carbon metabolism, and enzymatic capacity, while putrescine regulates signaling pathways, enzyme stability, and redox balance. Together, they promote sustained activation of the flavonoid biosynthetic pathway, thereby increasing flavonol accumulation throughout sesame growth and development. Overall, enhanced flavonol content under humic acid and putrescine applications reflects improved secondary metabolic efficiency, strengthened antioxidant defense, and greater physiological resilience in sesame, contributing to improved biochemical quality and stress tolerance of the crop (Muhammadamin & Mahmood's, 2023; Salamati et al., 2024; Weerasekara et al., 2021).

Table 4.3.8 Effect of different treatments on flavonols of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	8.16	8.59	8.48	8.15	8.75	8.37
HA 2 (500 ppm)	8.49	9.44	9.75	8.49	9.64	9.70
HA 3 (1000 ppm)	7.81	8.49	8.38	7.78	8.38	8.49
HA 4 (1500 ppm)	8.20	9.01	9.02	8.21	9.00	9.82
SEm ±	0.07	0.08	0.07	0.08	0.08	0.09
C.D. (p=0.05)	0.21	0.24	0.22	0.23	0.25	0.26
Factor (Putrescine)						
P 1 (0 ppm)	7.90	8.67	8.72	7.87	8.84	8.56
P 2 (50 ppm)	7.78	8.57	8.57	7.82	8.37	8.85
P 3 (100 ppm)	8.85	9.48	9.35	8.94	9.49	9.69
P 4 (150 ppm)	8.14	8.81	8.97	8.00	9.08	9.29
SEm ±	0.07	0.08	0.07	0.08	0.08	0.09
C.D. (p=0.05)	0.21	0.24	0.22	0.23	0.25	0.26
Interaction	NS	NS	0.44	NS	NS	0.52

Table 4.3.8a Interaction effect of humic acid and putrescine application on flavonols at 60 DAS (2023 and 2024)

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	7.51	7.76	9.08	9.56	8.48	7.18	7.47	9.16	9.65	8.37
HA2	9.70	9.81	10.41	9.06	9.75	9.26	9.69	10.46	9.41	9.70
HA3	7.82	8.27	9.38	8.07	8.38	7.75	7.97	8.76	9.49	8.49
HA4	9.86	8.46	8.55	9.20	9.02	10.03	10.29	10.37	8.60	9.82
Mean	8.72	8.57	9.35	8.97		8.56	8.85	9.69	9.29	
SEm±	0.14					0.18				
C.D (P=0.05)	0.44					0.52				

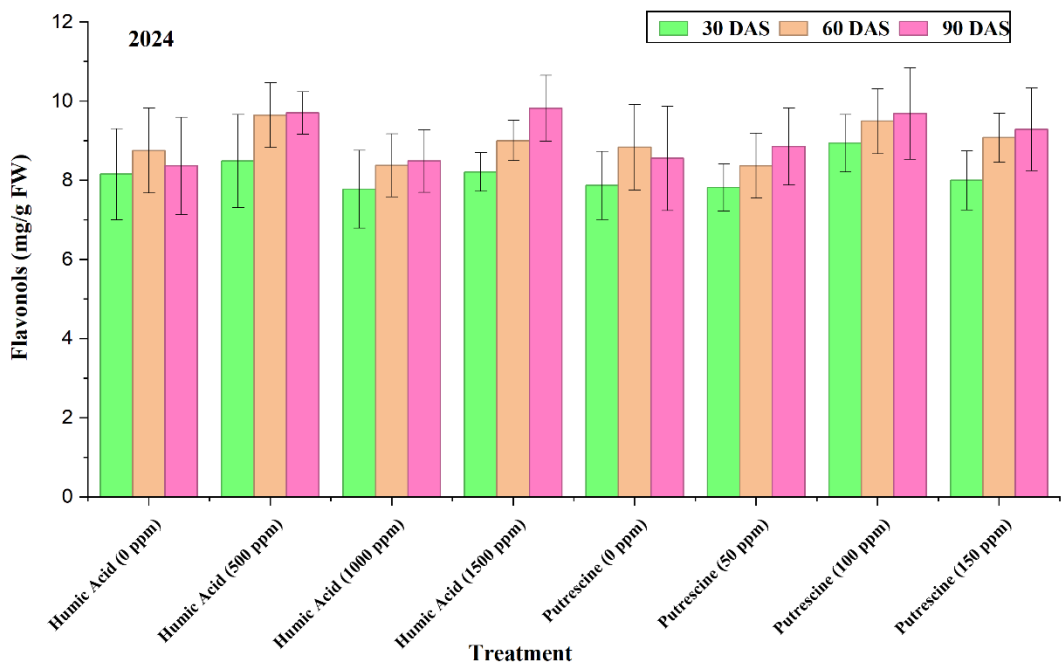
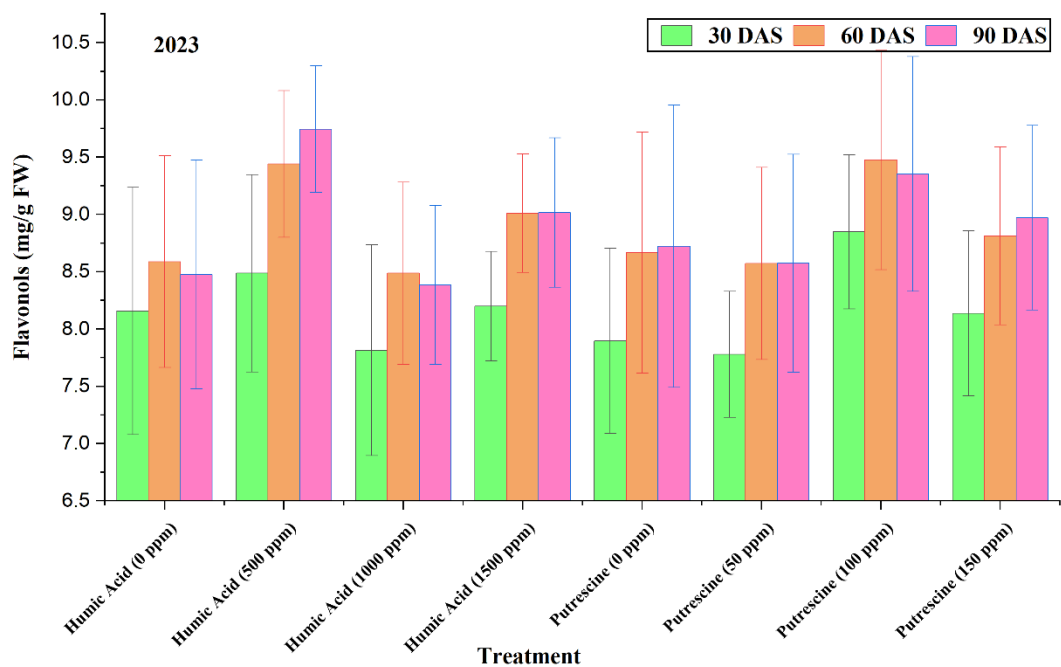


Figure 4.3.8 Effect of different treatments on flavonols of sesame during kharif 2023 & 2024

4.3.9 Flavonoids (mg/g Fresh Weight): The applied treatments exerted a significant influence on the flavonoids of sesame at different growth stages (30, 60, and 90 DAS) during the 2023 and 2024 seasons (Table 4.3.9 and 4. 3.9). In 2023, flavonoid content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, higher flavonoid levels were observed across treatments, reflecting active early vegetative growth and early induction of secondary-metabolite synthesis. The highest flavonoid content was recorded under HA4 (0.29), followed by HA3 (0.25) and HA2 (0.25), whereas HA1 (0.24) registered the lowest value, indicating a clear treatment-dependent response. At 60 DAS, flavonoid content increased further as the crop advanced toward the reproductive phase. Treatments HA4 (0.28) and HA3 (0.28) maintained significantly higher flavonoid levels, while HA2 (0.28) exhibited a moderate response, and HA1 (0.25) continued to record the minimum value. By 90 DAS, flavonoid content increased across all treatments as plants approached physiological maturity. Despite this overall increase, HA2 (0.30) and HA3 (0.28) sustained comparatively higher flavonoid levels than HA4 (0.26) and HA1 (0.23), suggesting enhanced stress tolerance and stimulation of secondary metabolism under humic acid application. A comparable response pattern was observed under putrescine treatments. At 30 DAS, flavonoid content was higher across treatments, with P4 recording the maximum value (0.27), followed by P3 (0.26) and P2 (0.25). At the same time, P1 (0.23) exhibited the lowest value, indicating a positive role of putrescine in promoting early flavonoid biosynthesis. At 60 DAS, flavonol content increased in all treatments, with the highest values observed under P4 (0.30) and P1 (0.27), followed by P3 (0.26), whereas P2 (0.26) remained the lowest. At 90 DAS, flavonoid content declined slightly as the crop approached maturity; however, P4 (0.30) and P2 (0.29) maintained relatively higher flavonoid levels compared with P1 (0.25) and P3 (0.23). These trends indicate that higher putrescine concentrations promote sustained accumulation of flavonoids, thereby enhancing antioxidant capacity and stress adaptation during later growth stages. In 2024, flavonoid content in sesame varied significantly across growth stages under different humic acid treatments. At 30 DAS, higher flavonoid levels were observed across treatments, reflecting active early vegetative growth and early induction of secondary-metabolite synthesis. The highest flavonoid content was recorded under HA2 (0.28), followed by HA4 (0.26) and HA1

(0.25), whereas HA3 (0.21) registered the lowest value, indicating a clear treatment-dependent response. At 60 DAS, flavonoid content increased further as the crop advanced toward the reproductive phase. Treatments HA3 (0.33) and HA2 (0.30) maintained significantly higher flavonoid levels, while HA4 (0.25) exhibited a moderate response, and HA1 (0.22) continued to record the minimum value. By 90 DAS, flavonoid content increased across all treatments as plants approached physiological maturity. Despite this overall increase, HA2 (0.29) and HA4 (0.27) sustained comparatively higher flavonoid levels than HA3 (0.25) and HA1 (0.20), suggesting enhanced stress tolerance and stimulation of secondary metabolism under humic acid application. A comparable response pattern was observed under putrescine treatments. At 30 DAS, flavonoid content was higher across treatments, with P4 recording the maximum value (0.27), followed by P2 (0.25) and P3 (0.24). At the same time, P1 (0.24) exhibited the lowest value, indicating a positive role of putrescine in promoting early flavonoid biosynthesis. At 60 DAS, flavonoid content increased in all treatments, with the highest values observed under P4 (0.33) and P2 (0.29), followed by P3 (0.25), whereas P1 (0.24) remained the lowest. At 90 DAS, flavonoid content declined slightly as the crop approached maturity; however, P4 (0.35) and P1 (0.25) maintained relatively higher flavonoid levels compared with P2 (0.21) and P3 (0.20). These trends indicate that higher putrescine concentrations promote sustained flavonoid accumulation, thereby enhancing antioxidant capacity and stress adaptation during later growth stages.

Flavonoids are key secondary metabolites in plants, playing crucial roles in antioxidant defense, stress mitigation, UV protection, and regulation of growth and development. The observed enhancement of flavonoid accumulation in sesame following application of humic acid and putrescine across growth stages and seasons indicates that both biostimulants effectively stimulate secondary metabolic pathways, particularly those associated with the phenylpropanoid–flavonoid biosynthetic network. Higher flavonoid levels during early vegetative growth reflect the early activation of secondary metabolism, which is required to protect rapidly dividing tissues. Humic acid contributes to this response primarily by improving soil physicochemical properties and nutrient availability, particularly nitrogen, phosphorus, iron, and micronutrients that

serve as cofactors for enzymes involved in flavonoid biosynthesis. Improved nutrient uptake enhances carbon assimilation and stimulates the shikimate and phenylpropanoid pathways, increasing the availability of phenylalanine, the primary precursor for flavonoid synthesis. Additionally, humic substances function as signaling molecules that modulate gene expression in secondary metabolism, thereby increasing the activity of key enzymes such as phenylalanine ammonia-lyase, chalcone synthase, and flavanone synthase. As sesame progressed toward the reproductive phase, the sustained increase in flavonoid content under humic acid treatments suggests enhanced metabolic efficiency and improved assimilate partitioning toward protective compounds. Flavonoids are particularly important during flowering and seed development, where they mitigate oxidative stress arising from heightened metabolic activity. By enhancing photosynthetic efficiency and carbohydrate availability, humic acid provides sufficient carbon skeletons for continued flavonoid biosynthesis, thereby strengthening the plant's antioxidant defense system. The maintenance of higher flavonoid levels during later growth stages under humic acid application indicates delayed senescence and improved cellular protection. Flavonoids scavenge reactive oxygen species, stabilize membranes, and protect proteins and nucleic acids from oxidative damage. This protective function enhances physiological resilience and sustains metabolic activity during crop maturation. Application of putrescine also significantly enhanced flavonoid accumulation across growth stages, highlighting the regulatory role of polyamines in secondary metabolism. Polyamines function as signaling molecules that regulate transcription and enzymatic activity in the phenylpropanoid pathway. Putrescine induces PAL activity and modulates downstream flavonoid biosynthetic genes via calcium-dependent signaling and mitogen-activated protein kinase cascades, thereby stimulating flavonoid synthesis. Furthermore, putrescine stabilizes cellular membranes and maintains redox homeostasis by regulating reactive oxygen species levels. This redox balance creates a favorable intracellular environment for flavonoid accumulation, as moderate oxidative signaling is known to trigger secondary metabolite production. The sustained flavonoid levels observed under putrescine application during later growth stages suggest improved stress adaptation and enhanced antioxidant buffering capacity during senescence. The combined influence of humic acid and putrescine demonstrates a complementary mode of action. While humic acid primarily enhances

nutrient acquisition, photosynthetic performance, and metabolic capacity, putrescine regulates cellular signaling, enzyme stability, and oxidative balance. Together, these biostimulants promote continuous activation of flavonoid biosynthesis, resulting in improved antioxidant status, stress tolerance, and biochemical quality of sesame plants. Overall, the enhanced flavonoid accumulation observed under humic acid and putrescine treatments reflects improved secondary metabolic regulation, stronger antioxidant defense mechanisms, and greater physiological adaptability, all of which contribute to improved crop performance and resilience (Salamati et al., 2024; Raj et al., 2025; Hussein et al., 2023).

Table 4.3.9 Effect of different treatments on flavonoids of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Factor (Humic Acid)						
HA 1 (0ppm)	0.24	0.25	0.23	0.25	0.22	0.206
HA 2 (500 ppm)	0.25	0.28	0.30	0.28	0.30	0.299
HA 3 (1000 ppm)	0.25	0.28	0.28	0.21	0.33	0.25
HA 4 (1500 ppm)	0.29	0.28	0.26	0.26	0.25	0.273
SEm ±	0.01	0.02	0.02	0.02	0.02	0.02
C.D. (p=0.05)	NS	NS	NS	NS	NS	0.06
Factor (Putrescine)						
P 1 (0 ppm)	0.23	0.27	0.25	0.24	0.24	0.252
P 2 (50 ppm)	0.25	0.26	0.29	0.25	0.29	0.215
P 3 (100 ppm)	0.26	0.26	0.23	0.24	0.25	0.206
P 4 (150 ppm)	0.27	0.30	0.30	0.27	0.33	0.355
SEm ±	0.01	0.02	0.02	0.02	0.02	0.02
C.D. (p=0.05)	NS	NS	NS	NS	0.06	0.06
Interaction	NS	NS	NS	NS	NS	NS

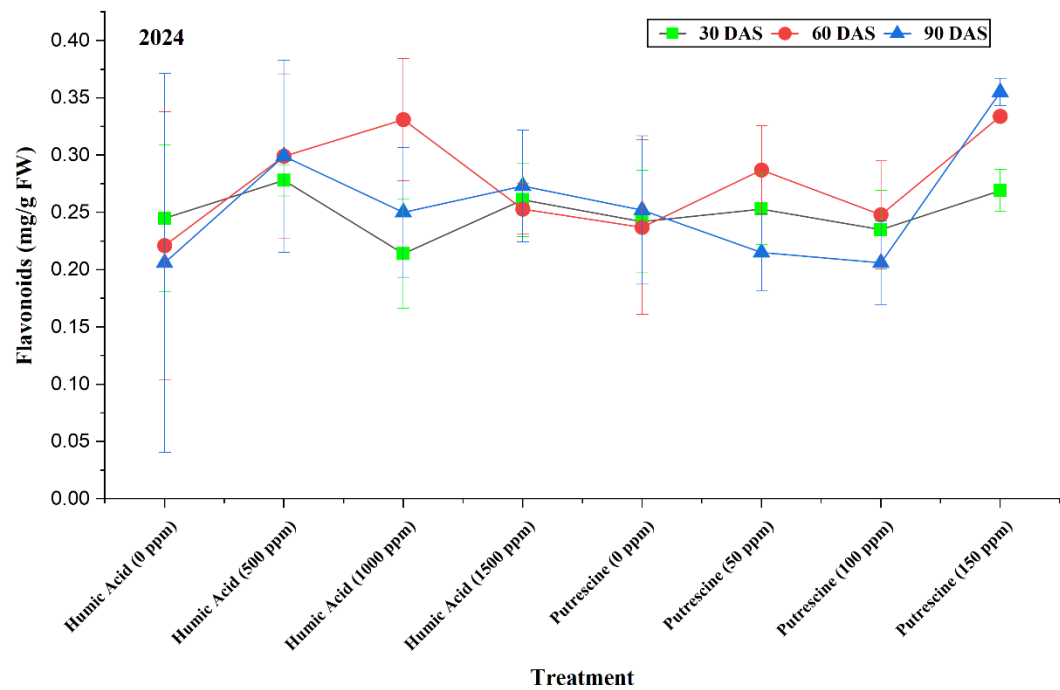
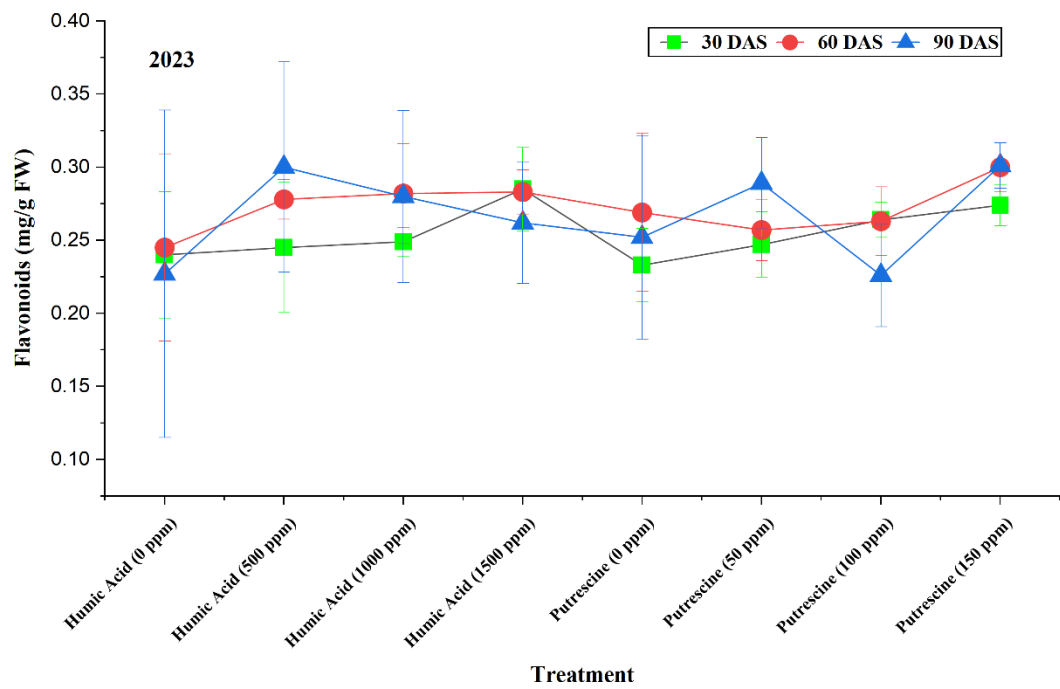


Figure 4.3.9 Effect of different treatments on flavonoids of sesame during kharif 2023 & 2024

4.4 Effects of humic acid and putrescine on yield parameters of sesame cultivation

4.4.1 Capsule length(mm): During the 2023 and 2024 growing seasons, sesame capsule length responded positively to humic acid and putrescine treatments, with higher concentrations resulting in better capsule length (Table 4.4.1 and Figure 4.4.1). In 2023, capsule length increased significantly with increasing humic acid levels, with HA4 recording the highest value (35.27 mm), followed by HA3 (29.29 mm) and HA2 (26.81 mm), while the shortest capsule length was observed in the untreated control, HA1 (23.73 mm). A similar pattern was observed under putrescine treatments, with P4 producing the maximum capsule length (30.33 mm), followed by P3 (29.47 mm) and P2 (28.23 mm), whereas P1 had the minimum value (27.08 mm). In 2024, the same trend was maintained. The most extended capsule length was recorded under HA4 (32.51 mm), followed by HA3 (28.12 mm) and HA2 (25.01 mm), whereas HA1 resulted in the shortest capsule length (21.61 mm). Among putrescine treatments, P4 again produced the highest capsule length (28.41 mm), followed by P3 (27.43 mm) and P2 (26.34 mm), with the lowest value recorded under P1 (25.07 mm). Increasing concentrations of humic acid and putrescine consistently increased capsule length across both seasons, indicating their positive effects on reproductive growth and capsule development in sesame.

4.4.1a Interaction between different treatments on the capsule length of sesame

The interaction effect of humic acid and putrescine on capsule length was significant in both 2023 and 2024 (Table 4.4.1a). In 2023, the maximum capsule length was recorded in HA4+P4 (39.18 mm), followed by HA4+P3 (36.48 mm), HA4+P2 (33.37 mm), and HA4+P1 (32.07 mm), while the minimum was observed in HA1+P1 (21.60 mm). A similar trend was observed in 2024, with HA4+P4 producing the highest capsule length (35.35 mm), followed by HA4+P3 (33.24 mm), HA4+P2 (31.23 mm), and HA4+P1 (30.20 mm). The lowest capsule length in both seasons was consistently recorded under HA1+P1 (19.43 mm).

Capsule length is a critical yield-determining trait in sesame, as it directly influences seed number, seed size, and overall productivity. The positive, concentration-dependent response of capsule length to applications of humic acid and putrescine across both

growing seasons highlights the pivotal role of these biostimulants in enhancing reproductive development under open-field conditions. Humic acid-mediated improvement in capsule length can be attributed to its well-documented effects on nutrient availability, root architecture, and hormonal regulation. Humic substances enhance the uptake and translocation of essential macronutrients such as nitrogen, phosphorus, and potassium, which are crucial for cell division and elongation during reproductive organ development. Recent studies have demonstrated that humic acid exhibits auxin- and cytokinin-like activity, stimulating sink strength and promoting assimilate partitioning toward developing reproductive structures, including capsules in oilseed crops. Improved photosynthetic efficiency and carbohydrate supply under humic acid application further support sustained capsule growth and elongation. Putrescine also significantly increased capsule length, underscoring the importance of polyamines in reproductive physiology. Putrescine regulates cell expansion, stabilizes membranes and nucleic acids, and enhances enzyme activity associated with flower and fruit development. Its involvement in modulating endogenous hormone balance—particularly gibberellins and cytokinins—contributes to improved floral retention and enhanced capsule elongation. Additionally, putrescine mitigates oxidative stress during reproductive stages by strengthening antioxidant defense systems, thereby maintaining cellular integrity and promoting uninterrupted capsule development. The consistency of the response across both seasons suggests that humic acid and putrescine improve reproductive efficiency by strengthening physiological resilience and optimizing source–sink relationships. Enhanced assimilate availability during the capsule initiation and elongation stages ensures better capsule structural development, ultimately contributing to improved yield attributes. Similar improvements in capsule or pod length under humic substances and polyamine treatments have been reported in sesame and other oilseed crops, reinforcing the broader applicability of these biostimulants in sustainable crop production systems (Rajapar et al., 2011; Anaz et al., 2021; Savita & Girijrsh, 2019).

4.4.2 Number of capsules per plant: During the 2023 and 2024 growing seasons, the number of capsules per plant in sesame responded positively to humic acid and putrescine applications, with higher concentrations resulting in increased capsule production (Table 4.4.1 and Figure 4.4.1). In 2023, the number of capsules per plant increased significantly with increasing humic acid levels. The highest value was recorded under HA4 (35.39), followed by HA3 (34.70) and HA2 (33.78), whereas the lowest number of capsules per plant was observed in the untreated control, HA1 (31.25). A similar trend was observed under putrescine treatments, with P4 producing the highest number of capsules per plant (36.48), followed by P3 (35.73) and P2 (34.75), whereas the lowest value was observed in P1 (28.16). In 2024, this pattern was consistently maintained. The highest number of capsules per plant was recorded under HA4 (33.39), followed by HA3 (33.70) and HA2 (31.81), whereas HA1 yielded the lowest number of capsules (29.63). Among putrescine treatments, P4 again recorded the highest number of capsules per plant (34.58), followed by P3 (34.05) and P2 (32.51), with the lowest value observed under P1 (27.41). Increasing concentrations of humic acid and putrescine consistently enhanced the number of capsules per plant across both seasons, highlighting their positive influence on reproductive growth and capsule development in sesame.

The number of capsules per plant is among the most important yield-determining traits in sesame because it reflects the efficiency of floral initiation, flower retention, and successful transformation of flowers into mature capsules. The consistent increase in capsule number with increasing concentrations of humic acid and putrescine during both growing seasons demonstrates that these biostimulants effectively promoted reproductive development and enhanced yield potential under field conditions. The beneficial effect of humic acid on capsule formation is associated with its multiple functions in improving soil fertility, nutrient availability, and plant physiological performance. Humic substances increase the uptake and utilization of nitrogen, phosphorus, and potassium, nutrients that are essential for reproductive differentiation, flowering, and capsule development. Furthermore, humic acid possesses hormone-like properties similar to auxins and cytokinins, which stimulate branching, floral initiation, and reproductive sink establishment. Enhanced root development, improved

photosynthetic efficiency, and greater assimilate production under humic acid treatment also provide a continuous supply of carbohydrates to developing reproductive organs, thereby increasing the number of capsules per plant. Foliar application of putrescine further enhanced capsule production, highlighting the significant role of polyamines in reproductive regulation. Putrescine promotes cell division, improves pollen viability, maintains floral organ integrity, and reduces flower abscission, thereby increasing successful flower-to-capsule conversion. It also regulates endogenous hormonal balance, stabilizes cellular membranes, and protects reproductive tissues against oxidative damage under field conditions. Recent investigations indicate that putrescine strengthens reproductive sink activity by enhancing carbohydrate translocation, improving antioxidant defense, and maintaining metabolic stability during flowering and capsule formation, resulting in greater capsule production in sesame and other oilseed crops. The similar response pattern observed across both seasons indicates that humic acid and putrescine consistently improved the physiological resilience of sesame plants under varying environmental conditions. Improved source–sink relationships, enhanced nutrient acquisition, efficient assimilate partitioning, balanced hormonal regulation, and strengthened reproductive metabolism collectively contributed to higher capsule numbers and ultimately improved seed yield. Similar increases in capsule or pod number under humic substances and polyamine treatments have been reported in sesame and other oilseed crops, supporting the consistency and agronomic relevance of these findings (Hatami, 2017; Kandil & Essam, 2015; Anaz et al., 2021).

4.4.3 Number of seeds per capsule: During the 2023 and 2024 growing seasons, the number of seeds per capsule in sesame responded positively to humic acid and putrescine applications, with higher concentrations resulting in increased seed per capsule (Table 4.4.1 and Figure 4.4.1). In 2023, the number of seeds per capsule increased significantly with increasing humic acid levels. The highest value was recorded under HA4 (32.72), followed by HA3 (32.43) and HA2 (32.58), whereas the lowest number of seeds per capsule was observed in the untreated control, HA1 (31.25). A similar trend was observed under putrescine treatments, with P4 producing the highest number of seeds per capsule (34.34), followed by P3 (33.17) and P2 (32.28), whereas the lowest value was observed under P1 (29.19). In 2024, this trend was consistently maintained. The highest number of seeds per capsule was recorded under HA4 (32.06), followed by HA2 (30.83) and HA3 (30.43), whereas HA1 yielded the lowest number of seeds per capsule (28.53). Among putrescine treatments, P4 again recorded the highest number of seeds per capsule (31.96), followed by P3 (31.42) and P2 (30.04), with the lowest value observed under P1 (28.44). Overall, increasing concentrations of humic acid and putrescine consistently enhanced the number of seeds per capsule across both seasons, underscoring their positive influence on reproductive development and seed formation in sesame.

The number of seeds per capsule is a critical yield component in sesame, as it directly reflects the success of ovule fertilization, seed setting efficiency, and assimilate availability during reproductive development. The consistent improvement in seed number per capsule under increasing concentrations of humic acid and putrescine across both growing seasons highlights the effectiveness of these biostimulants in enhancing reproductive efficiency under open-field conditions. The positive influence of humic acid on seed formation can be attributed to its role in improving nutrient uptake, photosynthetic efficiency, and hormonal balance during the flowering and seed-setting phases. Adequate availability of macro- and micronutrients, particularly nitrogen, boron, and zinc, is essential for pollen viability, fertilization, and embryo development. Humic acid enhances root growth and nutrient mobility in the rhizosphere, thereby improving the supply of assimilates to developing capsules.

Additionally, the hormone-like activity of humic substances promotes better flower retention and ovule development, ultimately resulting in a higher number of seeds per capsule. Application of putrescine further enhanced the seed set, underscoring the pivotal role of polyamines in reproductive physiology. Putrescine is known to regulate cell division, stabilize membranes, and protect reproductive tissues from oxidative damage during flowering and fertilization. Its involvement in pollen tube growth, fertilization efficiency, and early seed development contributes to improved seed formation within capsules. Moreover, putrescine enhances carbohydrate partitioning toward reproductive sinks, ensuring sufficient energy supply for developing seeds. The consistency of treatment responses across seasons suggests that humic acid and putrescine improve the physiological robustness of sesame plants, enabling them to maintain reproductive performance under varying environmental conditions. Enhanced source–sink relationships, improved hormonal regulation, and reduced reproductive stress collectively contribute to increased seed numbers per capsule. Similar improvements in seed set per reproductive unit have been reported in sesame and other oilseed crops following application of humic substances and polyamines, supporting the reliability of these findings (Vani et al., 2017; Farzi Aminabad et al., 2021; Wei et al., 2013).

Table 4.4.1 Effect of different treatments on capsule length (mm), number of capsules per plant, and number of seeds per capsule and of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	Capsule Length (mm)	No of Capsules per Plant	No of Seeds per Capsule	Capsule Length (mm)	No of Capsules per Plant	No of Seeds per Capsule
Factor (Humic Acid)						
HA 1 (0ppm)	23.73	31.25	31.25	21.61	29.63	28.53
HA 2 (500 ppm)	26.81	33.78	32.58	25.01	31.81	30.83
HA 3 (1000 ppm)	29.29	34.70	32.43	28.12	33.70	30.43
HA 4 (1500 ppm)	35.27	35.39	32.72	32.51	33.39	32.06
SEm ±	0.45	0.31	0.32	0.13	0.18	0.19
C.D. (p=0.05)	1.31	0.90	0.94	0.40	0.53	0.57
Factor (Putrescine)						
P 1 (0 ppm)	27.08	28.16	29.19	25.07	27.41	28.44
P 2 (50 ppm)	28.23	34.75	32.28	26.34	32.51	30.04
P 3 (100 ppm)	29.47	35.73	33.17	27.43	34.05	31.42
P 4 (150 ppm)	30.33	36.48	34.34	28.41	34.58	31.96
SEm ±	0.45	0.31	0.32	0.13	0.18	0.19
C.D. (p=0.05)	1.31	0.90	0.94	0.40	0.53	0.57
Interaction	2.62	NS	NS	0.80	NS	NS

Table 4.4.1a Interaction effect of humic acid and putrescine application on capsule length (m) at 2023 and 2024

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	21.60	23.91	24.08	25.34	23.73	19.43	21.13	22.54	23.34	21.61
HA2	26.32	26.88	26.87	27.19	26.81	24.33	25.23	25.18	25.32	25.01
HA3	28.32	28.77	30.44	29.62	29.29	26.32	27.77	28.77	29.62	28.12
HA4	32.07	33.37	36.48	39.18	35.27	30.20	31.23	33.24	35.35	32.51
Mean	27.08	28.23	29.47	30.33		25.07	26.34	27.43	28.41	
SEm±	0.90					0.26				
C.D (P=0.05)	2.62					0.80				

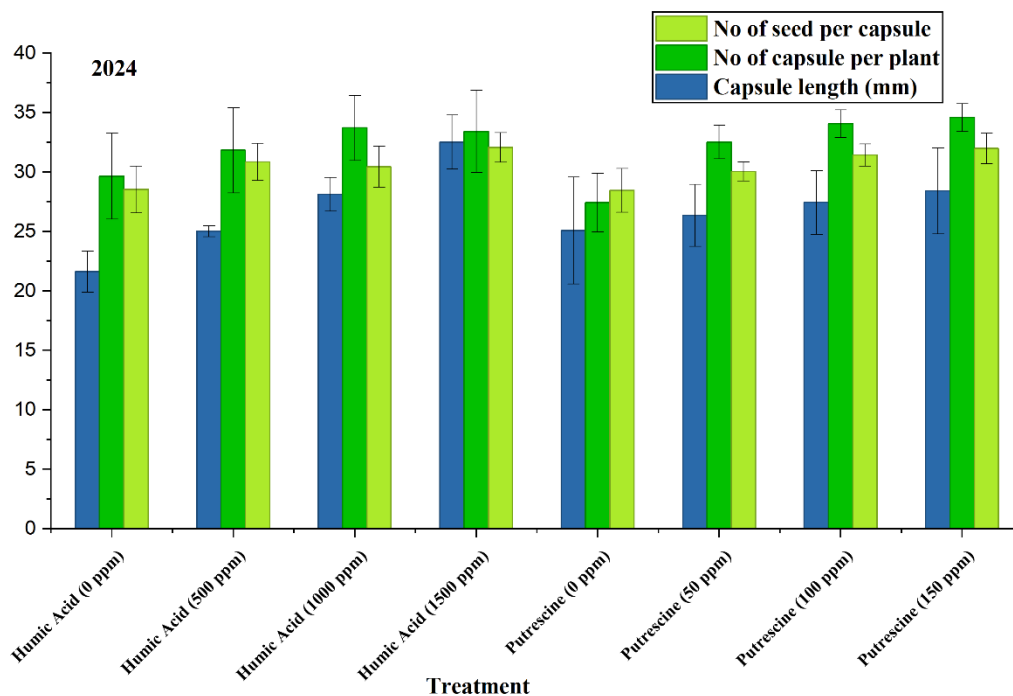
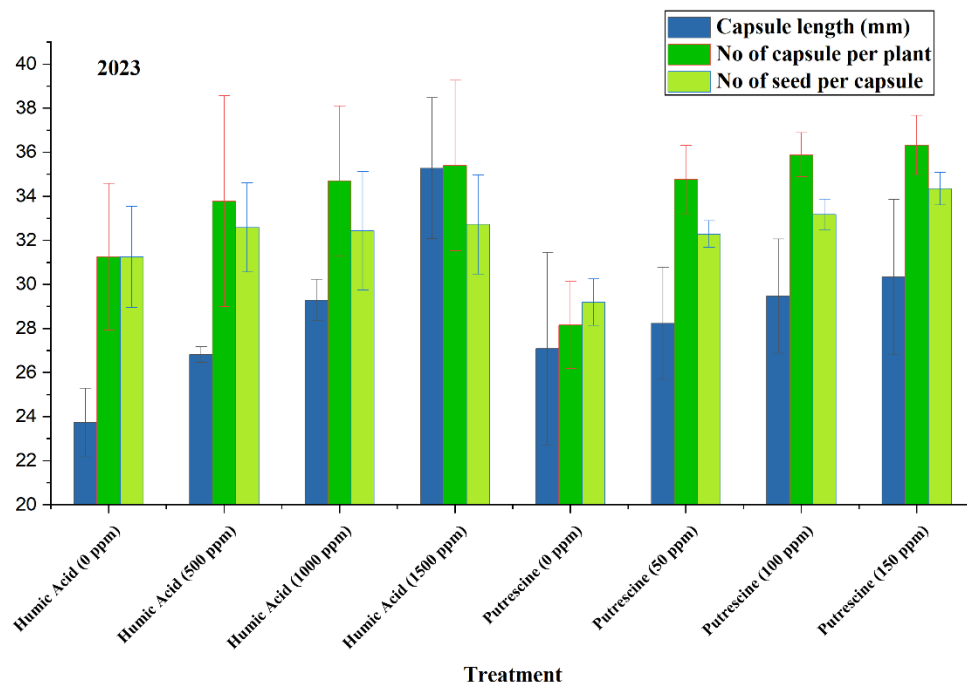


Figure 4.4.1 Effect of different treatments on capsule length (mm), number of capsules per plant, and number of seeds per capsule and of sesame during kharif 2023 & 2024

4.4.4 Test Weight (g): During the 2023 and 2024 growing seasons, the test weight of sesame responded positively to humic acid and putrescine applications, with higher concentrations resulting in increased seed weight (Table 4.4.2 and Figure 4.2.2). In 2023, test weight increased significantly with increasing humic acid levels. The maximum test weight was recorded for HA4 (2.90 g), followed by HA3 (2.70 g) and HA2 (2.69 g), whereas the lowest test weight was observed in the untreated control, HA1 (2.62 g). A comparable response was observed under putrescine treatments, with P4 producing the highest test weight (2.79 g), followed by P3 (2.76 g) and P2 (2.70 g), whereas the lowest value was observed under P1 (2.64 g). In 2024, a similar trend was maintained. The highest test weight was recorded for HA4 (2.89 g), followed by HA3 (2.69 g) and HA2 (2.60 g), whereas HA1 yielded the lowest value (2.46 g). Among putrescine treatments, P4 again recorded the maximum test weight (2.81 g), followed by P3 (2.71 g) and P2 (2.61 g), with the lowest value observed under P1 (2.54 g). Overall, increasing concentrations of humic acid and putrescine consistently increased test weight across both seasons, highlighting their beneficial effects on seed development and yield attributes in sesame.

4.4.4a Interaction between different treatments on test weight (g) of sesame

The interaction effect of humic acid and putrescine on test weight was significant in both 2023 and 2024 (Table 4.4.2a). In 2023, the highest test weight was recorded under the HA4+P4 treatment (3.08 g), followed by HA4+P3 (3.01 g), HA4+P2 (2.77 g), and HA4+P1 (2.74 g). In contrast, the lowest test weight was observed under HA1+P1 (2.53 g). A similar response pattern was evident during the 2024 season. The HA4+P4 combination again produced the maximum test weight (3.06 g), followed by HA4+P3 (3.04 g), HA3+P3 (2.79 g), and HA4+P1 (2.76 g). Consistent across both seasons, the minimum test weight was recorded in the HA1+P1 treatment (2.30 g), indicating poor seed development in the absence of biostimulant application.

Test weight is a critical yield-determining trait in sesame, as it reflects the efficiency of assimilate accumulation, the duration of seed filling, and the overall physiological health of the plant during the reproductive phase. The consistent improvement in

thousand-seed weight observed under humic acid and putrescine applications across both growing seasons underscores the vital role of these biostimulants in enhancing seed development and sink strength under open-field conditions. Humic acid significantly influences seed weight by improving root architecture, nutrient uptake efficiency, and soil–plant interactions. Enhanced absorption of essential macronutrients, such as nitrogen, phosphorus, and potassium, and of micronutrients, such as zinc and boron, plays a pivotal role in enzymatic activity and carbohydrate metabolism during seed filling. Humic substances also stimulate plasma membrane H⁺-ATPase activity, thereby increasing nutrient translocation and improving photosynthetic performance. This enhanced source capacity ensures a continuous supply of assimilates to developing seeds, thereby increasing individual seed mass. In addition to nutritional effects, humic acid exhibits hormone-like activity, particularly auxin- and cytokinin-like responses, which delay leaf senescence and prolong the effective photosynthetic period. This extended seed-filling duration promotes greater deposition of storage compounds, such as lipids and proteins, in sesame seeds, thereby increasing thousand-seed weight. Similar improvements in seed weight following humic substance application have been reported in oilseed crops such as sunflower, soybean, and sesame, supporting the physiological basis of these findings. Application of putrescine further enhanced test weight by regulating cellular metabolism and protecting developing seeds from oxidative stress. As a key polyamine, putrescine stabilizes cell membranes, nucleic acids, and proteins, thereby maintaining metabolic integrity during the critical seed-filling stage. Its role in modulating antioxidant enzyme activity helps mitigate the accumulation of reactive oxygen species, which is particularly important under fluctuating field conditions. By preserving photosynthetic efficiency and enhancing carbon assimilation, putrescine strengthens source–sink relationships, allowing more assimilates to be allocated toward seed development. Moreover, putrescine has been shown to influence hormonal crosstalk involving gibberellins and cytokinins, which regulate cell expansion and reserve accumulation in developing seeds. Enhanced activity of sucrose synthase and starch- and oil-biosynthetic enzymes under polyamine application contributes to improved seed density and weight. These mechanisms collectively explain the observed increases in thousand-seed weight under higher putrescine concentrations. The consistency of

treatment effects across seasons indicates that humic acid and putrescine not only enhance seed filling under optimal conditions but also confer physiological resilience, enabling sesame plants to maintain efficient assimilate partitioning under variable environmental conditions. The combined improvement in nutrient acquisition, photosynthetic performance, antioxidant protection, and hormonal regulation ultimately results in heavier, better-filled seeds (Wei et al., 2013; Reddy et al., 2021; Hayati et al., 2022).

4.4.5 Biological yield (q ha^{-1}): During the 2023 and 2024 growing seasons, the biological yield of sesame responded positively to humic acid and putrescine applications, with higher concentrations of both biostimulants resulting in increased biomass production (Table 4.4.2 and Figure 4.4.2). In 2023, the biological yield of sesame increased significantly with increasing humic acid levels. The highest biological yield was recorded under HA4 (40.79 q ha^{-1}), followed by HA3 (35.53 q ha^{-1}) and HA2 (34.60 q ha^{-1}), whereas the lowest yield was observed in the untreated control, HA1 (29.71 q ha^{-1}). A similar trend was evident under putrescine treatments, with P4 producing the maximum biological yield (41.51 q ha^{-1}), followed by P3 (39.92 q ha^{-1}) and P2 (34.96 q ha^{-1}); the minimum yield was recorded under P1 (25.21 q ha^{-1}). In 2024, this pattern was consistently maintained. The highest biological yield was obtained with HA4 (37.67 q ha^{-1}), followed by HA3 (32.49 q ha^{-1}) and HA2 (30.82 q ha^{-1}), while HA1 resulted in the lowest biological yield (27.99 q ha^{-1}). Among putrescine treatments, P4 again recorded the highest biological yield (38.05 q ha^{-1}), followed by P3 (34.84 q ha^{-1}) and P2 (31.75 q ha^{-1}), whereas the lowest value was observed under P1 (24.32 q ha^{-1}). Overall, increasing concentrations of humic acid and putrescine consistently enhanced the biological yield of sesame across both seasons, underscoring their positive effects on biomass accumulation and overall crop productivity.

4.4.5a Interaction between different treatments on biological yield (q ha^{-1}) of sesame

The interaction effect of humic acid and putrescine on biological yield was significant in both 2023 and 2024 (Table 4.4.2b). In 2023, the highest biological yield was recorded under the HA4+P4 treatment (52.20 q ha^{-1}), followed by HA4+P3 (45.79 q ha^{-1}), HA3+P4 (41.14 q ha^{-1}), and HA2+P2 (40.30 q ha^{-1}). In contrast, the lowest biological yield weight was observed under HA1+P1 (21.21 q ha^{-1}). A similar response pattern was evident during the 2024 season. The HA4+P4 combination again produced the maximum biological yield (46.91 q ha^{-1}), followed by HA4+P3 (40.76 q ha^{-1}), HA3+P4 (37.95 q ha^{-1}), and HA4+P2 (35.47 q ha^{-1}). Consistent across both seasons, the minimum biological yield was recorded in the HA1+P1 treatment (20.99 q ha^{-1}), indicating poor seed development in the absence of biostimulant application.

Biological yield represents the cumulative outcome of vegetative growth, photosynthetic efficiency, nutrient acquisition, and assimilate partitioning throughout the crop life cycle. The consistent enhancement of biological yield in sesame under humic acid and putrescine applications across both growing seasons highlights the effectiveness of these biostimulants in promoting total biomass production under open-field conditions. Humic acid plays a pivotal role in improving biological yield primarily through its influence on soil–plant interactions and plant physiology. By enhancing soil structure, cation exchange capacity, and microbial activity, humic substances improve nutrient availability and uptake efficiency. Enhanced absorption of nitrogen, phosphorus, potassium, and essential micronutrients supports vigorous vegetative growth, leading to increased leaf area development and higher photosynthetic capacity. Improved photosynthesis directly translates into greater dry matter accumulation in stems, leaves, and reproductive organs, thereby increasing total biomass production. In addition to its nutritional effects, humic acid exhibits hormone-like activity, mimicking auxins and cytokinins, thereby stimulating cell division, cell elongation, and lateral root proliferation. A more extensive root system enhances water and nutrient uptake, particularly during critical growth phases, ensuring sustained growth even under fluctuating environmental conditions. Furthermore, humic acid delays leaf senescence by maintaining chlorophyll stability and photosystem integrity, prolonging the effective photosynthetic period, and contributing to cumulative biomass gain. Application of putrescine further increased biological yield by modulating metabolic and stress-related processes. As a key polyamine, putrescine stabilizes cellular membranes, nucleic acids, and proteins, thereby supporting efficient cellular function during both vegetative and reproductive stages. Its role in regulating antioxidant defense systems reduces oxidative damage caused by reactive oxygen species, thereby maintaining photosynthetic efficiency and metabolic activity for extended periods. This sustained physiological performance enhances dry matter accumulation across plant organs. Putrescine is also involved in hormonal crosstalk, interacting with auxins, gibberellins, and cytokinins to regulate growth dynamics. By promoting cell expansion and vascular differentiation, putrescine improves assimilate transport from source leaves to sink tissues, contributing to greater biomass accumulation. Enhanced activity of enzymes involved in carbon and nitrogen metabolism under polyamine application further supports efficient conversion

of assimilates into structural and storage biomass. The observed consistency of treatment effects across seasons indicates that both humic acid and putrescine enhance growth under favorable conditions and improve crop resilience to seasonal variability. Their combined influence on nutrient uptake, photosynthesis, antioxidant protection, and hormonal regulation creates an integrated growth-promoting environment that maximizes biomass production (Reddy et al., 2021; Hayati et al., 2022; Gajanan et al., 2022).

Table 4.4.2 Effect of different treatments on test weight (g) and biological yield (q ha⁻¹) of sesame during kharif 2023 & 2024

Treatments	2023		2024	
	Weight of 1000 Seeds (g)	Biological Yield (q ha ⁻¹)	Weight of 1000 Seeds (g)	Biological Yield (q ha ⁻¹)
Factor (Humic Acid)				
HA 1 (0ppm)	2.62	29.71	2.46	27.99
HA 2 (500 ppm)	2.69	34.60	2.69	30.82
HA 3 (1000 ppm)	2.70	35.53	2.6	32.49
HA 4 (1500 ppm)	2.90	40.79	2.89	37.67
SEm ±	0.01	0.60	0.01	0.25
C.D. (p=0.05)	0.05	1.74	0.05	0.75
Factor (Putrescine)				
P 1 (0 ppm)	2.64	25.21	2.54	24.32
P 2 (50 ppm)	2.70	34.96	2.61	31.75
P 3 (100 ppm)	2.76	39.92	2.71	34.84
P 4 (150 ppm)	2.79	41.54	2.81	38.05
SEm ±	0.01	0.60	0.01	0.25
C.D. (p=0.05)	0.05	1.74	0.05	0.75
Interaction	0.10	3.48	0.10	1.50

Table 4.4.2a Interaction effect of humic acid and putrescine application on test weight (g) at 2023 and 2024

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	2.53	2.64	2.66	2.64	2.62	2.30	2.40	2.49	2.64	2.46
HA2	2.64	2.65	2.73	2.73	2.69	2.61	2.66	2.75	2.76	2.69
HA3	2.67	2.76	2.66	2.71	2.70	2.50	2.68	2.57	2.79	2.64
HA4	2.74	2.77	3.01	3.08	2.90	2.76	2.72	3.04	3.06	2.89
Mean	2.64	2.70	2.76	2.79		2.54	2.61	2.71	2.81	
SEm±	0.03					0.03				
C.D (P=0.05)	0.10					0.10				

Table 4.4.2b Interaction effect of humic acid and putrescine application on biological yield (q ha⁻¹) of sesame at 2023 and 2024

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	21.21	31.87	33.23	32.54	29.71	20.99	28.50	30.3	32.16	27.99
HA2	24.40	34.80	38.89	40.30	34.60	23.12	30.76	34.21	35.18	30.82
HA3	26.79	36.44	38.75	41.14	35.53	25.65	32.29	34.08	37.95	32.49
HA4	28.46	36.73	45.79	52.20	40.79	27.53	35.47	40.76	46.91	37.67
Mean	25.21	34.96	39.92	41.54		24.32	31.75	34.84	38.05	
SEm±	1.20					0.51				
C.D (P=0.05)	3.48					1.50				

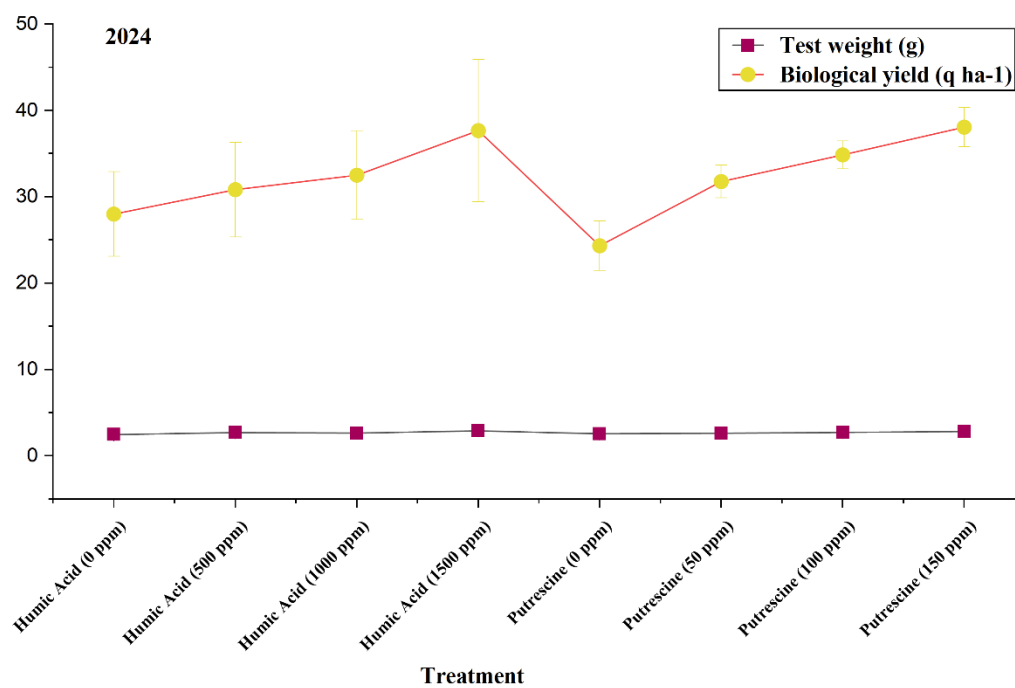
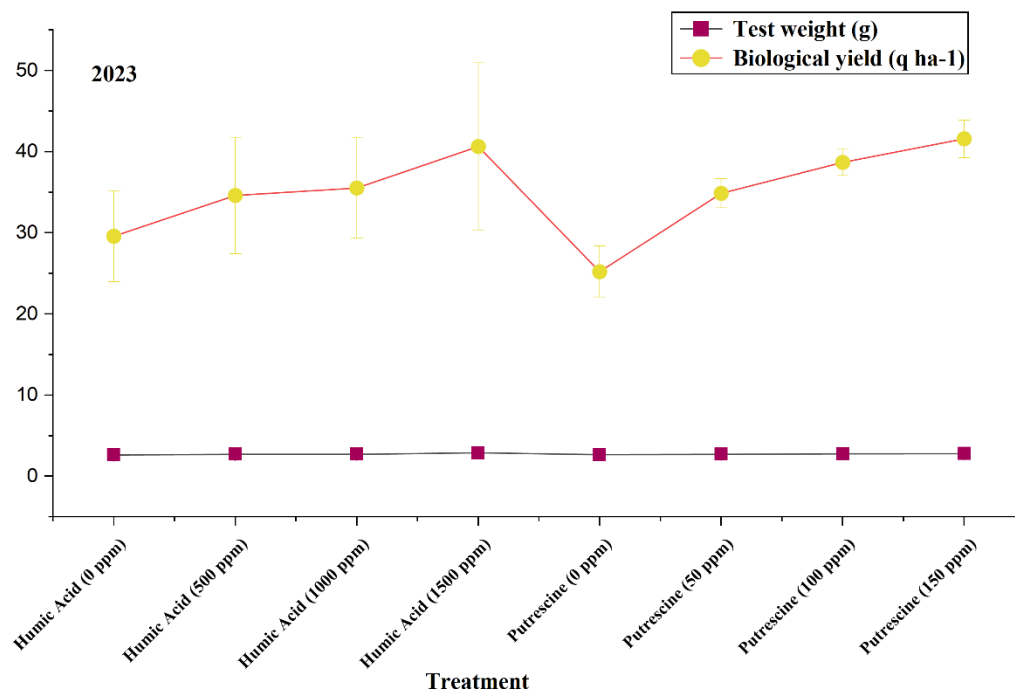


Figure 4.4.2 Effect of different treatments on test weight (g) and biological yield (q ha⁻¹) of sesame during kharif 2023 & 2024

4.4.6 Seed yield (q ha⁻¹): During the 2023 and 2024 growing seasons, seed yield of sesame responded positively to humic acid and putrescine applications, with higher concentrations of both biostimulants resulting in increased yield (Table 4.4.3 and Figure 4.4.3). In 2023, seed yield increased significantly with increasing humic acid levels. The highest seed yield was recorded under HA4 (7.26 q ha⁻¹), followed by HA3 (6.45 q ha⁻¹) and HA2 (6.28 q ha⁻¹), while the lowest yield was observed in the untreated control, HA1 (5.36 q ha⁻¹). A similar response was observed under putrescine treatments, with P4 producing the maximum seed yield (7.52 q ha⁻¹), followed by P3 (6.92 q ha⁻¹) and P2 (6.35 q ha⁻¹); the minimum yield was recorded under P1 (4.54 q ha⁻¹). In 2024, the same trend was maintained. The highest seed yield was obtained under HA4 (6.79 q ha⁻¹), followed by HA3 (5.91 q ha⁻¹) and HA2 (5.50 q ha⁻¹), whereas HA1 resulted in the lowest seed yield (4.51 q ha⁻¹). Among putrescine treatments, P4 again recorded the highest seed yield (6.79 q ha⁻¹), followed by P3 (6.26 q ha⁻¹) and P2 (5.40 q ha⁻¹), with the lowest value observed under P1 (4.27 q ha⁻¹). Overall, increasing concentrations of humic acid and putrescine consistently enhanced seed yield in sesame across both seasons, highlighting their beneficial effects on assimilate production and overall crop productivity.

4.4.6a Interaction between different treatments on seed yield (q ha⁻¹) of sesame

The interaction effect of humic acid and putrescine on seed yield was significant in both 2023 and 2024 (Table 4.4.3a). In 2023, the highest seed yield was recorded under the HA4+P4 treatment (9.48 q ha⁻¹), followed by HA4+P3 (7.72 q ha⁻¹), HA3+P4 (7.47 q ha⁻¹), and HA2+P4 (7.35 q ha⁻¹). In contrast, the lowest seed yield was observed under HA1+P1 (3.83 q ha⁻¹). A similar response pattern was evident during the 2024 season. The HA4+P4 combination again produced the maximum seed yield (8.19 q ha⁻¹), followed by HA4+P3 (7.34 q ha⁻¹), HA3+P4 (7.28 q ha⁻¹), and HA4+P2 (6.40 q ha⁻¹). Consistent across both seasons, the minimum seed yield was recorded in the HA1+P1 treatment (3.11 q ha⁻¹), indicating poor yield development in the absence of biostimulant application.

Seed yield is the ultimate expression of successful integration among vegetative growth, physiological efficiency, reproductive development, and assimilate

partitioning. The consistent increase in sesame seed yield with the application of humic acid and putrescine across both growing seasons clearly demonstrates the effectiveness of these biostimulants in improving crop productivity under open-field conditions. Humic acid contributes to seed-yield improvement primarily through its multifaceted effects on soil fertility, nutrient availability, and plant physiological performance. By improving soil aggregation, microbial activity, and cation exchange capacity, humic substances enhance the availability and uptake of essential macro- and micronutrients. Improved nutrient acquisition supports robust vegetative growth, higher leaf area index, and prolonged photosynthetic activity, which collectively increase assimilate production during critical growth stages. Enhanced photosynthate supply strengthens sink development, resulting in improved capsule formation, seed filling, and final seed yield. In addition, humic acid exhibits hormone-like activity, stimulating auxin- and cytokinin-mediated processes that promote root proliferation, shoot growth, and reproductive organ differentiation. A well-developed root system enhances water and nutrient uptake efficiency, particularly during flowering and seed-filling stages, ensuring uninterrupted assimilate flow to developing seeds. Humic acid also delays leaf senescence by stabilizing chlorophyll and protecting photosynthetic machinery, thereby extending the duration of effective carbon assimilation and contributing to higher seed yield. Putrescine further increased seed yield by regulating cellular metabolism, stress tolerance, and reproductive development. As a polyamine, putrescine stabilizes membranes, nucleic acids, and proteins, maintaining cellular integrity during rapid growth and seed development. It enhances antioxidant defense systems by regulating enzymatic and non-enzymatic antioxidants, thereby reducing oxidative damage and preserving photosynthetic efficiency under fluctuating environmental conditions. Sustained photosynthesis ensures adequate assimilate supply for reproductive sinks, resulting in improved seed formation and yield stability. Putrescine also plays a key role in hormonal crosstalk, interacting with auxins, gibberellins, and cytokinins to regulate flowering, capsule development, and seed filling. Enhanced vascular differentiation and phloem loading under polyamine influence improve source–sink translocation efficiency, ensuring efficient movement of carbohydrates and nitrogenous compounds to developing seed. Moreover, putrescine stimulates enzymes involved in carbon and nitrogen metabolism, improving the conversion of assimilates into storage

compounds such as oils and proteins within seeds. The consistent seed yield improvement across both seasons suggests that humic acid and putrescine not only enhance yield under optimal conditions but also increase crop resilience to seasonal variability. Their combined effects on nutrient uptake, photosynthetic performance, antioxidant protection, and assimilate partitioning create a favorable physiological environment for sustained reproductive success. The increased seed yield of sesame under applications of humic acid and putrescine can be attributed to improved soil–plant interactions, enhanced photosynthesis, prolonged assimilate availability, efficient source–sink dynamics, and strengthened stress-tolerance mechanisms. These findings reinforce the role of humic acid and putrescine as sustainable biostimulants for improving sesame productivity under open-field cultivation (Hayati et al., 2022; Noveyri et al., 2022; Salama, 2023).

4.4.7 Stalk yield (q ha⁻¹): During the 2023 and 2024 growing seasons, the stalk yield of sesame responded positively to humic acid and putrescine applications, with higher concentrations of both biostimulants resulting in increased biomass production (Table 4.4.3 and Figure 4.4.3). In 2023, stalk yield increased significantly with increasing levels of humic acid. The highest stalk yield was recorded under HA4 (33.37 q ha⁻¹), followed by HA3 (29.07 q ha⁻¹) and HA2 (28.32 q ha⁻¹), whereas the lowest yield was observed in the untreated control, HA1 (24.35 q ha⁻¹). A similar response was evident under putrescine treatments, with P4 producing the maximum stalk yield (34.01 q ha⁻¹), followed by P3 (31.82 q ha⁻¹) and P2 (28.60 q ha⁻¹), while the minimum yield was recorded under P1 (20.68 q ha⁻¹). In 2024, the same trend was maintained. The highest stalk yield was obtained with HA4 (30.87 q ha⁻¹), followed by HA3 (26.57 q ha⁻¹) and HA2 (25.31 q ha⁻¹), whereas HA1 resulted in the lowest stalk yield (23.47 q ha⁻¹). Among putrescine treatments, P4 again recorded the highest stalk yield (31.26 q ha⁻¹), followed by P3 (28.57 q ha⁻¹) and P2 (26.35 q ha⁻¹), with the lowest value observed under P1 (20.05 q ha⁻¹). Overall, increasing concentrations of humic acid and putrescine consistently enhanced stalk yield across both seasons, highlighting their beneficial role in improving biomass accumulation and overall crop productivity in sesame.

4.4.7a Interaction between different treatments on stalk yield (q ha⁻¹) of sesame

The interaction effect of humic acid and putrescine on stalk yield was significant in both 2023 and 2024 (Table 4.4.3b). In 2023, the highest stalk yield was recorded under the HA4+P4 treatment (42.71 q ha⁻¹), followed by HA4+P3 (37.40 q ha⁻¹), HA3+P4 (33.67 q ha⁻¹), and HA2+P4 (32.95 q ha⁻¹). In contrast, the lowest stalk yield weight was observed under HA1+P1 (17.39 q ha⁻¹). A similar response pattern was evident during the 2024 season. The HA4+P4 combination again produced the maximum stalk yield (38.71 q ha⁻¹), followed by HA4+P3 (33.41 q ha⁻¹), HA3+P4 (30.67 q ha⁻¹), and HA4+P2 (29.06 q ha⁻¹). Consistent across both seasons, the minimum stalk yield was recorded in the HA1+P1 treatment (17.87 q ha⁻¹), indicating poor stalk yield development in the absence of biostimulant application.

Stalk yield represents a major component of biological yield in sesame and reflects the cumulative effects of vegetative growth, photosynthetic efficiency, nutrient uptake, and

biomass partitioning. The consistent enhancement of stalk yield under humic acid and putrescine applications across both growing seasons highlights the strong influence of these biostimulants on structural biomass accumulation and overall crop vigor under open-field conditions. Humic acid plays a pivotal role in improving stalk yield by enhancing soil physicochemical properties and stimulating plant physiological processes. Its application improves soil structure, aeration, and water-holding capacity, which facilitates better root penetration and expansion. A well-developed root system improves the uptake of nitrogen, phosphorus, potassium, and micronutrients, which are essential for cell division, cell elongation, and secondary wall thickening in stems. Improved nutrient availability supports greater internodal elongation and stem girth, directly contributing to increased stalk biomass. Additionally, humic substances exhibit hormone-like activity, particularly mimicking auxins and cytokinins, which stimulate cambial activity and vascular differentiation. Enhanced xylem and phloem development improves translocation efficiency and mechanical strength of the stem, resulting in higher dry matter accumulation in stalk tissues. Humic acid also enhances chlorophyll synthesis and delays leaf senescence, prolonging the photosynthetically active period and increasing carbon assimilation that is subsequently allocated to vegetative organs, including stems. Putrescine further amplified stalk yield through its regulatory role in cellular metabolism and structural development. As a polyamine, putrescine stabilizes cell membranes, nucleic acids, and proteins, promoting sustained cell expansion and lignification in stem tissues. It enhances the activity of enzymes involved in carbon metabolism and cell wall biosynthesis, leading to increased deposition of cellulose, hemicellulose, and lignin in stalks. This structural reinforcement not only improves stem biomass but also enhances lodging resistance, indirectly supporting yield stability. Putrescine also contributes to improved stalk yield by mitigating oxidative stress through activation of antioxidant defense systems. Reduced oxidative damage preserves photosynthetic efficiency and metabolic activity during peak vegetative growth, ensuring continuous assimilate supply for stem elongation and thickening. Furthermore, putrescine interacts with endogenous phytohormones such as gibberellins and auxins, promoting internodal elongation and enhancing stem growth dynamics. The combined improvement in stalk yield across seasons indicates that humic acid and putrescine enhance the source capacity of sesame

plants by strengthening vegetative growth and increasing total dry matter production. Enhanced stalk biomass also reflects efficient partitioning of assimilates between vegetative and reproductive organs, suggesting a balanced growth pattern rather than excessive reproductive allocation at the expense of plant structure. Overall, the increased stalk yield observed under higher concentrations of humic acid and putrescine can be attributed to improved soil–plant interactions, enhanced nutrient uptake, prolonged photosynthetic activity, strengthened antioxidant protection, and optimized hormonal regulation of stem growth. These findings underscore the potential of humic acid and putrescine as sustainable agronomic tools for improving biomass production and total productivity of sesame under open-field cultivation (Desoky et al., 2023; Salama, 2023; Gholipour, 2020).

4.4.8 Harvest Index: During the 2023 and 2024 growing seasons, the harvest index of sesame responded positively to humic acid and putrescine applications, with higher concentrations of both biostimulants resulting in improved partitioning of assimilates toward economic yield (Table 4.4.3 and Figure 4.4.3). In 2023, the harvest index increased marginally but significantly with increasing levels of humic acid. The highest harvest index was recorded under HA4 (18.18), followed closely by HA3 (18.16) and HA2 (18.12), while the lowest value was observed in the untreated control, HA1 (18.05). A comparable trend was observed under putrescine treatments, with P3 producing the maximum harvest index (18.21), followed by P2 (18.18) and P4 (18.12); the minimum harvest index was recorded under P1 (18.00). In 2024, a similar pattern was maintained. The highest harvest index was obtained with HA3 (18.17), followed by HA4 (18.13) and HA2 (17.81), whereas HA1 resulted in the lowest harvest index (16.00). Among putrescine treatments, P3 again recorded the highest harvest index (17.93), followed by P4 (17.83) and P1 (17.42), while the lowest value was observed under P2 (16.95). Overall, increasing concentrations of humic acid and putrescine consistently enhanced the harvest index across both seasons, indicating improved assimilate allocation to seed yield and greater reproductive efficiency in sesame.

Harvest index (HI) is a critical indicator of crop efficiency, reflecting the proportion of total biomass that is successfully partitioned into economic yield. The observed improvement in harvest index of sesame under humic acid and putrescine treatments across both growing seasons indicates enhanced assimilate allocation toward reproductive organs, particularly seeds, rather than excessive vegetative growth. Such improvements in HI are agronomically desirable, as they signify greater conversion efficiency of photosynthates into harvestable yield. Humic acid application plays a key role in improving harvest index through its multifaceted effects on plant nutrition, physiology, and source–sink dynamics. By improving soil structure, cation exchange capacity, and microbial activity, humic acid enhances the availability and uptake of essential nutrients, including nitrogen, phosphorus, and potassium. Improved nutrient status supports balanced vegetative growth while simultaneously strengthening reproductive sinks, leading to more efficient translocation of assimilates from leaves and stems to developing capsules and seeds. This optimized source–sink relationship

contributes directly to improved harvest index. In addition, humic substances exhibit hormone-like properties that influence endogenous auxin, cytokinin, and gibberellin signaling pathways. These hormonal interactions promote timely flowering, improved capsule development, and efficient seed filling, ensuring that assimilates produced during photosynthesis are preferentially directed toward reproductive organs. Enhanced photosynthetic efficiency and delayed senescence under humic acid treatments further prolong the assimilate supply during the critical seed-filling stage, resulting in improved biomass partitioning toward seed yield rather than structural tissues. Putrescine also contributed significantly to improvements in harvest index by regulating metabolic efficiency and stress tolerance. As a polyamine, putrescine stabilizes cell membranes, chloroplast structures, and nucleic acids, thereby sustaining photosynthetic activity and enzymatic function during reproductive growth. This ensures continuous carbon assimilation and efficient remobilization of stored assimilates from vegetative tissues to developing seeds, which is a key determinant of harvest index. Furthermore, putrescine synergistically interacts with phytohormones involved in sink development, particularly cytokinins and abscisic acid, which regulate seed development and assimilate unloading into reproductive tissues. Enhanced sink strength under putrescine application improves seed-filling efficiency and reduces assimilate losses to nonproductive plant parts, thereby increasing harvest index. Its role in mitigating oxidative stress through activation of antioxidant defense systems further preserves metabolic activity during late reproductive stages, supporting efficient biomass conversion into seed. The consistent improvement in harvest index across seasons suggests that both humic acid and putrescine promote a favorable balance between vegetative growth and reproductive development. Rather than merely increasing total biomass, these biostimulants enhance the efficiency with which biomass is converted into economic yield. This balanced growth pattern is significant in sesame, where excessive vegetative growth can sometimes limit reproductive output. These findings highlight the potential of humic acid and putrescine as practical agronomic tools to improve yield efficiency and reproductive performance in sesame under open-field cultivation (Shah et al., 2023; Gholipour, 2020; Luiz et al., 2022).

Table 4.4.3 Effect of different treatments on seed yield (q ha⁻¹), stalk yield (q ha⁻¹), and harvest index of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	Seed yield (q ha ⁻¹)	Stalk yield (q ha ⁻¹)	Harvest index	Seed yield (q ha ⁻¹)	Stalk yield (q ha ⁻¹)	Harvest index
Factor (Humic Acid)						
HA 1 (0ppm)	5.36	24.35	18.05	4.51	23.47	16.00
HA 2 (500 ppm)	6.28	28.32	18.12	5.50	25.31	17.81
HA 3 (1000 ppm)	6.45	29.07	18.16	5.91	26.57	18.17
HA 4 (1500 ppm)	7.26	33.37	18.18	6.79	30.87	18.13
SEm ±	0.23	0.88	0.11	0.08	0.18	0.20
C.D. (p=0.05)	0.70	2.66	NS	0.23	0.53	NS
Factor (Putrescine)						
P 1 (0 ppm)	4.54	20.68	18.00	4.27	20.05	17.42
P 2 (50 ppm)	6.35	28.60	18.18	5.40	26.35	16.95
P 3 (100 ppm)	6.92	31.82	18.21	6.26	28.57	17.93
P 4 (150 ppm)	7.52	34.01	18.12	6.79	31.26	17.83
SEm ±	0.23	0.88	0.11	0.08	0.18	0.20
C.D. (p=0.05)	0.70	2.66	N.S	0.23	0.53	NS
Interaction	1.40	5.32	N.S	0.46	1.06	NS

Table 4.4.3a Interaction effect of humic acid and putrescine application on seed yield (q ha⁻¹) of sesame at 2023 and 2024

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	3.83	5.75	6.03	5.82	5.36	3.11	4.39	5.10	5.45	4.51
HA2	4.35	6.36	7.05	7.35	6.28	4.07	5.32	6.37	6.23	5.50
HA3	4.81	6.64	6.90	7.47	6.45	4.67	5.48	6.23	7.28	5.91
HA4	5.16	6.67	7.72	9.48	7.26	5.23	6.40	7.34	8.19	6.79
Mean	4.54	6.35	6.92	7.53		4.27	5.40	6.26	6.79	
SEm±	0.47					0.16				
C.D (P=0.05)	1.40					0.46				

Table 4.4.3b Interaction effect of humic acid and putrescine application on stalk yield (q ha⁻¹) of sesame at 2023 and 2024

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	17.39	26.11	27.20	26.71	24.35	17.87	24.11	25.20	26.72	23.47
HA2	20.04	28.44	31.84	32.95	28.32	19.04	25.44	27.84	28.94	25.31
HA3	21.98	29.80	30.85	33.67	29.07	20.98	26.80	27.85	30.67	26.57
HA4	23.30	30.06	37.40	42.71	33.37	22.30	29.06	33.41	38.71	30.87
Mean	20.68	28.60	31.82	34.01		20.05	26.35	28.57	31.26	
SEm±	1.60					0.36				
C.D (P=0.05)	5.32					1.06				

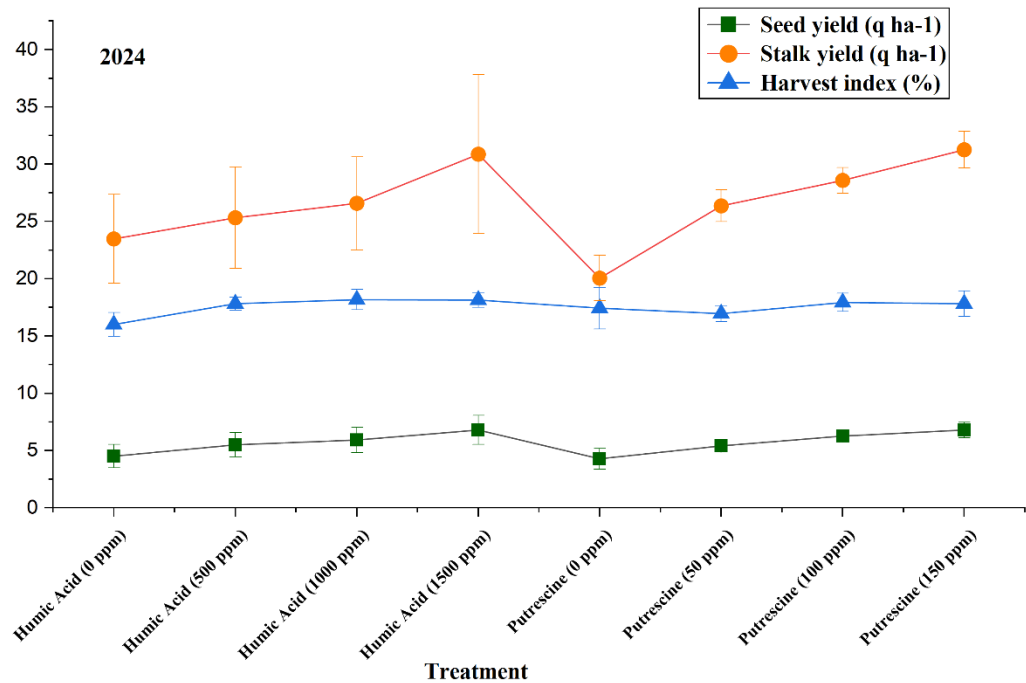
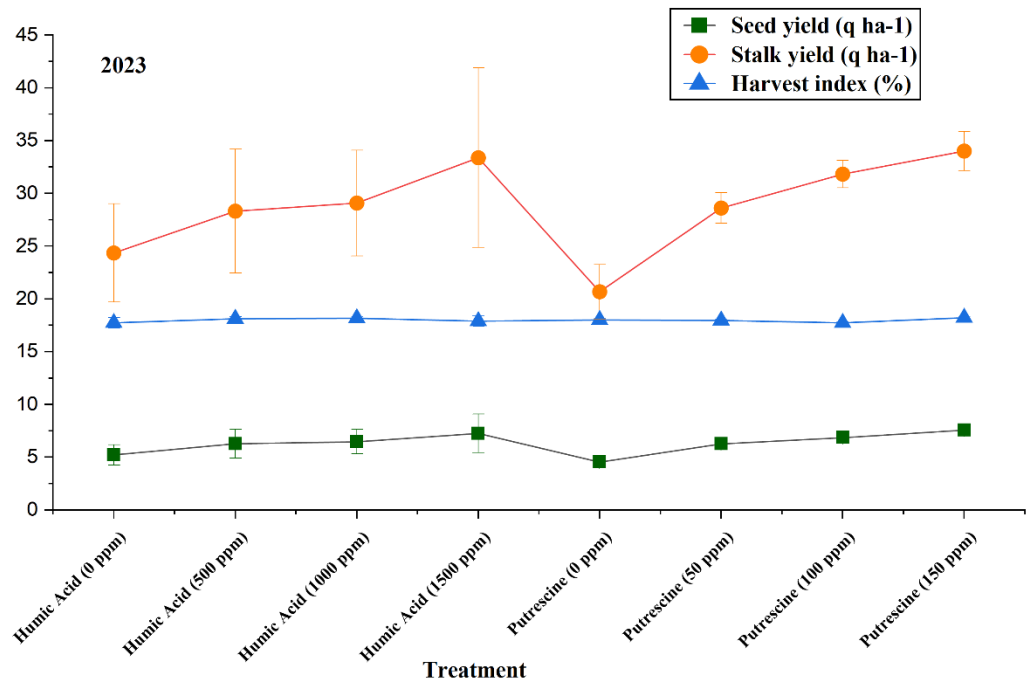


Figure 4.3.3 Effect of different treatments on seed yield (q ha⁻¹), stalk yield (q ha⁻¹), and harvest index of sesame during kharif 2023 & 2024

4.4.9 Oil content (100 g of harvested seeds): During the 2023 and 2024 growing seasons, the oil content of sesame seeds responded positively to the application of humic acid and putrescine, with higher concentrations of both biostimulants resulting in increased oil accumulation per 100 g of seed (Table 4.4.4 and Figure 4.4.4). In 2023, the oil content of sesame seeds increased significantly with increasing humic acid levels. The highest oil content was recorded under HA4 (38.40%), followed by HA3 (35.70%) and HA2 (34.40%), whereas the lowest value was observed in the untreated control, HA1 (32.40%). A comparable trend was observed under putrescine treatments, with P4 exhibiting the highest oil content (37.20%), followed by P3 (36.30%) and P2 (34.50%), whereas the lowest oil content was observed in the control treatment (P1; 32.90%). In 2024, a similar response pattern was maintained. The highest oil content was again recorded under HA4 (39.20%), followed by HA3 (34.97%) and HA2 (33.97%), whereas the lowest oil content was observed under HA1 (32.08%). Among the putrescine treatments, P4 produced the highest oil content (37.34%), followed by P3 (36.29%) and P2 (33.87%), whereas the lowest value was recorded for P1 (32.08%). Overall, increasing concentrations of humic acid and putrescine consistently enhanced the oil content of sesame seeds across both growing seasons. This improvement may be attributed to enhanced assimilate availability, improved photosynthetic efficiency, and more effective translocation of metabolites toward lipid biosynthesis, resulting in superior seed quality.

Oil content is a key quality attribute of sesame and reflects the efficiency of carbon assimilation, metabolite partitioning, and lipid biosynthesis during seed development. The consistent increase in oil content under applications of humic acid and putrescine across both growing seasons indicates that these biostimulants positively influenced metabolic pathways associated with seed quality, particularly lipid accumulation. Humic acid plays a central role in improving oil content through its effects on soil fertility, nutrient availability, and plant physiological processes. By enhancing soil structure, microbial activity, and cation exchange capacity, humic acid improves the uptake of essential nutrients, including nitrogen, phosphorus, sulfur, and micronutrients, that are directly involved in fatty acid synthesis and oil accumulation. Improved phosphorus availability supports ATP generation and carbon metabolism,

whereas sulfur is critical for enzyme activation and oil biosynthesis. Enhanced nutrient nutrition ensures an adequate supply of precursors required for lipid biosynthesis in developing seeds. In addition to nutritional effects, humic acid exhibits hormone-like activity that modulates endogenous auxins, cytokinins, and gibberellins. These hormonal interactions promote efficient photosynthesis, delay leaf senescence, and prolong the seed-filling period. Extended photosynthetic activity increases assimilate availability, particularly sucrose, which serves as the primary carbon source for fatty acid synthesis in oilseeds. Improved source–sink relationships under humic acid application facilitate effective translocation of assimilates from leaves to developing seeds, thereby enhancing oil deposition. Putrescine, a low-molecular-weight polyamine, also plays a crucial role in increasing oil content by modulating cellular metabolism and stress responses. Polyamines are known to stabilize cellular membranes and chloroplast structures, protecting photosynthetic machinery and maintaining higher photosynthetic efficiency during reproductive growth. Sustained photosynthesis ensures a continuous carbon supply for oil synthesis during seed development. Furthermore, putrescine influences key enzymatic activities involved in fatty acid biosynthesis by regulating gene expression associated with acetyl-CoA carboxylase, fatty acid synthase, and desaturase enzymes. These enzymes catalyze the conversion of carbohydrates into fatty acids and, subsequently, into triacylglycerols, the main storage lipids in sesame seeds. Enhanced enzymatic efficiency under putrescine application promotes greater lipid accumulation and improved oil quality. Putrescine also contributes to enhanced oil content by mitigating oxidative stress during seed development. By activating antioxidant defense systems, polyamines reduce lipid peroxidation and preserve membrane integrity in developing seeds. This protective role ensures efficient lipid accumulation and minimizes degradation of oil reserves, particularly under fluctuating environmental conditions. The combined effects of humic acid and putrescine result in improved photosynthetic performance, enhanced assimilate production, efficient translocation of metabolites, and optimized lipid biosynthetic activity. Rather than merely increasing seed size or biomass, these biostimulants enhance the biochemical efficiency of oil synthesis, thereby improving seed quality. The consistent response across seasons suggests that humic acid and putrescine can reliably increase oil content in sesame under diverse field conditions.

The enhancement of oil content observed under humic acid and putrescine treatments can be attributed to improved nutrient acquisition, hormonal regulation of reproductive development, sustained photosynthetic efficiency, strengthened source–sink relationships, and direct stimulation of lipid biosynthesis pathways. These findings underscore the potential of humic acid and putrescine as effective agronomic interventions to improve both yield and oil quality in sesame (El-Khayat, 2024; Ayoubizadeh et al., 2020; Javed et al., 2022).

4.4.10 Oil cake yield (100 g of harvested seeds): During the 2023 and 2024 growing seasons, the oil cake content of sesame seeds was significantly affected by the application of humic acid and putrescine (Table 4.4.4 and Figure 4.4.4). In contrast to seed oil content, oil cake percentage declined consistently with increasing concentrations of both biostimulants. In 2023, oil cake content decreased significantly with increasing humic acid levels. The highest oil cake content was observed in the untreated control (HA1, 61.50%), followed by HA2 (60.00%) and HA3 (58.70%), whereas the lowest value was recorded for HA4 (56.50%). A similar pattern was observed under putrescine treatments, with the control treatment P1 recording the highest oil cake content (61.50%), followed by P2 (59.90%) and P3 (58.10%), whereas the lowest oil cake content was observed under P4 (57.20%). In 2024, the same trend was maintained. The highest oil cake content was again recorded in the control treatment (HA1; 61.62%), followed by HA2 (59.76%) and HA3 (59.17%), whereas the lowest value was recorded under HA4 (55.90%). Among putrescine treatments, P1 showed the highest oil cake content (61.08%), followed by P2 (60.56%) and P3 (58.01%), whereas the lowest oil cake content was recorded under P4 (56.79%). Increasing concentrations of humic acid and putrescine resulted in a progressive reduction in oil cake content, which corresponded with the observed increase in seed oil percentage. This inverse relationship suggests a shift in assimilate partitioning toward enhanced lipid biosynthesis, thereby improving seed quality in sesame.

Oil cake content represents the non-lipid fraction of sesame seeds and is inversely related to seed oil concentration. The observed decline in oil cake percentage with increasing levels of humic acid and putrescine across both growing seasons indicates a pronounced shift in assimilate partitioning toward lipid accumulation rather than structural or storage carbohydrates and proteins. This response highlights the role of these biostimulants in enhancing seed quality by optimizing carbon and energy allocation during seed development. Humic acid influences oil cake content primarily through its effects on nutrient acquisition, photosynthetic efficiency, and carbon metabolism. Improved availability of nitrogen, phosphorus, sulfur, and micronutrients under humic acid application enhances overall metabolic efficiency and promotes the synthesis of high-energy compounds. During seed filling, enhanced phosphorus

availability supports ATP production, whereas sulfur is essential for activating enzymes involved in fatty acid biosynthesis. As lipid synthesis is an energetically demanding process, improved nutrient status favors oil accumulation over the formation of structural seed components, resulting in a relative reduction in oil cake fraction. Another key mechanism involves the hormone-like activity of humic substances. Humic acid modulates endogenous plant growth regulators, such as auxins and cytokinins, thereby strengthening source-sink relationships and prolonging the effective seed-filling period. Extended assimilate supply to developing seeds promotes the conversion of carbohydrates into fatty acids and triacylglycerols rather than their deposition as cell wall materials or storage proteins. Consequently, the proportion of residual oil cake decreases as lipid accumulation increases. Putrescine, as a polyamine, plays a critical role in regulating cellular metabolism and carbon partitioning during reproductive development. Polyamines are known to influence the expression of genes involved in lipid biosynthesis while simultaneously regulating nitrogen metabolism. By stabilizing membranes and protecting chloroplasts from oxidative damage, putrescine maintains higher photosynthetic efficiency, ensuring a continuous supply of carbon skeletons for oil synthesis. This metabolic prioritization leads to increased lipid deposition and a corresponding decline in the relative proportion of oil cake constituents. Additionally, putrescine modulates enzyme activities involved in acetyl-CoA production and fatty acid elongation, thereby enhancing the conversion of sugars into fatty acids. As more assimilated carbon is diverted toward lipid synthesis, less carbon remains available for protein and fiber accumulation, which constitute major components of oil cake. This metabolic redirection explains the consistent reduction in oil cake content with increasing putrescine levels. The antioxidative role of putrescine further contributes to reduced oil cake content by minimizing lipid degradation during seed development. Enhanced antioxidant activity limits oxidative stress and preserves newly synthesized lipids, thereby increasing oil concentration and proportionally decreasing the residual oil cake fraction. The inverse relationship between oil content and oil cake percentage observed in this study reflects improved carbon utilization efficiency rather than a decline in overall seed quality. While oil cake remains nutritionally valuable, the shift toward higher oil accumulation is desirable in sesame cultivated for edible oil production. The consistency of response across seasons

suggests that humic acid and putrescine reliably regulate assimilate partitioning under open-field conditions. The reduction in oil cake content under humic acid and putrescine treatments can be attributed to enhanced nutrient uptake, hormonal regulation of seed development, improved photosynthetic performance, optimized carbon partitioning, and direct stimulation of lipid biosynthetic pathways. These mechanisms collectively promote superior oil accumulation at the expense of non-lipid seed components, thereby improving the economic and industrial value of sesame seeds (Javed et al., 2022; Lotfi et al., 2015; Nouriyani et al., 2019).

4.4.11 Crude protein of oil cake: During the 2023 and 2024 growing seasons, the crude protein content of sesame oil cake was significantly influenced by humic acid and putrescine applications, with progressively higher concentrations of both biostimulants leading to a marked increase in crude protein accumulation (Table 4.4.4 and Figure 4.4.4). In 2023, the crude protein content of sesame oil cake increased significantly with increasing humic acid levels. The highest crude protein content was recorded under HA4 (41.25%), followed by HA3 (40.92%) and HA2 (37.97%), whereas the lowest value was observed in the untreated control, HA1 (36.63%). A similar trend was observed under putrescine treatments, with P4 exhibiting the highest crude protein content (41.00%), followed by P3 (40.34%) and P2 (38.14%), whereas the lowest value was in the control treatment (P1; 37.29%). In 2024, a comparable response pattern was maintained. The highest crude protein content was again recorded under HA4 (39.98%), followed by HA3 (38.33%) and HA2 (34.00%), whereas the lowest value was observed under HA1 (33.15%). Among putrescine treatments, P4 produced the highest crude protein content (38.56%), followed by P3 (37.63%) and P2 (35.72%), whereas the lowest value was recorded for P1 (34.55%). Increasing concentrations of humic acid and putrescine consistently enhanced the crude protein content of sesame oil cake across both seasons. This improvement may be attributed to enhanced nitrogen assimilation, improved photosynthetic efficiency, and more effective translocation of assimilates toward protein synthesis, resulting in improved nutritional quality of sesame oil cake.

4.4.11a Interaction between different treatments on crude protein of oil cake of sesame

The interaction effect of humic acid and putrescine on crude protein of oil cake was significant in both 2023 and 2024 (Table 4.4.4a). In 2023, the highest crude protein of oil cake was recorded under the HA4+P4 treatment (44.41%), followed by HA4+P3 (42.66%), HA3+P4 (42.65%), and HA3+P3 (41.77%). In contrast, the lowest crude protein content in oil cake was observed under HA1+P1 (34.48%). A similar response pattern was evident during the 2024 season. The HA4+P3 combination again produced the highest crude protein content in the oil cake (41.45%), followed by HA3+P4 (40.81%), HA4+P4 (40.54%), and HA4+P1 (39.47%). Consistent across both seasons,

the lowest crude protein content of oil cake was observed in the HA1+P1 treatment (32.47%), indicating poor crude protein development in the absence of biostimulant application.

Crude protein content of sesame oil cake is an important indicator of its nutritional quality, particularly for livestock feed and various industrial applications. The consistent increase in crude protein content observed following humic acid and putrescine treatments during both growing seasons demonstrates that these biostimulants play a significant role in regulating nitrogen assimilation, amino acid metabolism, and protein accumulation during seed development. Unlike oil content, which reflects carbon allocation toward lipid synthesis, crude protein deposition is primarily controlled by nitrogen availability and metabolic efficiency, both of which are enhanced by humic acid and putrescine. Humic acid improves crude protein accumulation mainly by increasing nitrogen uptake and utilization efficiency. Humic substances stimulate root development, expand root surface area, and enhance membrane permeability, thereby facilitating greater absorption of nitrate and ammonium ions from the soil. Moreover, humic acid activates important enzymes involved in nitrogen metabolism, including nitrate reductase and glutamine synthetase, promoting the conversion of inorganic nitrogen into amino acids and proteins. Enhanced nitrogen assimilation consequently increases the availability of nitrogen-containing metabolites required for protein biosynthesis in developing seeds and the resulting oil cake. Another important mechanism involves the indirect influence of humic acid on photosynthesis and carbon–nitrogen balance. Enhanced chlorophyll formation and photosynthetic activity increase the production of carbon skeletons required for amino acid biosynthesis. The balanced availability of carbon and nitrogen promotes efficient protein accumulation while minimizing nitrogen losses or excessive carbohydrate storage. Consequently, the oil cake fraction becomes enriched with crude protein despite simultaneous improvements in seed oil content. Putrescine, an important naturally occurring polyamine, complements this response by regulating cellular metabolism and gene expression associated with nitrogen utilization. Polyamines are closely linked with nitrogen-rich metabolites and function as regulators of nitrogen remobilization during reproductive growth. Putrescine enhances amino acid

synthesis by stabilizing enzymes involved in nitrogen metabolism and protein biosynthesis. It also promotes phloem loading and translocation of nitrogenous compounds from vegetative tissues to developing seeds, thereby increasing protein deposition within the oil cake. Furthermore, putrescine reduces oxidative stress through enhanced antioxidant enzyme activity, protecting newly synthesized proteins from degradation during seed filling. Reduced protein oxidation allows greater retention of storage proteins in the oil cake fraction. Polyamines additionally delay leaf senescence by stabilizing cellular membranes and nucleic acids, extending the duration of nitrogen assimilation and protein deposition during later stages of crop development. The simultaneous enhancement of seed oil and crude protein content under humic acid and putrescine application indicates an overall improvement in assimilate production rather than competition between carbon and nitrogen allocation. This reflects enhanced metabolic efficiency, whereby lipid and protein biosynthetic pathways are simultaneously stimulated through improved nutrient availability, hormonal regulation, and stress tolerance. From an agronomic perspective, increasing the crude protein concentration of sesame oil cake substantially improves its value as a nutritious livestock feed resource. The consistent responses obtained across both seasons confirm the effectiveness of humic acid and putrescine as reliable agronomic inputs for enhancing seed productivity, oil quality, and the nutritional value of sesame by-products. Overall, the higher crude protein content of sesame oil cake following humic acid and putrescine application resulted from improved nitrogen uptake, enhanced enzymatic nitrogen assimilation, balanced carbon–nitrogen metabolism, superior photosynthetic efficiency, efficient assimilate translocation, and protection of proteins against oxidative degradation. These mechanisms collectively contribute to the superior nutritional quality and greater economic value of sesame oil cake (Zhu et al., 2023; Li et al., 2025; Anbhazhagan et al., 2024).

Table 4.4.4 Effect of different treatments on oil content, oil cake yield, and crude protein of oil cake of sesame during kharif 2023 & 2024

Treatments	2023			2024		
	Oil content	Oil cake yield	Crude protein of oil cake	Oil content	Oil cake yield	Crude protein of oil cake
Factor (Humic Acid)						
HA 1 (0ppm)	32.4	61.5	36.63	32.08	61.62	33.15
HA 2 (500 ppm)	34.4	60.0	37.97	33.97	59.76	34.00
HA 3 (1000 ppm)	35.7	58.7	40.92	34.97	59.17	38.33
HA 4 (1500 ppm)	38.4	56.5	41.25	39.20	55.90	39.98
SEm ±	0.30	0.31	0.27	0.28	0.45	0.34
C.D. (p=0.05)	1.11	0.89	0.79	0.82	1.32	0.99
Factor (Putrescine)						
P 1 (0 ppm)	32.9	61.5	37.29	32.72	61.08	34.55
P 2 (50 ppm)	34.5	59.9	38.14	33.87	60.56	35.72
P 3 (100 ppm)	36.3	58.1	40.34	36.29	58.01	37.63
P 4 (150 ppm)	37.2	57.2	41.00	37.34	56.79	38.56
SEm ±	0.30	0.31	0.27	0.28	0.45	0.34
C.D. (p=0.05)	1.11	0.89	0.79	0.82	1.32	0.99
Interaction	NS	NS	1.59	NS	NS	1.99

Table 4.4.4a Interaction effect of humic acid and putrescine application on crude protein of oil cake of sesame in 2023 and 2024

P HA	2023					2024				
	P1	P2	P3	P4	Mean	P1	P2	P3	P4	Mean
HA1	34.48	36.11	37.48	38.48	36.64	31.25	32.47	33.47	35.41	33.15
HA2	36.48	37.44	39.48	38.49	37.97	32.90	33.47	36.14	33.48	34.00
HA3	38.74	40.54	41.77	42.65	40.93	34.58	38.47	39.46	40.81	38.33
HA4	39.47	38.48	42.66	44.41	41.26	39.47	38.48	41.45	40.54	39.98
Mean	37.29	38.14	40.35	41.01		34.55	35.72	37.63	37.56	
SEm±	0.54					0.68				
C.D (P=0.05)	1.59					1.99				

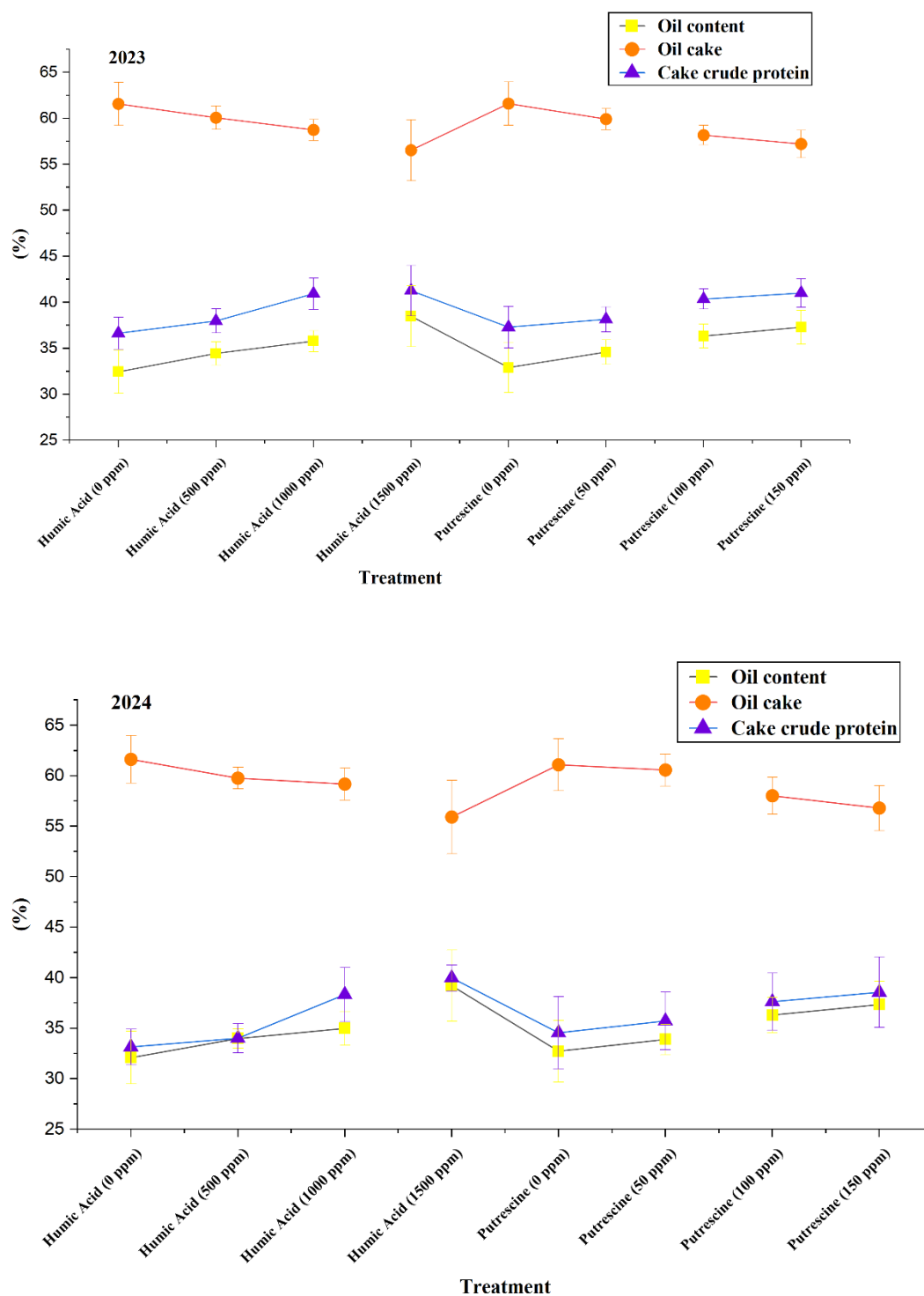


Figure 4.4.4 Effect of different treatments on oil content (100 g of harvested seeds), oil cake yield (100 g of harvested seeds), and crude protein of oil cake of sesame during kharif 2023 & 2024

4.5 Effects of humic acid and putrescine on Quality Parameters of Oil of sesame cultivation

4.5.1 Peroxide value (milli eq. iodine/g of oil): During the 2023 and 2024 growing seasons, the peroxide value of sesame oil was significantly influenced by the application of humic acid and putrescine, with increasing concentrations of both biostimulants resulting in a progressive rise in peroxide value (Table 4.5.1 and Figure 4.5.1). In 2023, the peroxide value increased significantly with increasing humic acid levels. The highest peroxide value was recorded under HA4 (0.33), followed by HA3 (0.31) and HA2 (0.31), whereas the untreated control, HA1 (0.23), registered the lowest value. A similar trend was observed under putrescine treatments, with P4 exhibiting the highest peroxide value (0.31), followed by P1 (0.30) and P2 (0.29), whereas P3 showed the lowest value (0.28). A comparable response pattern was maintained in 2024. The highest peroxide value was again recorded under HA4 (0.31), followed by HA3 (0.29) and HA2 (0.26), whereas the lowest value was recorded in the control treatment HA1 (0.20). Among putrescine treatments, P4 produced the highest peroxide value (0.29), followed by P1 (0.27) and P3 (0.26), whereas the lowest value was recorded for P2 (0.26). Overall, increasing concentrations of humic acid and putrescine consistently elevated the peroxide value of sesame oil across both seasons. This response may be associated with enhanced metabolic activity and increased incorporation of unsaturated fatty acids into oil fractions, reflecting changes in oil composition and oxidative status under biostimulant application.

Peroxide value is a key indicator of the initial stage of lipid oxidation and reflects the presence of primary oxidation products, particularly hydroperoxides, in oil. The observed increase in the peroxide value of sesame oil under application of humic acid and putrescine across both growing seasons indicates that these biostimulants significantly affected oil composition and oxidative dynamics during seed development. The increase in peroxide value under humic acid treatments is primarily attributable to its enhancement of nutrient availability, metabolic activity, and lipid biosynthesis. Humic acid enhances the uptake of nitrogen, sulfur, magnesium, and micronutrients, which are essential for enzymatic reactions involved in fatty acid synthesis and oil accumulation. Improved photosynthetic efficiency and assimilate

availability under humic acid application increase the synthesis of unsaturated fatty acids, particularly oleic and linoleic acids, which are more susceptible to oxidative reactions. As peroxide value is directly associated with the oxidation of unsaturated fatty acids, increased incorporation of these fatty acids into oil fractions likely contributed to the elevated peroxide levels observed. Moreover, humic substances function as signaling molecules that regulate gene expression involved in carbon metabolism and lipid biosynthesis. Enhanced activity of key enzymes such as acetyl-CoA carboxylase and fatty acid desaturases under biostimulant application can increase the degree of unsaturation in seed oil. While this improves nutritional quality, it also predisposes the oil to greater peroxide formation during the early stages of oxidation, thereby increasing the peroxide value. Application of putrescine also resulted in increased peroxide value, reflecting its influence on membrane dynamics, lipid metabolism, and oxidative balance. Polyamines such as putrescine regulate cell division, membrane stability, and stress signaling, particularly during reproductive development. Putrescine modulates fatty acid desaturation by influencing membrane-bound desaturase activity, thereby increasing the synthesis of polyunsaturated fatty acids. These fatty acids are highly reactive with oxygen, leading to increased formation of lipid hydroperoxides and a corresponding rise in peroxide value. In addition, putrescine plays a dual role in oxidative metabolism. While it enhances antioxidant defense mechanisms at the cellular level, its involvement in reactive oxygen species signaling during seed filling can promote controlled lipid oxidation processes. This controlled oxidative environment may facilitate oil maturation and stabilization of oil bodies. Still, it can also lead to a moderate increase in primary oxidation products, as reflected by a higher peroxide value. In oilseed crops, moderate increases in peroxide value within acceptable limits often indicate enhanced metabolic activity and improved synthesis of nutritionally desirable fatty acids. Overall, the elevated peroxide value observed under humic acid and putrescine treatments highlights their role in modifying lipid metabolism, fatty acid composition, and oxidative status of sesame oil. These changes suggest improved oil biosynthesis efficiency and enhanced incorporation of unsaturated fatty acids, which are essential for nutritional quality. However, they necessitate careful post-harvest handling to maintain oil stability (Dehsheikh et al., 2020; Vani et al., 2017; Choudhary et al., 2017).

4.5.2 Anisidine value (g/100 ml isooctane): During the 2023 and 2024 growing seasons, the anisidine value of sesame oil was significantly affected by the application of humic acid and putrescine. Increasing concentrations of both biostimulants resulted in a progressive increase in anisidine value, indicating a dose-dependent response (Table 4.5.1 and Figure 4.5.1). In 2023, the anisidine value increased significantly with increasing humic acid levels. The highest anisidine value was recorded under HA4 (1.07), followed by HA3 (0.63) and the untreated control HA1 (0.62), whereas HA2 (0.60) registered the lowest value. A similar trend was observed under putrescine treatments, with P2 exhibiting the highest anisidine value (0.80), followed by P3 (0.78) and P4 (0.71), whereas the untreated control P1 (0.63) had the lowest value. A comparable response pattern was observed in 2024. The highest anisidine value was again recorded under HA4 (1.56), followed by HA3 (1.09) and HA2 (0.90), whereas the lowest value occurred in the untreated control HA1 (0.73). Among the putrescine treatments, P3 produced the maximum anisidine value (1.18), followed closely by P4 (1.17) and P2 (1.09), while P1 recorded the lowest value (0.84). Overall, increasing concentrations of humic acid and putrescine consistently elevated the anisidine value of sesame oil across both seasons. This increase may be attributed to enhanced metabolic activity and greater accumulation of secondary oxidation products, reflecting changes in oil composition and oxidative status under biostimulant application.

Anisidine value is an important indicator used to evaluate the extent of secondary lipid oxidation in edible oils, primarily reflecting the accumulation of aldehydes generated through the decomposition of lipid hydroperoxides. The observed increase in the anisidine value of sesame oil following humic acid and putrescine application during both growing seasons indicates that these biostimulants significantly influenced the oxidative characteristics and compositional changes of the oil throughout seed development and maturation. The increase in anisidine value under humic acid treatment can be attributed to its positive effects on plant metabolism, nutrient acquisition, and lipid biosynthesis. Humic acid stimulates root development and enhances the uptake of nitrogen, sulfur, and essential micronutrients required for enzymatic reactions associated with fatty acid synthesis. Greater metabolic activity supporting lipid production under humic acid application frequently promotes

increased synthesis of unsaturated fatty acids. Although these fatty acids improve the nutritional quality of sesame oil, they are inherently more susceptible to oxidation, resulting in the generation of secondary oxidation products such as aldehydes, which are reflected by increased anisidine values. Moreover, humic substances regulate metabolic processes by influencing the expression of genes associated with carbon partitioning and lipid biosynthesis. Enhanced activity of fatty acid desaturase enzymes under humic acid treatment increases the degree of unsaturation within sesame oil. During seed maturation, extraction, or storage, hydroperoxides derived from unsaturated fatty acids readily decompose into aldehydes and ketones, thereby contributing to higher anisidine values. This response reflects alterations in fatty acid composition rather than deterioration of oil quality, because oils containing greater proportions of unsaturated fatty acids are naturally more susceptible to secondary oxidation. Application of putrescine also increased anisidine values, emphasizing its influence on oxidative metabolism and lipid stability. As a naturally occurring polyamine, putrescine participates in cellular signaling, membrane stabilization, and regulation of stress responses during seed development. It interacts with membrane lipids and influences fatty acid desaturation as well as oil body formation. Furthermore, putrescine exerts a complex effect on reactive oxygen species homeostasis. Importantly, increases in anisidine value should be interpreted together with peroxide value because secondary oxidation products generally accumulate following primary oxidation, particularly in oils enriched with unsaturated fatty acids. In oilseed crops such as sesame, moderate increases in anisidine value within acceptable quality limits indicate active lipid metabolism and greater oil unsaturation rather than severe oxidative deterioration. Overall, humic acid and putrescine modified the oxidative characteristics of sesame oil by altering fatty acid composition, stimulating metabolic activity, and regulating oxidative signaling pathways. These physiological changes increased the formation of secondary oxidation products, reflected by higher anisidine values, while simultaneously supporting enhanced oil biosynthesis and improved nutritional quality. Proper post-harvest handling and storage conditions are therefore essential to preserve oil stability under enhanced biostimulant-induced oil production systems (Vani et al., 2017; Choudhary et al., 2017; Seervi et al., 2018).

4.5.3 Totex value (mg/g): During the 2023 and 2024 growing seasons, the totex value of sesame oil was significantly influenced by the application of humic acid and putrescine. Increasing concentrations of both biostimulants resulted in a progressive increase in the total oxalate (totox) value, indicating a clear dose-dependent response (Table 4.5.1 and Figure 4.5.1). In 2023, the TOTOX value increased significantly with increasing humic acid levels. The highest total toxin value was recorded for HA4 (3.36), followed by HA3 (2.05) and HA2 (1.56), whereas the untreated control, HA1 (1.36), recorded the lowest value. A similar trend was observed under putrescine treatments, with P3 exhibiting the highest tototox value (2.44), followed by P4 (2.13) and P2 (1.90), whereas the untreated control P1 (1.87) recorded the lowest value. A comparable response pattern was observed in 2024. The highest total toxin value was again recorded under HA4 (2.18), followed by HA3 (1.67) and HA2 (1.49), whereas the lowest value was in the untreated control HA1 (1.14). Among the putrescine treatments, P4 produced the maximum tototox value (1.76), followed closely by P3 (1.71) and P2 (1.62), whereas P1 recorded the lowest value (1.39). Overall, increasing concentrations of humic acid and putrescine consistently elevated the tototox value of sesame oil across both seasons. This increase may be attributed to enhanced metabolic activity and greater accumulation of both primary and secondary lipid oxidation products, reflecting changes in oil composition and oxidative stability under biostimulant application.

The Totox value, which integrates both peroxide and anisidine values, provides a comprehensive assessment of the overall oxidative status and stability of edible oils. The observed increase in the total tocopherol content of sesame oil under application of humic acid and putrescine across both growing seasons indicates that these biostimulants significantly influenced lipid metabolism, fatty acid composition, and oxidative processes during seed development and oil accumulation. The increase in total oxalate (totox) under humic acid treatments is primarily attributable to enhanced metabolic activity and improved nutrient availability. Humic acid is known to stimulate root growth, nutrient uptake, and photosynthetic efficiency, thereby increasing assimilate production and carbon flux toward oil biosynthesis. Enhanced lipid synthesis often leads to higher proportions of unsaturated fatty acids, which, although nutritionally desirable, are inherently more susceptible to oxidation. The oxidation of

these unsaturated lipids produces primary oxidation products (hydroperoxides) that subsequently decompose into secondary oxidation compounds, collectively contributing to elevated total oxides (TOX) values. Moreover, humic acid regulates enzymatic activities involved in fatty acid desaturation and oil body formation. Increased activity of desaturase enzymes enhances oil unsaturation levels, thereby increasing oxidative sensitivity. While humic substances also enhance antioxidant systems in plant tissues, accelerated lipid biosynthesis during seed filling may exceed the protective capacity of endogenous antioxidants at the oil level, leading to higher cumulative oxidation indices, such as the tottox value. The application of putrescine also contributed to an increased total oxidant value, reflecting its complex role in regulating oxidative metabolism and membrane dynamics. As a polyamine, putrescine interacts with membrane phospholipids and influences lipid remodeling and oil body stability during seed maturation. In addition, putrescine modulates antioxidant enzyme systems and stress-responsive pathways, promoting controlled oxidative conditions that are essential for normal seed maturation and oil biosynthesis. This response reflects a shift in oil oxidative dynamics rather than a deterioration of oil quality, particularly when values remain within acceptable limits for edible oils. The consistency of the observed response across both seasons suggests that the increase in total tox value was primarily driven by biostimulant-induced metabolic regulation rather than by environmental variability. Importantly, higher toluene values in this context indicate enhanced lipid biosynthesis and greater oil unsaturation, characteristics often associated with improved nutritional quality but reduced oxidative stability. These findings highlight the need for optimized post-harvest processing and storage practices to preserve oil quality when biostimulants are used to enhance productivity. Overall, the application of humic acid and putrescine altered the oxidative profile of sesame oil by enhancing fatty acid biosynthesis, increasing lipid unsaturation, and modulating oxidative signaling pathways. These combined effects resulted in greater accumulation of both primary and secondary lipid oxidation products, as reflected by increased total oxylipin (tottox) values, while simultaneously contributing to improved oil yield and biochemical quality (Seervi et al., 2018; Choudhary et al., 2017; Farzi aminabad et al., 2021).

Table 4.5.1 Effect of different treatments on peroxide value, anisidine value, and totex value of sesame oil during kharif 2023 & 2024

Treatments	2023			2024		
	Peroxide value	Anisidine value	Totex Value	Peroxide value	Anisidine value	Totex Value
Factor (Humic Acid)						
HA 1 (0ppm)	0.23	0.62	1.36	0.20	0.73	1.14
HA 2 (500 ppm)	0.31	0.60	1.56	0.29	0.90	1.49
HA 3 (1000 ppm)	0.31	0.63	2.05	0.29	1.09	1.67
HA 4 (1500 ppm)	0.33	1.07	3.36	0.31	1.56	2.18
SEm ±	0.003	0.006	0.015	0.003	0.01	0.009
C.D. (p=0.05)	0.007	0.017	0.044	0.01	0.02	0.027
Factor (Putrescine)						
P 1 (0 ppm)	0.30	0.63	1.87	0.27	0.84	1.39
P 2 (50 ppm)	0.29	0.80	1.90	0.26	1.09	1.62
P 3 (100 ppm)	0.28	0.78	2.44	0.26	1.18	1.71
P 4 (150 ppm)	0.31	0.71	2.13	0.29	1.17	1.76
SEm ±	0.003	0.006	0.015	0.003	0.01	0.009
C.D. (p=0.05)	0.007	0.017	0.044	0.01	0.02	0.027
Interaction	NS	NS	NS	NS	NS	NS

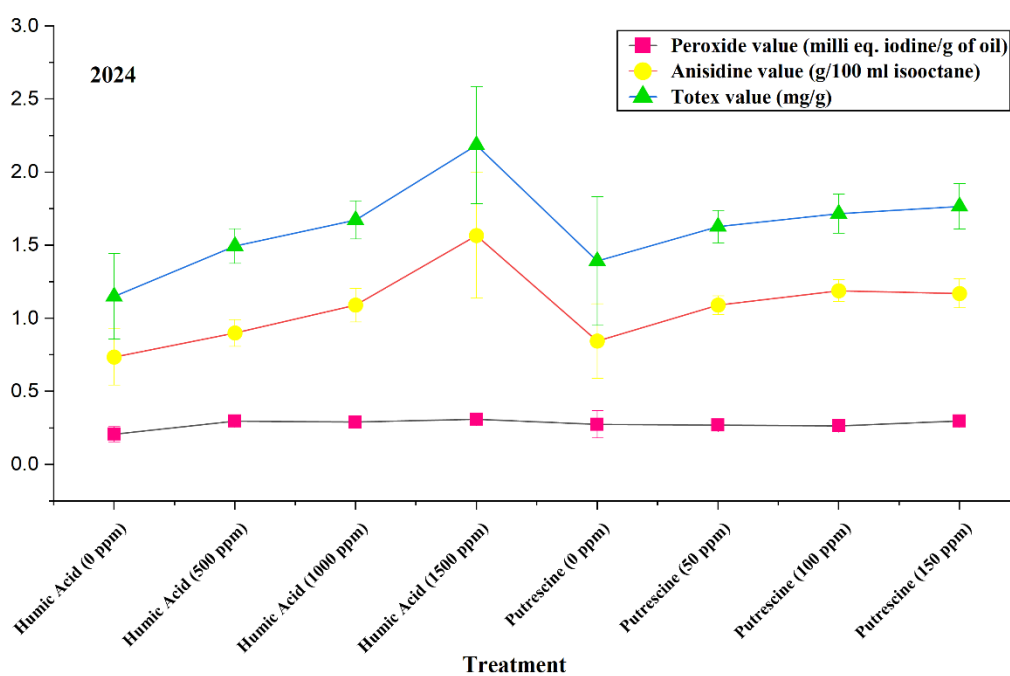
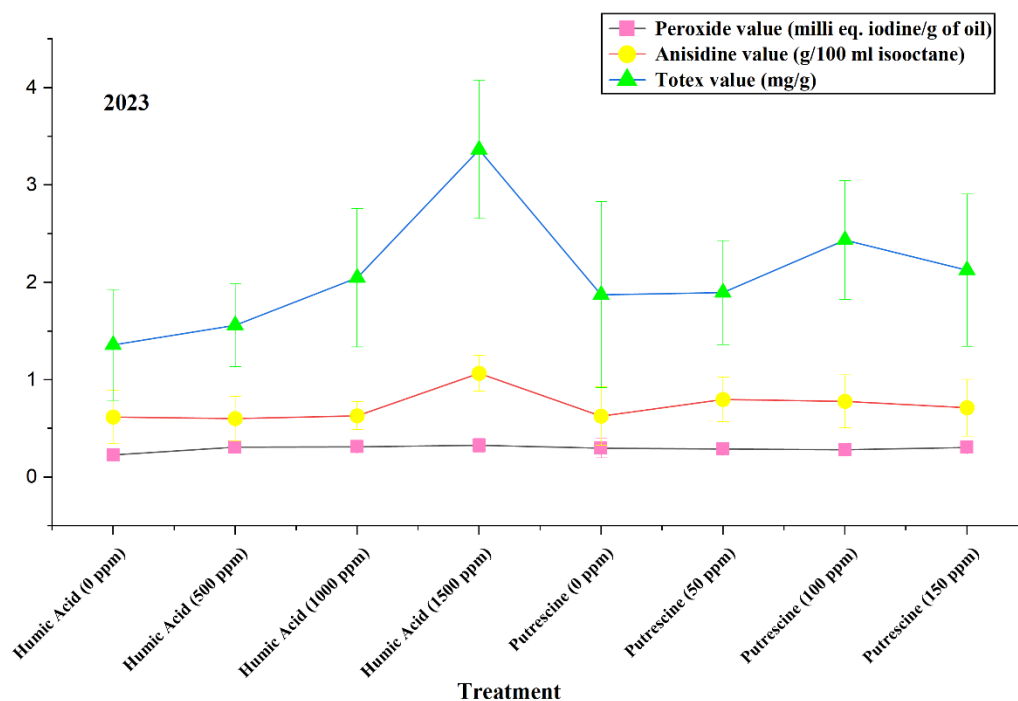


Figure 4.5.1 Effect of different treatments on peroxide value, anisidine value, and totex value of sesame oil during kharif 2023 & 2024

4.5.4 Acid Value (mg KOH /g): During the 2023 and 2024 growing seasons, the acid value of sesame oil was significantly influenced by the application of humic acid and putrescine (Table 4.5.2 and Figure 4.5.2). Increasing concentrations of both biostimulants resulted in a progressive reduction in acid value, indicating a clear dose-dependent improvement in oil quality. In 2023, the acid value decreased significantly with increasing humic acid levels. The highest acid value was recorded in the untreated control HA1 (4.59), followed by HA2 (4.01) and HA3 (3.16), while HA4 (2.09) registered the lowest value. A similar trend was observed under putrescine treatments, with the untreated control P1 recording the maximum acid value (4.70), followed by P2 (3.17) and P3 (3.11), whereas P4 (2.87) recorded the minimum value. A comparable response pattern was observed in 2024. The highest acid value was again recorded in the untreated control HA1 (5.24), followed by HA2 (4.50) and HA3 (3.47), whereas the lowest value was recorded for HA4 (2.86). Among the putrescine treatments, the untreated control P1 produced the maximum acid value (5.15), followed by P2 (3.93) and P3 (3.57), whereas P4 recorded the lowest value (3.43). Overall, increasing concentrations of humic acid and putrescine consistently reduced the acid value of sesame oil across both seasons. This reduction indicates lower accumulation of free fatty acids and improved oil stability, likely due to enhanced physiological regulation of lipid metabolism and reduced hydrolytic degradation under biostimulant application.

Acid value is a critical indicator of oil quality, reflecting the extent of free fatty acid formation due to lipid hydrolysis and enzymatic degradation during seed development and oil extraction. The observed reduction in the acid value of sesame oil under application of humic acid and putrescine across both growing seasons clearly demonstrates the positive role of these biostimulants in improving oil stability and preserving lipid integrity. The decrease in acid value under humic acid treatments can be attributed to the multifaceted effects of humic acid on plant physiology and lipid metabolism. Humic acid enhances nutrient uptake, particularly nitrogen, potassium, calcium, and magnesium, which are essential for maintaining membrane stability and enzyme regulation during seed development. Improved nutrient availability strengthens cellular membranes and oil bodies, thereby reducing the susceptibility of triacylglycerols to lipase-mediated hydrolysis. This stabilization limits the release of

free fatty acids, resulting in lower acid values in the extracted oil. In addition, humic acid improves photosynthetic efficiency and assimilate partitioning toward developing seeds, ensuring synchronized seed maturation and uniform oil biosynthesis. Well-coordinated seed filling reduces metabolic stress and prevents premature lipid breakdown, a common cause of elevated free fatty acid levels. Humic substances also regulate endogenous hormone-like activity, particularly auxins and cytokinins, which delay senescence and reduce oxidative and hydrolytic damage to storage lipids during seed maturation. The application of putrescine further reduced the acid value by enhancing membrane stabilization and mitigating stress. As a polyamine, putrescine binds to negatively charged phospholipids and proteins, enhancing membrane rigidity and protecting oil bodies from structural disruption. This interaction suppresses the activity of lipolytic enzymes, thereby reducing hydrolytic cleavage of triglycerides into free fatty acids. Moreover, putrescine modulates reactive oxygen species homeostasis by activating antioxidant defense mechanisms and maintaining redox balance during seed development. Reduced oxidative stress minimizes lipid peroxidation and subsequent secondary hydrolytic reactions that elevate acid value. Polyamines also regulate gene expression in lipid metabolism, promoting efficient oil synthesis while limiting lipid degradation pathways. The consistent reduction in acid value across both seasons indicates that the observed improvement in oil quality was primarily driven by biostimulant-mediated physiological regulation rather than seasonal environmental variability. Lower acid values indicate reduced accumulation of free fatty acids, improved oil stability, and greater suitability of sesame oil for storage and consumption. Notably, the simultaneous increase in metabolic activity and reduction in lipid hydrolysis suggests that humic acid and putrescine promote efficient oil biosynthesis while preserving lipid integrity. Overall, the application of humic acid and putrescine improved sesame oil quality by stabilizing membrane structures, regulating lipid metabolic pathways, reducing enzymatic hydrolysis of triglycerides, and enhancing antioxidant protection during seed maturation. These mechanisms collectively resulted in lower acid values, reflecting superior oil stability and enhanced nutritional quality (Farzi aminabad et al., 2018; Seervi et al., 2018; Alizadeh et al., 2022).

4.5.5 Iodine Value (g/100g oil): During the 2023 and 2024 growing seasons, the iodine value of sesame oil was significantly influenced by the application of humic acid and putrescine (Table 4.5.2 and Figure 4.5.2). Variations in iodine value across treatments indicate that these biostimulants modulated the degree of unsaturation in sesame oil in a dose-dependent manner. In 2023, the iodine value varied significantly among humic acid treatments. The highest iodine value was recorded for HA2 (1.87), followed by HA4 (1.53) and HA3 (0.87), whereas the untreated control, HA1 (0.79), had the lowest value, indicating comparatively lower unsaturation. Under putrescine treatment, the untreated control P1 recorded the highest iodine value (1.34), followed by P4 (1.30) and P2 (1.22), whereas P3 (1.21) showed the lowest value. A comparable response pattern was observed in 2024. The highest iodine value was again recorded for HA2 (1.56), followed by HA4 (1.54) and HA3 (1.28), whereas the lowest value was in the untreated control HA1 (0.74). Among the putrescine treatments, P4 had the highest iodine value (1.32), followed by the untreated controls, P1 (1.30) and P3 (1.27), whereas P2 had the lowest value (1.24). Overall, the application of humic acid and putrescine significantly altered the iodine value of sesame oil across both seasons, reflecting changes in the degree of fatty acid unsaturation. Higher iodine values under specific biostimulant treatments suggest enhanced synthesis or retention of unsaturated fatty acids, thereby improving the nutritional quality of sesame oil.

Iodine value is a key indicator of oil quality, reflecting the degree of unsaturation of fatty acids present in the oil. Higher iodine values indicate a greater proportion of unsaturated fatty acids, which are nutritionally desirable due to their beneficial effects on human health. The variation in iodine value of sesame oil under humic acid and putrescine application across both growing seasons clearly indicates that these biostimulants modulated fatty acid biosynthesis and desaturation processes in a dose-dependent manner. The increase in iodine value under humic acid application can be attributed to its regulatory effects on plant metabolism, nutrient uptake, and on enzymatic activities involved in lipid biosynthesis. Humic acid enhances the availability and translocation of essential nutrients, including nitrogen, sulfur, iron, and magnesium, which are crucial cofactors for enzymes involved in fatty acid synthesis and desaturation. Improved nutrient availability enhances desaturase activity,

increasing the conversion of saturated fatty acids to mono- and polyunsaturated fatty acids, thereby elevating the iodine value. Humic substances also enhance photosynthetic efficiency and carbon assimilation, thereby increasing the supply of photosynthates and reducing power (NADPH) required for fatty acid elongation and desaturation in developing seeds. Enhanced assimilate flow toward seed tissues promotes the synthesis of structurally complex lipids with higher degrees of unsaturation. Furthermore, humic acid exerts hormone-like effects, particularly auxin- and cytokinin-like activities, which regulate seed development and delay senescence. This prolongs the active period of lipid biosynthesis and favors the accumulation of unsaturated fatty acids in sesame oil. Application of putrescine affected the iodine value by virtue of its polyamine role in stabilizing membranes, mitigating stress, and regulating lipid metabolic pathways. Polyamines interact with membrane phospholipids and fatty acids, maintaining membrane fluidity and structural integrity during seed development. This favorable membrane environment supports the proper functioning of fatty acid desaturase enzymes located in plastids and endoplasmic reticulum, thereby promoting unsaturated fatty acid synthesis. In addition, putrescine plays a critical role in modulating reactive oxygen species homeostasis and protecting lipid molecules from oxidative degradation. By enhancing antioxidant defense systems, putrescine reduces premature oxidation of unsaturated fatty acids during oil accumulation, allowing greater retention of double bonds and consequently higher iodine values. At the same time, excessive or suboptimal levels may alter metabolic balance and enzyme efficiency. The consistency of treatment effects across both seasons indicates that biostimulant-induced changes in iodine value were primarily governed by internal physiological regulation rather than environmental fluctuations. Overall, the application of humic acid and putrescine improved the nutritional quality of sesame oil by modulating fatty acid composition toward greater unsaturation. This improvement was achieved through enhanced nutrient uptake, stimulation of desaturase activity, improved photosynthetic performance, protection of lipids from oxidative damage, and regulation of lipid metabolic pathways. These findings highlight the potential of humic acid and putrescine as practical agronomic tools for improving sesame oil quality while maintaining metabolic stability (Alizadeh et al., 2022; Nassar et al., 2020; Kazami & Eskandari et al., 2024).

4.5.6 Refractive Index: During the 2023 and 2024 growing seasons, the refractive index of sesame oil was marginally but significantly influenced by the application of humic acid and putrescine (Table 4.5.2 and Figure 4.5.2). Although the numerical differences among treatments were minor, the observed variation in refractive index reflects subtle changes in oil composition, particularly the degree of fatty acid unsaturation. In 2023, the refractive index differed slightly among humic acid treatments. The highest refractive index was recorded in the untreated control HA1 (1.474), followed closely by HA2 and HA3 (1.473 each), whereas HA4 (1.473) exhibited the lowest value, indicating a comparatively lower level of unsaturation with higher humic acid application. Under putrescine treatment, the untreated control P1 showed the highest refractive index (1.473), followed by P2 and P3 (1.473), whereas P4 (1.473) showed the lowest value. A similar trend was observed in 2024. The highest refractive index was again recorded for the untreated control HA1 (1.473), followed by HA2 and HA3 (1.473), whereas HA4 (1.473) had the lowest value. Among putrescine treatments, P4 showed the highest refractive index (1.474), followed by the untreated controls P1 and P3 (1.473), whereas P2 exhibited the lowest value (1.473). Overall, the application of humic acid and putrescine caused only slight variations in the refractive index of sesame oil across both seasons. These minor shifts suggest modest alterations in fatty acid composition, particularly in levels of unsaturation, under biostimulant application. However, the narrow range of refractive index values indicates that the fundamental physicochemical characteristics of sesame oil remained largely stable across treatments.

The refractive index of edible oils is a sensitive physicochemical parameter that reflects the structural characteristics of fatty acids, particularly chain length, degree of unsaturation, and overall molecular arrangement. Humic acid application influenced the refractive index primarily by modulating nutrient uptake, carbon assimilation, and lipid metabolism during seed development. Humic substances enhance the availability and mobility of essential nutrients such as nitrogen, sulfur, iron, and magnesium, which act as cofactors for enzymes involved in fatty acid synthesis and desaturation. These nutrients regulate the balance between saturated and unsaturated fatty acids, which in turn determines the optical density and refractive behavior of oils. Slight reductions in

refractive index under higher humic acid concentrations suggest a fine adjustment in fatty acid composition, possibly due to a relative stabilization of fatty acid profiles rather than drastic increases in unsaturation. Additionally, humic acid improves photosynthetic efficiency and assimilate partitioning toward developing seeds, ensuring a steady supply of carbon skeletons for lipid biosynthesis. Enhanced metabolic coordination under humic acid treatment promotes uniform fatty acid chain assembly and reduces compositional heterogeneity within the oil fraction. This metabolic stability explains the narrow range of refractive index values observed across humic acid treatments, indicating preservation of the intrinsic physicochemical identity of sesame oil. Application of putrescine influenced the refractive index through its regulatory roles in membrane integrity, lipid stabilization, and oxidative balance. Polyamines, such as putrescine, interact electrostatically with membrane phospholipids and fatty acids, thereby maintaining membrane fluidity and structural organization during seed filling. This interaction supports optimal functioning of lipid-synthesizing enzymes while preventing excessive alterations in fatty acid unsaturation. As a result, putrescine-treated plants exhibited only minor shifts in refractive index, reflecting controlled modulation of oil composition rather than drastic structural changes. The stability of refractive index across putrescine treatments suggests that polyamine-induced metabolic adjustments were balanced and did not compromise the fundamental quality attributes of sesame oil. The consistency of refractive index trends across both seasons highlights the robustness of sesame oil composition under biostimulant application, despite variations in environmental conditions. The marginal yet significant differences indicate that humic acid and putrescine fine-tuned fatty acid metabolism without altering the oil's core physicochemical characteristics. Such stability is desirable from both nutritional and industrial perspectives, as it ensures oil uniformity while allowing physiological improvements in plant performance. Overall, the slight variations in refractive index observed under humic acid and putrescine treatments reflect subtle, well-regulated changes in fatty acid composition associated with enhanced metabolic efficiency and lipid stability. These findings confirm that biostimulant application can improve plant physiological processes and oil quality attributes while preserving the inherent physicochemical identity of sesame oil (Kazami & Eskandari et al., 2024; Nassar et al., 2020; Nargesi et al., 2022).

Table 4.5.2 Effect of different treatments on acid value, iodine value, and refractive index of sesame oil during kharif 2023 & 2024

Treatments	2023			2024		
	Acid value	Iodine value	Refractive index	Acid value	Iodine value	Refractive index
Factor (Humic Acid)						
HA 1 (0ppm)	4.59	0.79	1.474	5.24	0.74	1.473
HA 2 (500 ppm)	4.01	1.87	1.473	4.50	1.28	1.473
HA 3 (1000 ppm)	3.16	0.87	1.473	3.47	1.56	1.473
HA 4 (1500 ppm)	2.09	1.53	1.473	2.86	1.54	1.473
SEm ±	0.03	0.01	0.010	0.03	0.01	0.01
C.D. (p=0.05)	0.08	0.03	0.030	0.08	0.03	NS
Factor (Putrescine)						
P 1 (0 ppm)	4.70	1.34	1.473	5.15	1.30	1.473
P 2 (50 ppm)	3.17	1.22	1.473	3.93	1.24	1.473
P 3 (100 ppm)	3.11	1.21	1.473	3.57	1.27	1.472
P 4 (150 ppm)	2.87	1.30	1.473	3.43	1.32	1.474
SEm ±	0.03	0.01	0.010	0.03	0.01	0.01
C.D. (p=0.05)	0.08	0.03	0.030	0.08	0.03	NS
Interaction	NS	NS	NS	NS	NS	NS

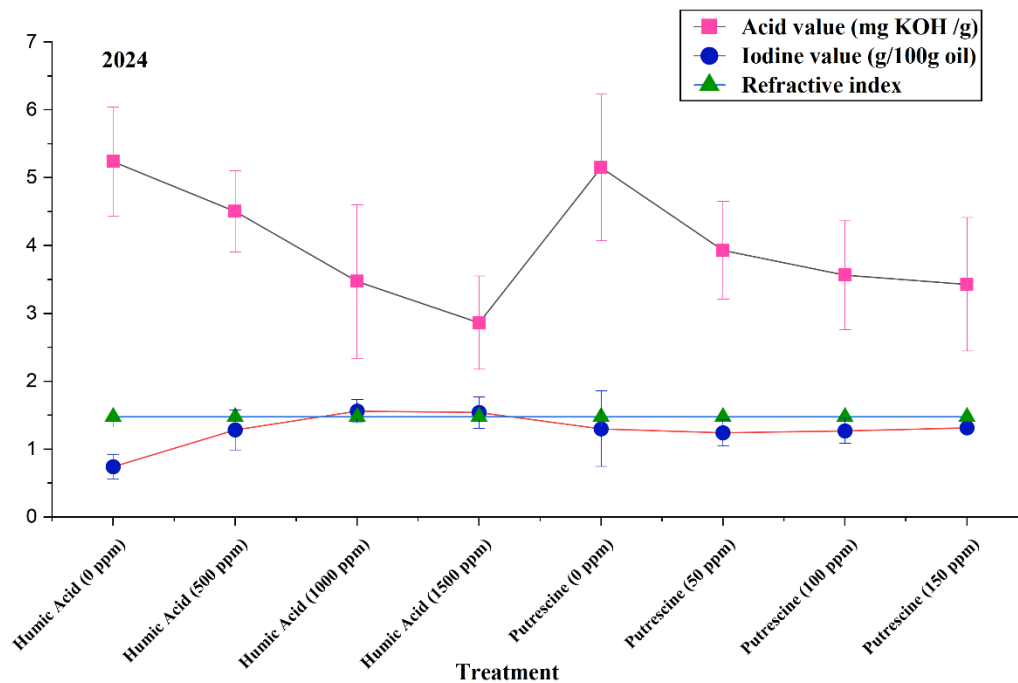
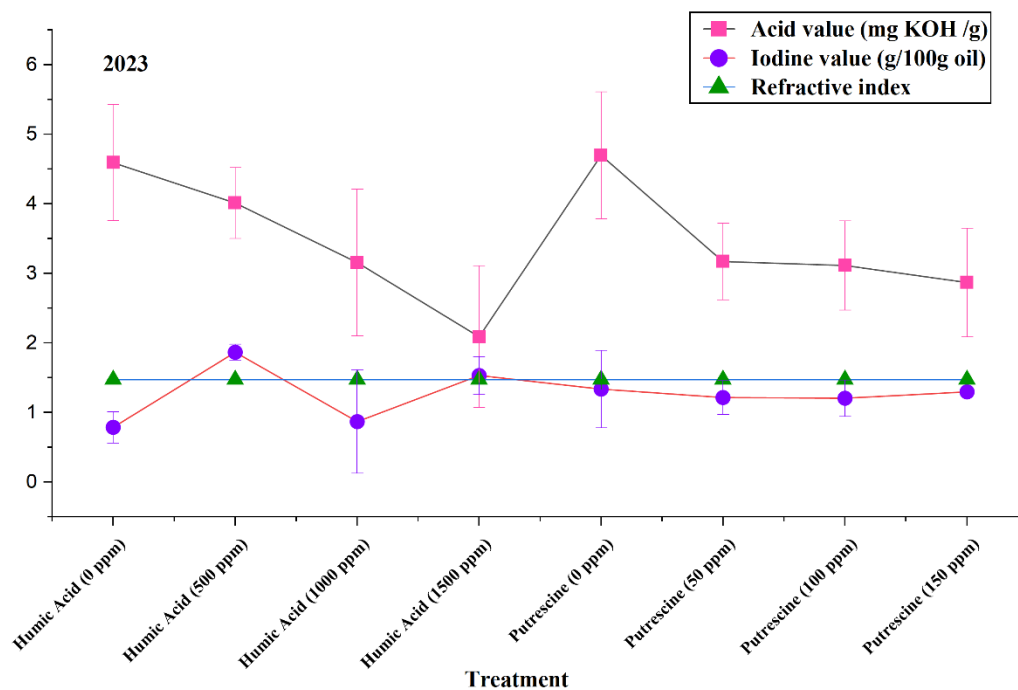


Figure 4.5.2 Effect of different treatments on acid value, iodine value, and refractive index of sesame oil during kharif 2023 & 2024

4.5.7 Density (g ml⁻¹): During the 2023 and 2024 growing seasons, the density of sesame oil was significantly influenced by the application of humic acid and putrescine (Table 4.5.3 and Figure 4.5.3). Although the magnitude of variation was minor, differences in oil density among treatments suggest subtle modifications in oil composition associated with biostimulant application. In 2023, oil density varied significantly among humic acid treatments. The highest density was recorded under HA4 (0.933), followed by HA3 (0.925) and HA2 (0.924), whereas the untreated control HA1 (0.918) exhibited the lowest value. Under putrescine treatments, P2 recorded the highest oil density (0.927), followed by the untreated control P1 (0.926) and P4 (0.925), whereas P3 (0.922) recorded the lowest value. A similar trend was observed in 2024. The highest oil density was again recorded under HA4 (0.927), followed by HA3 (0.922) and HA2 (0.920), whereas the lowest value occurred in the untreated control HA1 (0.917). Among the putrescine treatments, P2 produced the highest oil density (0.924), followed by the untreated controls P1 and P4 (0.922 each), whereas P3 (0.920) had the lowest value. Overall, the application of humic acid and putrescine produced modest yet significant changes in sesame oil density across both seasons. These variations likely reflect minor shifts in fatty acid composition and molecular packing rather than significant alterations in oil structure. Higher density values under specific biostimulant treatments may be associated with changes in lipid profile, contributing marginally to the physicochemical quality of sesame oil.

The observed modest increases in sesame oil density following application of humic acid (HA) and putrescine (Put) across the 2023 and 2024 seasons likely reflect subtle alterations in seed metabolism that affect oil *composition* rather than gross oil structure. Oil density is influenced by the proportions of fatty acids and minor lipid components, and shifts in these constituents — particularly between unsaturated and saturated fatty acids—can alter density without significant changes in total oil content. Humic acids are complex organic molecules that act as biostimulants, influencing plant physiology both indirectly (via soil improvement) and directly (via effects on plant metabolism). HA can enhance nutrient availability by complexing soil minerals and increasing cation exchange capacity, thereby facilitating the uptake of macro- and micronutrients critical for metabolic pathways, including lipid biosynthesis. HA also exhibits hormone-like

activity, modulating phytohormone networks, including auxins and cytokinins, that influence growth and metabolic processes associated with seed development and composition. This hormone-like behavior has been demonstrated in crop systems, where HA upregulates auxin and cytokinin biosynthesis pathways, thereby increasing metabolic vigor and nutrient distribution in plants. Enhanced photosynthetic efficiency increases the supply of carbon substrates, such as acetyl-CoA, and reducing equivalents (NADPH), which are essential for fatty acid synthesis. HA has also been shown to mitigate stress responses by stimulating antioxidant systems, thereby reducing lipid peroxidation and stabilizing oil quality under suboptimal conditions. In the context of sesame, these mechanisms may have resulted in slight but consistent density differences among HA treatments. Higher HA concentrations (e.g., HA4) possibly supported greater nutrient assimilation and metabolic flux through lipid biosynthetic pathways, yielding oil with a composition richer in specific fatty acids or minor compounds that slightly increase density. Putrescine, a polyamine, plays a distinct but complementary role. Polyamines are low-molecular-weight organic cations involved in stress tolerance, cell division, and metabolic regulation. Exogenous Put application increases endogenous polyamine levels, which can modulate metabolic pathways through effects on gene expression and enzyme activities involved in lipid metabolism and stress responses. In oilseeds, Put may enhance osmotic adjustment and membrane stability, improving seed filling and fatty acid synthesis under field conditions. Polyamines also have antioxidant properties that protect against oxidative damage; these properties can maintain the integrity of lipid biosynthetic enzymes and membranes, leading to slight changes in the oil profile that affect density. Similar patterns across seasons indicate that the biostimulant effects of HA and Put are reproducible and not tied to a single environmental condition, although their impact was negligible. For instance, slight increases in long-chain or unsaturated fatty acids could marginally increase density, reflect improved metabolic efficiency, and alter seed biochemistry. Overall, the results support the hypothesis that applications of humic acid and putrescine can enhance physiological processes that indirectly influence oil physicochemical quality, consistent with studies reporting that biostimulants modify oil composition and metabolic profiles in other oilseed and aromatic crops (Nargesi et al., 2022; Amiri et al., 2020; Khoung et al., 2022).

4.5.8 Viscosity ($\text{mm}^2 \text{s}^{-1}$): During the 2023 and 2024 growing seasons, the viscosity of sesame oil was significantly influenced by the application of humic acid and putrescine (Table 4.5.3 and Figure 4.5.3). Although the magnitude of variation was minor, differences in oil viscosity among treatments indicate subtle modifications in oil composition associated with biostimulant application. In 2023, oil viscosity varied significantly among humic acid treatments. The highest viscosity was recorded under HA4 (4.25), followed by HA3 (4.21) and HA2 (4.21), whereas the untreated control, HA1 (4.18), exhibited the lowest value. Under putrescine treatments, P2 showed the highest viscosity (4.22), followed by the untreated control (P1, 4.21) and P4 (4.21), whereas P3 (4.20) showed the lowest value. A similar trend was observed in 2024. The highest viscosity was again recorded under HA4 (4.22), followed by HA3 (4.19) and HA2 (4.19), whereas the lowest value occurred in the untreated control, HA1 (4.17). Among the putrescine treatments, P2 produced the maximum viscosity (4.20), followed by P1 and P4 (4.19 each), while P3 (4.19) recorded the minimum value. Overall, the application of humic acid and putrescine caused modest but significant changes in sesame oil viscosity across both seasons. These variations likely reflect minor alterations in fatty acid composition and molecular packing rather than substantial changes in oil structure. Slightly higher viscosity values under specific biostimulant treatments may be associated with changes in lipid profile, thereby contributing marginally to the physicochemical quality of sesame oil.

The observed small but statistically significant increases in sesame oil viscosity following the application of humic acid and putrescine across both growing seasons suggest that biostimulant treatments subtly influenced seed lipid chemistry. Oil viscosity in edible oils is primarily determined by fatty acid composition (chain length and degree of unsaturation), as well as by the relative abundance of minor components, such as sterols and polar lipids. Higher viscosity typically correlates with a greater proportion of long-chain or more saturated fatty acids, as well as with changes in oil microstructure and intermolecular interactions arising from lipid profile adjustments. Humic acid (HA) functions as a multifunctional biostimulant that can modulate plant physiology and metabolism through multiple pathways. As a complex organic substance, HA improves soil structure, enhances nutrient chelation, and increases root

absorption of macro- and micronutrients. This enhanced nutrient status supports more efficient carbon and nitrogen metabolism during seed development, thereby altering the balance between the synthesis of saturated and unsaturated fatty acids in oilseeds. In oil crops such as rapeseed, HA application has been associated with changes in oil yield and fatty acid composition, suggesting that biostimulants can influence the metabolic networks underlying lipid assembly. While the quantitative shifts in sesame fatty acid profiles under HA treatment in your study were modest, even small increases in specific fatty acids can subtly change the oil's physicochemical properties, including viscosity. Metabolically, HA may influence key enzymes involved in lipid biosynthesis. For instance, by improving plant nutritional status and enhancing photosynthetic efficiency, HA can increase the availability of acetyl-CoA and reducing equivalents (NADPH), which are essential precursors for fatty acid elongation and desaturation. It may also indirectly alter phytohormone signalling (e.g., auxin-cytokinin balance), thereby affecting gene expression in lipid metabolic pathways during the seed-filling stage. Putrescine can modulate fatty acid biosynthesis by influencing transcriptional and translational regulation of metabolic enzymes, thereby affecting the balance between saturated and unsaturated fatty acids in developing seeds. In oilseed crops under water deficit, foliar polyamine sprays have been reported to increase the proportion of unsaturated fatty acids, particularly oleic acid, and to reduce saturated fatty acids, such as palmitic acid. Such compositional shifts improve oil quality and can also influence viscosity since oils richer in unsaturated fatty acids generally exhibit lower viscosity than those richer in saturated lipids. Although your results showed only slight viscosity changes, they could reflect modest shifts in fatty acid ratios or in minor lipid components influenced by putrescine-mediated metabolic regulation. Finally, both HA and putrescine are known to enhance plant stress resilience, nutrient uptake, and metabolic efficiency. These systemic improvements in plant physiological status during critical phases of seed development likely contributed to the observed changes in oil viscosity by fine-tuning lipid biosynthesis pathways. Such subtle modifications underscore the capacity of biostimulants to modulate oil quality traits even when effects on yield or major constituents are limited (Khoung et al., 2022; Jalilian et al., 2022; Asajri et al., 2024).

4.5.9 Fatty Acid Content: During the 2023 and 2024 growing seasons, the fatty acid content of sesame oil was significantly influenced by the application of humic acid and putrescine (Table 4.5.3 and Figure 4.5.3). Variations in fatty acid content across treatments indicate that these biostimulants modulated the degree of unsaturation in sesame oil in a dose-dependent manner. In 2023, fatty acid content varied significantly among humic acid treatments. The highest content was recorded under HA4 (40.02%), followed by HA3 (39.52%) and the untreated control, HA1 (38.44%), whereas HA2 (36.54%) registered the lowest value, indicating comparatively lower unsaturation. Under putrescine treatments, P4 showed the highest fatty acid content (39.41%), followed by P3 (39.29%) and the untreated control (P1) (38.85%), whereas P2 (36.98%) exhibited the lowest value. A similar trend was observed in 2024. The highest fatty acid content was again recorded under HA4 (39.99%), followed by HA3 (39.49%) and HA2 (39.00%), whereas the lowest value was observed in the untreated control, HA1 (38.40%). Among putrescine treatments, P2 produced the maximum fatty acid content (39.45%), followed by P4 (39.38%) and P3 (39.24%), while the untreated control, P1 (38.82%), recorded the lowest value. Overall, the application of humic acid and putrescine significantly influenced the fatty acid content of sesame oil across both seasons.

Higher fatty acid content under specific biostimulant treatments suggests enhanced synthesis or retention of unsaturated fatty acids, thereby improving the nutritional quality and stability of sesame oil. The significant modulation of sesame oil fatty acid content observed with the application of humic acid (HA) and putrescine (Put) across both growing seasons suggests that these biostimulants influenced the underlying physiological and biochemical pathways governing lipid metabolism during seed development. Rather than altering total oil quantity, the treatments appear to have modulated the degree of unsaturation—a key quality parameter that determines nutritional value and oxidative stability of edible oils. Humic acids are complex organic molecules derived from soil organic matter that have long been recognized as effective plant biostimulants. They act through multiple, interlinked mechanisms that influence plant nutrient status, hormonal balance, carbon partitioning, and metabolic profiles. At the soil-plant interface, HA enhances nutrient chelation and availability, particularly of

macro- and micronutrients that are critical cofactors for enzymatic activities involved in lipid biosynthesis. Improved nutrient uptake can lead to more efficient carbon assimilation and an enhanced supply of substrates (e.g., acetyl-CoA, malonyl-CoA) necessary for fatty acid synthesis during seed filling. HA's influence on root architecture and increased membrane H⁺-ATPase activity also support enhanced nutrient uptake and translocation, thereby optimizing conditions under which metabolic pathways operate at peak efficiency. Beyond nutrient effects, humic acids exhibit hormone-like activity, interacting with auxin and cytokinin signaling pathways. This can upregulate gene expression patterns associated with primary metabolism, including lipid biosynthetic enzymes. Studies in other crops have shown changes in overall metabolic fingerprints following HA application, with alterations in lipid profiles and related metabolites, indicating that humic substances can directly or indirectly modulate fatty acid synthesis and distribution within seed tissues. While most work has focused on growth outcomes, these metabolomic changes suggest that HA can influence the balance between saturated and unsaturated fatty acids by modulating regulatory networks and metabolic fluxes that govern desaturase activity—a key determinant of unsaturation. Putrescine, a low-molecular-weight polyamine, contributes to plant growth and stress responses by stabilizing membranes, regulating gene expression, and modulating antioxidant systems. Polyamines, such as Putrescine, are known to influence lipid metabolism and membrane integrity, particularly under conditions of oxidative stress. They can affect the expression and activity of desaturase enzymes that catalyze the introduction of double bonds into fatty acid chains, ultimately shaping the profile of monounsaturated and polyunsaturated fatty acids in seeds. Although specific studies linking putrescine to fatty acid biosynthesis in sesame are limited, research in other species indicates that polyamine application can alter the expression of genes involved in lipid biosynthetic pathways and enhance stress resistance, thereby affecting carbon partitioning among lipid classes. Mechanistically, both HA and Put may also impact oxidative status within developing seeds. Improved antioxidant capacity reduces lipid peroxidation, thereby increasing the retention of unsaturated fatty acids, which are typically more susceptible to oxidation than their saturated counterparts. This would manifest as higher relative proportions of unsaturated fatty acids and slightly elevated total fatty acid content under biostimulant treatments, as seen in your results.

It is important to note that while the magnitude of changes in fatty acid content was modest, they were consistent and statistically significant, indicating dose-dependent modulation of metabolic pathways rather than random variation. Such subtler shifts are often characteristic of biostimulant effects, which operate through fine-tuning endogenous processes rather than imposing drastic changes. Energy metabolism, nutrient allocation, stress signaling, and enzyme regulation collectively contribute to the synthesis and accumulation of lipids in plant seeds, and biostimulants such as HA and Put provide multifaceted enhancement of these systems. Overall, the enhanced fatty acid content and improved unsaturation patterns under specific humic acid and putrescine treatments reflect integrated physiological adjustments that support lipid biosynthesis and quality, aligning with current understanding of biostimulant influences on plant metabolic networks (Zhao et al., 2025; Sagun et al., 2023; Lal & Bhatla, 2023).

Table 4.5.3 Effect of different treatments on density, viscosity, and fatty acid content of sesame oil during kharif 2023 & 2024

Treatments	2023			2024		
	Density	Viscosity	Fatty Acid Content	Density	Viscosity	Fatty Acid Content
Factor (Humic Acid)						
HA 1 (0ppm)	0.918	4.18	38.44	0.917	4.17	38.40
HA 2 (500 ppm)	0.924	4.21	36.54	0.920	4.19	39.00
HA 3 (1000 ppm)	0.925	4.21	39.52	0.922	4.19	39.49
HA 4 (1500 ppm)	0.933	4.25	40.02	0.927	4.22	39.99
SEm ±	0.008	0.02	0.30	0.008	0.03	0.34
C.D. (p=0.05)	NS	NS	0.87	NS	NS	1.01
Factor (Putrescine)						
P 1 (0 ppm)	0.926	4.21	38.85	0.922	4.19	38.82
P 2 (50 ppm)	0.927	4.22	36.98	0.924	4.20	39.45
P 3 (100 ppm)	0.922	4.20	39.29	0.920	4.19	39.24
P 4 (150 ppm)	0.925	4.21	39.41	0.922	4.19	39.38
SEm ±	0.008	0.02	0.30	0.008	0.03	0.34
C.D. (p=0.05)	NS	NS	0.87	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS

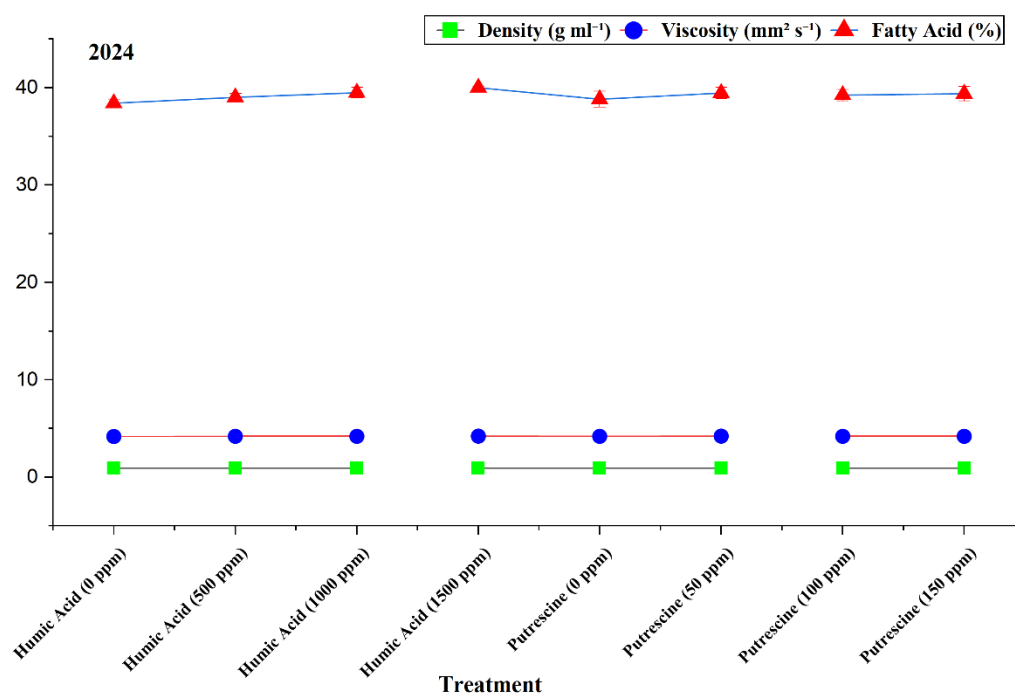
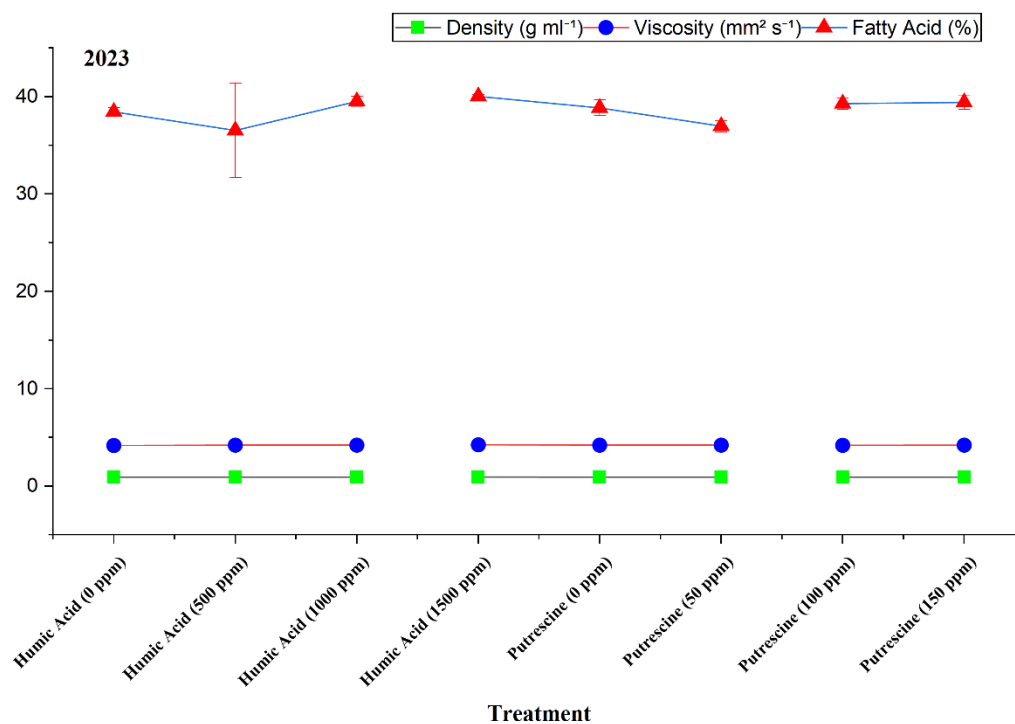


Figure 4.5.3 Effect of different treatments on density, viscosity, and fatty acid content of sesame oil during kharif 2023 & 2024

4.6 Effects of humic acid and putrescine on the economics of sesame cultivation

4.6.1 Cost of cultivation (₹ ha⁻¹): The effect of individual and combined applications of humic acid and putrescine on the cost of cultivation of sesame at harvest (Table 4.6.1). The cultivation cost was calculated using data from both experimental years (2023 and 2024). Significant variation in cultivation costs was observed among treatments, attributable to differences in input levels. The highest cultivation cost was recorded for treatment T16 (₹35174 ha⁻¹), which involved the combined application of humic acid at 1500 ppm and putrescine at 150 ppm. In contrast, the lowest cultivation cost was observed under the control treatment (T1; ₹22,475 ha⁻¹). Specifically, the cost of cultivation under treatments T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, and T15 was ₹26555, ₹30635, ₹34715, ₹22,628, ₹26708, ₹30788, ₹34868, ₹22,781, ₹26861, ₹30941, ₹35021, ₹22934, ₹27014, and ₹31094 ha⁻¹, respectively. Overall, the increase in cultivation costs with higher humic acid and putrescine levels can be attributed to additional expenditures on biostimulant inputs and their application, resulting in progressively higher production costs across treatments.

4.6.2 Gross returns (₹ ha⁻¹): The effect of humic acid and putrescine, individually and in their combinations, on gross return in sesame at harvest is shown in (Table 4.6.1). In 2023 and 2024, there was a significant difference in gross return in sesame. In 2023, the highest gross return was recorded under treatment T16, which combined humic acid (1500 ppm) and putrescine (150 ppm), yielding ₹86,138 ha⁻¹. In contrast, the lowest gross return was obtained from the control treatment T1, where no humic acid or putrescine was applied, with a gross return of ₹34,816 ha⁻¹. In 2024, the highest gross return was recorded under treatment T16, which combined humic acid (1500 ppm) and putrescine (150 ppm), yielding ₹79791 ha⁻¹. In contrast, the lowest gross return was obtained from the control treatment T1, in which no humic acid or putrescine was applied, at ₹30651 ha⁻¹.

4.6.3 Net returns (₹ ha⁻¹): The effect of humic acid and putrescine, individually and their combinations, on net return in sesame at harvest is shown in (Table 4.6.1). In 2023 and 2024, there was a significant difference in net return in sesame. In 2023, the highest net return was recorded under treatment T16, which combined humic acid (1500 ppm) and putrescine (150 ppm), yielding ₹50964 ha⁻¹. In contrast, the lowest net return was obtained from the control treatment T1, where no humic acid or putrescine was applied, with a gross return of ₹12341 ha⁻¹. In 2024, the highest net return was recorded under treatment T16, which combined humic acid (1500 ppm) and putrescine (150 ppm), yielding ₹44617 ha⁻¹. In contrast, the lowest net return was obtained from the control treatment T1, where no humic acid or putrescine was applied, with a gross return of ₹8176 ha⁻¹.

4.6.4 B: C ratio: The effect of individual and combined applications of humic acid and putrescine on the benefit–cost (B: C) ratio of sesame at harvest (Table 4.6.1). Significant differences in the B: C ratio were observed among treatments in both the 2023 and 2024 seasons. In 2023, the highest B: C ratio (2.45) was observed for treatment T16, which combined humic acid (1500 ppm) and putrescine (150 ppm). In contrast, the lowest B: C ratio (1.55) was observed in the control treatment T1, in which no humic acid or putrescine was applied. Similarly, in 2024, the maximum B: C ratio (2.31) was observed under treatment T14, which comprised humic acid (1500 ppm) and putrescine (50 ppm). The minimum B: C ratio (1.36) was again observed in the control treatment T1, indicating inferior economic performance in the absence of biostimulant application.

Table 4.6.1 Economic analysis

	Treatments Details	2023				2024			
		Cost of cultivation (₹ ha ⁻¹)	Gross returns (₹ ha ⁻¹)	Net returns (₹ ha ⁻¹)	B: C ratio	Cost of cultivation (₹ ha ⁻¹)	Gross returns (₹ ha ⁻¹)	Net returns (₹ ha ⁻¹)	B: C ratio
T ₁	HA (0 ppm) + P (0 ppm)	22475	34816	12341	1.55	22475	30651	8176	1.36
T ₂	HA(0 ppm) + P (50 ppm)	26555	49221	22666	1.85	26555	43158	16603	1.63
T ₃	HA (0 ppm) + P (100 ppm)	30635	52098	21463	1.70	30635	49764	19129	1.62
T ₄	HA (0 ppm) + P (150 ppm)	34715	54191	19476	1.56	34715	53179	18464	1.53
T ₅	HA (500 ppm) + P (0 ppm)	22628	39590	16962	1.75	22628	39678	17050	1.75
T ₆	HA (500 ppm) + P (50 ppm)	26708	57809	31101	2.16	26708	51835	25127	1.94
T ₇	HA (500 ppm) + P (100 ppm)	30788	64062	33274	2.08	30788	61851	31063	2.01
T ₈	HA (500 ppm) + P (150 ppm)	34868	66800	31932	1.92	34868	60688	25820	1.74
T ₉	HA (1000 ppm) + P (0 ppm)	22781	43759	20978	1.92	22781	45426	22645	1.99
T ₁₀	HA (1000 ppm) + P (50 ppm)	26861	60320	33459	2.25	26861	53523	26662	1.99
T ₁₁	HA (1000 ppm) + P (100 ppm)	30941	62646	31705	2.02	30941	60504	29563	1.96
T ₁₂	HA (1000 ppm) + P (150 ppm)	35021	67937	32916	1.94	35021	70550	35529	2.01
T ₁₃	HA (1500 ppm) + P (0 ppm)	22934	46905	23971	2.05	22934	50747	27813	2.21
T ₁₄	HA (1500 ppm) + P (50 ppm)	27014	60597	33583	2.24	27014	62279	35265	2.31
T ₁₅	HA (1500 ppm) + P (100 ppm)	31094	70478	39384	2.27	31094	71395	40301	2.30
T ₁₆	HA (1500 ppm) + P (150 ppm)	35174	86138	50964	2.45	35174	79791	44617	2.27

Table 4.6.2 Cost of cultivation

Particulars	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16
Field preparation	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000
Fertilizer application(labour)	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400
Urea	690	690	690	690	690	690	690	690	690	690	690	690	690	690	690	690
SSP	648	648	648	648	648	648	648	648	648	648	648	648	648	648	648	648
HUMIC ACID	0	0	0	0	150	150	150	150	300	300	300	300	450	450	450	450
PUTRESCINE	0	4000	8000	12000	0	4000	8000	12000	0	4000	8000	12000	0	4000	8000	12000
Seed	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Sowing with Seed drill/labour	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
hand weeding	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000

corazon	440	440	440	440	440	440	440	440	440	440	440	440	440	440	440	440
coloro	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25	656. 25
insecticide application	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700
Irrigation	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400	2400
Irrigation application	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700
Harvesting and threshing	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0	4,40 0
Transportation	441	521	601	681	444	524	604	684	447	527	607	687	450	530	610	690
Total (COC)	22,4 75	26,5 55	30,6 35	34,7 15	22,6 28	26,7 08	30,7 88	34,8 68	22,7 81	26,8 61	30,9 41	35,0 21	22,9 34	27,0 14	31,0 94	35,1 74

SUMMARY AND CONCLUSION

The present research work, entitled “Effect of Humic Acid and Putrescine Application on Growth, Yield, Oil Quality, and Economics in *Sesamum indicum*,” was conducted during the Kharif season of 2023-2024 at the Lovely Professional University field in Jalandhar, Punjab. The total experimental area covered approximately 1100 m². The experiment involved 16 treatments, arranged in a randomized design with three replications, yielding 48 experimental plots. The treatments included the following: humic acid (0, 500, 1000, and 150 ppm) and putrescine (0, 50, 100, and 150 ppm), applied as foliar sprays at 15 and 45 days after sowing (DAS). Growth, biochemical, yield, and quality parameters were measured at three growth stages (30, 60, and 90 DAS) across all treatments. The results of the investigation are summarized below:

5.1 Morphological and Physiological Parameters of Sesame

1. The combined use of humic acid (1500 ppm) and putrescine (150 ppm) was found to significantly enhance the height of the plants, their stem girth, internodal length, and primary branches when compared to the plants that were not treated and those that were treated separately.
2. Sesame plants sprayed with humic acid (1500ppm) and putrescine (150ppm) showed a significant increase in the number of leaves, the leaf area, and the leaf area index (LAI). The increased light interception and photosynthetic efficiency resulted from increased canopy growth.
3. Humic acid (1500 ppm) and putrescine (150 ppm) applied jointly gave the highest level of dry matter accumulation per plant when compared to all the other treatments. It exhibited improved nutrient uptake, increased photosynthetic synthesis, and assimilate translocation, which were effective in producing more biomass.
4. The combined action of the (1500ppm) and putrescine (150ppm) had a considerable positive effect on the parameters of crop growth (CGR) as well as on relative growth rate (RGR) and net assimilation rate (NAR). The interaction

process increased the efficiency of biomass accumulation and photosynthetic performance.

5. Humic acid (1500 ppm) was used together with putrescine (150 ppm), which enhanced early flowering and physiological maturity at the appropriate time compared to the control. Better nutrient supply and metabolism enhanced crop development without affecting the growth time.

5.2 Sesame Biochemical Parameters

1. Humic acid (1500ppm) combined with putrescine (150ppm) was found to have significant improvements in total chlorophyll content, total soluble sugars, and total soluble proteins with the control and the individual treatment. Higher chlorophyll concentration enhanced photosynthetic efficiency, resulting in greater carbohydrate production.
2. The index of stability of membranes (MSI) in the case of the combination use of humic acid (1500ppm) and putrescine (150ppm) was considerably high, which implies enhanced cellular integrity. The improved MSI indicates reduced membrane damage and protection against oxidative stress.
3. Applied humic acid (1500 ppm) and putrescine (150 ppm), used in combination with each other, significantly increased total phenol. The increase in phenolic content indicates activation of secondary metabolic pathways involved in stress and antioxidant defense.
4. The sum of the soluble proline content was significantly greater in the combined treatment applied humic acid (1500 ppm) and putrescine (150 ppm), which means that osmotic adjustment and mitigation of stress were better. Moreover, PAL activity increased markedly, indicating stimulated phenylpropanoid metabolism.
5. Applied humic acid (1500 ppm) and putrescine (150 ppm) produced remarkably greater amounts of flavonol and total flavonoid than the control. The high concentration of flavonoids contributes to greater antioxidant capacity and resistance to oxidative stress.

5.3 Yield Contributing Parameters of Sesame

6. The putrescine concentration (150 ppm) combined with humic acid (1500 ppm) had a significant positive effect on yield parameters like number of capsules per plant, capsule length, and number of seeds per capsule. The increased availability of assimilates and effective translocation resulting from biostimulant application led to improved capsule development.
7. Weight of 1000 seeds and yield of seed (q ha^{-1}) were significantly better in the Applied humic acid (1500 ppm) and putrescine (150 ppm) than in the control and in the treatments. The development of photosynthesis and nutrient uptake was enhanced, leading to greater seed filling and biomass partitioning.
8. The combined use of humic acid (1500 ppm) and putrescine (150 ppm) showed a significant increase in stalk yield and biological yield. The increased biomass production resulted from greater dry matter accumulation and balanced relationships between sources and sinks. An increased harvest index with such treatment implies increased efficiency in terms of total biomass into economic yield.
9. The level of oil in harvested seeds and oil cake yield was significantly improved in the presence of both the humic acid (1500 ppm) and putrescine (150 ppm). Due to better physiological activity and lipid metabolism, there were increased levels of oil. The increase in oil cake is a measure of improved seed makeup and post-extraction by-product quality.
10. Applied humic acid (1500 ppm) and putrescine (150 ppm) treatment significantly improved the content of crude protein of oil cake. An increase in nitrogen assimilation and protein synthesis led to a nutritionally improved oil cake.

5.4 Quality Parameters of Sesame Oil

11. Humic acid (1500 ppm) and putrescine (150 ppm), when used together, had a significant impact on the peroxide and anisidine values of the sesame oil, which reported a positive impact on oxidative stability. Reduced primary and secondary lipid oxidation occurs at low peroxide and anisidine values under this treatment. This demonstrates that combining biostimulants improves the oil's shelf life and preserves its chemical properties.

12. The acid value was lowered, and the iodine value was kept at optimum levels with the combined treatment of humic acid (1500 ppm) and putrescine (150 ppm), indicating that free fatty acid was not built up, and the quality of the oil was also improved. The new treatment guarantees increased nutritional value and oil stability.
13. The use of the humic acid (1500 ppm) and putrescine (150 ppm) had a substantial effect on the values of the refractive index and the density of sesame oil. Minor changes in refractive index and density reflect modulation of the level of unsaturation and fatty acid composition.
14. A combination of humic acid (1500 ppm) and putrescine (150 ppm) moderately increased the totex value and viscosity of sesame oil, which is evidence of increased accumulation of secondary oxidation products and slight changes in molecular packing.
15. ❖ Fatty acids, and unsaturated fatty acids, were significantly enhanced whenever humic acid was applied (1500 ppm) in combination with putrescine (150 ppm). Improved nutrient absorption and metabolism lead to improved fatty acid production.

5.5 Economics

16. Humic acid (1500 ppm) and putrescine (150 ppm), when combined, slightly increased the overall cost of cultivation since there were extra input costs. Nevertheless, this investment was justified, as the biostimulants increased plant growth, yield, and oil quality.
17. The combined treatment of humic acid (1500 ppm) and putrescine (150 ppm) increased the gross returns and the net returns as an index of enhanced seed and oil yield. Increased growth parameters and oil quality directly affected marketable output. Although input costs increased, higher yields and quality ensured greater profits.
18. The ratio benefit-cost was greater during the treatment of HA (1500 ppm) + putrescine (150 ppm), which indicated the cost-effectiveness of biostimulants. Higher returns on unit investment indicate that such an integrated strategy enhances productivity and financial sustainability. It demonstrates that the integrated treatment approach is cost-effective for cultivating sesame.

Conclusion

1. The combined application of HA (1500 ppm) and putrescine (150 ppm) significantly enhanced plant growth, vegetative parameters, and yield components, resulting in higher plants, more branches, and higher seed and biological yield.
2. This treatment, humic acid (1500 ppm) and putrescine (150 ppm), improved leaf biochemical traits, including chlorophyll content, soluble sugars, proteins, phenols, proline, and antioxidant enzyme activity, indicating enhanced physiological and stress tolerance.
3. It was found that the quality of sesame oil was enhanced by the joint use of humic acid (1500 ppm) and putrescine (150 ppm), resulting in an improved level of fatty acids, increased oxidative stability, and positive physicochemical factors, including peroxide, acid, and Iodine values.
4. The combination of humic acid (1500 ppm) and putrescine (150 ppm) enhanced gross and net returns as well as a higher B: C ratio, which proved that the mixture is economical and sustainable in the practice of sesame farming.
5. Future studies should validate the optimum combination of humic acid (1500 ppm) and putrescine (150 ppm) across diverse agro-climatic regions, soil types, and sesame genotypes to confirm its wider applicability. Further investigations should focus on the molecular and transcriptomic mechanisms regulating nutrient uptake, antioxidant metabolism, fatty acid biosynthesis, and stress-responsive pathways under combined humic acid and putrescine application. Long-term field experiments are also needed to evaluate soil health, nutrient-use efficiency, carbon sequestration, and environmental sustainability. In addition, studies integrating these biostimulants with precision nutrient management, biofertilizers, and climate-resilient production systems will help develop sustainable strategies for improving sesame productivity, oil quality, and economic returns under changing climatic conditions.

BIBLIOGRAPHY

- Abdiani, N., Kolahi, M., Javaheriyani, M., & Sabaeian, M. (2024). Effect of storage conditions on nutritional value, oil content, and oil composition of sesame seeds. *Journal of Agriculture and Food Research*, 16, 101117.
<https://doi.org/10.1016/j.jafr.2024.101117>
- Aghajanzadeh, T. A., Shahkouhi, A., & Jazayeri, O. (2025). Putrescine Improves Growth and Modulates Nitrogen Metabolism in *Brassica napus* L. Under Zinc Toxicity. *Journal of Plant Growth Regulation*, 1-15.
<https://doi.org/10.1007/s00344-025-11885-3>
- Al Amin, M., Ali, M. A., Alam, M. S., Nahar, A., & Chew, S. C. (2023). Oxidative degradation of sunflower oil blended with roasted sesame oil during heating at frying temperature. *Grain & Oil Science and Technology*, 6(1), 34-42.
<https://doi.org/10.1016/j.gaost.2022.11.004>
- Alayunt, N. Ö. (2025). A Significant Need in the Medical Field: A New Method for Putrescine, Spermine and Spermidine Using High-Performance Liquid Chromatography (HPLC). *Processes*, 13(4), 941.
<https://doi.org/10.3390/pr13040941>
- Alizadeh, S., Roozbahani, A., Rad, A. H. S., & Seyedhadi, M. H. (2022). Foliar application of humic acids improves seed yield and oil quality of rapeseed (*Brassica napus* L.) genotypes at well-time and late planting dates. *Journal of Soil Science and Plant Nutrition*, 22(1), 549-559.
<https://doi.org/10.1007/s42729-021-00670-2>
- Alkan, K. K., Satilmis, F., Sonmez, G., Deniz, Y. E., Culha, M. H., Ciftci, M. F., ... & Alkan, H. (2025). Putrescine supplementation improves the developmental competence of in vitro produced bovine embryos. *Theriogenology*, 231, 133-143. <https://doi.org/10.1016/j.theriogenology.2024.10.017>
- Alsajri, F. A., Farhan, M., & Hilai, N. (2024). Evaluating the Efficiency of Potassium Fertilizer Sources and Levels on Sesame Growth and Yield in Two Different Gypsum Soils. *Tikrit Journal for Agricultural Sciences*, 24(1), 156-169.
<https://doi.org/10.25130/tjas.24.1.13>
- Aly, H., Abdelsalam, A. Effect of Humic Acid and Selenium on Seed Germination, Seedling Growth, and Priming with Mitigate Salt Stress Effect on Mustard (*Brassica juncea*) Seeds. *Russ J Plant Physiol* 72, 72 (2025).
<https://doi.org/10.1134/S1021443724609364>
- Amiri, M., Rad, A. H. S., Valadabadi, A., Sayfzadeh, S., & Zakerin, H. (2020). Response of rapeseed fatty acid composition to foliar application of humic

- acid under different plant densities. *Plant Soil Environ*, 66(6), 303-308.
<https://doi.org/10.17221/220/2020-PSE>
- Ampong, K., Thilakaranthna, M. S., & Gorim, L. Y. (2022). Understanding the role of humic acids on crop performance and soil health. *Frontiers in Agronomy*, 4, 848621. <https://doi.org/10.3389/fagro.2022.848621>
- Anbhzahagan, A., Ramah, K., Sakthivel, N., & Sivasakthivelan, P. (2024). Role of organic and inorganic nutrients in enhancing the production of soybean: a review. *Journal of Plant Nutrition*, 47(19), 3777-3792.
<https://doi.org/10.1080/01904167.2024.2384573>
- Angamuthu, M., Govindasamy, S., Kasirajan, S., Langyan, S., Rangan, P., Pradheep, K. (2025). Origin and History of Sesame and Its Uses. In: Langyan, S., Yadava, P., Yadav, R., Bhardwaj, R. (eds) *Sesame: Sustainable Production and Applications. Cereals, Pulses and Oilseeds*. Springer, Singapore.
https://doi.org/10.1007/978-981-96-0656-6_1
- Anielak, A. M., Świdarska-Dąbrowska, R., Łomińska-Płatek, D., Dąbrowski, T., & Piaskowski, K. (2025). Methods for Obtaining Humus Substances: Advantages and Disadvantages. *Applied Sciences*, 15(5), 2463.
<https://doi.org/10.3390/app15052463>
- Arora, P. K., Tripathi, S., Omar, R. A., Chauhan, P., Sinhal, V. K., Singh, A., ... & Singh, V. P. (2024). Next-generation fertilizers: the impact of bionanofertilizers on sustainable agriculture. *Microbial cell factories*, 23(1), 254. <https://doi.org/10.1186/s12934-024-02528-5>
- Ayoubizadeh, N., Laei, G., Amini Dehaghi, M., Masoud Sinaki, J., & Rezvan Bidokhti, S. (2020). Effect of drought stress and foliar nutrition of iron nano-chelate and fulvic acid on grain yield and fatty acids composition in seed oil of two sesame cultivars. *Journal of Crops Improvement*, 22(2), 231-243.
<https://doi.org/10.22059/jci.2020.281730.2220>
- Bai, Y. P., Zhang, T., Hu, Z. Y., Zhang, Y., Wang, D. G., Zhou, M. Y., ... & Kong, X. (2024). Sesamin ameliorates nonalcoholic hepatic steatosis by inhibiting CD36-mediated hepatocyte lipid accumulation in vitro and in vivo. *Biochemical Pharmacology*, 224, 116240.
<https://doi.org/10.1016/j.bcp.2024.116240>

- Banik, S. (2023). Diseases of Sesame (*Sesamum indicum* L.) and Their Integrated Management. In *Diseases of Oil Crops and Their Integrated Management* (pp. 142-164). CRC Press. <https://doi.org/10.1201/9781032627960>
- Bedasa, Y., Tekalign, T. Technical efficiency of smallholder farmers in sesame production (Ethiopia). *SN Bus Econ* 4, 3 (2024). <https://doi.org/10.1007/s43546-023-00604-3>
- Bhatt, P., & Singh, V. K. (2022). Effect of humic acid on soil properties and crop production—A review. *Indian Journal of Agricultural Sciences*, 92(12), 1423-1430. <https://doi.org/10.56093/ijas.v92i12.124948>
- Bhattacharya, R., Mondal, S.K. (2025). Soil Conservation Techniques and Adaptive Management Practices for Climate-Resilient Agriculture. In: Singh, A.K., Kumar, P., Singh, S.S. (eds) *Sustainable Agriculture Management in Semi-Arid Climates*. Springer, Cham. https://doi.org/10.1007/978-3-031-94062-0_6
- Bisht, D., Sharma, U., & Kumar, N. (2025). Anatomical Traits: Adaptations for Resource Acquisition and Allocation. *Enhancing Forest Resilience through Plant Functional Traits*, 32-45. <https://doi.org/10.1201/9781003545231>
- Chamoto, K., Zhang, B., Tajima, M., Honjo, T., & Fagarasan, S. (2024). Spermidine—an old molecule with a new age-defying immune function. *Trends in Cell Biology*, 34(5), 363-370. <https://doi.org/10.1016/j.tcb.2023.08.002>
- Chowardhara, B., Saha, B., Borgohain, P., Awasthi, J. P., Kityania, S., & Panda, S. K. (2022). Effect of ethanol, putrescine and acetic acid on cadmium accumulation and toxicity in Indian mustard. *South African Journal of Botany*, 147, 42-52. <https://doi.org/10.1016/j.sajb.2021.12.019>
- Datta, A., Choudhury, M., Sharma, P. C., Jat, H. S., Jat, M. L., & Kar, S. (2022). Stability of humic acid carbon under conservation agriculture practices. *Soil and Tillage Research*, 216, 105240. <https://doi.org/10.1016/j.still.2021.105240>
- Dawar, R., Nirmal, R. C., Yogi, A. K., Kakraliya, M., Vishwakarma, H., & Bana, R. S. (2025). Agro-Techniques for the Cultivation of Sesame. In *Sesame: Sustainable Production and Applications* (pp. 57-80). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-96-0656-6_4
- Decsi, K., Ahmed, M., Rizk, R., Abdul-Hamid, D., & Tóth, Z. (2024). Analysis of Plant Physiological Parameters and Gene Transcriptional Changes Under the Influence of Humic Acid and Humic Acid-Amino Acid Combinations in Maize. *International journal of molecular sciences*, 25(24), 13280. <https://doi.org/10.3390/ijms252413280>

- Desoky, E. S. M., Alharbi, K., Rady, M. M., Elnahal, A. S., Selem, E., Arnaout, S. M., & Mansour, E. (2023). Physiological, biochemical, anatomical, and agronomic responses of sesame to exogenously applied polyamines under different irrigation regimes. *Agronomy*, 13(3), 875.
<https://doi.org/10.3390/agronomy13030875>
- dos Reis, J.G.M., Aktas, E., Machado, S.T. (2025). Soybean Supply Chains, Markets, and Global Trade. In: Singh, K.P., Singh, N.K., T, A. (eds) Soybean Production Technology. Springer, Singapore. https://doi.org/10.1007/978-981-97-8677-0_19
- Dossa, K. F., Enete, A. A., Miassi, Y. E., & Omotayo, A. O. (2023). Economic analysis of sesame (*Sesamum indicum* L.) production in Northern Benin. *Frontiers in Sustainable Food Systems*, 6, 1015122.
<https://doi.org/10.3389/fsufs.2022.1015122>
- Dwivedi, S. K., Issar, K., & Tiwari, V. (2026). Nutrient requirements in health and disease. In *Handbook of Nutraceuticals: Science, Technology and Engineering* (pp. 151-175). Cham: Springer Nature Switzerland.
https://doi.org/10.1007/978-3-031-98791-5_3
- Edalateyan Kharazi, M., & Asgharzadeh, A. (2023). Effects of drought stress, salicylic acid, and polyamines on plant growth yield and oil and essential oil content of *Nigella sativa* L. *Iranian Journal of Medicinal and Aromatic Plants Research*, 39(2), 237-254. <https://doi.org/10.22092/ijmapr.2023.360004.3235>
- El-Khayat, L. A. (2024). EFFECT OF HUMIC ACID AND SOME MICRO NUTRIENTS ON GROWTH, SEED YIELD AND OIL CONTENT OF *BORAGO OFFICINALIS* L. PLANT. *Scientific Journal of Flowers and Ornamental Plants*, 11(2), 85-105. <https://doi.org/10.21608/sjfop.2024.392477>
- Ewais, M. A., El-Sherif, A. M., & Azmy, S. T. (2025). Competitive interactions and economic assessment of different intercropping systems of peanut and sesame with foliar application of humic acid. *Fayoum Journal of Agricultural Research and Development*, 39(1), 44-53.
<https://doi.org/10.21608/fjard.2025.327650.1074>
- Farooq, U., Ashraf, M. A., & Rasheed, R. (2025). Citrulline enhances salinity tolerance via photosynthesis, redox balance, osmotic and hormonal regulation, and nutrient assimilation in sunflower (*Helianthus annuus* L.). *Physiology and Molecular Biology of Plants*, 31(6), 1027-1052.
<https://doi.org/10.1007/s12298-025-01626-x>

- Farsaraei, S., Mehdizadeh, L. & Moghaddam, M. Seed Priming with Putrescine Alleviated Salinity Stress During Germination and Seedling Growth of Medicinal Pumpkin. *J Soil Sci Plant Nutr* **21**, 1782–1792 (2021).
<https://doi.org/10.1007/s42729-021-00479-z>
- Forotaghe, Z. A., Sour, M. K., Jahromi, M. G., & Torkashvand, A. M. (2021). Physiological and biochemical responses of onion plants to deficit irrigation and humic acid application. *Open agriculture*, 6(1), 728-737.
<https://doi.org/10.1515/opag-2021-0050>
- Gangurde, S.S., Bhat, R.S., Shirasawa, K., Varshney, R.K., Pandey, M.K. (2025). Breeding for High Oleate Oilseed Crops: Opportunities, Constraints, and Prospects. In: Pandey, M.K., Mallikarjuna, M.G., Lohithaswa, H.C., S. Aski, M., Gupta, S. (eds) Breeding Climate Resilient and Future Ready Oilseed Crops. Springer, Singapore. https://doi.org/10.1007/978-981-97-7744-0_15
- Ghareeb, E. A. T. E. S., Ismail, F. H. M., El-Nabarawy, A. A. E. F., & Eid, R. S. M. (2024). Enhancing Growth and Yield of Sesame Plant (*sesamum indicum* L.) by the Amendment of Benzyladenine, Yeast Extract and Mixed Amino Acids. *Annals of Agricultural Science, Moshtohor*, 62(3), 1-20.
<https://doi.org/10.21608/assjm.2024.308435.1305>
- Ghasemi, M., Zahedi, M., Gheysari, M. *et al.* Effects of inoculation with four mycorrhizal species on seed phenolic and fatty acids of sesame plants grown under different irrigation regimes. *Sci Rep* **13**, 16482 (2023).
<https://doi.org/10.1038/s41598-023-42375-9>
- Gholipour, S. (2020). Effect of putrescine and calcium nitrate foliar application on some physiological traits of (*Sesamum indicum* L.) under different moisture. *Journal of Plant Process and Function*, 9(39), 91-108.
<https://dor.isc.ac/dor/20.1001.1.23222727.1399.9.39.5.4>
- González-Hernández, A. I., Scalschi, L., Vicedo, B., Marcos-Barbero, E. L., Morcuende, R., & Camaño, G. (2022). Putrescine: a key metabolite involved in plant development, tolerance and resistance responses to stress. *International journal of molecular sciences*, 23(6), 2971.
<https://doi.org/10.3390/ijms23062971>
- Gupta, A., Kumar, P.S.S. (2025). Ameliorating Effects of Sesame Oil Against Toxicity. In: Langyan, S., Yadava, P., Yadav, R., Bhardwaj, R. (eds) Sesame: Sustainable Production and Applications. Cereals, Pulses and Oilseeds. Springer, Singapore. https://doi.org/10.1007/978-981-96-0656-6_10

- Hamam, M., Di Vita, G., Zanchini, R., Spina, D., Raimondo, M., Pilato, M., & D'Amico, M. (2022). Consumers' attitudes and purchase intention for a vitamin-enriched extra virgin olive oil. *Nutrients*, 14(8), 1658.
<https://doi.org/10.3390/nu14081658>
- Haque, M., Umadevi, M., Langyan, S. (2025). Challenges and Strategies for Global Sustainable Production, Market Dynamics, and Trade in Sesame. In: Langyan, S., Yadava, P., Yadav, R., Bhardwaj, R. (eds) Sesame: Sustainable Production and Applications. Cereals, Pulses and Oilseeds. Springer, Singapore.
https://doi.org/10.1007/978-981-96-0656-6_2
- Harshitha, G., Rao, C. B. B., Prakash, T. R., & Hussain, S. A. (2021). Effect of hormones on yield and economics of mustard (*Brassica juncea* L.) under southern Telangana Agro-climatic conditions. *International Journal of Environment and Climate Change*, 11(7), 89-94.
<http://library.go4subs.com/id/eprint/127>
- Hayati, A., Hosseini, S. M., Rahimi, M. M., & Kelidari, A. (2022). the effect of humic acid and nano-chelate iron on the absorption of some nutrients, grain yield, oil, and essential oil of (*Nigella sativa*) under drought stress. *Communications in Soil Science and Plant Analysis*, 53(14), 1744-1755.
<https://doi.org/10.1080/00103624.2022.2063319>
- Hilles, A. R., Waly, M. I., & Mahmood, S. (2026). Role of natural biostimulants (seaweed extracts, humic acids, beneficial bacteria, and fungi) in leguminous crops. In *Agricultural Biostimulants for Mitigation of Salt, Drought, and Heat Stress* (pp. 261-288). Academic Press. <https://doi.org/10.1016/B978-0-443-33457-3.00008-2>
- Huang, P., Wang, M., Lu, Z., Shi, S., Wei, X., Bi, C., ... & Wang, B. (2023). Putrescine accelerates the differentiation of bone marrow derived dendritic cells via inhibiting phosphorylation of STAT3 at Tyr705. *International Immunopharmacology*, 116, 109739.
<https://doi.org/10.1016/j.intimp.2023.109739>
- Hussein, H. A. A., Alshammari, S. O., Abd El-Sadek, M. E., Kenawy, S. K., & Badawy, A. A. (2023). The promotive effect of putrescine on growth, biochemical constituents, and yield of wheat (*Triticum aestivum* L.) plants under water stress. *Agriculture*, 13(3), 587.
<https://doi.org/10.3390/agriculture13030587>

- Irshad, S. S., Panwar, N., Bansal, L., Thirumurugan, S., & Kumar, S. (2024). Plant Resistance to Insects in Oilseed Crops. In *Plant Resistance to Insects in Major Field Crops* (pp. 183-214). Singapore: Springer Nature Singapore.
https://doi.org/10.1007/978-981-99-7520-4_8
- JACKSON, M.L., 1973, Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi, pp.498
- Jalilian, S., Mondani, F., Fatemi, A., & Bagheri, A. (2022). Evaluation of animal-and green manures co-application on soil micronutrients and yield of sesame (*Sesamum indicum* L.) in organic farm condition. *Journal of Plant Nutrition*, 45(15), 2348-2359. <https://doi.org/10.1080/01904167.2022.2063738>
- Javed, M., Tahir, M., Iqbal, A., Saleem, M. A., Naveed, M. T., & Kausar, M. (2022). Effect of humic acid application methods on yield and quality of Mungbean (*Vigna radiata* L.). <https://doi.org/10.55627/pbulletin.003.02.0928>
- Jeyaraj, S., & Beevy, S. S. (2024). Insights into the drought stress tolerance mechanisms of sesame: the queen of oilseeds. *Journal of Plant Growth Regulation*, 1-22. <https://doi.org/10.1007/s00344-024-11353-4>
- Joshi, K., Ahmed, S., Ge, L., Avestakh, A., Oloyede, B., Phuntumart, V., ... & Morris, P. F. (2024). Spatial organization of putrescine synthesis in plants. *Plant Science*, 349, 112232. <https://doi.org/10.1016/j.plantsci.2024.112232>
- Jumrani, J., & Meenakshi, J. V. (2023). How effective is a fat subsidy? Evidence from edible oil consumption in India. *Journal of Agricultural Economics*, 74(2), 327-348. <https://doi.org/10.1111/1477-9552.12510>
- Kabato, W., Getnet, G. T., Sinore, T., Nemeth, A., & Molnár, Z. (2025). Towards climate-smart agriculture: Strategies for sustainable agricultural production, food security, and greenhouse gas reduction. *Agronomy*, 15(3), 565.
<https://doi.org/10.3390/agronomy15030565>
- Karmakar, K., Patil, S. S., & Kundu, B. (2024). From Soil to Oil: The Crucial Roles of Potassium and Sulphur in Enhancing Oilseed Crop Quality. *Journal of Advances in Biology & Biotechnology*, 27(8), 335-346.
<https://doi.org/10.9734/jabb/2024/v27i81146>
- Kazemi, K., & Eskandari, H. (2024). Improvement of Water Productivity in the Condition of Irrigation Interruption in the Flowering Stage of Sesame in Response to the Application of Humic Acid and Silicon.
<https://doi.org/10.22084/ppt.2024.29909.2136>

- Khatrri, P., Kumar, P., Shakya, K. S., Kirlas, M. C., & Tiwari, K. K. (2024). Understanding the intertwined nature of rising multiple risks in modern agriculture and food system. *Environment, Development and Sustainability*, 26(9), 24107-24150. <https://doi.org/10.1007/s10668-023-03638-7>
- Khuong, N. Q., Thuc, L. V., Tran, N. T. B., Huu, T. N., & Sakagami, J. I. (2022). Foliar application of boron positively affects the growth, yield, and oil content of sesame (*Sesamum indicum* L.). *Open Agriculture*, 7(1), 30-38. <https://doi.org/10.1515/opag-2022-0067>
- Kibar, H., Kibar, B. Enhancing Cowpea Resilience: Role of Putrescine and Salicylic Acid in Mitigating Salt Stress Effects on Germination, Early Growth and Biochemical Content. *Biol Bull Russ Acad Sci* **52**, 69 (2025). <https://doi.org/10.1134/S1062359024612631>
- Kishk, A. M. (2024). Effect of plant biostimulants, harvesting dates, and storage periods on Sesame (*Sesamum indicum* L.) seed quality in newly reclaimed soils. *Egyptian Journal of Agronomy*, 46(2), 329-341. <https://doi.org/10.21608/agro.2024.304521.1464>
- Kochi, M. S., Dejam, M., Madandoust, M., & Baziar, M. R. (2025). Foliar Application of Salicylic Acid and Jasmonic Acid Enhances Drought Tolerance in Sesame (*Sesamum indicum* L.) by Modulating Antioxidant Defense, Photosynthetic Efficiency and Seed Yield. <https://doi.org/10.21203/rs.3.rs-6303656/v1>
- Kolesnikov, Y. S., Kretynin, S. V., Filepova, R., Dobrev, P. I., Martinec, J., & Kravets, V. S. (2024). Polyamines metabolism and their biological role in plant cells: what do we really know?. *Phytochemistry Reviews*, 23(4), 997-1026. <https://doi.org/10.1007/s11101-024-09913-3>
- Lal, M. A., & Bhatla, S. C. (2023). Lipid metabolism. In *Plant physiology, development and metabolism* (pp. 261-294). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-5736-1_10
- Latino, M. E., De Devitiis, B., Corallo, A., Viscecchia, R., & Bimbo, F. (2022). Consumer acceptance and preference for olive oil attributes—a review. *Foods*, 11(23), 3805. <https://doi.org/10.3390/foods11233805>
- Li, H., Tahir ul Qamar, M., Yang, L., Liang, J., You, J., & Wang, L. (2023). Current progress, applications and challenges of multi-omics approaches in sesame

- genetic improvement. *International Journal of Molecular Sciences*, 24(4), 3105. <https://doi.org/10.3390/ijms24043105>
- Li, M., Luo, J., Nawaz, M. A., Stockmann, R., Buckow, R., Barrow, C., ... & Rasul Suleria, H. A. (2024). Phytochemistry, bioaccessibility, and bioactivities of sesame seeds: An overview. *Food Reviews International*, 40(1), 309-335. <https://doi.org/10.1080/87559129.2023.2168280>
- Li, S., Ye, X., Wen, X., Yang, X., Wang, L., Gao, K., ... & Jiang, Z. (2024). Arginine and its metabolites stimulate proliferation, differentiation, and physiological function of porcine trophoblast cells through β -catenin and mTOR pathways. *BMC Veterinary Research*, 20(1), 167. <https://doi.org/10.1186/s12917-024-04023-w>
- Li, X., Huang, W., Yang, Z., Hu, W., Zhou, Z., & Chen, B. (2025). Leaf and pod growth affect seed yield after shoot removal and different nitrogen rates of dual-purpose rapeseed (*Brassica napus* L.). *Journal of Integrative Agriculture*. <https://doi.org/10.1016/j.jia.2025.04.035>
- Li, Y., Fang, F., Wei, J., Cui, R., Li, G., Zheng, F., & Tan, D. (2021). Physiological effects of humic acid in peanut growing in continuous cropping soil. *Agronomy Journal*, 113(1), 550-559. <https://doi.org/10.1002/agj2.20482>
- Luiz, S. A. R., Yamashita, O. M., de Carvalho, M. A. C., Dresch, E., de Oliveira Domingues, S. C., Dutra, L. A., ... & Cândido, A. C. T. F. (2022). Humic acid and growth promoting microorganisms in the germination and initial development of white sesame plants. *Research, Society and Development*, 11(6), e15511629094-e15511629094. <https://doi.org/10.33448/rsd-v11i6.29094>
- Lukurugu, G. A., Nzunda, J., Kidunda, B. R., Chilala, R., Ngamba, Z. S., Minja, A., & Kapinga, F. A. (2023). Sesame production constraints, variety traits preference in the Southeastern Tanzania: Implication for genetic improvement. *Journal of Agriculture and Food Research*, 14, 100665. <https://doi.org/10.1016/j.jafr.2023.100665>
- Lumactud, R. A., Gorim, L. Y., & Thilakarathna, M. S. (2022). Impacts of humic-based products on the microbial community structure and functions toward sustainable agriculture. *Frontiers in Sustainable Food Systems*, 6, 977121. <https://doi.org/10.3389/fsufs.2022.977121>
- Luo, J. H., Li, J., Shen, Z. C., Lin, X. F., Chen, A. Q., Wang, Y. F., ... & Wang, X. Y. (2023). Advances in health-promoting effects of natural polysaccharides:

- Regulation on Nrf2 antioxidant pathway. *Frontiers in Nutrition*, 10, 1102146. <https://doi.org/10.3389/fnut.2023.1102146>
- Ma, S., Zhou, X., Jahan, M. S., Guo, S., Tian, M., Zhou, R., ... & Shu, S. (2022). Putrescine regulates stomatal opening of cucumber leaves under salt stress via the H₂O₂-mediated signaling pathway. *Plant Physiology and Biochemistry*, 170, 87-97. <https://doi.org/10.1016/j.plaphy.2021.11.028>
- Mabudi Bilasvar, H., Ghassemi-Golezani, K., & Mohammadi Nassab, A. D. (2022). Seed development, oil accumulation and fatty acid composition of drought stressed rapeseed plants affected by salicylic acid and putrescine. *Gesunde Pflanzen*, 74(2), 333-345. <https://doi.org/10.1007/s10343-021-00612-z>
- Maffia, A., Oliva, M., Marra, F., Mallamaci, C., Nardi, S., & Muscolo, A. (2025). Humic substances: Bridging ecology and agriculture for a greener future. *Agronomy*, 15(2), 410. <https://doi.org/10.3390/agronomy15020410>
- Mahdavi, S., Mohammadin, R. N., Ashouri, M., Amiri, E., & Doroudian, H. R. (2021). Effects of Irrigation Management and Humic Acid on Yield and Yield Components of Peanut (*Arachis hypogaea* L.). <https://sid.ir/paper/1001470/en>
- Mahmoud, N. E., & Abdelhameed, R. M. (2022). Postsynthetic modification of ti-based metal–organic frameworks with polyamines and its behavior on biochemical constituents of *Sesamum indicum* L. under heat stress conditions. *ACS Agricultural Science & Technology*, 2(5), 1023-1041. <https://doi.org/10.1021/acsagscitech.2c00169>
- Mathur, R. K., Sujatha, M., Bera, S. K., Rai, P. K., Babu, B. K., Suresh, K., ... & Singh, V. V. (2023). Oilseeds and Oil Palm. In *Trajectory of 75 years of Indian Agriculture after Independence* (pp. 231-264). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-7997-2_10
- Mekdad, A.A.A., El-Sherif, A.M.A., Rady, M.M. *et al.* Culture Management and Application of Humic Acid in Favor of *Helianthus annuus* L. Oil Yield and Nutritional Homeostasis in a Dry Environment. *J Soil Sci Plant Nutr* **22**, 71–86 (2022). <https://doi.org/10.1007/s42729-021-00636-4>
- Mizani, A., Masuod Sinaki, J., Rezvan, S., Abedini Esfahlani, M., & Damavandi, A. (2025). Assessment of foliar and nano-potassium fertilization on physiological and biochemical traits of sesame seed oil, fatty acids, and lignans under different irrigation regimes. *Journal of Plant Nutrition*, 1-26. <https://doi.org/10.1080/01904167.2025.2513410>
- Mohajeri, M., Ayatollahi, S. A., Khandan, M., Mokhtari, S., Hosseinzadeh, V., & Kobarfard, F. (2024). A simple, easy, and efficient HPTLC method for

- simultaneous determination of polyamines (putrescine, spermidine, and spermine) in plant tissues. *Journal of Food Composition and Analysis*, 126, 105835. <https://doi.org/10.1016/j.jfca.2023.105835>
- Morsy, H. Y., Shady, M. A., & Youssef, G. H. (2024). Humic acid and silicon applications for improving fertility of light texture soil and productivity wheat-sesame crops. *Journal of Soil Sciences and Agricultural Engineering*, 15(11), 321-330. <https://doi.org/10.21608/jssae.2024.324193.1256>
- Movahedi, M., Gholami, A., Omran, V.G. *et al.* Mitigating Drought Stress in Lentil (*Lens culinaris* Medik.) Grown Under Dryland Conditions Using *Pseudomonas fluorescens* Inoculation and Spermidine Foliar Application. *Int. J. Plant Prod.* **19**, 825–846 (2025). <https://doi.org/10.1007/s42106-025-00372-0>
- Muhammadamin, S. A., & Mahmood, B. J. (2023). Influence of Combination Between Fertilizer Treatments and Nipping on Growth, Yield and Quality of Sesame (*Sesamum indicum* L.). *Zanco Journal of Pure and Applied Sciences*, 35(1), 38-55. <https://doi.org/10.21271/ZJPAS.35.1.5>
- Nabi, F., Sarfaraz, A., Kama, R., Kanwal, R., & Li, H. (2025). Structure-based function of humic acid in abiotic stress alleviation in plants: a review. *Plants*, 14(13), 1916. <https://doi.org/10.3390/plants14131916>
- Najafi, R., Kappel, N., & Mozafarian, M. (2025). The Role of Exogenously Applied Polyamines to Improve Heat Tolerance in Tomatoes: A Review. *Agriculture*, 15(9), 988. <https://doi.org/10.3390/agriculture15090988>
- Nargesi, M. M., Sedaghatthoor, S., & Hashemabadi, D. (2022). Effect of foliar application of amino acid, humic acid and fulvic acid on the oil content and quality of olive. *Saudi Journal of Biological Sciences*, 29(5), 3473-3481. <https://doi.org/10.1016/j.sjbs.2022.02.034>
- Nassar, K. E. M., ELSonbaty, A. E., & El-Shaboury, H. A. (2020). Influence of potassium humate and micronutrients foliar nutrition on sesame productivity and quality under alluvial soils of Nile Delta. *Journal of Soil Sciences and Agricultural Engineering*, 11(12), 893-900. <https://doi.org/10.21608/jssae.2020.170646>
- Nassiri-Mahallati, M., Jahan, M. Using the AquaCrop model to simulate sesame performance in response to superabsorbent polymer and humic acid application under limited irrigation conditions. *Int J Biometeorol* **64**, 2105–2117 (2020). <https://doi.org/10.1007/s00484-020-02001-z>

- Ninama, A. R., Kumawat, P. D., Bhuriya, K. P., Solanki, B. P., & Muchhadiya, R. M. (2025). Effect of clipping, plant growth regulators and weed management on growth of summer sesame (*Sesamum indicum* L.). <https://www.doi.org/10.33545/2618060X.2025.v8.i6j.3105>
- Noveyri, S. G., Zamani, G. R., & Al-Ahmadi, M. J. (2022). The effect of putrescine and calcium nitrate on physiological properties and sesame (*Sesamum indicum* L.) yield under moisture stress. <https://doi.org/10.22077/escs.2020.3833.1922>
- Oboulbiga, E. B., Douamba, Z., Compaoré-Séréme, D., Semporé, J. N., Dabo, R., Semde, Z., ... & Dicko, M. H. (2023). Physicochemical, potential nutritional, antioxidant and health properties of sesame seed oil: a review. *Frontiers in nutrition*, 10, 1127926. <https://doi.org/10.3389/fnut.2023.1127926>
- Ovalde, A., Karlsons, A., Cekstere, G., Āboliņa, L., & Malecka, S. (2024). Foliar application <https://doi.org/10.21608/jssae.2024.324193.1256> of commercial humic substances for possible increase of nutrient status and yield of oilseed rape. *Environmental & Experimental Biology*, 22(1). <http://doi.org/10.22364/eeb.22.01>
- Piccolo, A., Drosos, M. The essential role of humified organic matter in preserving soil health. *Chem. Biol. Technol. Agric.* **12**, 21 (2025). <https://doi.org/10.1186/s40538-025-00730-0>
- Puente-Moreno, J., Garrido-Auñón, F., García-Pastor, M. E., Valero, D., & Serrano, M. (2025). The renaissance of polyamines: new roles in crop yield and quality properties in freshly fruit. *Agronomy*, 15(1), 201. <https://doi.org/10.3390/agronomy15010201>
- Raj, A., Kumar, P. and Choudhury, D. (2025) Impact of humic acid and putrescine application on disease severity and inhibition in sesame phyllody. *Biopestic. Int.*, 21, 49–55. <https://doi.org/10.59467/BI.2025.21.49>
- Rajput, A., Panhwar, Q. A., & Babar, H. (2024). Role of leguminous crops by enhancing soil fertility and plant nutrition. <https://doi.org/10.5772/intechopen.1006827>
- Rathor, P., Gorim, L. Y., & Thilakarathna, M. S. (2023). Plant physiological and molecular responses triggered by humic based biostimulants-a way forward to sustainable agriculture. *Plant and Soil*, 492(1), 31-60. <https://doi.org/10.1007/s11104-023-06156-7>

- Rauf, S., Basharat, T., Gebeyehu, A., Elsafy, M., Rahmatov, M., Ortiz, R., & Kaya, Y. (2024). Sesame, an underutilized oil seed crop: Breeding achievements and future challenges. *Plants*, 13(18), 2662. <https://doi.org/10.3390/plants13182662>
- Reddy, T. V., Patel, P. P., Patel, B. R., & Patel, G. N. (2021). Integrated nitrogen management, humic and fulvic acids on performance of sesame (*Sesamum indicum*). <https://doi.org/10.22271/09746315.2021.v17.i2.1449>
- Sagun, J. V., Yadav, U. P., & Alonso, A. P. (2023). Progress in understanding and improving oil content and quality in seeds. *Frontiers in plant science*, 14, 1116894. <https://doi.org/10.3389/fpls.2023.1116894>
- Salam, U., Ullah, S., Tang, Z. H., Elateeq, A. A., Khan, Y., Khan, J., ... & Ali, S. (2023). Plant metabolomics: an overview of the role of primary and secondary metabolites against different environmental stress factors. *Life*, 13(3), 706. <https://doi.org/10.3390/life13030706>
- Salama, S. H. (2023). Effect of Foliar Application with Putrescine and Benzyladenine on Vegetative Growth, Yield and Its Components and Seed Oil Contents of Chia (*Salvia Hispanica* L.) Plants Grown under Water Stress Conditions. *Annals of Agricultural Science, Moshtohor*, 61(3), 681-694. <https://doi.org/10.21608/assjm.2024.245537.1252>
- Salamati, N. , RAFIE, M. and omidvari, S. (2024). Investigation of the effects of Biostimulants and morpho-physiological traits of sesame with drip irrigation on water productivity indices. *Iranian Journal of Soil and Water Research*, 55(10), 1921-1937. <https://doi.org/10.22059/ijswr.2024.369953.669623>
- Sangeetha, A., Subrahmaniyan, K., Mahalingam, A., Veeramani, P., Rajavel, M., Harisudan, C., ... & Baskaran, R. (2025). Sesame Stalk Compost in Soil Revitalization and Long-term Sustainable Crop Productivity in Organic Sesame (*Sesamum indicum* L.). *Communications in Soil Science and Plant Analysis*, 56(4), 567-591. <https://doi.org/10.1080/00103624.2024.2425413>
- Shah, S. H., Islam, S., Alamri, S., Parrey, Z. A., Mohammad, F., & Kalaji, H. M. (2023). Plant growth regulators mediated changes in the growth, photosynthesis, nutrient acquisition and productivity of mustard. *Agriculture*, 13(3), 570. <https://doi.org/10.3390/agriculture13030570>
- Shahdadi, M. M., Dejam, M., Madandoust, M., & Baziar, M. R. (2025). The Effect of Chemical and Biological Fertilizers on Yield, Yield Components and Protein

Content of Sesame Grain (*Sesamum indicum* L.) under Different Irrigation Regimes. *Iranian Journal of Field Crops Research*, 22(4).
<https://doi.org/10.22067/jcsc.2024.87509.1317>

- Sharma, P., Lakra, N., Ahlawat, Y., Zaid, A., Abd-ElGawad, A. M., Elansary, H. O., & Gupta, A. (2023). Putrescine mitigates high temperature effects by modulating morpho-physiological and biochemical attributes in *Brassica juncea* seedlings. *Agronomy*, 13(7), 1879. <https://doi.org/10.3390/agronomy13071879>
- Sharma, S., Kashyap, L., Goyal, A., Bala, M., & Singh, M. (2025). Techniques for Sesame Oil Extraction and Its Quality Analysis and Comparison with Other Oilseed Crops. In *Sesame: Sustainable Production and Applications* (pp. 107-134). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-96-0656-6_6
- Singh, S., Sharma, S., Sarma, S. J., & Brar, S. K. (2023). A comprehensive review of castor oil-derived renewable and sustainable industrial products. *Environmental Progress & Sustainable Energy*, 42(2), e14008. <https://doi.org/10.1002/ep.14008>
- Singhal, R. K., Kumar, M., Bose, B., Mondal, S., Srivastava, S., Dhankher, O. P., & Tripathi, R. D. (2023). Heavy metal (loid) s phytotoxicity in crops and its mitigation through seed priming technology. *International journal of phytoremediation*, 25(2), 187-206. <https://doi.org/10.1080/15226514.2022.2068502>
- Srivastava, K., & Srivastava, A. (2023). Advances in Oilseeds Production Technologies. In *Advances in Crop Production and Climate Change* (pp. 143-183). CRC Press. <https://doi.org/10.1201/9781003281948>
- Subrahmaniyan, K., Veeramani, P., Mahalingam, A., Ranjith, R., Harishma, P. H., & Abarajitha, P. (2024). A comprehensive review on nutritional and antioxidant properties of Sesame (*Sesamum indicum* L.) seed oil with its therapeutic utilization as phytomedicine. *Annals of Phytomedicine*, 13(2), 1-9. <http://dx.doi.org/10.54085/ap.2024.13.2.71>
- Suganthi, D., Kumar, R., Rojh, P. *et al.* Supply Response to Policy Changes: Evidence from Indian Oilseeds Sector. *J. Quant. Econ.* **23**, 577–599 (2025). <https://doi.org/10.1007/s40953-024-00434-6>
- Sumara, A., Stachniuk, A., Montowska, M., Kotecka-Majchrzak, K., Grywalska, E., Mitura, P., ... & Fornal, E. (2023). Comprehensive review of seven plant seed oils: chemical composition, nutritional properties, and biomedical

- functions. *Food reviews international*, 39(8), 5402-5422.
<https://doi.org/10.1080/87559129.2022.2067560>
- Swamikannu, N., Okori, P., Mushoriwa, H., Kumar, A. A., Gupta, S. K., Kumar, S., ... & Kumar, A. (2024). Outlook on Global Oilseed Sector: Research for Development Opportunities. <https://hdl.handle.net/10568/168977>
- Tang, S., Wang, P., Xing, H., Huang, Z., Liu, P., Li, T., & Huang, J. (2024). Consumer Preferences and Willingness to Pay for High-Quality Vegetable Oils: A Cross-Sectional Analysis of Chinese Residents. *Foods*, 13(8), 1168.
<https://doi.org/10.3390/foods13081168>
- Tyagi, A., Ali, S., Ramakrishna, G., Singh, A., Park, S., Mahmoudi, H., & Bae, H. (2023). Revisiting the role of polyamines in plant growth and abiotic stress resilience: mechanisms, crosstalk, and future perspectives. *Journal of Plant Growth Regulation*, 42(8), 5074-5098. <https://doi.org/10.1007/s00344-022-10847-3>
- Upadhyay, A. K., & Sahu, R. (2012). Determination of total nitrogen in soil and plant. *Laboratory manual on advances in agro-technologies for improving soil, plant and atmosphere systems*, 18-19.
- Wahid, N., Wahab, S., Iqbal, S., Kanwal, Khan, M. N., Hayat, K., ... & Ali, A. (2025). Biostimulants as a Sustainable Strategy to Augment Salinity Tolerance in Oilseed Crops. In *Oilseed Crops Under Abiotic Stress: Mitigation Strategies and Future Perspectives* (pp. 1-50). Singapore: Springer Nature Singapore.
https://doi.org/10.1007/978-981-96-8346-8_1
- Wang, X., Zhang, J., Shen, J., Zhang, L., Wei, P., Liu, A., & Song, H. (2024). The alleviating effect on the growth, chlorophyll synthesis, and biochemical defense system in sunflowers under cadmium stress achieved through foliar application of humic acid. *BMC Plant Biology*, 24(1), 792.
<https://doi.org/10.1186/s12870-024-05516-4>
- Weerasekara, I., Sinniah, U. R., Namasivayam, P., Nazli, M. H., Abdurahman, S. A., & Ghazali, M. N. (2021). Priming with humic acid to reverse ageing damage in soybean [*Glycine max* (L.) Merrill.] seeds. *Agriculture*, 11(10), 966.
<https://doi.org/10.3390/agriculture11100966>
- Yadav, R., Kalia, S., Rangan, P., Pradheep, K., Rao, G. P., Kaur, V., ... & Siddique, K. H. (2022). Current research trends and prospects for yield and quality improvement in sesame, an important oilseed crop. *Frontiers in Plant Science*, 13, 863521. <https://doi.org/10.3389/fpls.2022.863521>

- Yadav, S., Langyan, S. (2025). Toward Sustainable Agriculture of Sesame Crop: Challenges and Recommendations. In: Langyan, S., Yadava, P., Yadav, R., Bhardwaj, R. (eds) Sesame: Sustainable Production and Applications. Cereals, Pulses and Oilseeds. Springer, Singapore. https://doi.org/10.1007/978-981-96-0656-6_13
- Yang, H., Fang, Y., Liang, Z., Qin, T., Liu, J. H., & Liu, T. (2024). Polyamines: pleiotropic molecules regulating plant development and enhancing crop yield and quality. *Plant Biotechnology Journal*, 22(11), 3194-3201. <https://doi.org/10.1111/pbi.14440>
- Yang, M., Zhou, D., Hang, H., Chen, S., Liu, H., Su, J., ... & Zhao, G. (2024). Effects of balancing exchangeable cations Ca, Mg, and K on the growth of tomato seedlings (*Solanum lycopersicum* L.) based on increased soil cation exchange capacity. *Agronomy*, 14(3), 629. <https://doi.org/10.3390/agronomy14030629>
- Yang, Y., Wang, L., Deng, Q., Liu, Y., & Zhou, Q. (2025). What contributes to the richness and stability of the sesame flavor?. *Comprehensive Reviews in Food Science and Food Safety*, 24(3), e70155. <https://doi.org/10.1111/1541-4337.70155>
- Zhao, Q., Wang, F., Xiong, A., Li, S., Wang, Y., Lei, X., ... & Zhang, J. (2025). Regulation of fatty acid synthesis in oilseed crops: multidimensional insights and strategies for enhancing oil quality. *Critical Reviews in Biotechnology*, 45(8), 1737-1753. <https://doi.org/10.1080/07388551.2025.2529591>
- Zhu, J., Dai, W., Chen, B., Cai, G., Wu, X., & Yan, G. (2023). Research progress on the effect of nitrogen on rapeseed between seed yield and oil content and its regulation mechanism. *International Journal of Molecular Sciences*, 24(19), 14504. <https://doi.org/10.3390/ijms241914504>
- Zio, S., Tarnagda, B., Tapsoba, F., Zongo, C., & Savadogo, A. (2024). Health interest of cholesterol and phytosterols and their contribution to one health approach. *Heliyon*, 10(21). <https://doi.org/10.1016/j.heliyon.2024.e40132>

LIST OF PUBLICATIONS

RESEARCH/REVIEW

1. **Raj, A.** and Kumar, P. (2026) Biotic and abiotic elicitation strategies for improved bioactive compound production in sesame in vitro cultures. *Biopestic. Int.*, 22, 1–5. <https://doi.org/10.59467/BI.2026.22.X>
2. Singh, R., Chaudhary, M. and **Raj, A.** (2026) Effect of integrated nutrient management on growth, productivity and profitability of baby corn (*Zea mays* L.) *Biopestic. Int.*, 22, 1–5. <https://doi.org/10.59467/BI.2026.22.X>
3. **Amit, R.**, Sujatha, H. T., Ramandeep, S., Manisha, C., Maharabam, A. D., and Surya, D. S. (2026). Influence of foliar application of urea and micronutrients on growth, yield attributes and nutrient uptake of green gram (*Vigna radiata* L.). *Plant Science Today*, 13(sp2). <https://doi.org/10.14719/pst.12718>
4. Chhajaj AK, Gour VS, Kumar P, **Raj A**, Tayal P, Rathi D, Sahil M, Kothari SL. Biotechnology of millets: Current scenario and future prospects (under agricultural biotechnology for climate-resilient crops). *J Appl Biol Biotech* 2026. Article in Press. <http://doi.org/10.7324/JABB.2026.273153>
5. **Raj, A.**, Kumar, P. and Choudhury, D. (2025) Agrochemical effects on growth traits, physiology, and yield outcomes in sesame (*Sesamum indicum* L.). *Biopestic. Int.*, 21, 143–150. <https://doi.org/10.59467/BI.2025.21.143>
6. **Raj, A.**, Kumar, P. and Choudhury, D. (2025) Impact of humic acid and putrescine application on disease severity and inhibition in sesame phyllody. *Biopestic. Int.*, 21, 49–55. <https://doi.org/10.59467/BI.2025.21.49>
7. Goutam, E., Kumar, A., Tripathi, V., Bharti, Kumar, L., **Raj, A.** (2024). Unveiling mechanisms for induced systemic resistance, resistance breeding and molecular marker-assisted breeding against Phomopsis blight of *Solanum melongena*. *Plant Pathology*, 73(4), 777-790.
8. Patel, M., **Raj, A.**, Devi, M. A., Singh, S. D., Patel, V. K. (2024). Crop residues management: A sustainable approach for rice and wheat production in Indo-Gangetic plains. *International Journal of Research in Agronomy*, 7(6), 24-32.

9. Sachin, S. H., Korav, S. B., **Raj, A.** (2022). Performance of soybean as influenced by foliar application of macro and micro nutrients. *The Pharma Innovation Journal*, 11(8), 967-972.

BOOK CHAPTERS (SPRINGER/ ELSEVIER PUBLISHERS)

1. Kumar, P., **Raj, A.**, & Upadhyay, S. K. (2026). Osmotic regulation and nutrient role. In *Mineral Nutrients to Mitigate Abiotic Stress in Crops* (pp. 435-461). Academic Press. <https://doi.org/10.1016/B978-0-443-44712-9.00016-5>
2. Kumar, P., **Raj, A.**, Dey, S. R., Singh, J., and Gogia, N. (2026). Light and Plant Life: Photoperiodism and Photomorphogenesis. In *Introduction to Rhythms in Plants* (pp. 161-200). Singapore: Springer Nature Singapore. <https://doi.org/10.1007/978-981-95-3592-77>
3. Kumar, P., **Raj, A.**, Patel, M., and Morya, S. (2026). Tools and technologies for metabolic engineering for bioproduction or biosynthesis. In *Novel Bioproducts through Metabolic Engineering of Secondary Metabolites* (pp. 165-194). Academic Press. <https://doi.org/10.1016/B978-0-443-40529-7.00016-0>
4. **Raj, A.**, and Kumar, P. (2026). Rodenticides and their impact on brain function. In N. Gogia and P. Kumar (Eds.), *Pesticides and neurotoxicant exposures* (pp. 213–227). Academic Press. <https://doi.org/10.1016/B978-0-443-44287-2.00014-4>
5. **Raj, A.**, and Kumar, P. (2026). Oxidative stress, reactive oxygen species, and mitochondrial dysfunction in pesticide toxicity. In N. Gogia and P. Kumar (Eds.), *Pesticides and neurotoxicant exposures* (pp. 245–259). Academic Press. <https://doi.org/10.1016/B978-0-443-44287-2.00017-X>
6. **Raj, A.**, and Kumar, P. (2026). Attention-deficit hyperactivity disorder and pesticide exposure. In N. Gogia and P. Kumar (Eds.), *Pesticides and neurotoxicant exposures* (pp. 389–404). Academic Press. <https://doi.org/10.1016/B978-0-443-44287-2.00027-2>
7. **Raj, A.**, and Kumar, P. (2026). Autophagy, apoptosis, and cell death pathways in pesticide toxicity. In N. Gogia and P. Kumar (Eds.), *Pesticides and neurotoxicant exposures* (pp. 329–344). Academic Press. <https://doi.org/10.1016/B978-0-443-44287-2.00023-5>

8. **Raj, A.**, and Kumar, P. (2026). Blood–brain barrier disruption mechanisms by pesticides. In N. Gogia and P. Kumar (Eds.), *Pesticides and neurotoxicant exposures* (pp. 313–327). Academic Press. <https://doi.org/10.1016/B978-0-443-44287-2.00021-1>
9. Kumar, P., and **Raj, A.** (2025). Nanoencapsulation techniques for controlled release of agrochemicals. In *Sustainable Era of Nanomaterials: Opening Up a Path for a Green Future* (pp. 147-184). Singapore: Springer Nature Singapore. <https://doi.org/10.1007/978-981-96-4471-18>
10. Kumar, P., Choudhury, D., Dey, S. R., and **Raj, A.** (2025). Nanofertilizers/nanopesticides for national economy In *Nanofertilizers for Sustainable Agriculture: Assessing Impacts on Health, Environment, and Economy* (pp. 407-434). Cham: Springer Nature Switzerland. <https://doi.org/10.1007/978-3-031-78649-517>
11. Kumar, P., and **Raj, A.** (2025). Degradation of misfolded proteins by ubiquitin proteasome system and autophagy lysosomal proteolytic pathways. In *Protein misfolding in neurodegenerative diseases* (pp. 253-289). Academic Press. <https://doi.org/10.1016/B978-0-443-18716-2.00007-7>
12. **Raj, A.**, and Kumar, P. (2025). Advances in methods to detect and study protein aggregation. In *Protein misfolding in neurodegenerative diseases* (pp. 51-65). Academic Press. <https://doi.org/10.1016/B978-0-443-18716-2.00003-X>
13. **Raj, A.**, Kumar, P., and Choudhury, D. (2025). Protein aggregation and its presence and confirmation. In *Protein Misfolding in Neurodegenerative Diseases* (pp. 27-50). Academic Press. <https://doi.org/10.1016/B978-0-443-18716-2.00002-8>
14. **Raj, A.**, Kumar, P., Choudhury, D., and Dey, S. R. (2025). Non-neurological diseases versus protein misfolding: Conflicts and opportunities. In *Protein Misfolding in Neurodegenerative Diseases* (pp. 525-549). Academic Press. <https://doi.org/10.1016/B978-0-443-18716-2.00015-6>
15. **Raj, A.**, and Kumar, P. (2025). Advancements in drug delivery technologies to treat protein misfolding diseases. In *Protein Misfolding in Neurodegenerative Diseases* (pp. 381-412). Academic Press. <https://doi.org/10.1016/B978-0-443-18716-2.00011-9>

16. Kumar, P., **Raj, A.**, Verma, K. K., Kumari, A., Shanthi, R., Singh, S. K., and Garg, V. K. (2025). Utilization of sugarcane germplasm for breeding climate-resilient varieties: a critical research topic. In *Revolutionizing Sugarcane Molecular Breeding and Biotechnological Approaches: Current Status and Future Strategies* (pp. 67-111). Singapore: Springer Nature Singapore. <https://doi.org/10.1007/978-981-96-6871-73>
17. **Raj, A.**, Kumar, P., Verma, K. K., Kumari, A., Enrique, R., and Sharma, R. (2025). Sugarcane Breeding: Current Status and Breeding Priorities. *Revolutionizing Sugarcane Molecular Breeding and Biotechnological Approaches: Current Status and Future Strategies*, 179-199. <https://doi.org/10.1007/978-981-96-6871-77>
18. Kumar, P., **Raj, A.**, Suneel Kumar, V. V., Tshering, U., and Daza. (2025). Longevity and Sustainability in Design: Approaches to Reducing Waste Through Durable Products. In *Sustainable Urban Future: Embracing Circular Economy in Waste Management* (pp. 155-184). Singapore: Springer Nature Singapore. <https://doi.org/10.1007/978-981-95-2976-68>
19. Kumar, P., **Raj, A.**, Bathula, J., Reddy, P. V., Kumar, A. L., and Kale, I. (2025). Rooting for Growth: Mycorrhizal Impact on Forestry Ecosystems. In *Sustainable Mycorrhizal Cultivation: Innovations for Hillside Farming Systems* (pp. 221-245). Singapore: Springer Nature Singapore. <https://doi.org/10.1007/978-981-95-1778-79>
20. Kumar, P., Agarwal, K., Choudhury, D., and **Raj, A.** (2025). Electro-Fermentation for the Circular Carbon Economy. In *Electro-Fermentation for Synthesis of Chemicals, Biochemicals and Biofuels* (pp. 377-415). Singapore: Springer Nature Singapore. <https://doi.org/10.1007/978-981-96-6358-316>
21. **Raj, A.**, and Kumar, P. (2025). Heavy metals and amyotrophic lateral sclerosis. In *Heavy Metal Toxicity and Neurodegeneration* (pp. 263-280). Academic Press. <https://doi.org/10.1016/B978-0-443-36575-1.00002-9>
22. **Raj, A.**, and Kumar, P. (2025). Parkinson's disease and heavy metal exposure. In *Heavy Metal Toxicity and Neurodegeneration* (pp. 231-249). Academic Press. <https://doi.org/10.1016/B978-0-443-36575-1.00010-8>

23. Kumar, P., **Raj, A.**, Patel, M., and Tshering, U. (2025). Biological and Biomedical Applications of Zirconia. In Handbook of Zirconia-Based Materials (pp. 310-342). CRC Press. <https://doi.org/10.1201/9781003519300-20>
24. **Raj, A.**, Goutam, E., Kumar, P., and Singh, J. (2024). Polyphenol oxidase: an enzyme of plants. Polyphenol oxidases: Function, wastewater remediation, and biosensors, 105 128.
25. Kumar, P., **Raj, A.**, and Kumar, V. A. (2024). Approach to reduce agricultural waste via sustainable agricultural practices. In Valorization of biomass wastes for environmental sustainability: green practices for the rural circular economy (pp. 21-50). Cham: Springer Nature Switzerland. <https://doi.org/10.1007/978-3-031-52485-12>

PATENT PUBLISHED

1. Kumar, P., **Raj, A.**, 2025. Method for enhancing maize yield through application of boron, 6-benzylaminopurine, oleic acid, and salicylic acid. Indian Patent Application No. 202511073177 A. Lovely Professional University, India. Filed: 01/08/2025; Published: 22/08/2025.
2. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Formulation for enhancing carbon enrichment in soil to promote sustainable agricultural practices. Indian Patent Application No. 202411102293 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
3. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Formulation for abiotic stress removal in plants. Indian Patent Application No. 202411102317 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
4. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Method and composition for enhancing protein content in crops under abiotic stress conditions. Indian Patent Application No. 202411102318 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
5. Kumar, P., **Raj, A.**, Rao, P.P., 2025. System for enhancing nutrient uptake efficiency in crop roots. Indian Patent Application No. 202411102313 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.

6. Kumar, P., **Raj, A.**, Rao, P.P., 2025. System for comprehensive analysis of lung function and health. Indian Patent Application No. 202411102321 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
7. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Novel formulation for effective control of soil salinity and improvement of crop yield. Indian Patent Application No. 202411102358 A. Lovely Professional University, India. Filed: 24/12/2024; Published: 03/01/2025.
8. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Formulation for enhancing crop resilience and yield through antioxidant gene expression. Indian Patent Application No. 202411102287 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
9. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Controlled release system of nutrients and water in crop rhizospheres for enhanced growth and sustainability. Indian Patent Application No. 202411102294 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
10. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Formulation for mitigating fluoride-indexed toxicity symptoms and enhancing crop yield. Indian Patent Application No. 202411102315 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
11. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Formulation for enhanced phytoremediation and crop protection. Indian Patent Application No. 202411102316 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
12. Kumar, P., **Raj, A.**, Rao, P.P., 2025. System for mitigating fluoride toxicity in soil and plants. Indian Patent Application No. 202411102320 A. Lovely Professional University, India. Filed: 23/12/2024; Published: 03/01/2025.
13. Kumar, P., **Raj, A.**, Rao, P.P., 2025. Automated graded biomass processing system. Indian Patent Application No. 202411103519 A. Lovely Professional University, India. Filed: 27/12/2024; Published: 10/01/2025.
14. Choudhury, D., Kumar, P., **Raj, A.**, 2025. Method for managing sesame phyllody disease using *Beauveria bassiana*-based biological formulations for vector control. Indian Patent Application No. 202511107294 A. Lovely Professional University, India. Filed: 06/11/2025; Published: 26/12/2025.

15. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Innovative nanomaterials for enhanced stability and transport of ice crystals. Indian Patent Application No. 202411089873 A. Lovely Professional University, India. Filed: 20/11/2024; Published: 06/12/2024.
16. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Controlled release pesticide filter bag for minimizing soil contamination. Indian Patent Application No. 202411090307 A. Lovely Professional University, India. Filed: 21/11/2024; Published: 06/12/2024.
17. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Innovative method for converting food waste into nutritional feed for cattle. Indian Patent Application No. 202411090310 A. Lovely Professional University, India. Filed: 21/11/2024; Published: 06/12/2024.
18. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Artificial soil environment for enhanced nitrogen fixation and climate change mitigation. Indian Patent Application No. 202411090311 A. Lovely Professional University, India. Filed: 21/11/2024; Published: 06/12/2024.
19. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Innovative nanomaterials for enhanced biological and nucleic acid pesticide formulations. Indian Patent Application No. 202411089869 A. Lovely Professional University, India. Filed: 20/11/2024; Published: 06/12/2024.
20. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Innovative reactive oxygen species (ROS) scavenger for climate change resilience. Indian Patent Application No. 202411090314 A. Lovely Professional University, India. Filed: 21/11/2024; Published: 06/12/2024.
21. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Soil nutrient trapping bag for enhanced nutrient retention and release in agricultural crops. Indian Patent Application No. 202411089879 A. Lovely Professional University, India. Filed: 20/11/2024; Published: 06/12/2024.
22. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Nanobiointeraction-based adaptive system for enhancing agricultural crop resilience in changing climate conditions. Indian Patent Application No. 202411089884 A. Lovely Professional University, India. Filed: 20/11/2024; Published: 06/12/2024.

23. Kumar, P., **Raj, A.**, Rao, P.P., 2024. Plant-based system for greenhouse gas emission control and carbon sequestration. Indian Patent Application No. 202411090313 A. Lovely Professional University, India. Filed: 21/11/2024; Published: 06/12/2024.

GRANTED COPYRIGHTS

1. **Raj, Amit.**, Kumar, P., 2026. Impact of agrochemical treatments on morpho physiological traits and yield performance in sesame (*Sesamum indicum* L.). Reg No. LD-52549/2025-CO, Date: 2025, Copyright Office, Government of India.
2. Dey, Shipa Rani., **Raj, Amit.**, Kumar, P., 2024. Protein folding, misfolding and aggregation. Reg No. L-154329/2023, Date- 24/09/2024, Diary Number: 24639/2024 CO/L, Copyright Office Govt. of India.
3. Dey, Shipa Rani., **Raj, Amit.**, Kumar, P., 2023. Molecular Chaperon Network to Treat Protein Misfolding Diseases. Reg No. L- 135419/2023, Date- 03/11/2023, Diary Number: 20422/2023-CO/L, Copyright Office Govt. of India.
4. Dey, Shipa Rani., **Raj, Amit.**, Kumar, P., 2023. Advancements in Drug Delivery Technologies to Treat Protein Misfolding Diseases. Reg No. L-136458/2023, Date- 21/11/2023, Diary Number: 20423/2023-CO/L, Copyright Office Govt. of India.
5. Dey, Shipa Rani., **Raj, Amit.**, Kumar, P., 2023. Phytoremediation: An Alternative Tool Towards Clean and Green Environment. Reg No. L-135406/2023, Date- 03/11/2023, Diary Number: 20520/2023-CO/L, Copyright Office Govt. of India.
6. Dey, Shipa Rani., **Raj, Amit.**, Kumar, P., 2023. Avenue of phytoremediation strategies for fluorine toxicity. Reg No. L-137652/2023, Date- 12/11/2023, Diary Number: 22961/2023-CO/L, Copyright Office Govt. of India.
7. Goutam, E., Kumar, A., Tripathi, V., Bharti., Kumar, L., **Raj, Amit.**, 2023. Pathogenesis, prevalence and overcoming measure of Phomopsis blight in brinjal. Reg No. L-124991/2023, Date- 12/06/2023, Diary Number: 26933/2022-CO/L, Copyright Office Govt. of India.

CONFERENCES

1. Oral Presentation presented a paper entitled Impact of humic acid and putrescine application on growth and yield in sesamum indicum at The International Conference on Recent Trends in Smart and Sustainable Agriculture for Food and Nutritional Security (SSAFNS-2024), 2024, held at Lovely Professional University, Phagwara, India.
2. Oral Presentation presented a paper entitled Impact of humic acid and putrescine application on growth and oil quality of sesame at The 7th International Conference on Advances in Agriculture Technology and Sciences (ICAATAS-2024), 2024, held at The Neotia University, West Bengal, India.
3. Oral Presentation presented a paper entitled Impact of humic acid and putrescine application on growth and yield in sesamum indicum at The International Conference on Recent Trends in Smart and Sustainable Agriculture for Food and Nutritional Security (SSAFNS-2025), 2025, held at Lovely Professional University, Phagwara, India.