Comparative Study of Bio-degradation of Pesticides in the Presence of Metals Ions and Humic acid

A

Thesis

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(BIOTECHNOLOGY)

By

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DECLARATION

I hereby declare that the thesis entitled, **"Comparative study of bio-degradation of Pesticides in the presence of metals ions and humic acid"** submitted for PhD Biotechnology degree to Department of Biotechnology, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for the award of any other degree.

Simranjeet Singh
Reg. No. 11209166

CERTIFICATE

This is to certify that **Mr Simranjeet Singh** has completed the PhD Biotechnology titled **"Comparative study of bio-degradation of Pesticides in the presence of metals ions and humic acid"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma.

The thesis is fit for the submission for the partial fulfilment of the condition for the award of the degree of PhD in Biotechnology.

Signature of Supervisor

(Dr Joginder Singh Panwar)
Associate Professor
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ABSTRACT

This thesis addresses "***Comparative study of bio-degradation of Pesticides in the presence of metals ions and humic acid***" of six different pesticides (acephate, atrazine, carbendazim, glyphosate, monocrotophos and phorate). Within the scope of the thesis, following four points are the highlights of thesis:

1) Isolation and identification of beneficial indigenous bacteria that survives in the high concentration of the pesticides.
2) Influence of metal ions (Cu^{++} and Fe^{++}) and soil components (humic acid) on biodegradation and their mode of bonding.
3) Influence of six different pesticides on the functional attributes of the plant growth promoting rhizobacteria.
4) Influence of six different pesticides on wheat growth parameters, chlorophyll and total carotenoids with respect to metal ions and humic acid.

<u>In the whole thesis, following points are the main outcomes</u>

It was observed that in the presence of Cu^{++}, Fe^{++} and humic acid, the rate of biodegradation of six pesticides depends on the chelating activity of the ligand and availability of coordinating sites of the pesticides. The observed rate of degradation was slow in the presence of humic acid, because of its neutralization effect or steric hindrance of bulky molecule humic acid.

19 different bacteria of the genus *Actinomyces, Bacillus, Pseudomonas, Rhizobium* and *Streptomyces* were isolated, identified on the basis of 16s rRNA sequencing and submitted to NCBI for accession numbers. Furthermore, they are assessed to check their role in biodegradation of six pesticides in the presence of Cu^{++} and Fe^{++} and humic acid.

In biodegradation of acephate, four different metabolites were observed metamidophos m/z at 141, phosphoramic acid m/z ratio at 96, phosphenamide at m/z 78 and one unidentified compound at m/z 62. The biodegradation of acephate was highly facilitated by the addition of Cu^{++} because Cu^{++} facilitates the regular decomposition due to its paramagnetic in nature & it assists in decomposition through -N-CO- bond. In biodegradation of atrazine, two major metabolites were identified as ((ethylamino) methylamino) methanediol m/z at 120, and aminomethanediol at m/z 63. Glyphosate degraded into methyl phosphonic acid; m/z 97 and phosphoric acid; m/z 79 after 7 days inoculated with bacterial strains. Similarly, in case of

glyphosate and carbendazim, Cu^{++} spurs the degradation rate. In addition to Fe^{++} and humic acid to pesticides initially, its gets interacted with it and make complex suitable for decomposition by microorganisms. In some cases, they both also assist in the faster degradation of the pesticides. In case of atrazine, monocrotophos and phorate no such results were observed by the addition of Cu^{++}, Fe^{++} or humic acid.

As carbendazim, glyphosate and acephate behave as strong chelating agents, their biodegradation rate is highly influenced by Cu^{++}, Fe^{++} and humic acid. Monocrotophos and phorate act as intermediate chelating agents, so there is no much difference in the degradation rate while atrazine acts as weak chelating agents and degradation rate is almost similar to the samples treated without humic acid and metal ions.

Similar experiments were performed to check the influence of these pesticides on plant growth promoting attributes of the rhizobacteria. Highest production of Indole acetic acid was observed in Strains *Rhizobium leguminosarum* GP3, *Rhizobium leguminosarum* MC3 and *Rhizobium leguminosarum* strain RK4 respectively, with production concentration up to 95, 94.5 and 89 µg/mL as compared to standard strains *Rhizobium leguminosarum* of 48.1 and *E. coli* 10 µg/mL. Out of 19 isolates, only 15 strains were capable to solubilize phosphorous both qualitatively and quantitatively. Highest solubilization efficacy was observed in most of the species of *Rhizobium* and *Pseudomonas*. Species of *Rhizobium* and *Pseudomonas* also possess a positive effect of sidherophoric production. However, additions of Cu^{++} possess a negative impact on hormones production while Fe^{++} and humic acid induces hormonal production. The concentration in the production of various hormones like indole acetic acid, solubilization of phosphate, siderophore production etc. is decreased by increasing the concentration of pesticide (2x) at significant level $p < 0.05$.

The negative effect of six different pesticides on the plant growth is in the order of glyphosate > phorate > monocrotophos > acephate > atrazine > carbendazim. All the selected pesticide hinders the growth rate when applied in a concentration above the recommended dose. Similar effects were observed on the concentrations of photosynthetic pigments production by the plant. Glyphosate was found to exert a most toxic effect on all the parameters including germination percentage, chlorophyll content and carotenoid content. With the addition of metal ions and humic acid, there is threshold increase in germination percentage rate of the wheat in all the cases at significant level $p < 0.05$. The effect of pesticides on radical and plumule germination is almost similar to the germination percentage test at two different concentrations of pesticides.

Effects of six pesticides, metal ions and humic acid on wheat growth parameters, chlorophyll content and total carotenoids were also carried out. With the addition of metal ions and humic acid, increase in plant growth and chlorophyll content were observed at significant level $p < 0.05$. It also reflects the negative impact of pesticides on wheat growth germination and on its parameters.

In future, detailed molecular levels studies are required to be conducted to find the exact mechanism behind the neutralization effect, the role of various enzymes to increase the efficacy of biodegradation rate and predicting other biochemical pathways of pesticide degradation.

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(**Simranjeet Singh**)

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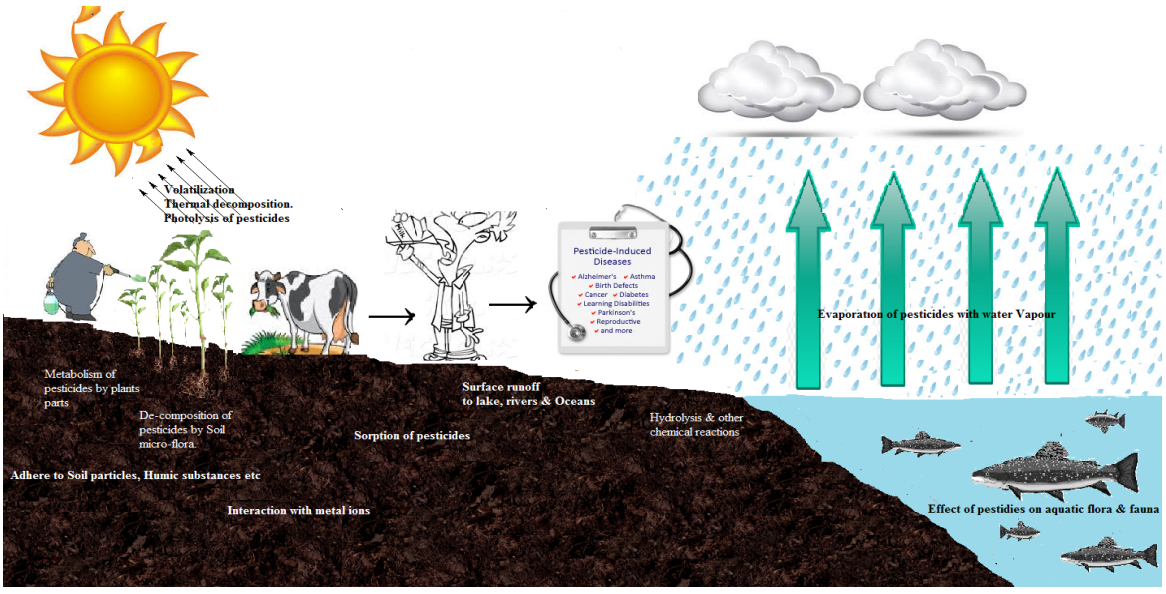
INTRODUCTION

1.1 Introduction

There is tremendous pressure to increase the production of food grains and fruits as a human population have crossed 7.4 billion. Being the second most populous country in the world, India has a current population of more than 1.3 billion. India is an agricultural dominated country providing employment to 70% of the total population and contributes 27% to GDP of the country. India has a total area of 3,287,260 km^2 in which land shares for 2,973,190 km^2 and water 314,070 km^2(De et al., 2014). The major portion of land is under agriculture (60.5% of total land area), under forests (22.8% of total land area) and other lands (16.7% of the total land area including habitat etc) (Tiwana et al., 2009). Growing population has posed extra burden effect on agricultural production to fulfil the country's food bowl. Out of total food production, 33% is eaten and damaged by 10,000 species of insects and 30,000 species of weeds (Dhaliwal et al., 2010). So there is a need to increase the protection of the crop production by providing additional chemicals agents that increase the productivity by protecting crops from harmful pests, weeds etc (Singh et al., 2017). The natural resources are being depleted rapidly; agriculture thereby needs to employ the use of certain synthetically produced compounds, termed in a broader sense as 'pesticides'. 'Pesticides' broader term covers the ample range of compounds like herbicide, nematicides, fungicide, insecticides and others (Aktar *et al.,* 2009). For this reason, pesticides now become a vital part of modern agriculture (Figure 1).

Figure 1: Pesticide cycle: translocation of different pesticides across biotic and abiotic factors in an ecosystem



Pesticides consumption throughout the world per year is near about 200,000 tonnes, which accounts 23% consumed in the United States, 45% of the total in Europe, 32% in other parts of the world. In India, the share is only 3.73%. The worldwide consumption is 47.5% of herbicides are 29.5% of insecticides and for fungicides, it is 17.5% and for others 5.5% only (De *et al.,* 2014). In India, 67.13% are of insecticides, 24.25% are for fungicides 0.43% is in the form of herbicides, and less than 3% are of others of the total consumed pesticides. The convention of pesticides in India per hectare is only 0.52 kg, while in other countries like Japan and Korea, it is 6.7 and 11.90 kg/hectare (De et al., 2014).

High pesticides consuming states in India include Andhra Pradesh, Uttar Pradesh and Punjab (Abhilash & Singh 2009). In terms of national land mass, Punjab shares only 1.5% and consumes 17% of the total pesticides usage in India (56.23metric tons) (Mittal et al.. 2014).

Punjab is classified into three regions based on socio-culturally, Malwa, the south region of the river Sutlej; Doaba, the region which lies between River Beas and South of Sutlej River and Majha the area between river Beas and river Ravi. The state has an area of 50,362 km^2 and it produces 22% wheat, 13% of cotton and 12% rice of the total share (Punjab 2006). The per hectare usage of pesticides is 923g/hectare which is country's highest as compared to a national average of 520g/hectare (Gruere 2011).

1.2 Definition of pesticides and their classification

Pesticides may be classified in a number of ways depending upon their chemical structure, target pest species, mode of action, toxicity level etc. According to USEPA (United state Environment Protection Agency), the classification of pesticides was based on the chemically related structure of pesticides which includes Organophosphates, Carbamates, Organochlorine, Pyrethoid, Sulphonylurea etc. In India, the pesticides regulations are governed by the Central Insecticides Board and Registration Committee (CIBRC) and the Food Safety and Standards Authority of India (FSSAI). 234 pesticides have been registered by CIBRC so far.

Table 1: Diverse classes of pesticides with specific groups, structure and general application

Classes	Groups	Structure	Uses	References
Based on chemical related structure	**Organophosphates**	Phosphorous is connected to terminal oxygen by a double bond. Two lipophilic groups, a phosphoryl group all bonded to phosphorous	These compounds have been used to control crop pests, mosquitoes, mites, flies of cattle's, and aphids etc	Casida 2016
	Carbamates	Having general formula is $R_1NHC(O)OR_2$, where R_1 and R_2 are aliphatic and/or aromatic moieties.	It is used to kill or incapacitate target organism being derived from carbamic acid	Singh et al., 2016
	Organochlorine pesticides	Organochlorines contain chlorinated hydrocarbons with minimum one covalently bond atom of chlorine.	It is extensively used to control pests like mosquito, aphids etc.	Kumar et al., 2015
	Pyrethoid	These are extracted from the flower of *Chrysanthemum*. It is a single pesticide active ingredient; contain six components pyrethrin 2, cinerin 2, cinerin 1, pyrethrin 1, jasmolin 2 and jasmolin 1 all have insecticidal activity	It is used till 1st Century and is largely used in agriculture, mosquito control, lawn etc.	Amweg et al., 2015.
Target pest species	**Algicides**	It contains the active ingredient sodium carbonate peroxyhydrate with copper chelates or Copper sulphate.	Control of Algae	Browne et al., 2015
	Avicides	Usually contains fenthion or 4-aminopyridine or alphachloralose compound	Control of Birds	Tuzimski et al., 2015
	Bactericides	Biological agents, antibiotics, essential oils were used as bactericides.	Control of Bacteria	Redondo et al., 2015
	Fungicides	Aerosol, chemical compounds were used containing an inert ingredient and active ingredient	Control of Fungi & Oomycetes	Singh et al., 2016
	Herbicides	Contains Phenoxy compounds, Phenyl acetic acid, Benzoic acid, Pthalic acid and many other nitrogen derivatives.	Control of Weeds	Kumar et al., 2015
	Insecticides	Natural insecticides include nicotine, pyrethrum, rotenone Synthetic	Control of Insects & Aphids	Kumar et al., 2014

		includes OP's Organochlorines, Carbamates etc		
	Miticides	Commonly used miticides include Azobenzene, dicofol, ovex, and tetradifon	Control of Mites	Rangel et al., 2015
	Molluscicides	Metaldehyde, Methiocarb, Ach inhibitors and metal salts such as Fe(III) phosphate and aluminium sulphate	Control of Slugs & Snails	Wang et al., 2016
	Nematicides	aldicarb, carbofuran, phorate, fensulfothion, DBCP essential oils etc were used as nematicides and chemical composition differs from compound to compound	Control of Nematodes	Chin-Pampillo et al., 2015
	Rodenticides	Anticoagulants, metal phosphides, hypercalcemis and other like arsenic, barium, sodium flouroacetate etc.	Control of Rodents	Gartiser et al., 2015
	Virucides	Commonly used virucides include H_2O_2, hypochlorites, ferric ions ethanol, lipids azodicarbonamide, curdline sulphate, disulfate benzamides, benziothiazolones etc	Control of Viruses	Wang et al., 2015
Based on work	**Contact pesticides**	These are the pesticides which control large number of pest when they come in contactwith the pesticide sprayed areas.	For the control of insects, weeds and usually used as herbicides.	Sharma 2015
	Systemic pesticides	Commonly called translocated pesticides which are translocated to different zones of plants.	Used as pest controller not inly in plants but also in animals to control lices, grubs etc	Van Lexmond et al., 2015
	Fumigants	These are pesticides that are applied in the form of gas, either applied as a solid, liquid or in gaseous form.	For the control of flies, bees, mosquito in the fields or in house hold to impede their mode on infection	Alavanja et al., 2014

The intensive use of pesticides contributes to soil pollution and results in the abatement of beneficial soil micro-organisms. The repetitive use of pesticides, however, contributes to soil pollution leading to declination in the number of significant soil micro-organism (Singh et al., 2016). Agriculture is largely responsible for causing sediment deposition and erosion of nutrient-rich layer through intensive management of pesticides. Moreover, use of specific nutrients causes an imbalance in the supply of soil nutrients leading to soil degradation and loss of equilibrium of stable soil (Dudal, 1981). The changing nature of pesticides has led to the evolution of advanced pesticides which become troublesome for pest control in agriculture and thereby increasing the resistivity of pesticides. The agricultural practices utilize pesticides that acted on multiple sites inhibiting the growth of pests but due to the emergence of resistance to pests, modern pesticides act on one particular metabolic pathway.

Soils exhibiting enhanced biodegradation of pesticides, or soils that were heavily contaminated with high concentrations of such compounds, have commonly been used as sources for the isolation of microorganisms with increased capability to rapidly degrade these compounds. The isolation in selective mineral salts media, where the pesticides act as the sole source of carbon, nitrogen or phosphorus source, has been used in the majority of cases in order to isolate microbial population capability of degrading pesticides. In most cases, the isolated microbial population is able to utilize the pesticide as a source of a single element (C, N, P or S). Numerous microbial populations, including bacteria, fungus have been reported so far in the degradation studies of the pesticides.

Humic substances also known as “Black gold of agriculture” are the main component of natural organic matter found in soils, water, lake sediments coals etc. that affect physical and chemical properties and improve soil fertility (Khaleda et al., 2017). Humic substances are complex and heterogeneous mixtures formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification) (Kamyab et al., 2014). Plant lignin and its transformation products, as well as polysaccharides, melanin, cutin, proteins, lipids, nucleic acids, fine char particles, etc., are important components taking part in this process. Humic substances in soils and sediments can be divided into three main fractions: humic acids (HA or HAs), fulvic acids (FA or FAs) and humin. Humic substances are highly chemically reactive. Humic acids are insoluble at low pH, and they are precipitated by adding strong acid (adjust to pH 1 with HCl) (Trevisan et al., 2010).

Humates and metal ions possess the ability to form complexes with pesticides resulting in the abatement of the toxic effect of these pesticides. Some studies have been reported that the pesticides sorbed into humates showing the neutralization effect of some pesticides (Shehta *et al.* 2014). But the mechanism is still unknown about the neutralization effect. The present study will be conducted to check the degradation and the uptake behaviour of pesticides in the absence and presence of metals ions and humates with isolated rhizobacteria and crops.

REVIEW OF LITERATURE

2.1 Pesticides and Environment Issues

The difference between the pesticide usage in India for the agricultural and non-agricultural sector is only 27% (PPQS 2013). Accumulation of pesticides in water and food led to serious implications to non-target plants, animals and human health. Dreaded conditions like hormone disruption, abnormalities in the reproductive system, immuno-suppression, cancer etc are a sign of their long-term exposure to pesticides (Singh et al., 2016; Hernandez et al., 2013; Goulson 2013). 51% of the food products in India are widely contained residues of different pesticides and more than 20% of that are beyond the MRL values (maximum residue level) (Thakre et al., 2013).

In the environment, contamination of pesticides is a major concern worldwide, because of high toxicity and adverse effect on human life and ecosystem. Agriculture is largely responsible for causing sediment deposition and erosion of nutrient-rich layer through intensive management of pesticides. In addition utilization of particular supplements cause unevenness in the supply of soil supplements prompting soil debasement and loss of harmony of stable soil (Jacobsen and Hajelmso 2014). Now a day's "Food bowl of the country" were also known as the 'Cancer capital of India" because there is an increase in threefold in a number of cancer cases in Punjab (Sengupta 2013). In last ten years, In India, there is 3% inclination in cancer death cases from 2005 to 2030 (WHO). The incidence, prevalence and suspects of Cancer rates per lakh population are highest in Malwa region, followed by Doaba and Majha region (Mittal et al., 2014). Report on cancer by Centre for Science and Environment investigated and found residues of pesticides in the blood samples of patients in the different villages of Punjab (Bedi et al., 2015). In Muktsar district, there is a spurt in cancer cases in a single year from 30 to 191in the year 2001 to 2002 (Mittal et al., 2014). During the same period, same observations were studied in Faridkot district with an increase in 19 to 141 cancer cases (Mittal et al., 2014).

Near about 500,000 people are either killed or incapacitated by poisoning every year in e world (Ashghar et al., 2016). Around 2 % globally, and 20 % food products in India were searched out, to be defiled with pesticide residues above endurance level (Thakre et al., 2013). Pesticide poisoning emerges either from regular intake of pesticides in minute quantities "chronic" or in a single dose "acute". Acute include hypersensitivity, giddiness, allergies, dermal abrasions, double vision, headache, etc. while Chronic includes damages to central and peripheral nervous system, sarcoma, soft tissue sarcomas, brain, stomach cancers, bone cancers, reproductive disorders, lymphomas, leukemia, death, immune system disruption and birth defects. LD50 and LC50 are the most common toxicity terms for

defining levels of toxicity (Mostafalou & Abdollahi 2013). Pesticide poisoning in India was first reported in 1958 in Kerala where wheat flour contaminated with parathion kills more than 100 people (Idrovo 2014). Recently, cooking oil contaminated with Monocrotophos in a midday meal scheme, in a Mashrakh village in Bihar has led to the death of 25 children after eating food (Tomer et al., 2015). Let us discuss the toxicity of major classes of pesticides one by one.

Organophosphates were having high mammalian, chronic and acute toxicity level and in developing countries, it is estimated around 2 million deaths and 3.2 million poisonings annually (Khan et al., 2016). Additionally, a wide range of nerve and muscular disorders is seen in many cases when exposed to OPs (Kumar et al., 2015). Carbamates results in the hydrolysis of acetylcholine to choline, acetic acid and also have an adverse effect on the activity of inhibiting other esterases also (Singh et al., 2016). Regular contact with these chlorinated hydrocarbons over a short period may produce a headache, weakness in muscles, sweating and headache and protracted exposure to Organochlorine pesticide damage the liver, kidney, CNS, TSH and bladder (Mahugija *et al.* 2014). They have an adverse effect on cellular respiration, especially when they come in contact with the human population. Long-term exposure causes damage to the immune system, skin sensitization and cancer (Li *et al.* 2014). Pyrethroids are axonic toxic substances and cause loss of motion of a living being. The compound causes' loss of motion by keeping the sodium diverts open in the neuronal films of a living being. The sodium channel comprises a layer protein with a hydrophilic inside; this inside is viable a minor gap which is formed precisely right to strip away the incompletely charged water atoms from a sodium particle and make a thermodynamically great route for sodium particles to go through the membrane, enter the axon, and proliferate an activity potentially. At the point when the poison keeps the directs in their open express, the nerves can't de-energize, so the organism is incapacitated. The pyrethroids are intense inhibitors of mitochondrial complex I (Li et al. 2014).

2.2 Mineralization of pesticides

Pesticides applied to various agricultural fields are highly persistent in the environment resulting in the contamination of air, soil and water bodies. Most of the pesticides have a longer half-life period posing threat to soil micro-flora also. The residues of pesticides accumulate in the food chain resulting in various acute and chronic diseases. To overcome these problems there is great need to develop an economically feasible, safe and convenient method for the removal of pesticides from water supplies and food products. Several

techniques have been developed for degradation of pesticides viz; Chemical, photolytic and biological.

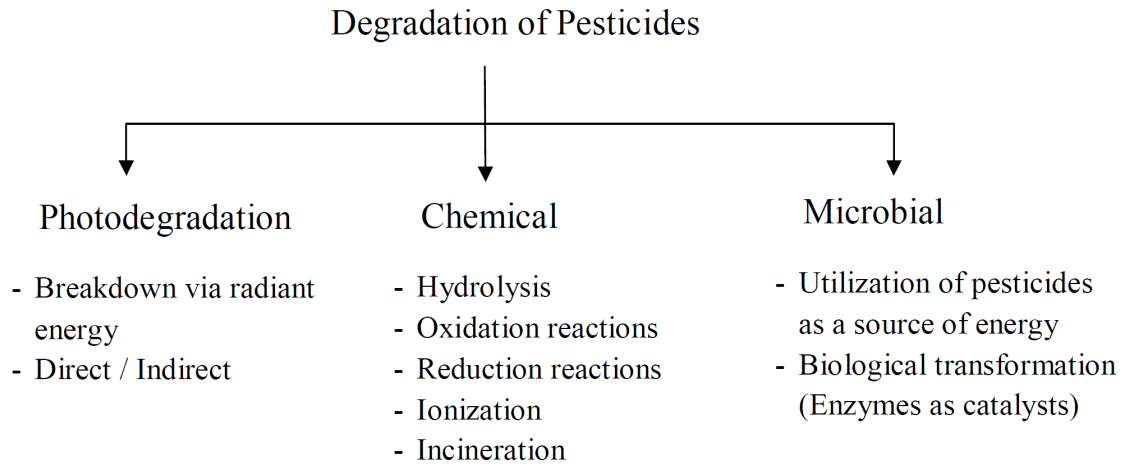


Figure 2: Mineralization of pesticides: Photo, chemical and microbial

Chemical degradation involves the disintegration of pesticides by chemical processes. Five major reactions are involved in chemical degradation of pesticides namely Dehalogenation, Elimination, Hydrolysis, Oxidation-reduction, reduction and substitution reactions. Organic matter, clay, pH and temperature influence the rate of degradation. In organo-phosphorous pesticides disintegrates by chlorine present in the environment resulting in the formation of P=O group by P=S group via oxidation reaction. Most of the organochlorine pesticides do not undergo chemical degradation (Kumar et al., 2014). It involves the substitution of Chlorine with hydroxyl of CN group. Carbamates rapidly disintegrate involving Fe (II) in suboxic and anoxic suspensions of soil by redox reaction forming a precursor complex of thiol and nitrile. Hydrolysis is often seen by pyrethroid pesticides through hydroxylation reactions involving an attack on the ester linkage resulting in the formation of metabolites (Singh et al., 2016).

Photolysis of pesticides involves degradation by photons. They break down pesticides in water, soil, air and even on foliage. pH, fulvic acid, humic acid, light intensity, time of exposure, the presence of ions in nature and depth of water influences the photo-degradation rate (Mathon et al., 2016). A free radical formation takes place by the cleavage of a homolytic bond in a photochemical reaction. Organo-phosphorous pesticides disintegrate via photolytic by cyclization, dimerization, dehalogenation, dehydrohalogenation, isomerisation, cleavage, oxidation of thioether group etc. In organochlorine pesticides, dechlorination, dimerization and isomerisation are normal reactions in a photolysis way (Catalá-Icardo et al., 2016). In the presence of light, carbamates undergo methylation, oxidation of aliphatic side

chain, hydrolysis, N-dealkylation, oxidation via thioester linkage reactions resulting in the formation of phenol ester. In pyrethroid pesticides, photolytic degradation undergoes by oxidation into COOH group by C=O group, CN group hydration, oxidation of halogenated chain etc.

Soils exhibiting enhanced degradation of pesticides, or soils that were heavily contaminated with high concentrations of such compounds, have commonly been used as sources for the isolation of microorganisms with increased capability to rapidly degrade these compounds. The isolation of selective mineral salts media, where the pesticides act as the energy has been used in the majority of cases in order to isolate microbial population capability of degrading pesticides. In most cases, the isolated microbial population is capable to make use of the pesticide as the energy source of a single element (C, N, P or S). Numerous microbial populations, including bacteria, fungus have been reported so far in the degradation studies of the pesticides.

Several biological techniques have been developed for biodegradation of pesticides. Biodegradation is an eco-friendly technique using naturally occurring plants and micro-organism to degrade, digest and convert organic compound into harmLess bio-products by using pesticides as an energy source of phosphorous, nitrogen and carbon. Four major types of mechanism are involved in the metabolism of organophosphophates. The principal reactions involved in the microbial degradation of organophosphates involve oxidation, alkylation, dealkylation, and hydrolysis. Hydrolysis of P-O-aryl and P-O-alkyl bonds is one of the major steps in degradation of organophosphorous compounds. Reductive de-chlorination is the major mechanism in the biodegradation of organochlorines. Carbamates undergo hydrolysis, biotransformation, metabolic and oxidation reactions in micro organisms whereas pyrethroids are disintegrated by the microbial population through oxidation-reduction, conjugation and ester hydrolysis mechanism.

2.3 Complexation of pesticides with metal ions and humic acid

Most of the metal in the transition series of the periodic table form complexes with pesticides. Many studies have been reported to date the strong adsorption of pesticides with metals ions The mechanism of interaction in ancient times were sustained using Infrared spectroscopy is through hydrogen bonding. An increase in Cu concentration increases complexation of the pesticides. Pesticide adsorption is enhanced by three ways, firstly Copper is coordinated with pesticide, Secondly, pesticide copper complexes have a higher ability to adsorbed on soil rather than free pesticide and finally copper acting as a bridge between the pesticides and soil

and low pH also enhances pesticides adsorption (Rojas et al., 2015). There are also other studies have been reported the adsorption of other metals ions such as Zinc. Low pH influences the zinc adsorption on sites of goethite through pesticides. Fourier transmission Infrared spectroscopy shows adsorption by coordination through carboxylate group (Okada et al., 2016). Presently days, impersonation of the pesticides connections with particles of metals in xylem was appeared by in which PC model is utilized to evaluate the level of complexation between the chelating operators in phloem which incorporates cysteine glutamic corrosive, citrate, histidine and nicotianamine (Harris and Smith 2016). Past Studies indicates Glyphosate ties to Fe in both states Fe^{3+}, Fe^{2+} Copper $^{2+}$, Ca^{2+}, Mn^{2+} and Mg^{2+} in the scope of pH scope of 8 to 6.5. At a pH of ordinary phloem of 8, just 1.5 mM glyphosate ties 8.4% of the aggregate Fe3+, 3.4% of the aggregate Mn^{2+}, and 2.3% of the aggregate Mg^{2+}, however, has no impact on the speciation of Ca^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{2+} and when pH declined to 6.5, there are some significant changes of the metal particles among the natural chelators. Computations demonstrated that 90% of the glyphosate in phloem is not bound to any metal particle and it is autonomous on the pH and their solvency limits (Harris et al., 2012).

A present study indicates neutralize of the antimicrobial impact of glyphosate by humic substances in vitro is appeared by Shehata et al., 2014 in which MIC of the glyphosate was done at various groupings of the humic acid substances at different pH. Findings conclude the complexation of glyphosate with humic acid resulting in the abatement of antimicrobial effect. Still, further research ought to be done to check the conduct of debasement of pesticides with various buildings of metals and humates. Moreover, pH, Temperature and grouping of the chelators assume an imperative part in adsorption of metals particles and humates with pesticides.

Pesticides frame the stable edifices when they showered onto the soil particles. These pesticides bring about the arrangement of additional stable buildings with soil particles creating supplement inadequacy to plants. A few pesticides like Glyphosate has been accounted for to shape edifices of different dependable qualities with divalent metal cations because of the nearness of amino, carboxylic and phosphonic moieties in its atom, where each could arrange independently to metal particles or in blends as a bidentate or tridentate ligand. Because of such collaboration, the nearness of a few divalent cations including Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+} and Zn^{2+} in splash arrangements was appeared to bring down the herbicidal impact of glyphosate. The development of inadequately solvent glyphosate-metal buildings may likewise be significant in the rhizosphere, decreasing the root uptake and translocation of these supplements inside plant tissues

As per chelating and HSAB principle, above mentioned pesticides having the binding sites, they can bind through N, O and S atoms. The solubility of particular metal complex or metal salt is governed by the pH factor of soil.

2.4 Biology and Ecology of Pesticides Biodegradation

Use of microorganisms to clean up the areas that have been contaminated by the use of pesticides is an easy feasible and eco-friendly method. Microbes usually develop metabolic pathways that transform pesticides into useful bioproducts. Most of the microorganism uses these pesticides as an energy source (carbon, phosphorous or nitrogen). Four major reactions are involved in microbial degradation involve alkylation, dealkylation, hydrolysis and oxidation (Matsumura 2012). Usually, Organophosphates include the chlorinated derivatives of diphenyl ethane, the group of hexachlorocyclohexane, the group of cyclodiene and chlorinated hydrocarbons (Chandra and Kumar 2015). These groups either contain P=O or P=S and being esters they have many sites which are vulnerable to hydrolysis. It occurs through hydrolysis of P-O-aryl and P-O-alkyl bonds are considered the most important step in detoxification. Organochlorine pesticides were first introduced in 1931 and are highly persistent in soil water etc. Reductive de-chlorination is the major mechanism in the biodegradation of organochlorines. The reaction involves the replacement of hydrogen atom by a aliphatic chlorine. The degradation is preceded by successive reductive de-chlorination reactions. Aerobic decomposition is 10 times faster than anaerobic micro-sites. Carbamates are long persistent in nature. Being toxic in nature they transformed by microorganisms by a number of processes like biotransformation, hydrolysis, oxidation, metabolic and reduction. Pyrethroids are disintegrated by the microbial population through oxidation-reduction, conjugation and ester hydrolysis mechanism.

Generally, literature survey reveals the biodegradation of most the pesticides were done in pure cultures. Cultures were isolated from soil samples by media enrichment technique in which microorganism uses pesticides as a sole source of energy. In media enrichment, only that cultures or bacterial population will survive which can resist or transform the pesticides via metabolic pathways.

2.5 Foraging activities of rhizobacteria in a Heterogeneous Soil Environment

Usually, rhizobacteria act as metal scavengers and exert their beneficial effects to associated plants through indirect or direct mechanisms. PGPR promotes the development of plants by inducing the various compounds which are essential for the growth of plants. They increase

the growth of plants by defending from harmful pathogens and enhance the production of various hormones like phosphate solubilization, siderophore production, cytokinins, auxins, and gibberellins nitrogen fixation, and uptake of essential major and micronutrients. Some strains have the potential to produce antibiotics (Ahemad et al., 2016). Out of these, Phosphate solubilisation & Siderophore production is very important as both play an eminent role in the uptake of various metal ions & phosphates which is essential for plant growth. Many rhizobacteria are reported to date which converts the unavailable form of various major elements (Phosphorous and Iron) into an available form which is essential for the plant to sustain good growth. It includes Phosphate solubilisation, Siderophore production etc (Bhattacharya and Jha 2012). These rhizobacteria convert the insoluble form of elements to a soluble form by exchange reaction mechanism or chelation mechanism and by secretion of certain protons or organic acids or by acidification methods.

2.6 Interaction between rhizobacteria and pesticides

Microorganism plays a significant role in the transformation of a pesticide and its residues from the soil. When a microbe or microbial population is regularly exposed to pesticide they adapt susceptibility against the toxic effect of pesticides. This adaptation may take quite rapidly or take some time depending on the metabolism of the microbial population. Factors like the structure of pesticides, type of soil, moisture content in soil, pH, salinity, biotic components of soil are the certain factor which influences the degradation rate of pesticides. The pesticides are transformed via different mechanisms like conjugation, hydrolysis, oxidation, reduction etc. Recent studies suggest that microbes usually use these pesticides as an energy source and transform them into useful bioproducts. These mechanisms involve various types of enzymes to start out this transformation reaction. Enzymes such as oxidoreductases, mono-oxygenases, cytochrome P450 oxidoreductases, dioxyenases, hydrolases, lyases, haloalkane dehydrochlorinases were particularly used by the microbial population for the transformation of pesticides in soil. Out of these oxidoreducatases are hydrolyases are commonly found in most of the microbial population. Major classes of pesticides containing peptide bonds, ureas, thioesters, esters, carbon-halide bonds are transformed by the hydrolase enzyme via the hydrolytic mechanism. The oxidation-reduction transformation reactions are usually catalyzed by oxidases using electron acceptor as oxygen and reduced it to hydrogen peroxide or water. Literature available shows lack of information related to environmental fate and pessimistic effects of pesticides in relation to microbial population, their interaction and complete remediation of their bio-transformed product.

2.7 Pesticides and Soil Biodiversity

The repetitive use of pesticides, however, contributes to soil pollution leading to declination in the number of significant soil micro-organisms called Plant growth promoting rhizobacteria. They are the beneficial rhizobacteria which form a symbiotic association with the linked plant resulting in the stimulation of host plant by inducing various growth hormones and decreases the occurrence of various plant diseases (Bhattacharyya and Jha 2012). Growth enhancement in plant usually occurs by ample diversity of various mechanisms like Siderophore production, indole acetic acid production, induction of 1-Aminocyclopropane-1- carboxylate deaminase (ACC), rhizosphere engineering, phosphate solubilization, quorum sensing (QS) signal interference, biological nitrogen fixation, production of volatile organic compounds (VOCs), interference with pathogen toxin production and many more which usually carried out by certain beneficial bacteria commonly known as Plant growth-promoting rhizobacteria (PGPR) (Bashan and de-Bashan 2010). They are classified into intracellular (iPGPR) and extracellular (ePGPR) on the basis of their degree of association (Kang et al., 2014). iPGPR usually present within the nodules of the root cells and ePGPR locates on rhizosphere or in between root cortex. These PGPR transform the unsolvable form of elements to solvable form by exchange reaction mechanism or chelation mechanism (Bhattacharyya & Jha (2012) and by secretion of certain protons or organic acids or by acidification methods (Majeed et al., 2015).

Pesticides hindermost of the functions of the PGPR which helps plants in sustaining various hormonal balances in plants. The uses of various pesticides, insecticides chemicals in agriculture sector implement crop production but in adverse have some negative effects on beneficial soil microflora (Newmann et al., 2016) Some pesticides are high persistence in soils having a negative impact on the survival and function of the rhizobacteria (Ahemad and Khan 2012).

The uses of various pesticides, insecticides chemicals in agriculture sector implement crop production, but in adverse have some negative effects on beneficial soil microflora (Decourtye et al., 2004). Some pesticides are high persistence in soils having a negative impact on the survival and function of the rhizobacteria (Ahemad and Khan 2012). Pesticides reduce the allocation of enzyme Photosynthate to the nodules for fixation of nitrogen in the environment (Datta et al., 2009). It also disrupts the signalling between rhizobium Nod D receptors and phytochemicals derived from leguminous plants (Fox et al, 2007).

Moreover, divalent metal ions, zero-valent iron and soil's humates are used for environmental remediation, including decontamination of pesticides (Shehata et al., 2014; Edwards and

Brown 2013; Fjordboge et al., 2013; Noubactep, 2008; Smolen and Stone 1997). Divalent metals and humic acid neutralise the antimicrobial effect of pesticides when performed in vitro (Shehta et al., 2014) thus showing a positive sign to deteriorate the adverse of pesticides against beneficial PGPRs. The current study was designed to check the effect of divalent metals Cu^{++} & Fe^{++} and humic acid against six different pesticides (Acephate, Atrazine, Carbendazim, Glyphosate, Monocrotophos and phorate) on the PGPR activities of the isolated rhizobacterial species from pesticide using agricultural fields of the Kapurthala region Punjab.

2.8 Pesticides Effect on Plant Growth Parameters

Plant protection has become a necessity of present-day life due to the stress of feeding more than 6 billion people from limited land invested in agriculture (Wise 2013). With the increase in population, the chemical pressure on the environment is also expected to rise (Habiba et al., 2015). The issue is becoming more complicated, an entire population is not only demanding the desired quantity of food, but they all require healthy and nutritious food (Enserink 2008). Studies revealed that almost one billion people are undernourished with lack of sufficient protein, fats, carbohydrates and micronutrients (such as iron, zinc and vitamin A) in their diets (Evenson and Gollin 2003). Birth defect increased chances of infection and diseases, as well as mental impairment; overall a negative impact on global health is the resultant of the dietary deficiencies. Empowerment of agricultural system is required as it is expected that within next four decades, additional two billion people will require nutritious food. But, the literature indicates lack of micronutrients in soil leading to less/ infertility of land and the exact reason behind this is still unknown. More than 3 million tons of pesticides are applied every year on almost 5 billion hectares of the world's agricultural land just to save food from pests (Kramer 2007). These pesticides directly or indirectly reach to the soil surface, where they interact with soil organic/ inorganic matter. Pesticides are not completely safe. However, before application of pesticides on to the land, they undergo several checks to protect environmental/ biological health. But unfortunately, the interaction of these pesticides with soil inorganic/ organic components (which are the backbone of soil fertility) is not the part of these checks. Pesticides because of the presence of one or more coordination sites can interact with metal ions of soil (bounded metal ions or free metal ions), soils oxides, organic matter etc (Duke et al., 2012). For example, glyphosate shows strong coordination chemistry with metal ions at variable pH values because it has three functional groups (P-O-, NH and COOH) (Duke et al., 2012). Like glyphosate, all pesticide contain one or more than one 121

active/ coordination site. These active/ coordination sites make the coordination chemistry of pesticides richer than their pesticidal application. If this coordination will affect the liability of metal ion (from soil to plant), plant health will be extremely affected. A significant decrease in root and shoot dry matter production, chlorophyll concentrations of young leaves, photosynthetic parameters and nutrients in tissues were evaluated in various studies involving the application of glyphosate (an organophosphate pesticide) on the soil. Micronutrients deficiencies were increasingly observed in agricultural land with frequent glyphosate applications. These facts are not limited to glyphosate only. Acephate (ACP), Atrazine (Atr), carbendazim (CB), glyphosate (Gly), monocrotophos (MC) and phorate (PR) are widely used pesticides for agricultural applications worldwide. These are known to inhibit the reversible hydrolysis of acetylcholine, and therefore the persistence of these pesticides for a longer time in the environment is hazardous.

In literature, there is a dearth of studies on "Acephate (Acp), Atrazine (Atr), carbendazim (CB), monocrotophos (MC) and phorate (PR) on plant growth". Only a few studies on Glyphosate have been reported but the further effect of additional applications of metal ions and humic acid is not reported. Sublethal or excess dose level applications of Acephate (ACP), Atrazine (Atr), carbendazim (CB), glyphosate (Gly), monocrotophos (MC) and phorate (PR) in mixed form may synergistically affect plant growth.

The abatement in soil fertility due to regular use of pesticides has an adverse effect on PGPR activities of the Rhizobacteria. Pesticide induces the growth promoting mechanism by inhibiting various enzymes which is essential for growth of plants. The permeability of the plant cell and transcuticular diffusion is affected by spraying of pesticides on them. Recent studies on the effect of pesticides on plant growth emphasis its effect on delay in seed germination experiments (Parween et al., 2016).

Most of the pesticides when sprayed on foliage weeds and crop plants affect the activity and growth of various beneficial microbial communities. These pesticides directly or indirectly reach to the soil surface, where they interact with soil organic/ inorganic matter. Pesticides are not completely safe. However, before application of pesticides on to the land, they undergo several checks to protect environmental/ biological health. But unfortunately, the interaction of these pesticides with soil inorganic/ organic components (which are the backbone of soil fertility) is not the part of these checks. Pesticides because of the presence of one or more coordination sites can interact with metal ions of soil (bounded metal ions or free metal ions), soils oxides, organic matter etc. For example, glyphosate shows strong coordination chemistry with metal ions at variable pH values because it has three functional

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Significant decrease in shoot and root dry matter production, the concentration of chlorophyll content, photosynthetic parameters and nutrients in tissues were evaluated in various studies involving the application of glyphosate (an organophosphate pesticide) on the soil. Recent studies reveal that the higher dose of pesticides decreases the potential of PGPR strains to induce the growth promoting mechanism and production of enzymes (Chennappa 2014; Ahemad et al., 2012; Myresiotis *et al.* 2012; Berg 2009). Pesticides also affect the permeability and transcuticular diffusion of the plant cell and delayed seed germination (Fantke *et al.* 2013).

2.9 Need and Goal for bioremediation

Various pesticides both organic and inorganic with various lethal properties were brought into the ecosystem through anthropogenic activities. Toxicity of pesticides varies depending upon their classification based on GHS (Globally Harmonized System of Classification) and Labelling of Chemicals by W.H.O. Many of the pesticides have adverse effects on human life and ecosystem. They directly reach into the various ecosystems like soil, water and land. Pesticides deteriorate the quality of soil resulting in the abatement of various beneficial microorganisms which induces the production of various hormones which act as growth regulators for plants. Some pesticides are so toxic in nature leading to the number of disorders like hypersensitivity, giddiness, allergies, dermal abrasions, double vision, headache, etc. while other includes damages to central and peripheral nervous system, bone cancers, sarcoma, leukemia, soft tissue sarcomas, lymphomas, brain, stomach cancers, disorders of the reproductive system, defects in birth, immune system disruption etc.

Keeping this point, we need to solve this environmental problem. It is not easy to remove the pesticides from the environment because most of the pesticides are soluble and highly persistent in nature. Various methods have been proposed to remove pesticides in the ecosystem such as treatment with various chemicals and methods likes volatilization, incineration etc. Chemical method is feasible involving use of acids and bases for the removal of pesticides but are challenging a too as large number of alkali and acid are formed during removal. Volatilization method results in the emission of toxic gases which could deteriorate the quality of air. Incineration has met serious public opposition because of its

potential toxic emissions, and high economic costs. Overall all of these methods are expensive, harmful and inefficient because the contaminated soil has to be excavated from a site and moved to a storage area where it can be processed. Due to environmental concerns associated with the accumulation of pesticides in food products and water supplies, there is great need to develop a safe, convenient and economically feasible method for clean-up of pesticides. Several biological techniques have been developed for biodegradation of pesticides. Biodegradation is an eco-friendly technique using naturally occurring plants and micro-organism to degrade, digest and convert organic compound into harmLess bio-products. Microbial metabolism is the most important pesticide removal method in which soils, as the degrading micro-organism obtains carbon, Nitrogen, phosphorous or energy from the pesticide molecules resulting in the formation of useful metabolites.

This technique relies on the capability of microorganisms to convert organic contaminants to simple and harmless compounds to the environment. Bioremediation allows the destruction of many organic contaminants at a reduced cost and thus overcomes the limitations of conventional methods for hazardous compounds disposal. Bioremediation has progressed to be an exceptional virtual technology which can be harnessed for detoxification and degradation of wide range pollutant. It forms to be an efficient and cheap prospect for decontamination of polluted ecosystems and destruction of pesticides. It has emerged as a potential alternative because it is environmentally friendly, economical and efficient. However, the biodegradation process of many pesticides has not been fully investigated.

2.10 Pesticides Selected for Present Study

Four Organophosphate (Acephate, Glyphosate, Monocrotophos and phorate) one triazine (Atrazine), one carbamate (Carbendazim) were selected for this study. These all six pesticides were selected on the following basis:

1) From a commercial point of view, all pesticides are the top-selling pesticides worldwide including India.

(2) From the coordination chemistry point of view; glyphosate & Carbendazim can behave strong complexing ligand, atrazine & acephate and phorate can behave as an intermediate complexing ligand, and monocrotophos can behave as a weak complexing ligand.

(3) From the application point of view, glyphosate & Atrazine is a herbicide, acephate and monocrotophos phorate are insecticides and Carbendazim is a fungicide.

(4) From recommendation point of view, 262 brands of pesticides are used in Kapurthala district (survey report of KVK), Out of which only 46 were recommended by the State agricultural university while rest of the brands are not recommended.

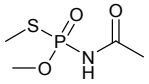
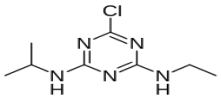
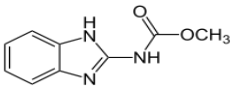
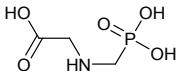
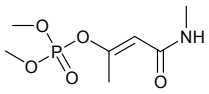
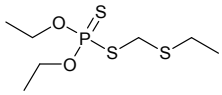
Chemical Structure	Description
Acephate 	CAS No. 30560-19-1 IUPAC Name: (*RS*)-(*O,S*-dimethyl acetylphosphoramidothioate) Toxicity: Acute toxicity; LD_{50} Oral - rat - 700 mg/kg; LD_{50} Dermal - rabbit - 2.000 mg/kg; Log Kow: -0.90; Log Koc: 1.34.
Atrazine 	CAS No. 1912-24-9 IUPAC Name: 2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine Toxicity: Acute toxicity; LD_{50} Oral - rat - 672 to 3,000 mg/kg; LC_{50} Log Kow: 2.61.
Carbendazim 	CAS No. 10605-21-7 IUPAC Name: methyl benzimidazol-2-ylcarbamate. Toxicity: Acute toxicity; LD_{50} Oral - rat - 50 mg/kg; LC_{50} Log Kow: 1.52.
Glyphosate 	CAS No. 1071-83-6 IUPAC Name: N-(phosphonomethyl)glycine Toxicity: Acute toxicity; LD_{50} Oral - rat - 5.000 mg/kg; LD_{50} Dermal - rabbit - 5.000 mg/kg; LD_{50} Intraperitoneal - rat - 235 mg/kg; Log Kow: -4.47; Log Koc: 1.27.
Monocrotophos 	CAS No. 6923-22-4 IUPAC Name: dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate Toxicity: Acute toxicity; LD_{50} Dermal - rabbit - 270 mg/kg; Log Kow: -0.35; Log Koc: 2.34.
Phorate 	CAS No. 298-02-2 IUPAC Name: O,O-diethyl S-ethylthiomethyl phosphorodithioate Toxicity: Acute toxicity; LC_{50} Inhalation - rat - 1 h - 11 mg/m^3; Log Kow: 3.56; Log Koc: 2.64.

Table 2: Pesticides selected for the current study.

HYPOTHESIS

3.1 Hypothesis

As pesticides are used to protect the agricultural crops from various pests, weeds etc but the residues in air and persistence of these pesticides in the soil affects the life cycle of non targeted species also. As per literature, the degradation of the pesticides governed by the factors likes microbial composition, pH, temperature etc. When pesticides are mixed with the soil they adsorbed on the soil surface and form the stable complexes hence the effectiveness of soil and pesticides itself reduces. These metal complexes are decomposed if competitive chelation took place by the other ligand having greater stability and complex formation ability. Humic substances (HS) symbolize a significant proportion of organic fraction in soils. One of the most significant characteristics of HS is their ability to interact with metal ions to form water-soluble and colloidal complexes. The capacity of HS to interact with metals is recognized to their high contents of oxygen-containing functional groups, such as carboxyl (COOH), hydroxyl (OH), and carbonyl (C=O). Interaction of pesticides with soil metal ions was studied at different pH, indicating that there was complexation taken place with metal ions and coordination sphere filled by the hydrogen bonding with water. Free pesticides also extracted from the soil, these complexes effect the soil and plant activities. A combination of antagonistic and synergistic effects was observed with the selected organophosphate and carbamate insecticides examined. Humic acid significantly reduced the toxicity of azinophos-methyl, chlorpyrifos, and carbofuran, while enhanced toxicity was observed with methyl parathion and carbaryl. These results indicate that humic-pesticide interactions can alter the toxicity of agricultural chemicals. Moreover, the influence of humic materials on the toxicity of these chemicals is dependent on temporal relationships and HA concentration. As per chelating and HSAB principle, above mentioned pesticides having the binding sites, they can bind through N, O and S atoms. The solubility of particular metal complex or metal salt is governed by the pH factor of soil. All the pesticides including insecticides, weedicides and herbicides influence the soil fertility (Aktar et al., 2009). These contaminants once interacted with soil's organic matter (humates) and inorganic matter (essential metal ions) they show the least mobility, bioavailability and less accessible to microbial degradation. The interactions of contaminants with soil at the molecular level are central to their bioavailability, bioaccumulation, transport, and toxicity in the environment (Farenhorst 2006). Ultimately this study will be executed to explore interactions and degradation of world top selling organophosphate pesticides namely Acephate, Atrazine, Glyphosate, Monocrotophos

Carbendazim and Phorate. Recent studies revealed that humic acid and metal ions possess the ability to form complexes with pesticides resulting in the abatement of the toxic effect of these pesticides. Some studies have been reported that the pesticides sorbed into humic acid showing the neutralization effect of some pesticides.

The current study will investigate the degradation behaviour of highly toxic carcinogenic pesticides of different families (Acephate, Atrazine, Glyphosate, Monocrotophos Carbendazim and Phorate) used in day to day life, firstly with native rhizobacteria, then the effect of pesticides on PGPR activities of the isolated strains and uptake behaviour of pesticides by plants. All the experiment will be carried out in the absence and presence of humic acid and metal ions Cu^{++} and Fe^{++} as humic acid and metal ions neutralize the antimicrobial effect of pesticides. Still, mechanism is unknown as we are familiar that humic acid are large complexes and they might bond with the pesticides resulting in the neutralization effect or they may play an eminent role in degradation by blocking the activated sites of the pesticides.

OBJECTIVES

4. Aim and Objectives

4.1 Background

Pesticides play an important role in crop production and facilitate the adoption of conservation agriculture to farmers exerting an adverse effect on soil and water ecosystem. Removal of pesticides by physical and chemical methods disposes of a large ample of acid and alkali in the environment. Bioremediation is advantageous involving the complete detoxification of the large variety of organic compounds without affecting ecosystem as microbial population obtain nitrogen, carbon or any other source from the molecules of pesticides. Bioremediation is widely acceptable by the statutory regulatory and public authorities because of its lesser impact on the natural ecosystem. Economical and efficient methods for removal of pesticides and its metabolites more rapidly from the ecosystem need to be devised.

Keeping this view in mind, the current study was designed to build up an eco-friendly, efficient and appropriate method to minimize the impact of pesticides and its residues in the Kapurthala district of Punjab, India.

4.2 Explanation

According to the literature surveyed, it is clear that if favourable nutritional and environmental conditions occur, the bacteria readily incorporate the simple organic substances into their cells and oxidize them. However, degradation of complex organic compounds with longer molecular structures is slower. Some compounds are so complex that they cannot be degraded at all, which are termed as recalcitrant or refractory compounds.

Still others may be toxic and thus inhibit the growth of microorganisms and their metabolic activity. Such compounds need special techniques or integration of physicochemical and biological techniques for effective remediation. Many native bacterial isolates generate the enzymes that catalyze the degradative reactions and play a significant role in bioremediation. The microorganisms like bacteria use organic substances as a source of carbon and energy. Thus while transforming the contaminant bacteria gain energy and raw material for their multiplication and maintenance. There is a great need to develop safe, convenient and economically feasible methods for pesticide remediation because of the environmental concerns associated with the accumulation of pesticides in food products and water supplies. Biological techniques involving biodegradation of organic compounds by microorganisms have been developed for this. Many bacteria such as *Pseudomonas* sp., *Bacillus* sp., *Arthrobacter* sp. etc. are widely detected in the pesticide-contaminated soil due to their

extensive biodegradation capacities. The metabolic range of naturally occurring microbiota may not be capable of degrading certain compounds or certain classes of compounds. Supplementation with the specialized microbes may become a necessity. One way of developing such specialized microbes is by repeatedly exposing them to a higher concentration of contaminants. Often the microorganisms with specialized degradation ability can also be enriched from the contaminated site. Most research reports pesticide remediation in soil has been concerned only with single pesticides. Pesticides, however, are more commonly found in mixtures in contaminated sites. Very few studies have examined the degradation of mixtures of pesticides. It is important to examine how the clean-up process is affected by environmental factors and soil amendments. It is also important to examine what enzymatic changes are triggered in the bioremediation of pesticide-contaminated soils. Further, it is also important to look at the effects of water availability on bioremediation.

4.3 Aim

Biodegradation is gaining significant attention these days due to its economical and eco-friendly nature. Therefore, the present study aims to provide a pragmatic view of the processes involved in biodegradation along with the issues to be considered when dealing with a proposal for biodegradation of pesticide-contaminated soil. Use of metal ions and humic acid boost this study to check the interaction between pesticides and their role in degradation.

4.4 Objectives

Looking at the aim of bioremediation of pesticide-contaminated soil using native bacterial isolates; following objectives were assessed in this work.

1. To isolate, and characterize rhizobacteria from agricultural field applied with pesticides (Acephate, Atrazine, Carbendazim, Glyphosate, Monocrotophos and Phorate) near Kapurthala district of Punjab.
2. To study the biodegradation potential of pesticides by rhizobacteria in the presence of Fe^{++}, Cu^{++} and humic acid.
3. To determine the effect of pesticides on functional attributes of rhizobacteria in the presence of Fe^{++}, Cu^{++} and humic acid.
4. To determine the bioremediation potential of rhizobacteria with selected crop against different pesticides in presence of Fe^{++}, Cu^{++} and humic acid.

Methods & Materials

5. Experimental Design

5.1 Site Selection

The site under study is a small district of Punjab Kapurthala region of Punjab. This district is located at an elevation height of 225m above sea level with coordinates 31.38°N 75.38°E. The fields in these villages are continuously using various pesticides from the last few decades to protect their crops from various pests, weeds etc. Fifty-eight soil samples were taken from different locations of the fields used for agricultural production of wheat, maize, rice, sugarcane, spinach, potato, cucumber, turnip, pumpkin, beet, peas, brinjal, onion, cauliflower, carrot, brussels sprouts capsicum, cabbage from twenty-nine villages viz. Noorpurdona, Dhariwal Dona, Kharsona, Dhadwandi, Tiba, Mothanwal, Talwandi Chodrian, Bholana Kanjali, Kokalpur, Dhambadshahpur, Nadala, Begowal, Dhilwan Dhapai, Aujla, Iban wadala, Kharamaja, Khojawal, Kadupur, Dhapai, Kadupur, Palali, Ranipur, Jagatpur Jattan, Bhularai, Chak Hakim, and Hardaspur of district Kapurthala, Punjab. Many pesticides have been sprayed every year at unknown concentration leading to the depletion of plant growth promoting rhizobacteria, the fertility of the soil and having an adverse effect on human health & population.

5.1.1 Sample Collection

Soil samples were collected in sterilized ziplock bags of approximately 100 grams, from fifty -eight different sites of the twenty-nine agricultural fields and kept in a refrigerator at 4^{o}C for further studies.

5.1.2 Isolation of bacteria by media enrichment technique

Different soil samples were taken from the site. To isolate bacteria having the potential of degrading pesticide (Acephate, Atrazine, Glyphosate, Monocrotophos Carbendazim and Phorate) a known amount of soil sample will be placed in six different conical flasks of 250 mL each containing six different minimal supplemented with an adequate concentration of the pesticide (1000 mg/kg). The mixture will be incubated in an orbital shaker incubator at 30^{0}C and 110 rotations per minute for 4 days. After incubation, 1mL of the supernatant will be transferred into fresh minimal media containing pesticide (Acephate, Atrazine, Glyphosate, Monocrotophos Carbendazim and Phorate) and incubated at 4 days under the

same circumstances as above for 3-4 times repeatedly (Jariyal et al., 2014; Ramu et al., 2014; Fang et al., 2010; Gundi et al., 2006; Singh et al., 2006).

5.1.3 Plating Procedure

The culture will be successively grown and cultivated in mineral salts medium with pesticides (Acephate, Atrazine, Glyphosate, Monocrotophos Carbendazim and Phorate) as the source of carbon, nitrogen, phosphorus and energy and incubated at $30^{o}C$ for 3-4 days (Jariyal et al., 2014; Ramu et al., 2014; Fang et al., 2010; Gundi et al., 2006; Singh et al., 2004).

5.1.4 Molecular Identification of isolates

16S rDNA analyses of the bacterial isolates were conducted at Samved Biotech Pvt. Ltd. (Ahmadabad, India). A fragment of 16S rDNA gene was amplified by PCR, forward and reverse DNA sequencing reaction of PCR amplicon was carried out with universal primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (ACCTTGTTACGACTT) (Saiki et al, 1985; Watanabe et al, 2000). All the steps, including extraction of DNA, PCR amplification, construction of 16s rDNA clone libraries, sequencing and phylogenetic analysis were carried out as per protocol given by Sun et al. (2010).

5.2 Biodegradation of pesticides

5.2.1 Degradation of pesticides by the isolates in mineral medium & preparation of Inoculum for degradation studies

To exploit the capability of the isolates to disintegrate pesticides, isolated strains were grown in broths and suspension culture was inoculated with isolates in each flask to reach a biomass level of 10^{7} cells/mL using 0.5 McFarland standards. The mineral medium was prepared with pesticide 1000 mg/L as the sole source of carbon, carbon and nitrogen and carbon and .phosphorous The minimal medium will be equipped with pesticides at 100mg/L in the presence of Fe^{++}, Cu^{++} and humic acid respectively.

5.2.2 Degradation of pesticides by the isolates in Minimal media

To check the degradation ability of the isolates to degrade pesticides (Acephate, Atrazine, Glyphosate, Monocrotophos Carbendazim and Phorate) the minimal medium will be amended with pesticides as a source of carbon, nitrogen, phosphorous and energy. To study

the effect of isolates on the degradation of pesticides, the minimal medium will be equipped with pesticides at different concentrations in the absence and presence Fe^{++}, Cu^{++} and humic acid respectively. All flasks will be incubated in a rotator shaker at 30^0C and 120 rotations per minute. At the regular period, 20mL of the broth were worn-out to determine growth by a UV-VIS spectrophotometer (1800UV Shimadzu) at 600 nm using triple distilled water as a control. All the experiment was carried out in triplicate to ensure accuracy (Ramu *et al.,* 2014; Jariyal *et al.,* 2014; Fang *et al.,* 2010; Gundi *et al.,* 2006; Singh *et al.,* 2004).

5.2.3 Determination of pesticidal metabolites by ESI-MS, HPLC and GC-MS

For ESI-MS analysis, 100 mL of the spent medium was cleared up by centrifugation at 5000 rpm, trailed by filtration through a Whatmann 1 filter paper. The cleared up medium was then removed thrice with an equivalent volume of ethyl acetic acid derivation. The extricated organic phase was permitted to air dry, and the rest was dissolved in a minimal volume (250 mL) of water and about 1 µl was analysed in a mass spectrophotometer with flow rates from 0.05- 5.0 mL/min (Micromass Q-TOF micro; Waters, ESI positive mode). The samples for HPLC was prepared by using liquid-liquid extraction method in which 50 mL of the culture medium was entirely transferred to a separatory funnel and extracted 3 times with 50 mL chloroform. After dehydration through anhydrous sodium sulphate, the organic phases were collected and concentrated to almost dryness with a slight N2 stream. The contents were dissolved in methanol followed by HPLC analysis with Agilent Technologies 1200 equipped with diode array detector. Chromatographic separation was achieved on an Eclipse XDB-C18 column at room temperature and the samples were detected by measuring absorbance at 281 nm with an elution of methanol and water mixture (45:55 v/v) at a flow rate of 0.8 mL min-1 (You et al. 2010). For GC-MS analysis, 100 mL of the spent medium was centrifuged at 9000 X g for 20 min keeping 4 ºC for clarification and filtration was done with the Whatman filter paper No. 1. The clarified medium was then extracted with addition of ethyl acetate in 1:1 ratio. The extracted organic material was kept for air drying and concentrates up to 0.1mL. Gas chromatography (GC-MS) equipped with electron capture detector and DB5 capillary column of dimensions (30m X 0.25 mm X 0.25 µm) comprising splitless injector which is operated at 70 eV. Helium was employed as a carrier gas with a flow rate of 1.5 mL per min. The temperature was adjusted to 280^OC and 300^OC for transfer line and to trap ion respectively. The concentration of atrazine was assessed on the basis of comparison of peaks in the abiotic control with respect to the samples. For recognition of metabolites, comparison

of mass spectra of the standards was done with mass spectra of the products (Ramu *et al.,* 2014; Jariyal *et al.,* 2014; Fang *et al.,* 2010; Gundi *et al.,* 2006; Singh *et al.,* 2004).

5.3 Impact of pesticides on PGPR traits of the isolated strains

5.3.1 Indole acetic acid estimation test and effect of six different pesticides, Cu^{++}, Fe^{++} and humic acid in its production

Production of indole acetic acid (IAA) in the isolated strains will be determined by inoculation of culture into Luria Bertani (LB) broth amended with 50 microgram per millilitre L-tryptophan with and without different concentrations of each pesticide (500 mg/L and 1000mg/L) in the absence and presence of Cu^{++}, Fe^{++} and humic acid. The cultures will be kept at 30°C for 2-3 days and then centrifuged at cooling centrifuge at 10,000 g for 10 minutes. The supernatant will be further used and the concentration will be determined using Salkowski reagent at 530 nm (Colo *et al.* 2014).

A standard curve was prepared by taking different concentrations of standard IAA solution (0.1% to 1%) and the final volume was ended to 2 mL with distilled water. After that 4 mL of Salkowski reagent was added to the solution and incubated for 30 minutes at room temperature and absorbance was calculated at 530 nm and values were calculated by plotting a standard curve by using standard Indole acetic acid at 530 nm.

Standard strains of Rhizobium leguminosarium (positive control), and E. coli (Negative control) were procured from IMTECH (Institute of Microbial Technology) Chandigarh bearing MTCC no 10096 and 1696 were used to compare the result with the isolated strains quantitatively.

5.3.2 Phosphate solubilisation assay and effect of pesticides, Cu^{++}, Fe^{++} and humic acid in its production

For qualitative assay of Phosphate solubilisation activity, the isolates will be grown in Pikovskaya medium with and without different concentrations of each pesticide in the presence and absence of humic acid and Cu^{++}, Fe^{++}. Bacterial culture will be inoculated as spots and remain incubated at 30°C for 2-3 days. The appearance of the halo zone nearby the inoculated spot confirms that the isolate has the capability to solubilize the phosphates (Colo et al., 2014). All the experiments will be carried out in triplicates.

The values solubilizing efficiency was calculated using following formula:

Solubilizing efficiency = $\frac{Z-C}{C} X\ 100$

Z = zone of Solubilization (mm)

C = Diameter of colony (mm)

For quantitative estimation, the isolated strains were assessed by evaluation of solubilization of insoluble calcium phosphate into soluble form in PVK broth under agitated conditions along with different concentrations of pesticides (500 mg/L and 1000mg/L), metal ions and humic acid (100mg/L). One mL of inoculums from the test tubes with O.D. 0.5 (λ 600) was added to 100 mL Erlenmeyer flasks containing 25 mL of Pikovskaya's broth & further incubated at 30^{o}C till maximum solubilization was observed. Uninoculated flasks were taken as controls. The samples were centrifuged at 15,000 rpm for 10 minutes and supernatant was collected taken as a sample for a further test for phosphorus solubilised as given by John (1970). Standard strains of *Rhizobium leguminosarium* (positive control), and *E. coli* (Negative control) were procured from IMTECH (Institute of Microbial Technology) Chandigarh bearing MTCC no 10096 and 1696 to compare the result with the isolated strains both qualitatively and quantitatively.

5.3.3 Qualitative and quantitative estimation of Siderophores and effect of six different pesticides, Cu^{++}, Fe^{++} and humic acid in its production

For qualitative estimation of Siderophores, Chrome Azurole's (CAS) agar will be prepared and the cultures will be inoculated as spots with and without different concentrations of each pesticide along with metal ions and humic acid and incubated for 4–5 days at 28°C. The appearance of an orange or yellow ring around the colony is considered as a positive test (Joshi and Bhat. 2011).

The quantitative estimation of Siderophore by the isolated cultures was further calculated according to the protocol of Schwyn & Neilands (Schwyn and Neilands 1987). The pH was set to 6.8 by using Pipes buffer (0.1 M). 0.5mL of the CAS assay solution along with 5-sulfosalicylic acid (0.2 M) was added to the culture supernatant and allows mixing for a few minutes. Then absorbance was calculated at 630nm using CAS and 5-sulfosalicylic acid as a reference and was calculated as [(Ar - As)/Ar] x 100 = % Where As= O.D. of the sample and Ar is the O.D. of the standard. Same was repeated with and without different concentrations

(500 mg/L and 1000mg/L) of each pesticide in the presence and absence of Cu^{++}, Fe^{++} and humic acid (Hussein and Joo 2014).

Standard strains of *Pseudomonas fluorescens* (positive control) and *E. coli* (Negative control) were procured from IMTECH (Institute of Microbial Technology) Chandigarh bearing MTCC no 1749 and 1696 were used to compare the result with the isolated strains both qualitatively and quantitatively.

5.3.4 Qualitative estimation of cyanide production and effect of six different pesticides, Cu^{++}, Fe^{++} and humic acid in its production

This test will be determined by the qualitative method of spreading the culture on King's B medium in addition with 4.4 g glycine with and without different concentrations of each pesticide in the presence of Cu^{++} Fe^{++} ions and humic acid. A disc of Whatman filter paper soaked in the solution of picric acid (0.5%) and Na_2CO_3 (2%) will be positioned on the cover of the petri plates at 30 °C, for an incubation period of 4 days. The appearance of the brown orange colour of disc paper indicates HCN production. All the experiments were carried out in triplicates (Colo et al., 2014). Standard strains of *Pseudomonas fluorescens* (positive control) were procured from IMTECH (Institute of Microbial Technology) Chandigarh bearing MTCC no 1749 were used to compare the result with the isolated strains both qualitatively and quantitatively.

5.4 Effect of pesticides, Cu^{++}, Fe^{++} and humic acid on plant growth

5.4.1 Testing of Compatibility of selected bacterial cultures

Compatibility test for these isolates was performed following the method described by Nikam et al (2007) with slight modifications for in vitro testing. All the selected bacterial cultures were spread on the growth media and allowed to grow for 24 h at 37°c. Five mm size sterilized paper (Whatman paper no 1) discs impregnated with a bacterial suspension of individual isolates were placed at the distance of 5 mm from the periphery of petriplates already having growth of cultures inoculated in a different pattern to check the antagonistic effect of bacterial cultures on each other.

5.4.2 Seed Germination Assay

For experimentation work, damaged seeds were removed by inspecting and similar size seeds were selected and used for both the experimentation work (Filter paper method and pot experimentation method).

Before sowing, seeds were surface sterilized with 4% sodium hypochlorite solution for 30 min on a magnetic stirrer and rinsed with sterile distilled water; seeds were placed in sucrose solution (10%) for 15-20 minutes. After surface-sterilization, ten seeds were sown in a petriplate (equal distance from each other) containing triple layer of whatmann filter paper (Manmathan et al., 2013) and were treated with all the pesticides separately (Acephate, Atrazine, Carbendazim, Glyphosate, Monocrotophos and Phorate) at two different concentrations (recommended 1x and double of recommended 2x) along with same concentration of Cu^{++}, Fe^{++} and humic acid using distilled water (Acephate, Glyphosate, Monocrotophos) DMSO (Atrazine) and Ethanol (Carbendazim and Phorate) as a solvent and one group was left untreated and served as controls.

5.4.3 Effect of pesticides on length of radical and plumule treated with and without metal ions and humic acid

Similar experiments were done by inoculating the seeds into the bacterial suspension for 30 minutes after treating with sucrose solution (10%) and then suspended into the petriplates.

There were three major groups in this experiment; the first group consists of four different treatments in which the first was left untreated (control), second were amended with 100mg/L Cu^{++}, the third one with 100mg/L Fe^{++} and last one with 100mg/L of humic acid.

Second group was sub-divided into four parts containing 1x concentrations of each pesticide (recommended dose) separately along with same concentration of 100mg/L Cu^{++}, 100mg/L Fe^{++} and 100mg/L of humic acid.

The third group was the further division of the four parts containing 2x concentrations (double the recommended) of each pesticide separately along with the same concentration of 100mg/L Cu^{++}, 100mg/L Fe^{++} and 100mg/L of humic acid.

Plants were grown under natural light in greenhouse. After 3 days incubation, the number of germinated seeds was counted and the germination rate was calculated as

$$\text{Germination rate } \% - \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Moreover, the radical and plumule length were also calculated.

5.4.4 Soils collection and characterization for pot experimentation

The soil for pot experimentation was collected from the organic farming near Guru Hargobind Nagar Phagwara. They were continuously using this farm from past 13 years for

organic farming only. Stones and plant tissues were removed and set to air dry under laboratory conditions. The soils were passed through 2mm sieve and characterized for its physic-chemical properties and concentrations of nutrient analysis were analyzed at multiplex biotech private limited at Bangalore.

5.4.5 Effect of pesticides Cu^{++}, Fe^{++} and humic acid on plant growth parameters, chlorophyll and carotenoid content

In this experiment, more than 15 seeds treated with bacterial suspension and 15 untreated seeds were amended in 2kg of the autoclaved soil. For this experiment, same groups were set up as for the germination experiment (Dubey and Fulekar 2011). Three major groups were set up in this experiment; the first group consists of four different treatments in which the first was left untreated (control), second were amended with 100mg/L Cu^{++}, the third one with 100mg/L Fe^{++} and last one with 100mg/L of humic acid.

Second group was sub-divided into four parts containing 1x concentrations of each pesticide separately along with same concentration of 100mg/L Cu^{++}, 100mg/L Fe^{++} and 100mg/L of humic acid.

The third group was the further division of the four parts containing 2x concentrations of each pesticide separately along with the same concentration of 100mg/L Cu^{++}, 100mg/L Fe^{++} and 100mg/L of humic acid.

After seed germination, ten plants were selected according to their height and equidistance from each other and are used for further experimentation. The pesticides, humic acid and metal ions salts solution spray were sprayed when the plants were of 10 days old and distilled water was used as a solvent in whole experiments. From sowing to maturation no external mineral or nutrients were added. The plants were grown until grain maturation. Each treatment group consisted of three pot replicates with each containing 10 individual plants. Plants were watered with distilled water regularly throughout the experiment.

Physical parameters (plant height), Chlorophyll A, Chlorophyll B and carotenoids contents were also measured by following the protocol of Baghizadeh et al.,(2014).

The carotenoid and chlorophyll contents were extracted by using 80% acetone and 0.1 gram of freshly collected wheat plant and the values were calculated by using Lichtenthaler as follows

Chlorophyll a (µg/mL) = Ca = 12.25A663.2 – 2.79A646.8

Chlorophyll b (µg/mL) = Cb = 21.50 A646.8 – 5.10 A663.2

Carotenoids (µg/mL) = Tc = (1000-A470 – 1.8 Ca -85.02 Cb) /198 – 2.79A646.8.

Results and Discussion

6 Results and Discussion

6.1 Isolation and molecular characterization of the isolates

A total of 19 different bacterial strains were isolated by media enrichment technique utilizing pesticides as the sole source of carbon; carbon and nitrogen and carbon and sulphur. The soil sample was collected from the fields used for the cultivation of various cash crops, vegetables, fruits etc. The isolated bacterial strains were labelled as ACP1, ACP2, ACP3 in figure 2 (from acephate contaminated samples), GP1, GP2 and GP3 in figure 3 (from glyphosate contaminated samples), RK1, RK2, RK3 and RK4 in figure 4 (from atrazine contaminated samples), CB1, CB2, CB3, and CB4 in figure 5 (from carbendazim contaminated samples), MC1, MC2 and MC3 in figure 6 (from monocrotophos contaminated samples) and PR1 and PR2 in figure 7 (from phorate contaminated soils), respectively.

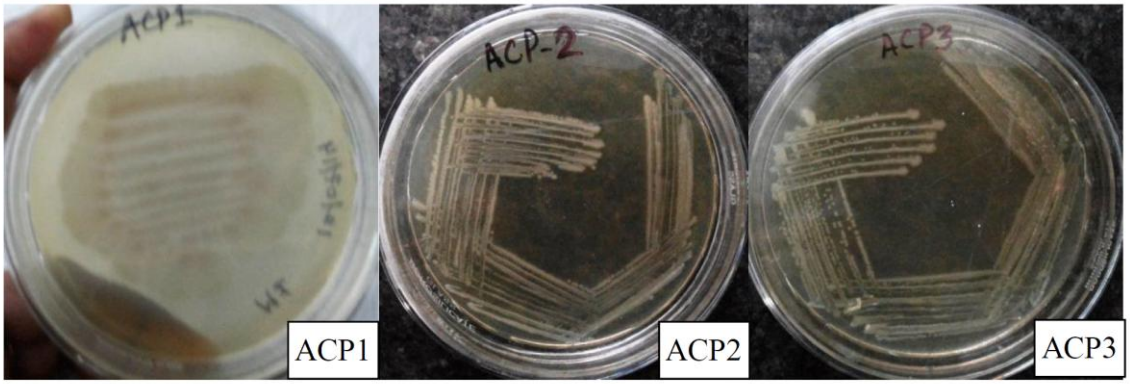


Figure 2: Rhizobacterial isolates obtained from acephate contaminated agriculture field soil samples (ACP1, ACP2, and ACP3).

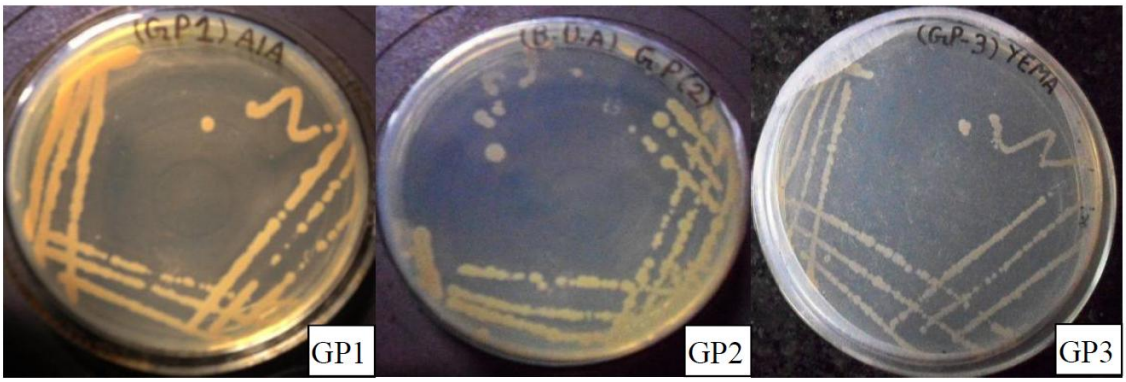


Figure 3: Rhizobacterial isolates obtained from glyphosate contaminated agriculture field soil samples (GP1, GP2, GP3)

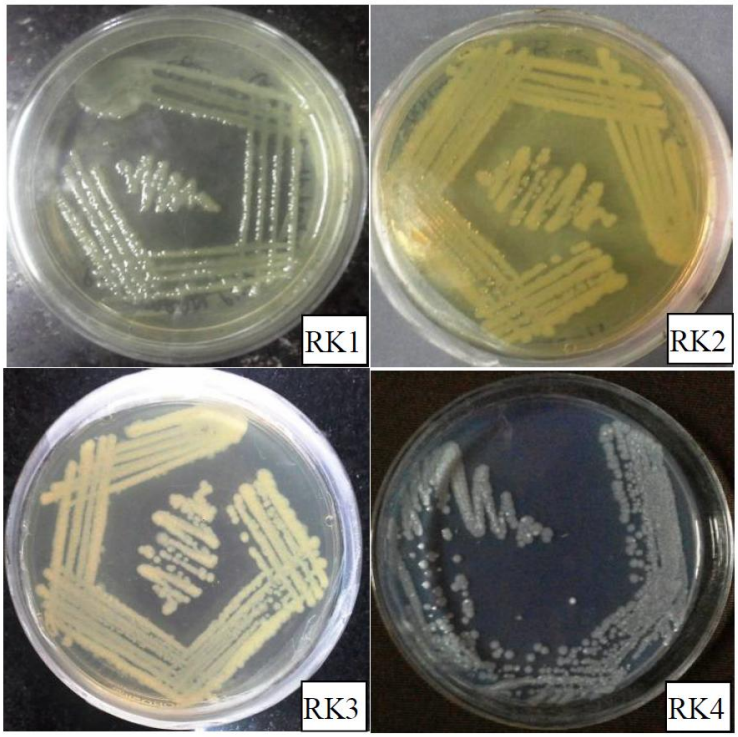


Figure 4: Rhizobacterial isolates obtained from atrazine contaminated agriculture field soil samples (RK1, RK2, RK3 and RK4)

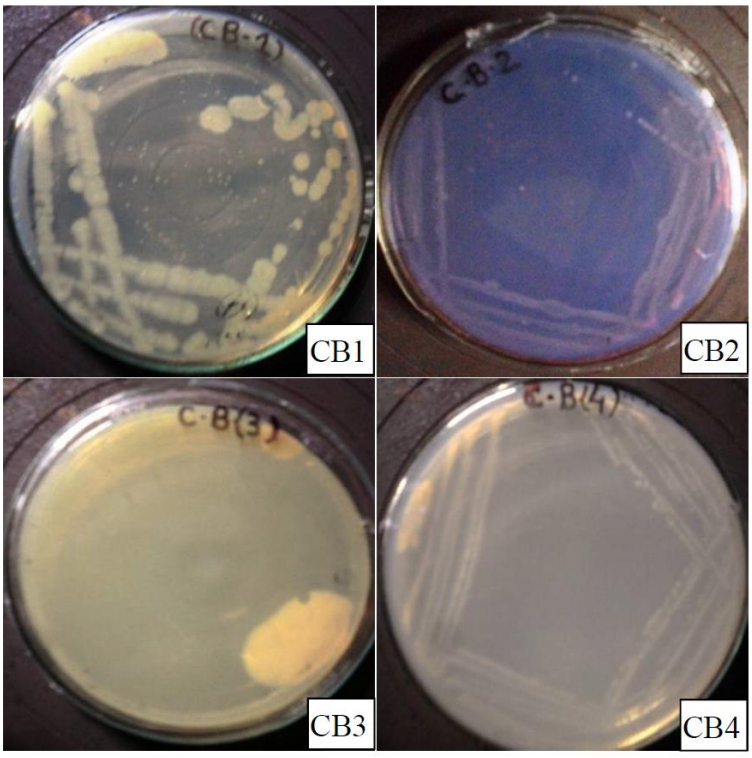


Figure 5: Rhizobacterial isolates obtained from carbendazim contaminated agriculture field soil samples (CB1, CB2, CB3 and CB4

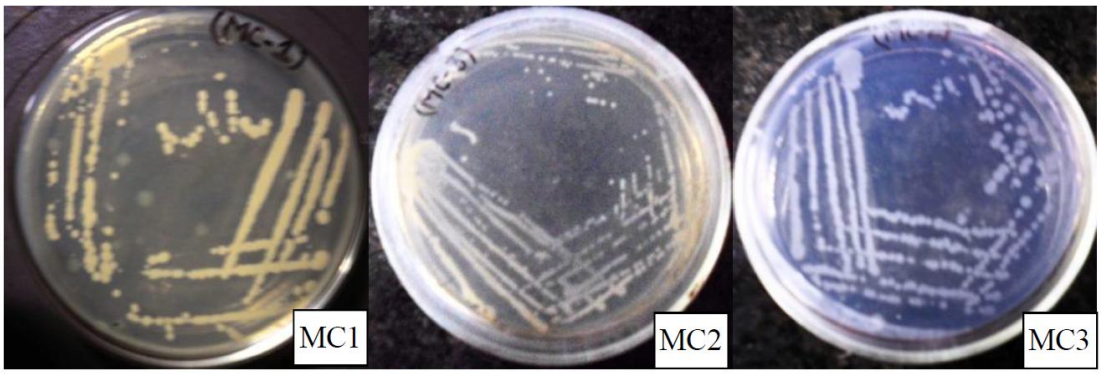


Figure 6: Rhizobacterial isolates obtained from monocrotophos contaminated agriculture field soil samples (MC1, MC2, and MC3)

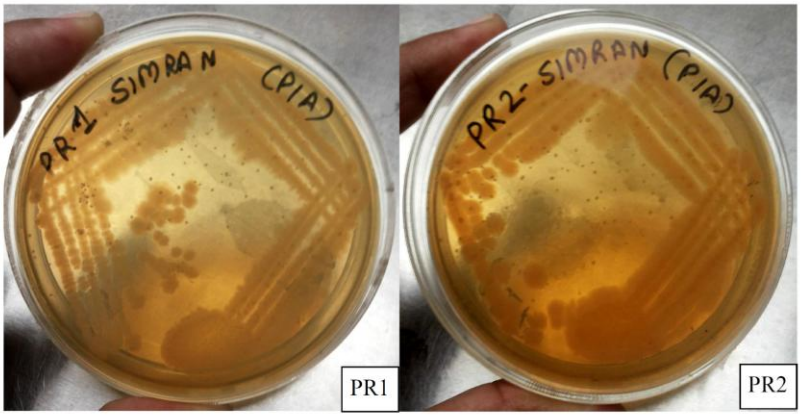


Figure 7: Rhizobacterial isolates obtained from phorate contaminated agriculture field soil samples (PR1 and PR2)

The very first step executed was the isolation of genomic DNA was done on 1.2% Agarose Gel, a single band of high-molecular-weight DNA has been observed. Fragment of 16S rDNA gene was amplified by PCR from the above-isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel which was continued with the purification of PCR amplicon to remove the undue contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 27F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The consensus sequence for rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.

Identification based on 16S rRNA gene sequencing reveals that the bacterial isolates were *Pseudomonas sp.* ACP1, *Pseudomonas sp* ACP2, *Pseudomonas sp* ACP3, *Streptomycetaceae bacterium* RK1, *Pseudomonas fluorescens strain* RK2, *Azotobacter chroococcum strain* RK3, *Rhizobium leguminosarum strain* RK4, *Actinomyces sp*. CB1, *Bacillus subtilis* CB2, *Pseudomonas aeruginosa* CB3, *Rhizobium leguminosarum* CB4, *Actinomyces sp*. GP1, *Bacillus subtilis* GP2, *Rhizobium leguminosarum* GP3, *Actinomyces sp.* MC1, *Bacillus subtilis* MC2, *Rhizobium leguminosarum* MC3, *Pseudomonas sp.* PR_01 and *Pseudomonas sp. PR_2* and the sequences were deposited in GeneBank under accession numbers shown in table 3.

Table 3: Identification based on 16S rRNA sequencing with accession numbers deposited in NCBI database (GenBank)

Isolates	Codes	Molecular Resemblance	Accession Number
1.	ACP1	*Pseudomonas* sp. ACP1	KP268769.1
2.	ACP2	*Pseudomonas sp* ACP2	KP268770.1
3.	ACP3	*Pseudomonas sp* ACP3	KP268771.1
4.	RK1	*Streptomycetaceae bacterium* RK1	KJ206091.1
5.	RK2	*Pseudomonas fluorescens* strain RK2	KJ466148.1
6.	RK3	*Azotobacter chroococcum* strain RK3	KJ511860.1
7.	RK4	*Rhizobium leguminosarum* strain RK4	KJ489410.1
8.	CB1	*Actinomyces* sp. CB1	KJ854399.1
9.	CB2	*Bacillus subtilis* CB2	KJ854400.1
10.	CB3	*Pseudomonas aeruginosa* CB3	KJ854401.1
11.	CB4	*Rhizobium leguminosarum* CB4	KJ854402.1
12.	GP1	*Actinomyces* sp. GP1	KJ854403.1
13.	GP2	*Bacillus subtilis* GP2	KJ854404.1
14.	GP3	*Rhizobium leguminosarum* GP3	KJ854405.1
15.	MC1	*Actinomyces* sp. MC1	KJ854396.1
16.	MC2	*Bacillus subtilis* MC2	KJ854397.1
17.	MC3	*Rhizobium leguminosarum* MC3	KJ854398.1
18.	PR1	*Pseudomonas* sp. PR_01	KP268772.1
19.	PR2	*Pseudomonas* sp. PR_2	KP268773.1

6.2 Biodegradation of pesticides

6.2.1 Cell growth

6.2.1.1 Bacterial cell growth of acephate degrading strains

In biodegradation assay, more than 95% degradation of 1000 mg/L acephate was observed within 14 days, while the cell density in 7 days increased from 0.012 to 0.036 (OD at 600 nm) in case of *Pseudomonas* sp. ACP1, 0.017 to 0.054 (OD at 600 nm) in *Pseudomonas* sp. ACP2 and 0.018 to 0.049 (OD at 600 nm) in *Pseudomonas* sp. ACP3, respectively. An increase in optical density (OD) at 600 nm has demonstrated the consumption of acephate as a source of carbon and phosphorus. With the addition 100 mg/L of Cu^{++} the cell density ranges in 7 days increased from 0.018 to 0.063 (OD at 600 nm) in case of ACP1, 0.021 to 0.058 (OD at 600 nm) in ACP2 and 0.018 to 0.039 (OD at 600 nm) in ACP3.

With the addition of 100 mg/L of Fe^{++} the rate of decomposition was approximately same as that of isolated strains + 1000 mg/L acephate experiment. Within 7 days, the cell density in case of Fe^{++} treated groups increased from 0.027 to 0.051 (OD at 600 nm) in case of ACP1, 0.014 to 0.050 (OD at 600 nm) in ACP2 and 0.017 to 0.032 (OD at 600 nm) in ACP3. In humic acid treated samples, the cell density groups increased from 0.032 to 0.055 (OD at 600 nm) in case of ACP1, 0.023 to 0.051 (OD at 600 nm) in ACP2 and 0.025 to 0.061 (OD at 600 nm) in ACP3 (figure 8).

Our study confirmed that all the isolated strains from acephate contaminated soils were capable of successfully removing acephate residues whilst the acephate is providing an energy source to them. These results indicate that further study on these organisms may help to understand the potential applications and metabolic versatility in bioremediation of acephate contaminated soil. Similar results were obtained by Ramu et al., (2014); Pinjari et al., (2012); Chai et al., (2010) in which the strains grown in minimal media supplemented with acephate achieves maximum growth. As the concentration of acephate was depleted in the minimal media solution, the growth rate slowed down reaching stationary phase, eventually reveal the declination of the cell density related to cell death.

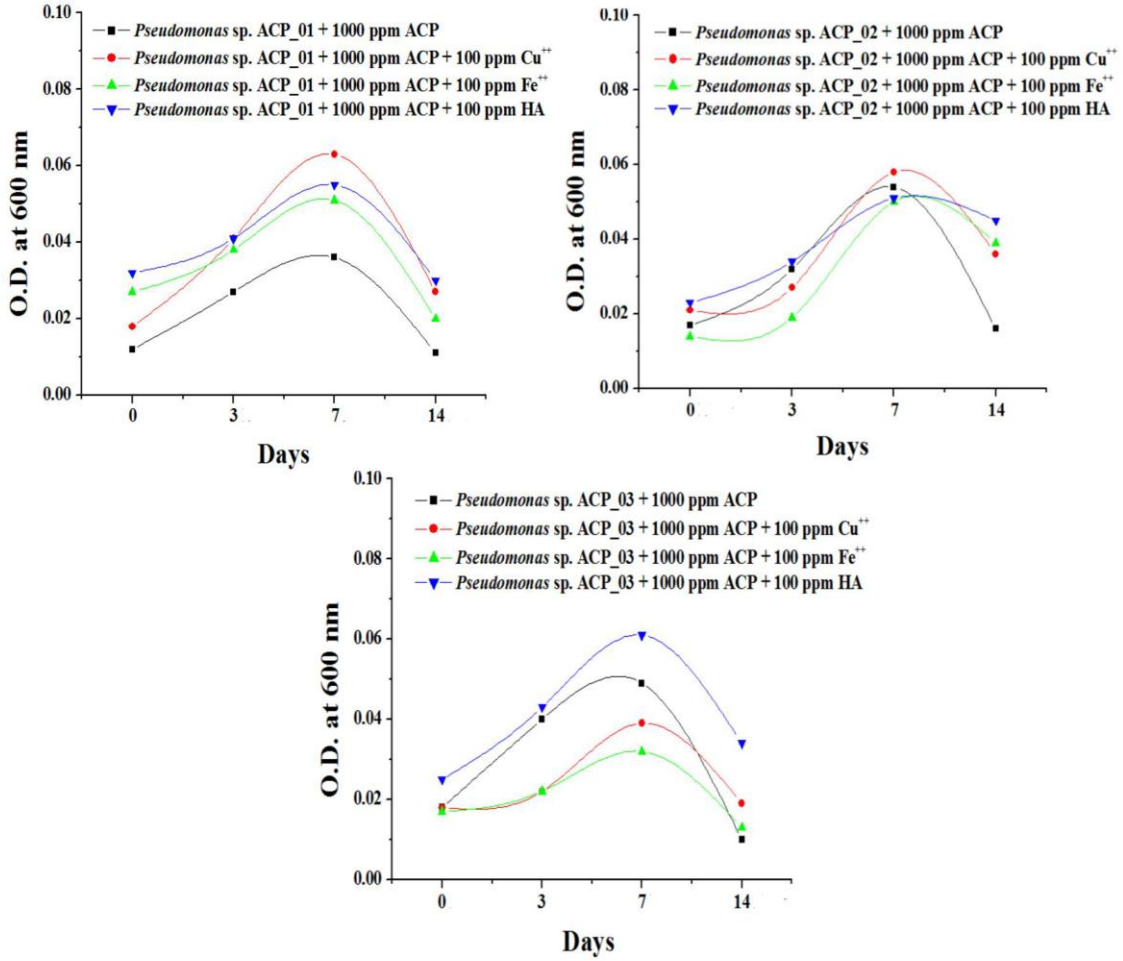


Figure 8: Effect of organophosphate acephate alone or in combination with humic acid (HA), Fe^{++} (as $FeCl_2$) and Cu^{++} (as $CuCl_2$) ions on the growth of bacterial isolates. Each isolate was grown in minimal salts medium and the growth was quantified by measuring absorbance at 600 nm. Here (A) *Pseudomonas* sp. ACP1, (B) *Pseudomonas* sp. ACP2 *&* (C) *Pseudomonas* sp. ACP3

6.2.1.2 Bacterial cell growth of atrazine degrading strains

More than 98% of the 1000 mg/L of atrazine was mineralized after 7 days of incubation with *Streptomycetaceae bacterium* RK1. However, remaining three strains *Pseudomonas fluorescens strain* RK2, *Azotobacter chroococcum strain* RK3, and *Rhizobium leguminosarum strain* RK4 shows the significant utilization of atrazine but low as compared to RK1. All the isolated cultures show threshold increase in their growth from 3rd day till 7th day. After 7 days of incubation, the relative growth of all the isolates starts decreasing. With the addition of Cu^{++}, the growth was relatively low in RK1, RK2, and RK3 except for RK4. In case of Fe^{++} treated groups, all the isolates showed least relative absorbance except RK2.

With the addition of 100 mg/L of humic acid, the growth of all isolates sharply increased as mentioned in figure 9.

Use of atrazine metabolites as carbon and nitrogen source was confirmed on the basis of the increase in optical density supplemented with 1000 mg/L. Our results obtained are similar to Yang et al., 2010; Singh et al., 2006; Radosevich et al., 1995, in which the strains utilizes atrazine as source of carbon and nitrogen up to a certain time period, the growth rate slowed down reaching stationary to cell death phase after certain period of time.

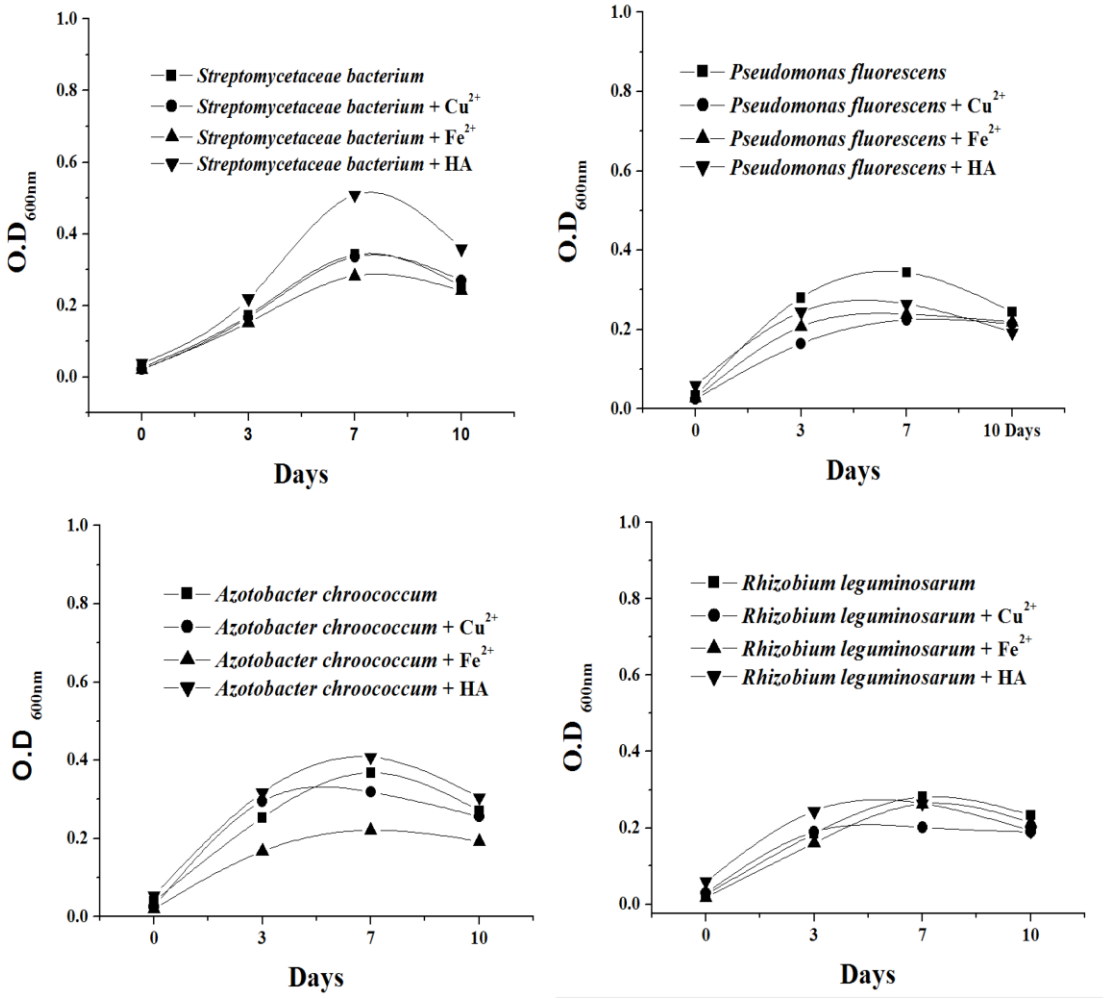


Figure 9: Effect of atrazine alone or in combination with humic acid (HA), Fe^{++} (as $FeCl_2$) and Cu^{++} (as $CuCl_2$) ions on the growth of bacterial isolates , RK1 = *Streptomycetaceae bacterium* RK1, RK2 = *Pseudomonas fluorescens* strain RK2, RK3 = *Azotobacter chroococcum* strain RK3, RK4 = *Rhizobium leguminosarum* strain RK4. Each isolate was grown in minimal salts medium and the growth was quantified by measuring absorbance at 600 nm

6.2.1.3 Bacterial cell growth of carbendazim degrading strains

The bacterial isolates were evaluated for their ability to utilize carbendazim as the sole source of carbon and energy. *Actinomyces* sp. CB1 incubated with carbendazim, showed a threshold increase in optical density from 0.026 to 0.51 indicating consumption of carbendazim as a source of carbon and energy (Figure 10). With the addition of 100 mg/L of Cu^{++}, the growth of *Actinomyces* sp. witnessed significant increase with a concomitant increase in carbendazim degradation as compared to carbendazim the only group. A similar pattern was observed with Fe^{++} and humic acid supplemented groups. In case of *B. subtilis* CB2, cell growth and carbendazim decomposition rate sharply increased when supplemented with Cu^{++}, Fe^{++} and humic acid. Comparable growth kinetics and degradation patterns were observed with *P. aeruginosa* CB3 and *R. leguminosarum* CB4.

These results were similar with the findings of Fang et al., 2010; Zhang et al., (2009) in which the isolated strains from carbendazim contaminated sites utilize carbendazim as a sole source of carbon and energy. A similar pattern of growth was observed in which growth rate increases, in the beginning, utilizing it as an energy source, then stationary and eventually reaches decline phase or death phase.

6.2.1.4 Bacterial cell growth of glyphosate degrading strains

The bacterial isolates were checked for their capability to utilize glyphosate as the sole source of carbon. *Actinomyces* sp. GP1 showed threshold increase in OD from 0.02 to 0.034 demonstrating utilization of glyphosate as a source of carbon. With the addition of 100 mg/L of Cu^{++}, the growth of *Actinomyces* sp. GP1 shows a significant increase as compared to glyphosate only. Similar patterns were observed with groups treated with Fe^{++} and humic acid. In case of *B. subtilis* GP2 and *R. leguminosarum* GP3, cell growth and glyphosate degradation rate sharply increased when supplemented with Cu^{++}, Fe^{++} and humic acid. Comparison of relative growth kinetics observed is displayed in figure 11.

During the growth of all the isolates strains, the growth curves obtained were similar to S shape. The continuation of growth phase to log phase then to stationary phase depicts the utilization of the glyphosate as energy source rapidly. Compared with the previous finding of Fan et al., (2012) there is threshold increase in optical density and these isolates has high environmental application and will be used for remediation of glyphosate contaminated sites.

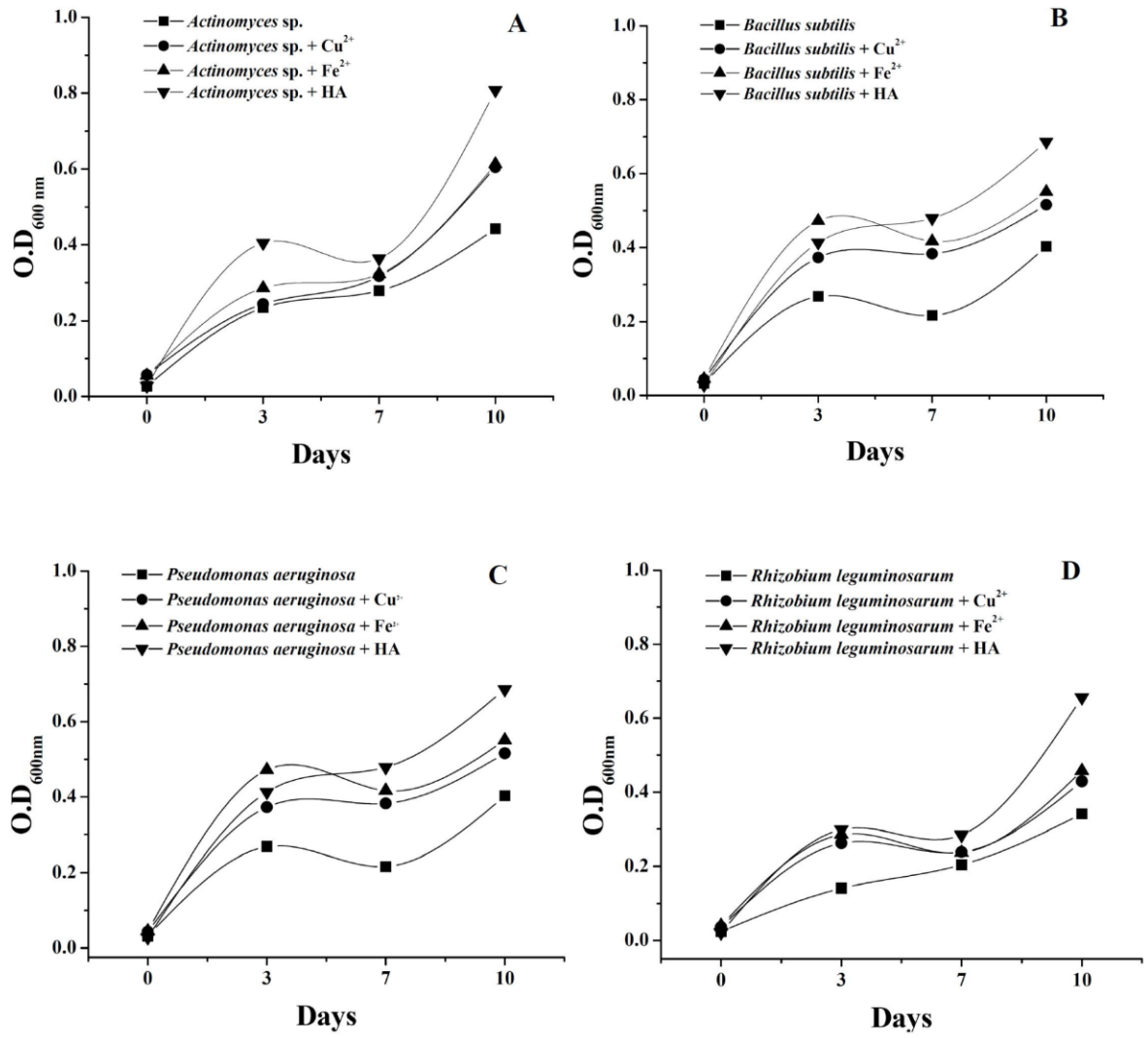


Figure 10: Effect of fungicide carbendazim alone or in combination with humic acid (HA), Fe^{++} (as $FeCl_2$) and Cu^{++} (as $CuCl_2$) ions on the growth of bacterial isolates. Each isolate was grown in minimal salts medium and the growth was quantified by measuring absorbance at 600 nm. Here (A) *Actinomyces* sp. CB1 (B) *Bacillus subtilis* CB2 (C) *Pseudomonas aeruginosa* CB3 and (D) *Rhizobium leguminosarum* CB4. Concentration of carbendazim used in degradation experiments was 1000 ppm whereas, HA, Fe^{++} and Cu^{++} were included at 100 ppm concentrations.

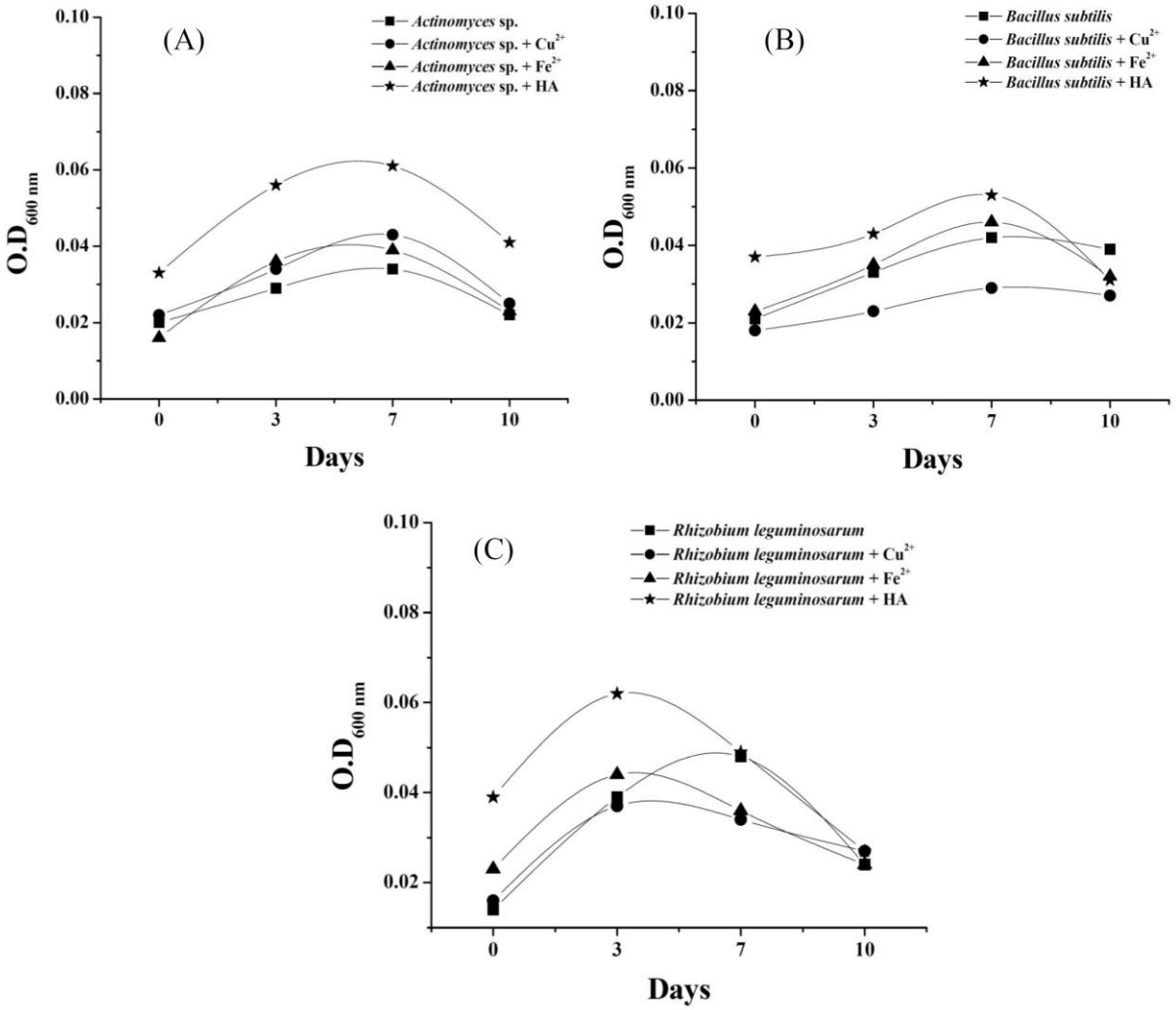


Figure 11: Effect of glyphosate singly and in combination with humic acid (HA), Fe^{++} (as $FeCl_2$ and Cu^{++} (as $CuCl_2$) on the growth of bacterial isolates. Each isolate was cultured in minimal salts medium and the growth was measured at different time points by recording OD_{600} nm. Here (A) represent *Actinomyces* sp. GP1, (B) *Bacillus subtilis* GP2 and (C) *Rhizobium leguminosarum* GP3. Concentration of glyphosate used in degradation experiments was 1000 mg/L whereas HA, Fe^{++} and Cu^{++} were included at 100 mg/L concentrations

6.2.1.5 Bacterial cell growth of monocrotophos degrading strains

The ability of the isolated strains to utilize monocrotophos as a sole carbon source was demonstrated by bacterial growth on minimal media supplemented with 1000 mg/L of monocrotophos, achieving a maximal growth rate. As the concentration of monocrotophos was depleted, cell growth slowed and reached a stationary cell density, ultimately demonstrating the decline in density associated with cell death. At the same time, the

concentration of the metabolites (E)-methyl 4-(methylamino)-4-oxobut-2-en-2-yl methylphosphonate, dimethyl phosphate, dimethyl phosphonate, methyl phosphonate and acetic acid increased through the first 7 days of growth. Upon reaching its maximum, the concentration did not change during further incubation. More than 90% degradation of monocrotophos was observed within 7 days, while the cell density increased from 0.013 to 0.037 (O.D. 600 nm). With the addition of 100 mg/L of Cu^{++}, Fe^{++} and humic acid similar patterns of cell growth was observed in all the three isolates (Figure 12).

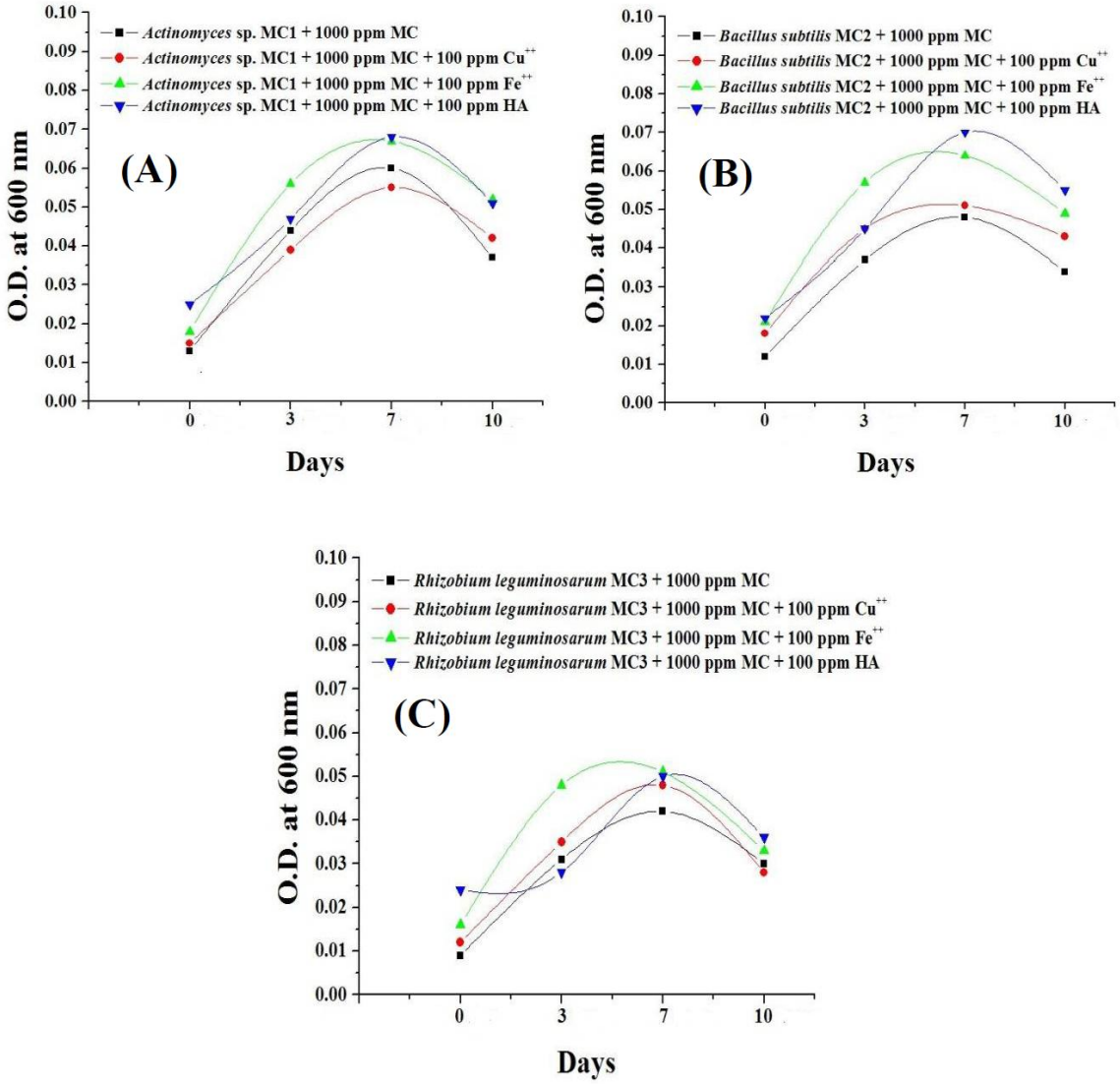


Figure 12: Effect of monocrotophos singly or in combination with humic acid (HA), Fe^{++} (as $FeCl_2$) and Cu^{++} (as $CuCl_2$) ions on the growth of bacterial isolates. Each isolate was cultured in minimal salts medium and the growth was quantified by measuring absorbance at 600 nm.

This is the first report in which addition of monocrotophos utilized by the *Rhizobium* species as a sole source of carbon. Two other species of the genus *Actinomyces* and *Bacillus* were also capable of degrading monocrotophos when monocrotophos was provided to them as an

energy source. This observation was in agreement with previously published work of Jia et al., (2006) and Bhadhabde et al., (2002) and this growth curve will help us to understand how the isolated strains are involved in degradation of monocrotophos from environmental samples.

6.2.1.6 Bacterial cell growth of phorate degrading strains

More than 98% of the 1000 mg/L of phorate was mineralized after 7 days of incubation with both the species of the pseudomonad genus. However, all the isolated cultures show threshold increase in their growth from 3rd day till 7th day. After 7 days of incubation, the relative growth of all the isolates starts decreasing. With the addition of Cu^{++}, the growth was relatively low in PR1 and PR2. In case of Fe^{++} treated groups, all the isolates show least relative absorbance except PR2. With the addition of 1000 mg/L of humic acid, the growth of all isolates sharply increased as mentioned in figure 13.

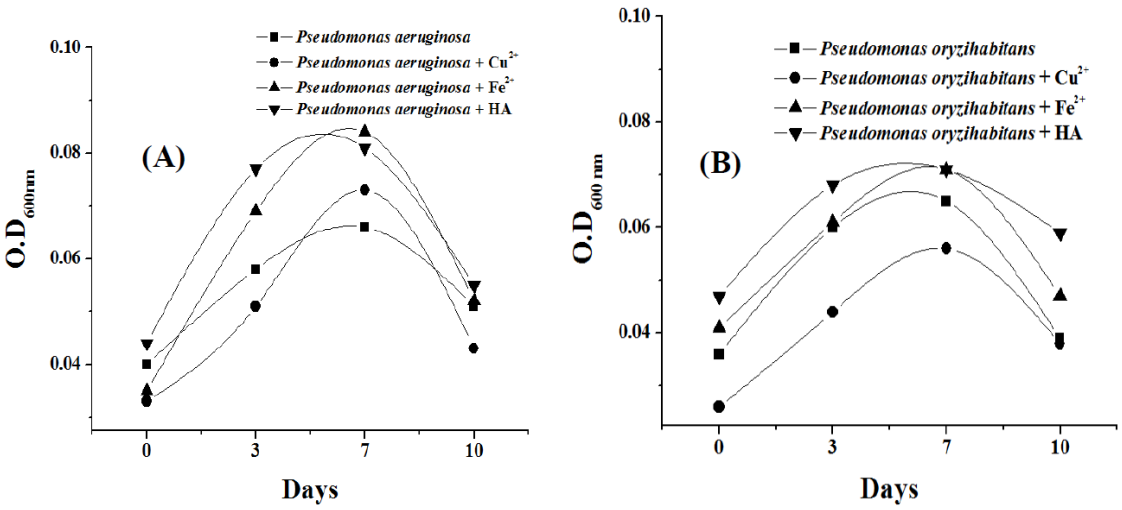


Figure 13: Effect of phorate singly or in combination with humic acid (HA), Fe^{++} (as $FeCl_2$ and Cu^{++} (as $CuCl_2$) on the growth of bacterial isolates. Each isolate was cultured in minimal salts medium and the growth was measured at different time points by recording OD_{600} nm. Here (A) represent *Pseudomonas* sp. PR_01, and (B) *Pseudomonas* sp. PR_02. Concentration of Phorate used in degradation experiments was 1000 mg/L whereas HA, Fe^{++} and Cu^{++} were included at 100 mg/L concentrations.

Thus, the observed growth curves by the isolated strains corroborate well with the earlier studies of Bano and Mussarat (2003) in which phorate was used as energy source. Increase in OD depicts the utilization of phorate by the isolated strains as an energy source. Thus, the multifarious biodegradation by the isolated strains demonstrates the agronomic and

environmental significance of the isolates for their possible exploitation in phorate removal from environmental samples.

6.2.2. Determination and degradation of pesticides metabolites

6.2.2.1 Degradation of acephate and identification of metabolites by ESI-MS

The mass/charge ratio (m/z) of acephate observed at m/z 183 i.e. {[M+H]+} and [M+H]+H_2O at m/z 198, four other metabolites were characterized at metamidophos m/z at 141, phosphoramic acid m/z ratio at 96, phosphenamide at m/z 78 and m/z 62. In addition, oxidation of the protonated acephate molecule at m/z 165, oxidation occurred during the ionization process resulting in a successive increase for first 7 days followed by a decrease in a peak at m/z 165 and OD at 600 nm. The percentage decrease in a peak at m/z 183 has confirmed that up to 14 days more than 95% acephate was decomposed. In case of ACP1, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (90%). On 7th days the acephate was decomposed into methamidophos at m/z 141 (78%), m/z phosphoramic acid at 96 (27%), phosphenamide at m/z 78 (43%).and m/z 62 (34%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed into methamidophos at m/z 141 (100%), and phosphenamide at m/z 78 (7%). In case of ACP2, on the 3rd day, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (87%). On 7th days the acephate was decomposed into methamidophos at m/z 141 (64%), m/z phosphoramic acid at 96 (62%), phosphenamide at m/z 78 (45%), and m/z 62 (22%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed into methamidophos at m/z 141 (94%), and phosphenamide at m/z 78 (10%). In case of third isolated strain ACP3, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (91%). On 7th days the acephate was decomposed into methamidophos at m/z 141 (87%), m/z phosphoramic acid at 96 (40%), phosphenamide at m/z 78 (53%), and m/z 62 (43%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed into methamidophos at m/z 141 (100%), and phosphenamide at m/z 78 (48%). Many bacteria have the potential to use specific pesticides as a sole of Carbon, phosphorous, nitrogen and Sulphur has been isolated (Ramu and Seetharaman 2014). The species of *Pseudomonas* have been isolated worldwide and some of them have shown positive results for degradation of acephate and its related compounds (Wang et al. 2010). The degradation pathway of acephate varies from species to

species suggesting two alternate degradation pathways. Some of the species follow the conversion of acephate to methamidophos which would be generated by Carboxyl esterase type enzyme and some acephate to O-methyl N- acetylphosphoramidate by the generation of phosphor-triesterase type enzyme to hydrolyse the bond of P-S (Singh and Walker 2006).

In case of Cu^{++} supplemented groups, the rate, as well as mode of bio-decomposition, was faster than to that of isolated strains + 1000 mg/L acephate. The percentage decrease in a peak at m/z 183 has confirmed that up to 14 days and in the presence of Cu^{++} ion more than 99% decomposition of acephate was observed. In case of ACP1, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (90%). On 7th days the acephate was totally decomposed into methamidophos at m/z 141 (24%), m/z phosphoramic acid at 96 (95%) and m/z 62 (100%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed

In second bacteria ACP2, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (56%) was decomposed into m/z 141 (100%). On 7th days the acephate was totally decomposed into phosphoramic acid at m/z 96 (100%), and phosphenamide at m/z 78 (24%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed. In case of third bacteria ACP3, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (50%) was decomposed into m/z 141 (100%). On 7th days, drastically, the acephate m/z at 183 (82%) was totally decomposed into methamidophos at m/z 141 (52%), m/z phosphoramic acid at 96 (74%) and m/z 62 (100%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed.

Here the formation of the stable complex at m/z 96 most prominently due to Cu-SH2 complex formation, which was confirmed by taking mass spectra of aliquot because it is a polar material. Here, phosphate to Cu complex formation was very consistent with a recent study of Yan et al., (2012), they reported that phosphate can promote the mineral dissolution, probably be due to the different affinities between metals (Fe>>Co>Ni>Cu) and phosphates. Preliminary studies have revealed that methamidophos and acephate highly persist on soils (Zhang et al. 2005; Battu et al. 2009). These are very toxic to soils microorganisms (Battu et al. 2009).

As per HSAB principle, soft metal ion Cu^{++} interacted with soft ligand S and leads to the formation of the stable complex. The rate of decomposition of acephate was not inhibited by the presence of Cu^{++} because of its paramagnetic (d9) nature. Once Cu^{++} interacted with S,

the methyl group would be cleaved and trans effect would allow the retention of NH_2 group with P and copper. In a net shell, Cu^{++} will facilitate the regular decomposition through -N-CO- bond. Amongst the Co^{++}, Cu^{++}, Ni^{++}, Pb^{++} and Zn^{++} divalent metals it was observed that, out of these metal ions, Most suitable rank was possessed by Cu^{++} for the following three catalytic mechanisms and also facilitated as the most efficient catalyst for the OPs (Blanchet and George 1982; Manzanilla-Cano 2004; Sarkouhi et al 2012). Due to the limitations associated with solubility at high pH, a decrease in the catalytic activity was observed for Cu^{++}.

In the presence of ferric ion (100 mg/L), the rate of bio-decomposition of acephate was decreased. The mode of decomposition was almost similar as that of without ferric ion. The percentage decrease in a peak at m/z 183 has confirmed that up to 14 days, acephate was decomposed.

In humic acid supplemented groups, it was observed that acephate gets associated with humic acid, and become non-available to strain until maximum humic acid was not consumed by strain as a carbon source. That's why only slow decomposition of acephate was observed up to 7 days. In case of ACP1, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (85%). On 7th days the acephate was decomposed into methamidophos at m/z 141 (72%), m/z phosphoramic acid at 96 (58%) and m/z 62 (78%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed into methamidophos at m/z 141 (53%), m/z phosphoramic acid at 96 (51%), m/z 78 (47%) and m/z 62 (91%). In second bacteria ACP2, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (85%). On 7th days the acephate was decomposed into methamidophos at m/z 141 (68%), m/z phosphoramic acid at 96 (67%) and m/z 62 (57%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed into methamidophos at m/z 141 (81%), and m/z 78 (3%). In case of ACP3, On 3rd days, from the mass analysis studies partial decomposition of acephate was observed, that is acephate at m/z 183 (100%) was decomposed into m/z 141 (85%). On 7th days the acephate was decomposed into methamidophos at m/z 141 (27%). It was observed that on the 14th days of the decomposition, acephate was totally decomposed into methamidophos at m/z 141 (88%), and m/z 78 (4%).

The slow decomposition of acephate is attributed to using of humic acids as a carbon source by strain as well as binding of acephate with humic acid. This thing also confirms the

selectivity of microorganisms towards their energy resources. The cause behind the selectivity may be toxicity of acephate, the toxicity of acephate is far greater than humic acid. This fact is equally applicable to other xenobiotics, and this is an important finding in terms of future perspectives.

Humic compounds are known as colloids that interact with pesticides through hydrogen bonding, charge transfer, hydrophobic bonding, and van der Waals bonding to form complexes of various stabilities (Beale et al 2013; Li et al 2003; Senesi 1992). Humic colloids are capable of absorbing both hydrophilic and hydrophobic compounds. Electron density and electro negativity play a crucial role in the binding mechanism of pesticides and humic acid. Results have suggested that in the water-soluble pesticides, HA attach because of the polar bonds and leads to the formation of H bonds prominently including dipole–dipole interactions (Senesi 1992). As per literature in the current study, the humic acid can form H-bonding between the N-H/C=O/P=O group of acephate and COOH/O-H/N-H groups of humic acid (Piccolo and Celano 1994). The aggregation of the humic molecule with increasing OPs or acephate content is probably due to an increase in hydrogen bond interactions between the P=O and C=O groups of acephate and the humic polymer (Miano and Sensei 1992). It was observed in past that the aggregation take place at a slower pace when is pH 4 and 5 whereas the pace is sharply increased by increasing pH (~8) where complete aggregation is achieved in 30 min (Brigante et at 2009). It was also observed that presence of monocarboxylic acids and anionic pesticides significantly increase the dissolution rate (Brigante et at 2009; Miano and Sensei 1992).

Table 4: Bio-decomposition behaviour of Acephate with Pseudomonad sps and different metabolites produced during degradation (methamidophos, phosphoramic acid, Phosphenamide) after 3, 7 and 14 days of degradation by ESI-MS

Metabolites observed after degradation at different time intervals (ESI-MS)			
Treatment	**3 days**	**7 days**	**14 days**
Pseudomonas sp. ACP_01 + 1000mg/L acephate	Methamidophos m/z at 141 (90%)	Methamidophos m/z at 141 (78%), Phosphoramic acid m/z at 96 (27%), Phosphenamide m/z at 78 (43%) and m/z at 62 (34%).	Methamidophos m/z at 141 (100%), and phosphenamide m/z at 78 (7%).
Pseudomonas sp. ACP_02 + 1000mg/L acephate	Methamidophos m/z at 141 (87%).	Methamidophos m/z at 141 (64%), Phosphoramic acid at m/z 96 (62%), Phosphenamide m/z at 78 (45%), and m/z at 62 (22%).	Methamidophos m/z at 141 (94%) and phosphenamide m/z at 78 (10%).
Pseudomonas sp. ACP_03 + 1000mg/L acephate	Methamidophos m/z at 141 (91%).	Methamidophos m/z at 141 (87%), Phosphoramic acid m/z at 96 (40%), Phosphenamide m/z at 78 (53%), and m/z at 62 (43%).	Methamidophos m/z at 141 (100%) and Phosphenamide m/z at 78 (48%).
Pseudomonas sp. ACP_01 + 1000mg/L Acephate + 100 mg/L Cu^{++}	Methamidophos m/z at 141 (90%).	Methamidophos m/z at 141 (24%), Phosphoramic acid m/z at 96 (95%) and m/z at 62 (100%).	Totally decomposed.
Pseudomonas sp. ACP_02 + 1000mg/L Acephate + 100 mg/L Cu^{++}	Methamidophos m/z at 141 (100%).	Phosphoramic acid m/z at 96 (100%), and Phosphenamide m/z at 78 (24%).	Totally decomposed
Pseudomonas sp. ACP_03 + 1000mg/L Acephate + 100 mg/L Cu^{++}	Methamidophos m/z at 141 (100%).	Methamidophos m/z at 141 (52%), Phosphoramic acid m/z at 96 (74%) and m/z at 62 (100%).	Totally decomposed
Pseudomonas sp. ACP_01 + 1000mg/L Acephate + 100 mg/L Fe^{++}	Methamidophos m/z at 141 (85%).	Methamidophos m/z at 141 (64%), Phosphoramic acid m/z at 96 (69%) and m/z at 62 (85%).	Methamidophos m/z at 141 (100%), and Phosphenamide m/z at 78 (3%).
Pseudomonas sp. ACP_02 + 1000mg/L Acephate + 100 mg/L Fe^{++}	Methamidophos m/z at 141 (85%).	Methamidophos m/z at 141 (64%), Phosphoramic acid m/z at 96 (43%) and m/z at 62 (65%).	Methamidophos m/z at 141 (61%), Phosphoramic acid m/z at 96 (47%), Phosphenamide m/z at 78 (47%) and m/z at 62 (65%).
Pseudomonas sp. ACP_03 + 1000mg/L Acephate + 100 mg/L Fe^{++}	Methamidophos m/z at 141 (85%).	Methamidophos at m/z 141 (57%), Phosphoramic acid m/z at 96 (45%) and m/z at 62 (33%).	Methamidophos m/z at 141 (100%), and Phosphenamide m/z at 78 (4%).
Pseudomonas sp. ACP_01 + 1000mg/L Acephate + 100 mg/L HA	Methamidophos m/z at 141 (85%).	Methamidophos at m/z 141 (72%), Phosphoramic acid m/z at 96 (58%) and m/z at 62 (78%).	Methamidophos at m/z 141 (53%), Phosphoramic acid m/z at 96 (51%), m/z at Phosphenamide 78 (47%) and m/z 62 (91%).
Pseudomonas sp. ACP_02 + 1000mg/L Acephate + 100 mg/L HA	Methamidophos m/z at 141 (85%)	Methamidophos m/z at 141 (68%), Phosphoramic acid m/z at 96 (67%) and m/z at 62 (57%).	Methamidophos at m/z 141 (81%), and m/z at 78 (3%).
Pseudomonas sp. ACP_03 + 1000mg/L Acephate + 100 mg/L HA	Methamidophos m/z at 141 (85%).	Methamidophos m/z at 141 (27%).	Methamidophos m/z at 141 (88%), and Phosphenamide m/z at 78 (4%).

6.2.2.2 Degradation of atrazine and identification of metabolites by GC-MS

The mass/ charge ratio (m/z) of atrazine observed at m/z 200 i.e. {[M+H]+} with retention time of 19.363 min. Two different metabolites were formed by all the isolated bacterial cultures after 7 days of degradation and were identified with mass charge ratio of 120 (M+1) and 63 and two other major metabolites were characterized and identified as ((ethylamino) methylamino)methanediol m/z at 120, and aminomethanediol at m/z 63. Oxidation occurred during ionization process the oxidation takes place which showed a successive increase for first 7 days followed by peak declination at m/z 200 and OD at 600 nm. Percentile peak declination at m/z 200 has confirmed that up to 7 days more than 95% atrazine was decomposed. In all isolated bacteria *Streptomycetaceae bacterium* RK1, *Pseudomonas fluorescens* strain RK2, *Azotobacter chroococcum* strain RK3 and *Rhizobium leguminosarum* strain RK4 same major metabolites were formed. The only difference is in their degradation percentage of major compound and formation of new compounds. In case of *Streptomycetaceae bacterium* RK1, the percentile peak declination at m/z 200 resulted as the formation of two metabolites (ethylamino)methylamino) methanediol m/z at 120 (8%) and aminomethanediol at m/z 63 (80%). With the addition of Cu^{++} and humic acid, the degradation percentage was very low (58% and 35% decrease in major peak m/z at 200). In Fe^{++} supplemented groups, the degradation percentage was higher almost 93% decrease in base peak.

Our findings indicated highest degradation of atrazine were common by the two isolates *Streptomycetaceae bacterium* RK1 and *Pseudomonas fluorescens* strain RK2 (98%) followed by *Azotobacter chroococcum* strain RK3 (95%) and *Rhizobium leguminosarum* strain RK4 (92%) on the incubation of 7 days showed the formation of same metabolites of different percentages. Effect of Cu^{++} on the degradation of atrazine is quite similar only in case of *Azotobacter chroococcum* strain RK3 and *Rhizobium leguminosarum* strain RK4 as compared to the un-supplemented group. The degradation percentage after 7 days were highest in *Pseudomonas fluorescens* strain RK2 (95%) > *Azotobacter chroococcum* strain RK3 (81%) > *Rhizobium leguminosarum* strain RK4 (78%) > and in *Streptomycetaceae bacterium* RK1 (58%). Effect of Fe^{++} on the degradation of atrazine was examined under the same circumstances and found that the degradation percentage was highest in *Streptomycetaceae bacterium* RK1 (82.46%) after 7 days as compared to other isolates *Pseudomonas fluorescens* strain RK2 (80%), *Azotobacter chroococcum* strain RK3 (72%) and *Rhizobium leguminosarum* strain RK4 (67%). Effect of humic acid on biodegradation of atrazine was found under the same circumstances and found the degradation percentage was

highest in *Pseudomonas fluorescens* strain RK2 (92%), *Azotobacter chroococcum* strain RK3 (88%), *Rhizobium leguminosarum* strain RK4 (44%) and *Streptomycetaceae bacterium* RK1 (35%).

This is the first study on atrazine interactions with metal ions and soil humic acid. Since atrazine contains NH, Cl and CH_3 coordinating sites, it is expected that it is involved in interaction with soil metal ions and soil humic contents. Atrazine can be degraded and detoxified rapidly by bacterial strains. In previous studies, some microbial species of *Bacillus subtilis Strain HB-6* (Wang et al., 2014) *Arthrobacter sp* (Wang et al., 2013) *E. cloacae strain JS08* (Solomon et al., 2013) *Arthrobacter strain DNS 10* (Zhang et al., 2011) *Arthrobacter* sp. *T3AB1* (Liu et al., 2010) *Klebsiella* sp. *A1 Comamonas* sp.*A2* (Yang et al., 2010) *Arthrobacter* sp. *GZK-1* (Getenga et al., 2009) showed degradation of atrazine showed degradation of atrazine under experimental and environmental conditions. Dechlorination, dealkylation and deamination are known to be the major routes for atrazine transformation. Some bacteria initiate degradation of atrazine involving the enzyme atrazine chlorohydrolase through the mechanism of hydrolytic dechlorination. Aminohydrolases catalyse two hydrolytic deamination reactions that hydroxyatrazine undergoes; N-isopropylammelide (Geetenga et al. 2009; Qinggyan et al. 2008) or N-ethylammelide (Topp et al. 2000) is formed as the intermediate metabolites. These ammelides are finally converted to cyanuric acid (Yang et al. 2010). Another route followed for atrazine degradation is N-dealkylation of the lateral ethyl and isopropyl chains to deethylatrazine, deisopropylatrazine, and deethyldeisopropylatrazine (Wang et al. 2011). These dealkylated atrazine metabolites undergo hydroxylation and cyanuric acid is formed as the ultimate metabolite (Vaishampayan et al., 2007). Atrazine contain one or more binding sites and they may interact with an essential metal ion of soil, organic matter etc (Trevisan et al., 2010). The interactions of the atrazine with soil at the molecular level are central to their bioavailability, bioaccumulation, and transport in the environment (Kutman et al. 2013).

Table 5: Fragmentation of atrazine into different metabolites after 7 days incubated with different isolated strains

Treated groups (atrazine (Atr) used 1000mg/L, metal ions (Cu^{++} and Fe^{++} used 100mg/L and HA used 100mg/L)	**Fragmentation into major metabolites in % age at given m/z after 7 days**		
	Atrazine m/z = 200	(Ethylamino)methylaminomethanediol m/z = 120	Aminomethanediol m/z = 63
Streptomycetaceae RK1 +Atr	1.89%	8.18%	80.15%
Streptomycetaceae RK1 +Atr +Cu^{++}	42%	29%	17%
Streptomycetaceae RK1 +Atr +Fe^{++}	7%	16%	51%
Streptomycetaceae RK1 +Atr+HA	65%	15%	11%
P.fluorescens strain 2+Atr	2%	22%	71%
P.fluorescens strain 2+Atr +Cu^{++}	5%	43%	41%
P.fluorescens strain 2+Atr +Fe^{++}	20%	43%	23%
P.fluorescens strain 2+Atr +HA	8%	54%	14%
A. chroococcum RK3+Atr	5%	6%	80%
A. chroococcum RK3+Atr + Cu^{++}	19%	13%	55%
A. chroococcum RK3+Atr +Fe^{++}	28%	14%	38%
A. chroococcum RK3+Atr +HA	12%	33%	40%
R. leguminosarum RK4+Atr	8 %	16%	87%
R. leguminosarum RK4+Atr +Cu^{++}	42%	33%	61%
R. leguminosarum RK4+Atr +Fe^{++}	36%	54%	47%
R. leguminosarum RK4+Atr +HA	35%	41%	53%

6.2.2.3 Degradation of carbendazim and identification of metabolites by HPLC

Degradation of carbendazim by bacterial isolates CB1 to CB4 was determined in minimal salts media at a concentration of 1000 mg/L. Our finding indicates the highest degradation of carbendazim (73.73%) by *P. aeruginosa* CB3 followed by CB2 (65.69%), CB1 (59.04%) and CB4 (55.29%) after 3 days incubation (Fig.14). On the other hand, the longer incubation period of 7 days resulted in highest degradation in CB1 (91.65%) followed by CB3 (87.35%), CB2 (81.85%) and CB4 (76.54%) (Fig. 15). Effect of Cu^{++} on the degradation of carbendazim is quite high as compared to unsupplemented group (Fig 14 and 15). The degradation percentage after 3 days were highest in isolate CB3 (93.73%) > CB1 (86.72%) > CB2 (85.4%) > and in CB4 (83%). After 7 days of incubation, maximum degradation of carbendazim was manifested by in CB3 (94.92%) followed by CB2 (89.61%), CB1 (87.89%) and least by CB4 (76.54%). These findings were in agreement with HPLC analyses (Fig. 16 A-D).

Effect of Fe^{++} on the degradation of carbendazim was examined under the same circumstances and found that the degradation percentage was highest in CB3 (82.46%) after 3 days as compared to other isolates CB2 (78.42%), CB1 (75.24%) and CB4 (57.85%). After an incubation of 7 days the degradation percentage was highest in CB1 (95.54%), CB3 (89.89%), CB2 (87.85%) and CB4 (79.47%). Addition of humic acid results in the removal of carbendazim at a slower rate after 3 days. The degradation percentage was highest in CB1 (82.18%), CB3 (81.9%), CB2 (67.33%) and CB4 (45.36%). After 7 days the degradation was highest in CB2 (98.92%) as compared to CB1 (94.92%), CB3 (92.12%) and CB4 (61.26%). These results indicate that the degradation was enhanced by the addition of Cu^{++}, Fe^{++} and humic acid in the reaction mixture. More than 98% of carbendazim were removed from the samples by the addition of humic acid after incubation for 7 days.

Our result indicates that carbendazim can be degraded and detoxified rapidly by bacterial strains. In previous studies, some species of *Rhodococcus* (Holtman and Kobayashi 1997 Zhang et al. 2013; Wang et al. 2010a,b; Xu et al. 2006; Xu et al. 2007; Jing-Liang et al. 2006; Xiao et al. 2013) *Pseudomonas* (Sun et al. 2014; Pandey et al. 2010; Fang et al. 2010; Kalwasinska et al. 2008a; Kalwasinska et al. 2008b) and *Bacillus* (Salunkhe et al. 2014) showed degradation of carbendazim under environmental and experimental conditions. They cleaved methyl carbamate side chain of carbendazim parent structure leading to the generation of 2-amino-benzimidazole, benzimidazole and 2-hydroxybenzimidazole derivatives. Encouragingly, the newly isolated strains endowed with superior bioremediation of carbendazim up to a concentration of 1000 mg/L under experimental conditions. Chemical control agents (insecticides, herbicides, fungicides) when applied to crops, interact with soil humates, clays and essential metal ions resulting in low mobility and less accessibility for microbial degradation (Beddington 2010; Caceres *et al.* 2010; Long et al. 2004). Pesticides may contain one or more than one coordination sites, and they can interact with metal ions of soil's (bounded metal ions or free metal ions), soils oxides, organic matter, etc. The interactions of the pesticides in soil at the molecular level are central to their bioavailability, bioaccumulation, and transport in the environment (Kutman *et al.* 2013; Huyee and Keiter 2009). There are limited studies on carbendazim interactions with metal ions and soil humic acid. Since carbendazim contains NH and CH_3 coordinating sites, it is expected that it is involved in interaction with soil metal ions and soil humic contents.

Metal ions and humic acid are known to form a complex with carbendazim and enhance its degradation as per as our assumption. Cu^{++} exhibited maximum degradation of carbendazim as compared to control and other samples because Cu has the ability to decompose CBZ

through chemical decomposition due to its paramagnetic nature and complex formation ability as compared to other metal ions, whereas after 7 days, humic acid plays an essential role in the removal of carbendazim from the samples. Hence, the addition of Fe^{++}, Cu^{++} and humic acid enhances the capability of bacterial isolates to exert degradation of carbendazim. Mechanistically, it is assumed that Fe^{++}, Cu^{++} and humic acid blocks the active sites of carbendazim and thus enhances its availability for bacterial catabolism. As we know, Humic Acid (HA) is a bulky molecule, initially, carbendazim (CBZ) gets interacted with it and make complex suitable for decomposition by microorganisms. HA interacts with CBZ and thus, can reduce the toxicity of CBZ due to hydrogen and Van der Waals interactions. As the all four bacterial strains exhibited the ability to survive and grow on carbendazim alone without the need for any supplements, it makes them ideally suitable for bioremediation under natural conditions. Therefore, the isolated strains definitely play a possible bioremediation role in the areas contaminated by carbendazim. Our study confirmed that the isolated strains were capable to remove carbendazim residues under various conditions by a high percentage.

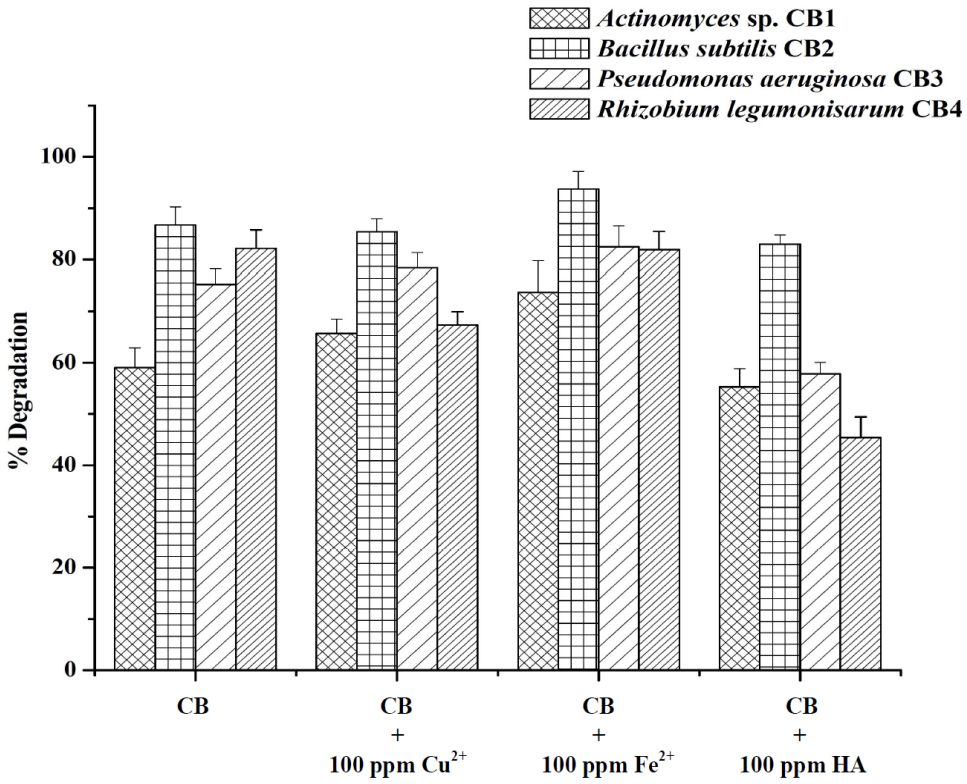


Figure 14: Degradation (%) of carbendazim (CB) by bacterial isolates inoculated singly and co-supplemented with Cu^{++} ($CuCl_2$) Fe^{++} ($FeCl_2$) and humic acid (HA) on day 3.

Where CB1 = *Actinomyces* sp. CB1, CB2 = *Bacillus subtilis* CB2, CB3 = *Pseudomonas aeruginosa* CB3, CB4 = *Rhizobium leguminosarum* CB4.

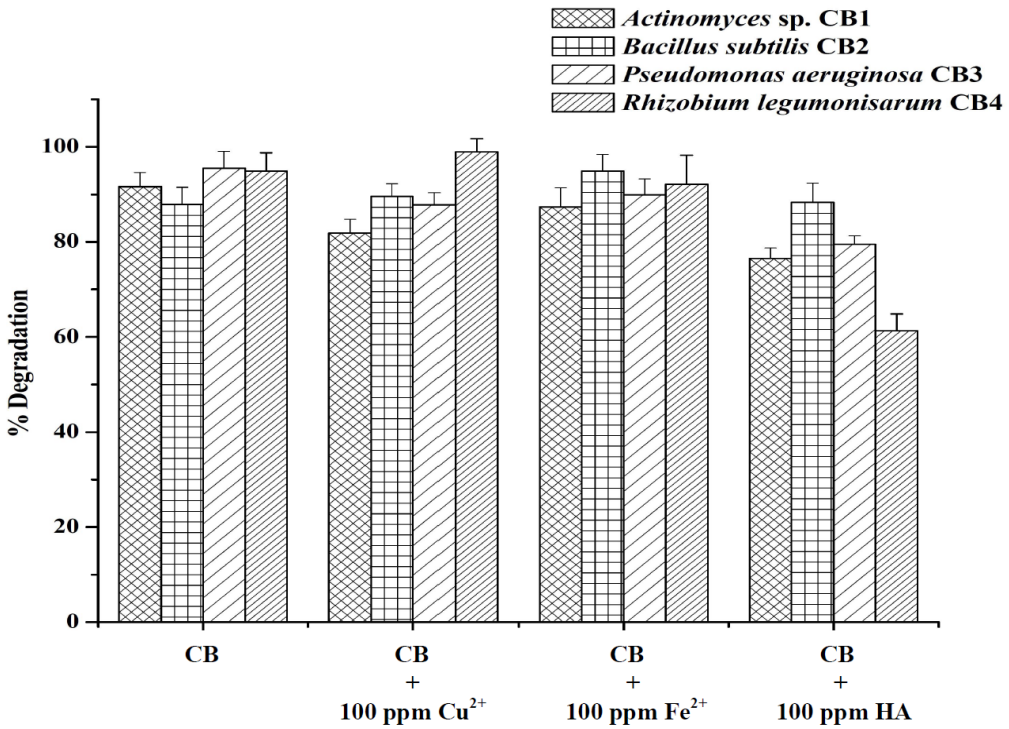


Figure 15: Degradation (%) of carbendazim (CB) by bacterial isolates inoculated singly and co-supplemented with humic acid (HA), Cu^{++} ($CuCl_2$) and Fe^{++} ($FeCl_2$) on day 7 (B) post-inoculation.

Where CB1 = *Actinomyces* sp. CB1, CB2 = *Bacillus subtilis* CB2, CB3 = *Pseudomonas aeruginosa* CB3, CB4 = *Rhizobium leguminosarum* CB4.

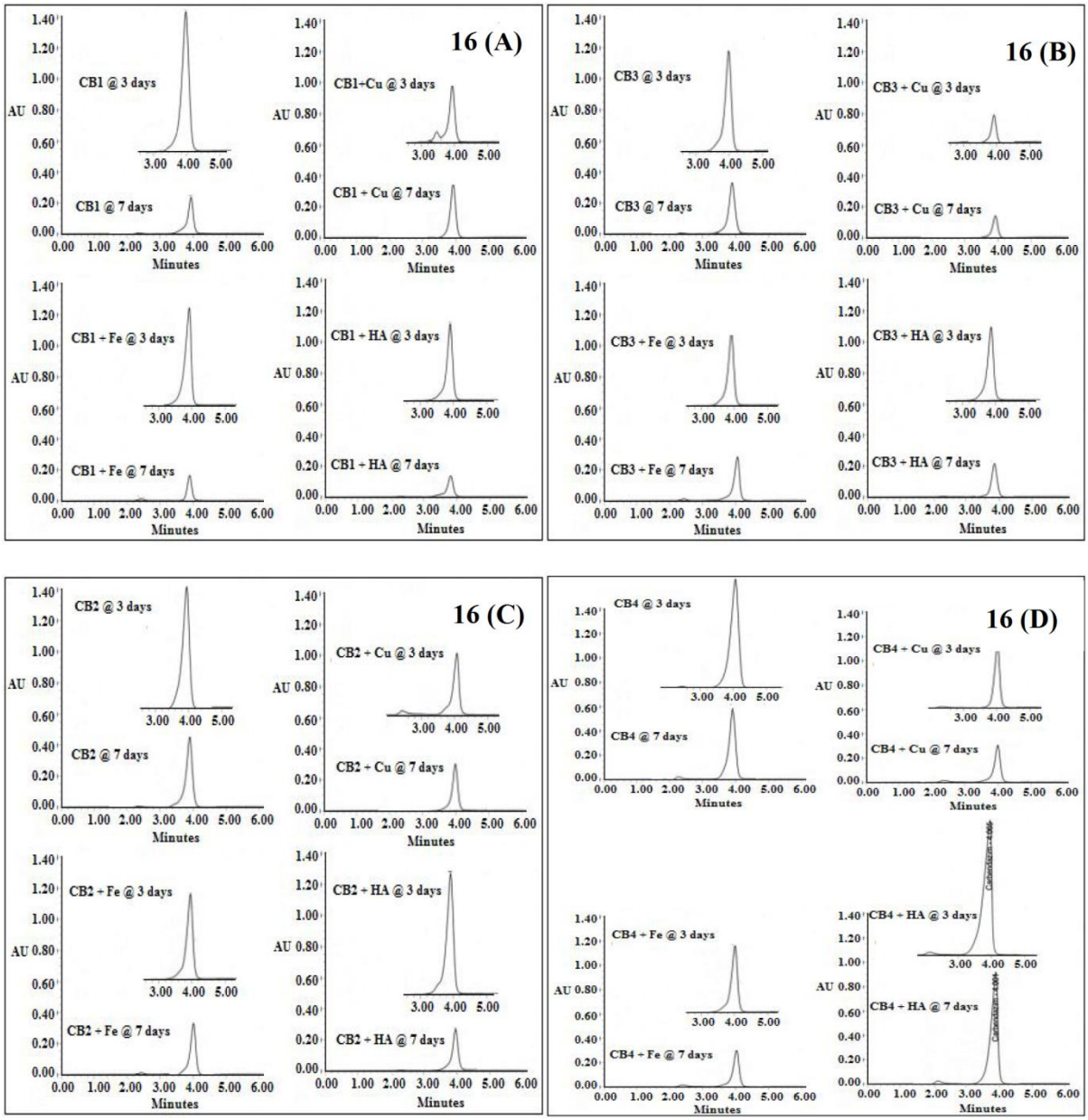


Figure 16 (A): HPLC analysis of carbendazim degradation (1000 mg/L) by *Actinomyces* sp. CB1 in minimal salts medium. (i) *Actinomyces* sp. CB1 on days 3, 7 post-inoculation. (ii) *Actinomyces* sp. CB1 supplemented with 100 mg/L Cu^{++} on days 3, 7 post-inoculation. (iii) *Actinomyces* sp. CB1 in minimal salts medium supplemented with Fe^{++} on days 3, 7 post-inoculation. (iv) *Actinomyces* sp. CB1 in minimal salts medium supplemented with Humic Acid on days 3, 7 post-inoculation.

Figure 16 (B): HPLC analysis of carbendazim degradation (1000 mg/L) by *Bacillus subtilis* CB2 in minimal salts medium. (i) *Bacillus subtilis* CB2 on days 3, 7 post-inoculation. (ii) *Bacillus subtilis* CB2 supplemented with 100 mg/L Cu^{++} on days 3, 7 post-inoculation. (iii) *Bacillus subtilis* CB2 in supplemented with Fe^{++} on days 3, 7 post-inoculation. (iv) *Bacillus subtilis* CB2 supplemented with Humic Acid on days 3, 7 post-inoculation.

Figure 16 (C): HPLC analysis of carbendazim degradation (1000 mg/L) in minimal salts medium. (i) *Pseudomonas aeruginosa* CB3 on days 3, 7 post-inoculation. (ii) *Pseudomonas aeruginosa* CB3 along with 100 mg/L Cu^{++} on days 3, 7 post-inoculation. (iii) *Pseudomonas aeruginosa* CB3 along with Fe^{++} on days 3, 7 post-inoculation. (iv) *Pseudomonas aeruginosa* CB3 along with Humic Acid on days 3, 7 post-inoculation.

Figure 16(D): HPLC analysis of carbendazim degradation (1000 mg/L) by *Rhizobium legumonisarum* CB4 in minimal salts medium. (i) *Rhizobium legumonisarum* CB4 on days 3, 7 post-inoculation (ii) *Rhizobium legumonisarum* CB4 supplemented with 100 mg/L Cu^{++} on days 3, 7 post-inoculation. (iii) *Rhizobium legumonisarum* CB4 supplemented with Fe^{++} on days 3, 7 post-inoculation. (iv) *Rhizobium legumonisarum* CB4 supplemented with Humic Acid on days 3, 7 post-inoculation.

Table 6: Effective degradation after three and seven days respectively, observed against fungicide carbendazim (1000 ppm) by bacterial isolates inoculated singly and co-supplemented with Cu^{++}, Fe^{++} and humic acid (100ppm) and metal ions

Isolates	Purity of sample	Standard area	Area after 3 days	Area after 7 days	Degradation after 3 days	Degradation after 7 days
CB1	98.0%	35201314	14715681	2999342	59.04%	91.65%
CB1 + Cu^{++}	98.0%	35201314	4770983	4352095	86.72%	87.89%
CB1 + Fe^{++}	98.0%	35201314	8777869	1605171	75.24%	95.54%
CB1 + HA	98.0%	35201314	6402374	1825419	82.18%	94.92%
CB2	98.0%	35201314	12325046	6521176	65.69%	81.85%
CB2 + Cu^{++}	98.0%	35201314	5245243	3732349	85.4%	89.61%
CB2 + Fe^{++}	98.0%	35201314	7754159	4364414	78.42%	87.85%
CB2 + HA	98.0%	35201314	11736166	3390967	67.33%	98.92%
CB3	98.0%	35201314	9439289	4546649	73.73%	87.35%
CB3 + Cu^{++}	98.0%	35201314	2255779	1827485	93.73%	94.92%
CB3 + Fe^{++}	98.0%	35201314	6302467	3632421	82.46%	89.89%
CB3 + HA	98.0%	35201314	6503247	2832715	81.9%	92.12%
CB4	98.0%	35201314	16061565	8426782	55.29%	76.54%
CB4 + Cu^{++}	98.0%	35201314	6108775	4202195	83%	88.31%
CB4 + Fe^{++}	98.0%	35201314	15142333	7374383	57.85%	79.47%
CB4 + HA	98.0%	35201314	19627779	13917767	45.36%	61.26%

CB1 - *Actinomyces* sp.; CB2 - *Bacillus subtilis*; CB3 - *Pseudomonas aeruginosa* and CB4 - *Rhizobium leguminosarum*; Cu - Copper; Fe - Iron and HA - Humic acid

Where CB1, CB2, CB3 and CB4 are different bacterial isolates. The degradation percentage was calculated by the given formula

$$\% \, degradation = 100 - Purity \; of \; sample \frac{Area \; of sample}{Area \; of standard}$$

6.2.2.4 Degradation of glyphosate and identification of metabolites by ESI-MS

Microbial degradation of glyphosate by GP1, GP2 and GP3 was studied in different experimental conditions. Our findings revealed the highest degradation of glyphosate by *R. leguminosarum* GP3 followed by *B. subtilis* GP2 (95%) and *Actinomyces* sp. GP1 (73%). After 7 days, the *Actinomyces* sp. GP1 decomposes 73% of the glyphosate into methyl phosphonic acid; m/z 97 (100%), and phosphoric acid; m/z 79 (47%). In *B. subtilis* GP2, the degradation was comparable high as 95% glyphosate was converted into methyl phosphonic acid; m/z 97 (100%), and phosphoric acid; m/z 79 (87%). In *R. leguminosarum* GP3, it was observed that on the 7 days of degradation, glyphosate was 95% decomposed into methyl phosphonic acid; m/z 97 (93%), and phosphoric acid; m/z 79 (100%) can be depicted in Table 6. Effect of Cu^{++} on the degradation of glyphosate was examined under the same conditions and found the degradation % was highest in *Actinomyces* sp. GP1 (97%) after 7 days as compared to *B. subtilis* GP2 (95%) and *R. leguminosarum* GP3 (95%). In case of *Actinomyces* sp. GP1, On the 7^{th} day, 97% degradation of glyphosate was observed i.e. glyphosate at m/z 169 (100%) was decomposed into methyl phosphonic acid; m/z 97 (100%) and phosphoric acid; m/z 79(51%). In *B. subtilis* GP2, 95% degraded glyphosate was converted into methyl phosphonic acid; m/z 97 (100%), and phosphoric acid; m/z 79 (53%). In *R. leguminosarum* GP3, it was observed that on day7, 95% glyphosate was decomposed into methyl phosphonic acid; m/z 97 (96%), and phosphoric acid; m/z 79 (100%). Mass spectrum of glyphosate degradation supplemented with copper (Cu^{2+}), iron (Fe^{2+}) and humic acid (HA) by screened rhizobacteria are presented in figure 17.

In the presence of a ferric ion, the rate of biodegradation of glyphosate was normal. The mode of degradation was almost similar to that of without ferric ion. The percentage decrease in a peak at m/z 169 has confirmed that up to 7 days; more than 85 to 97% of the glyphosate was decomposed by the bacterial isolates.

In case of *Actinomyces* sp. GP1, On 7^{th} day, from mass analysis studies, 97% degradation of Glyphosate was observed, that is Glyphosate at m/z 169 (100%) was decomposed into methyl phosphonic acid; m/z 97 (100%) and phosphoric acid; m/z 79(49%). In *B. subtilis* GP2, 88% degraded glyphosate was converted into methyl phosphonic acid; m/z 97 (100%), and

phosphoric acid; m/z 79 (95%). In *R. leguminosarum* GP3, it was observed that on the 7 days of degradation, Glyphosate was 93% decomposed into methyl phosphonic acid; m/z 97 (100%), and phosphoric acid; m/z 79 (91%).

In the presence of humic acid, biodegradation of glyphosate witnesses significant increase with a concomitant increase in glyphosate degradation ranging from 93% to 98%. In case of *Actinomyces* sp. GP1, on day 7, 98% degradation of glyphosate was observed i.e. glyphosate at m/z 169 (100%) was decomposed into methyl phosphonic acid; m/z 97 (100%) and phosphoric acid; m/z 79 (73%). In *B. subtilis* GP2, 93% degraded glyphosate was converted into methyl phosphonic acid; m/z 97 (93%), and phosphoric acid; m/z 79 (100%). In *R. leguminosarum*, it was observed that on the 7 days of degradation, Glyphosate was 95% decomposed into methyl phosphonic acid; m/z 97 (100%), and phosphoric acid; m/z 79 (95%) (Fig. 17).

Three effective bacterial strains were isolated, screened and purified through selective enrichment method capable of degrading glyphosate. Sequence homology of these isolated strains through 16S rRNA sequencing resembles with *Actinomyces* sp., *Bacillus subtilis* and *Rhizobium leguminosarum*. To the best of my knowledge, this is the first report on the degradation of glyphosate by these three isolates. Further, all the strains were checked out to confirm the growth on minimal media and activity against biodegradation of glyphosate. The degradation pathway in this study shared the same initial step as earlier reported (Fan et al. 2012), but two other different metabolites were observed at different m/z values of 97 and 79 and which were identified as methyl phosphonic acid and phosphoric acid. Only a few reports regarding glyphosate degradation have been published so far. It includes *Arthrobacter* sp. strain GLP-1, *Pseudomonas* sp. strain PG2982, *E. coli* and *Bacillus cereus* (Kent-Moor et al. 1983; Pipke and Amrhein, 1988; Fan et al. 2012). However, these three above mentioned strains can degrade glyphosate at a concentration of 1000 mg/L in minimal media. Due to the debate in sensitivity between microbial populations, glyphosate could derange the microbial community (Kruger et al. 2013, Shehata et al. 2014). Moreover, humic acid neutralizes the anti-microbial effect of glyphosate also.

The formation of aggregations through different interactions between pesticide, metal ions and humic acid, increase the stability of pesticide-humic acid complex or pesticide metal complex and change the conformations of pesticide as a result of which the lifespan of pesticide increases. Few results have indicated that humic-pesticide or metal pesticide interactions can abate the toxicity of agricultural chemicals (Wu and Laird 2004; Wuana and Okieimen 2011). They observed that humic acid significantly reduced the toxicity of

chlorpyrifos, carbofuran and azinophos-methyl, while humic acid enhances the toxicity was observed with carbaryl and methyl parathion. This was the first study on glyphosate interactions with metal ions and soil humic acid. Since glyphosate contains NH, P and O coordinating sites, it is expected that it can be involved in strong interaction with soil metal ions and soil humic contents. Glyphosate behaves as a strong ligand, the interaction between humates and essential metal ions are chemically thermodynamically four-membered rings. That's why there is a huge difference between the degradation percentage of the untreated and treated groups. Metal ions and humic acid play vital role in the degradation of glyphosate. Cu^{++} degrades maximum percentage of the glyphosate as compared to normal and other samples, whereas after 7 days humic acid takes over an essential role in the removal of glyphosate from the samples. Hence, the addition of metal ions (Cu^{++} and Fe^{++}) and humic acid increases the efficacy of the strains to degrade the glyphosate more rapidly. These metal ions and humic acids block the active sites of the glyphosate and make it available to the bacteria to be used as a source of energy. Simultaneously, due to the blocking of active sites as well as a change in conformation results in a reduction of toxicity towards the various microorganisms (Shehata et al. 2014), she also named this process as, neutralization effect by humic acid. The interactions of the pesticides with soil at the molecular level are means of their bioavailability, bioaccumulation, and transport in the environment (Kutman et al. 2013; Huyee and Keiter 2009). These three reported strains have a remarkable potential for application of removing excess glyphosate and its metabolites from pesticide-contaminated sites.

Table 7: Biodegradation of herbicide glyphosate by bacterial isolates on day 7 post-inoculation with/without the presence of humic acid and metal ions. Concentration of glyphosate used in degradation experiments was 1000 mg/L whereas HA, Fe^{++} and Cu^{++} were included at 100 mg/L concentrations

Experimental groups	**Formation of metabolites with m/z %**		
	Glyphosate (m/z =168)	**Methyl phosphonic acid (m/z = 97)**	**Phosphoric acid (79 m/z)**
Actinomyces sp. GP1	27%	100%	47%
Actinomyces sp. GP1 + Cu^{++}	3%	100%	51%
Actinomyces sp. GP1 + Fe^{++}	3%	100%	49%
Actinomyces sp. GP1 + HA	2%	100%	73%
Bacillus subtilis GP2	5%	100%	87%
Bacillus subtilis GP2 + Cu^{++}	5%	100%	53%
Bacillus subtilis GP2 + Fe^{++}	12%	100%	95%
Bacillus subtilis GP2 + HA	7%	93%	100%
Rhizobium leguminosarum GP3	5%	93%	100%
Rhizobium leguminosarum GP3 + Cu^{++}	5%	96%	100%
Rhizobium leguminosarum GP3 + Fe^{++}	7%	100%	91%
Rhizobium leguminosarum GP3 + HA	5%	100%	95%

HA: Humic acid; m/z %: mass/charge %; Fe^{++} as $FeCl_2$, Cu^{++} as $CuCl_2$.

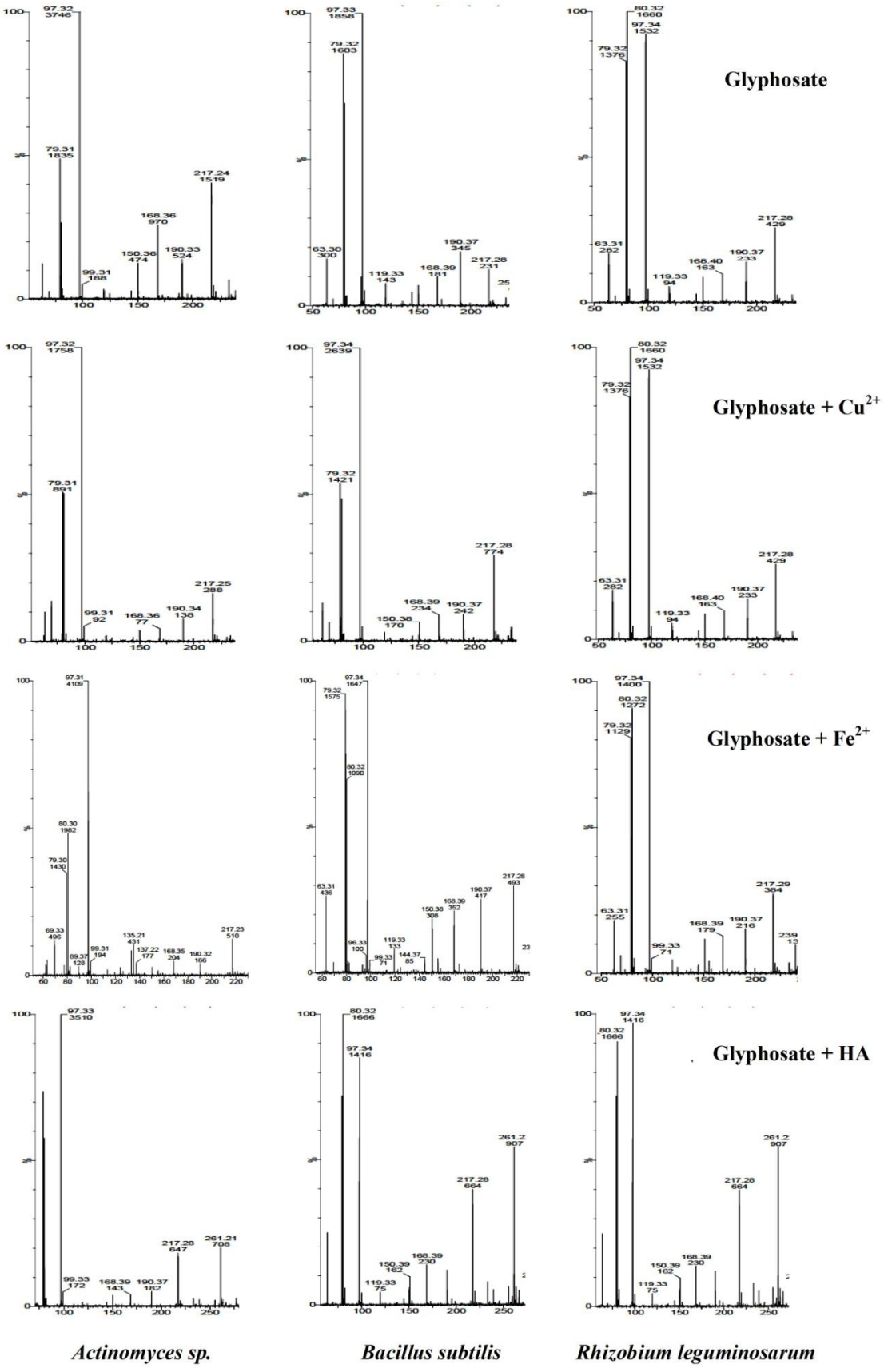


Figure 17: Mass spectrum of glyphosate degradation supplemented with copper (Cu^{++}), iron (Fe^{++}) and humic acid (HA) by screened rhizobacteria

6.2.2.5 Degradation of monocrotophos and identification of metabolites by ESI-MS

Two main peaks of phosphate and phosphonate with m/z of 96 and 79 were detected. Other metabolites were identified as (E)-methyl 4-(methylamino)-4-oxobut-2-en-2-yl methylphosphonate (m/z at 207), dimethyl phosphate (m/z at 124), methyl hydrogen phosphate (m/z at 110), phosphate (m/z at 97), phosphonate (m/z at 79) and acetic acid (m/z at 60) respectively (Table 7). Degradation of monocrotophos by isolates MC1, MC2 and MC3 was determined in minimal medium at a concentration of 1000 mg/L of monocrotophos. Our findings indicated highest degradation of monocrotophos (92%) by *Actinomyces* sp MC1 and MC2, and MC3 (90%) after 7 days incubation (Fig. 18). On the other hand, the effect of Cu^{++} on the degradation of monocrotophos is high as compared to the un-supplemented group. The degradation percentage after 7 days were highest in isolate MC2 (98%) > MC3 (93%) and in MC1 (91%) (Fig. 19). Effect of Fe^{++} and humic acid on the degradation of monocrotophos was examined under the same circumstances and found that the degradation percentage was highest in MC1 (97%) after 7 days as compared to other isolates MC2 (92%), and MC3 (90%) in Fe^{++} treated samples and highest in MC2 (95%), MC3 (92%) and MC1 (90%) (Fig. 20). More than 90% of carbendazim were removed from the samples by the addition of humic acid after incubation for 7 days (Fig 21).

Monocrotophos (m/z at 223) is first transformed into (E)-4-amino-4-oxobut-2-en-2-yl dimethyl phosphate (m/z at 208) by a de-methylation reaction on phosphate group; then it is converted into di methyl phosphate by deakylation (m/z at 124); di methyl phosphate is further de-methylated into methyl hydrogen phosphate (m/z at 110); di methyl phosphate is again de methylated in phosphate (m/z at 98); phosphate is converted into phosphonate (m/z at 79) by dehydration reaction removal of water; One other unidentified compound was also observed at m/z 60 which is formed after the degradation of the alkyl chain which might be either acetamide or acetic acid.

The degradation in this study shared the same initial step as earlier reported by Bhadbhade et al. (2002), but six other different metabolites were also observed at different m/z values.

This is the first study on monocrotophos interactions with metal ions and soil humic acid. Since monocrotophos contains NH, P and CH_3 coordinating sites, it is expected that it is involved in weak interaction with soil metal ions and soil humic contents.

As monocrotophos behaves as weak ligand so the interaction between humates and essential metal ions are chemically thermodynamically four-membered rings. That's why there is no such difference between the degradation percentage of the untreated and treated groups. The

interactions of the pesticides with soil at the molecular level are central to their bioavailability, bioaccumulation, and transport in the environment (Kutman et al. 2013).

Table 8: Bio-decomposition of Monocrotophos with bacterial species and visualization of different metabolites after 7 days of degradation by ESI-MS

Experimental Groups	**Formation of different metabolites with mass/charge ratio (m/z) % after 7 days**						
	223	**208**	**124**	**110**	**97**	**79**	**60**
MC1 + 1000 mg/L Monocrotophos (MC)	8%	40%	30%	22%	82%	100%	88%
MC1 + 1000 mg/L MC + 100 mg/L Cu^{++}	9%	50%	12%	22%	100%	98%	8%
MC1 + 1000 mg/L MC + 100 mg/L Fe^{++}	3%	32%	10%	13%	100%	88%	4%
MC1 + 1000 mg/L MC + 100 mg/L HA	10%	50%	18%	30%	96%	100%	7%
MC2 + 1000 mg/L MC	8%	55%	20%	20%	100%	70%	63%
MC2 + 1000 mg/L MC + 100 mg/L Cu^{++}	2%	44%	8%	10%	100%	65%	-
MC2 +1000 mg/L MC + 100 mg/L Fe^{++}	8%	70%	18%	30%	100%	83%	12%
MC2 + 1000 mg/L MC + 100 mg/L HA	5%	35%	8%	12%	100%	85%	-
MC3 + 1000 mg/L MC	10%	65%	20%	40%	100%	98%	8%
MC3 + 1000 mg/L MC + 100 mg/L Cu^{++}	7%	15%	5%	5%	90%	100%	8%
MC3 + 1000 mg/L MC + 100 mg/L Fe^{++}	10%	63%	20%	30%	100%	97%	15%
MC3 + 1000 mg/L MC + 100 mg/L HA	8%	70%	31%	34%	93%	100%	30%

where m/z at 223= Monocrotophos, 207= (E)-methyl 4-(methylamino)-4-oxobut-2-en-2-yl methylphosphonate, 124= dimethyl phosphate, 110= dimethyl phosphonate, 97= methyl phosphonate and 60= acetic acid. MC1 = Actinomyces sp. MC1, MC2 = Bacillus subtilis MC2, MC3 = Rhizobium leguminosarum MC3.

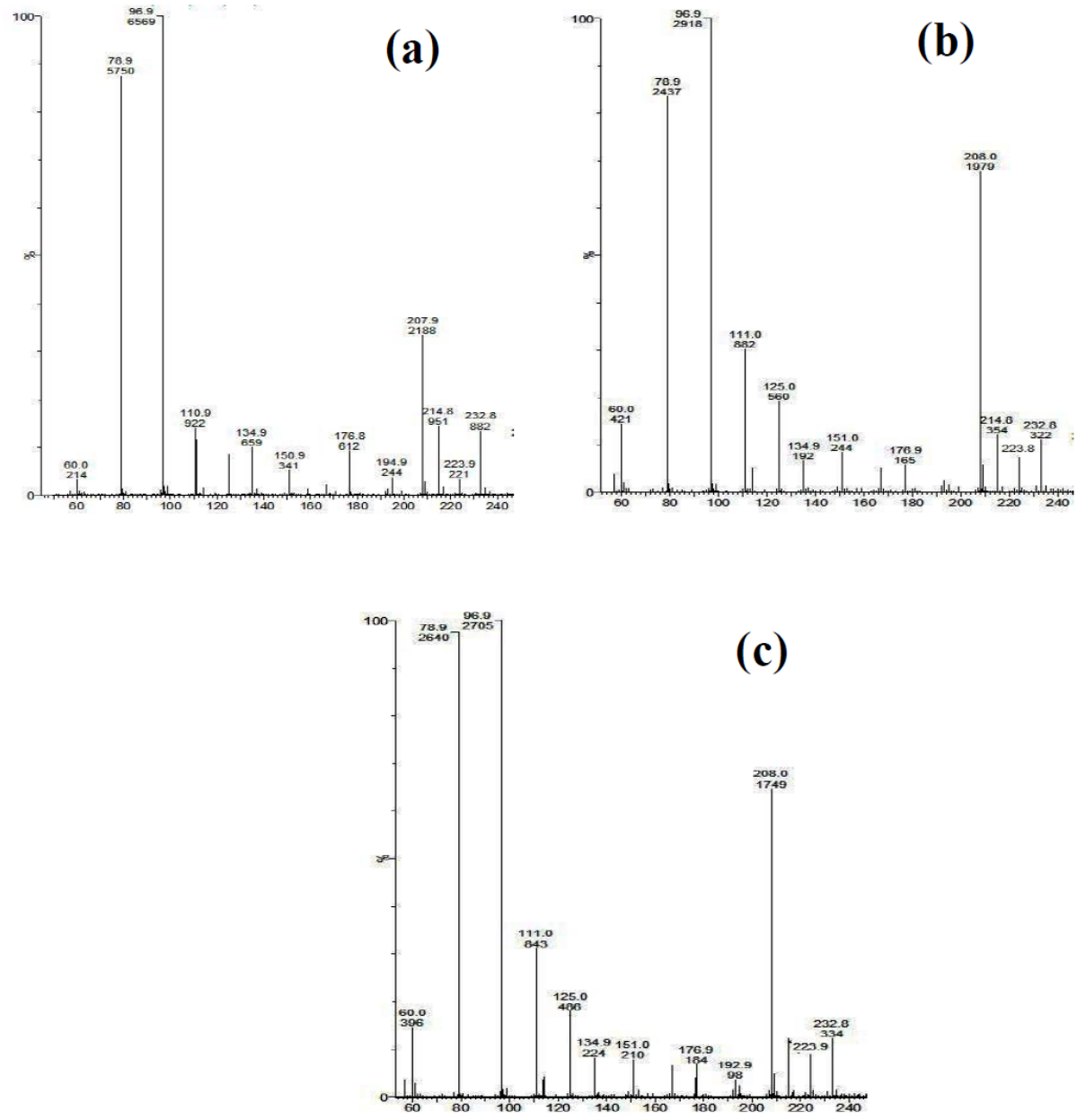


Figure 18: Mass spectrum of metabolites of monocrotophos (1000 mg/L + 100 mg/L Fe^{++}) degradation after 7 days (a) *Actinomyces* sp. MC1, (b) *Bacillus subtilis* MC2 and (c) *Rhizobium leguminosarum* MC3

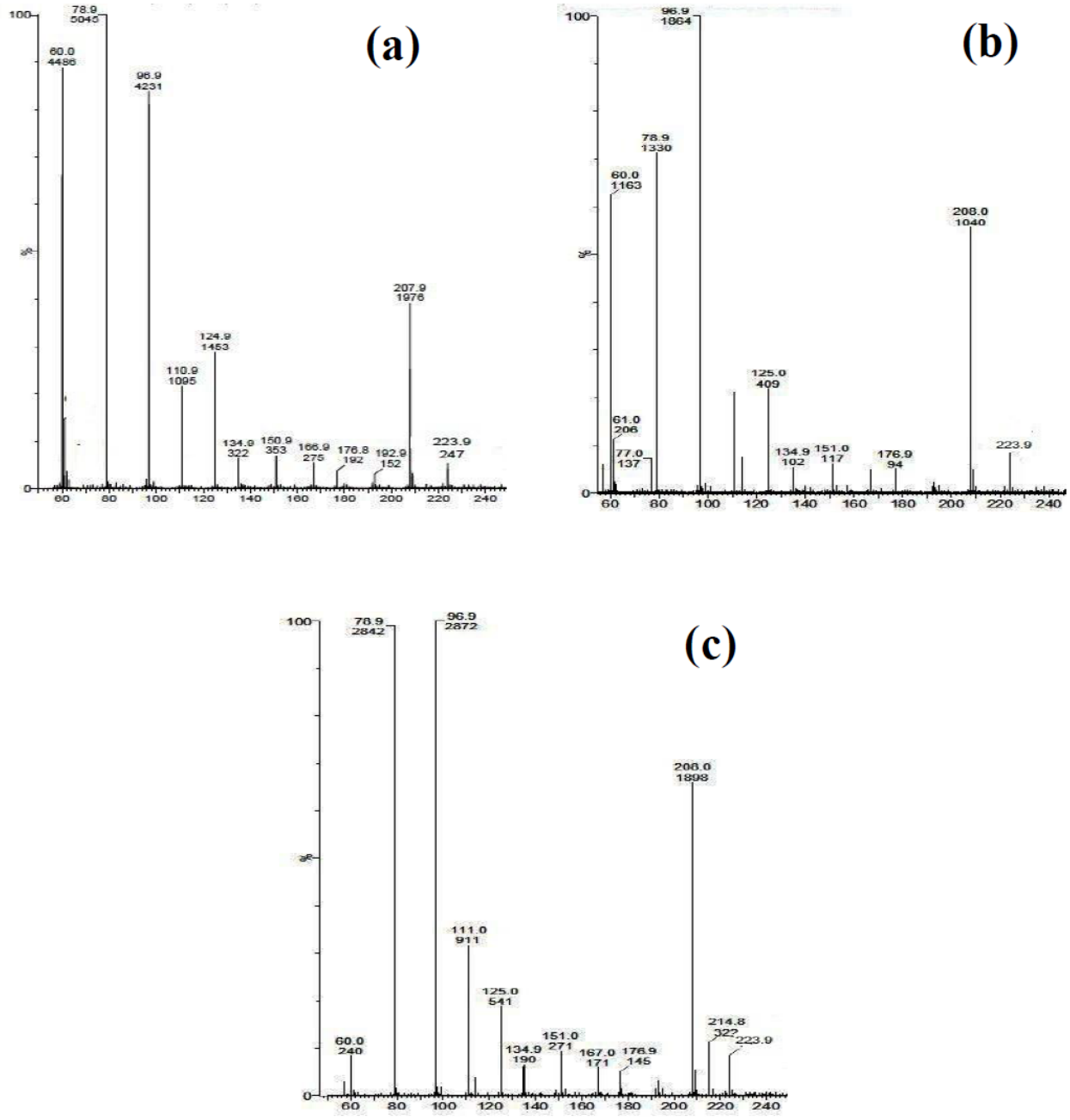


Figure 19: Mass spectrum of metabolites of monocrotophos (1000 mg/L + 100 mg/L Fe^{++}) degradation after 7 days (a) *Actinomyces* sp. MC1 , (b) *Bacillus subtilis* MC2 and (c) *Rhizobium leguminosarum* MC3

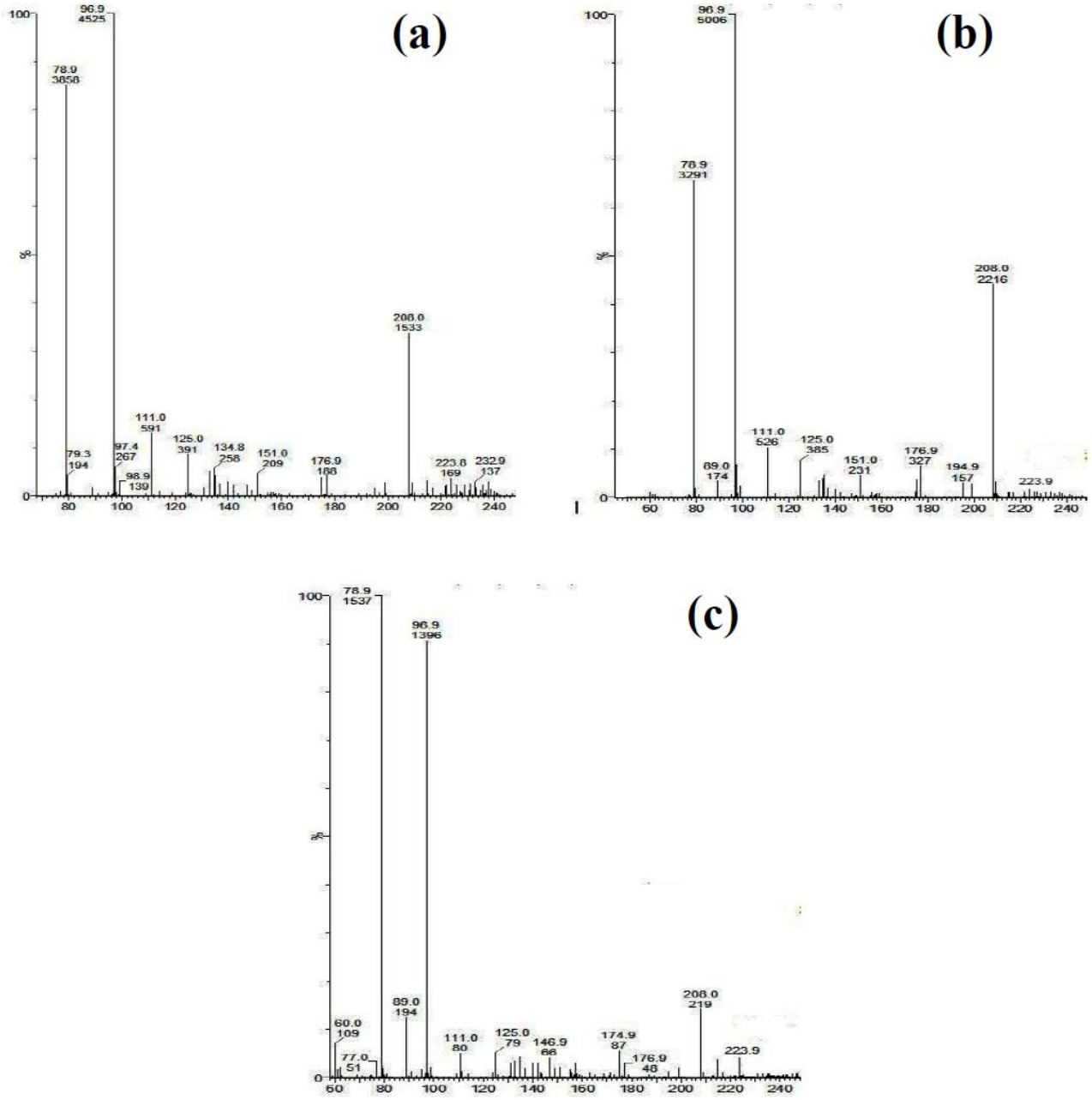


Figure 20: Mass spectrum of metabolites of monocrotophos (1000 mg/L + 100 mg/L Cu^{++}) degradation after 7 days (a) *Actinomyces* sp. MC1, (b) *Bacillus subtilis* MC2 and (c) *Rhizobium leguminosarum* MC3

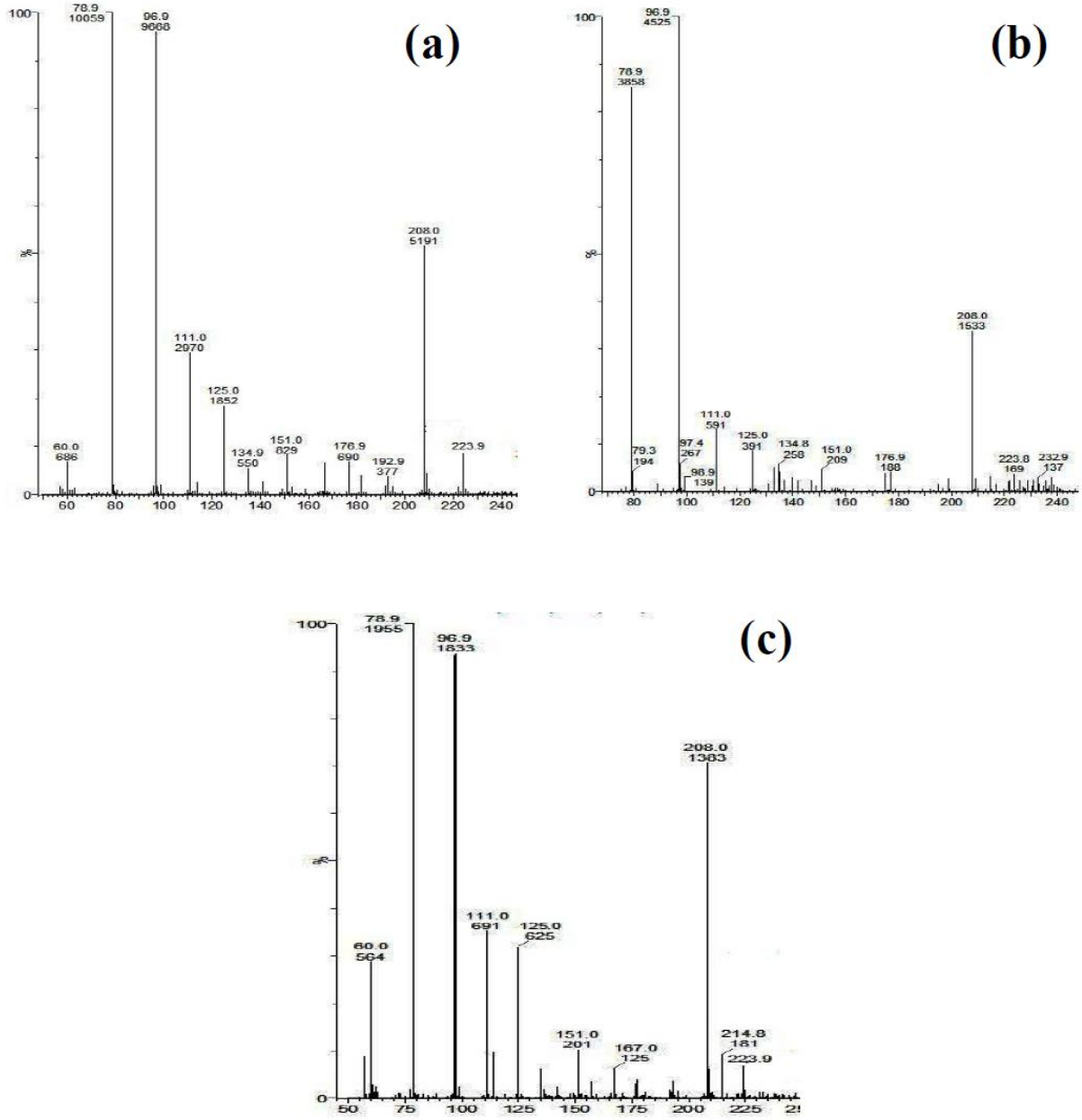


Figure 21: Mass spectrum of metabolites of monocrotophos (1000 mg/L + 100 mg/L Humic acid) degradation after 7 days (a) *Actinomyces* sp. MC1, (b) *Bacillus subtilis* MC2 and (c) *Rhizobium leguminosarum* MC3

6.2.2.6 Degradation of phorate and identification of metabolites by GC-MS

The mass/ charge ratio (m/z) of phorate observed at m/z 290 i.e. {$[M+H]^+$} with retention time of 19.363 min. Seven different metabolites were formed by all the isolated bacterial cultures after 3 and 7 days of degradation and were identified with mass charge ratio of 191(ethoxyphosphonothio)methanethiol, 175 S-mercaptomethyl O,O-dihydrogen phosphor

rodithioate), 149 (M-3) diethyl methylphosphonate, 75 methane dithoil , 57 (M+5)ethanethoil and two unidentified metabolites at m/z of 121 and 69 were formed. Oxidation occurred during ionization process the oxidation takes place which showed a successive increase for first 7 days followed by peak declination at m/z 290 and OD at 600 nm. Percentile peak declination at m/z 290 has confirmed that upto 14 days more than 99% phorate was decomposed. In all isolated bacteria PR1 and PR2 same major metabolites were formed. The only difference is in their degradation percentage of major compound and formation of new compounds.

In case of PR1, after 3 days the percentile peak declination at m/z 290 resulted as the formation of five metabolites m/z at 191(ethoxyphosphonothio)methanethiol (2.22%), 175 S-mercaptomethyl O,O-dihydrogen phosphor rodithioate (3.34%), 75 methane dithoil (74.02), 57 ethanethoil (9.32%) and one unidentified metabolite at m/z of 69(6.26%) were formed. The degradation percentage after 7 days result the formation of same metabolites with two more new metabolites at m/z unidentified metabolite 121 (0.71%) and 149 (M-3) diethyl methylphosphonate (1.06%).

With the addition of Cu^{++} the degradation percentage, was very high. The metabolites formed after 3 days was identified at m/z 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (6.27%), 149 (M-3) diethyl methylphosphonate (9.71%), 75 methane dithoil (60.02%), 57 ethanethoil (17.53%) and one unidentified metabolite at m/z of 69(6.48%) were formed. The percentage declination after 7 days in case of Cu^{++} supplemented groups results in the formation of six metabolites 191(ethoxyphosphonothio)methanethiol (3.49%), 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (7.15%), 149 (M-3) diethyl methylphosphonate (12.44%) 75 methane dithoil (15.42%) , 57 ethanethoil (32.72%) and one unidentified metabolites at m/z of 69(13.20%) were formed.

In Fe^{++} supplemented groups, the degradation percentage was higher more than 90% decrease in base peak after 3 days with m/z 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (6.55%), 75 methane dithoil (50.86%) , 57 ethanethoil (17.53%) and two unidentified metabolites at m/z of 121 (1.93%) and 69 (15.12%) were formed. After 7 days the metabolites formed were similar with two new compounds at m/z 191 and 121.

In humic acid treated groups, the isolate PR1 degrades phorate significantly. Six different metabolites were formed after 3 and 7 days at m/z 191(ethoxyphosphonothio)methanethiol, 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate), 149 (M-3) diethyl methylphosphonate, 75 methane dithoil, 57 (M+5)ethanethoil and one unidentified metabolite at m/z 69 were formed. The percentages of metabolites formed were depicted in table 8.

In case of PR2, after 3 days the percentile peak declination at m/z 290 resulted as the formation of all six metabolites m/z at 191(ethoxyphosphonothio)methanethiol (4.08%), 175 S-mercaptomethyl O,O-dihydrogen phosphor rodithioate (5.9%), 149 (M-3) diethyl methylphosphonate (15.52%) 75 methane dithoil (27.97) , 57 ethanethoil (21.38%) and two unidentified metabolites at m/z of 121 (1.28%) and 69(6.26%) were formed.

The degradation percentage after 7 days results in the formation of only two metabolites with m/z 57 ethanethoil (18.84%) and 149 (M-3) diethyl methylphosphonate (32.92%).

With the addition of Cu^{++} the degradation percentage was very hig has compared to PR1. The metabolites formed after 3 day was identified at m/z 191(ethoxyphosphonothio)methanethiol (6.24%), 175 S-mercaptomethyl O,O-dihydrogen phosphor rodithioate (11.20%), 149 (M-3) diethyl methylphosphonate (15.09%) 75 methane dithoil (27.97) , 57 ethanethoil (21.38%) and one unidentified metabolites at m/z 69(6.26%) were formed.

The percentage declination after 7 days in case of Cu^{++} supplemented groups results in the formation of only two metabolites 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (7.52%) and 75 methane dithoil (15.42%).

In Fe^{++} supplemented groups, the degradation percentage was higher more than 90% decrease in base peak after 3 days with m/z m/z 191(ethoxyphosphonothio)methanethiol (3.83%), 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (7.83%),149 (M-3) diethyl methylphosphonate (13.62%) 75 methane dithoil (16.90%) , 57 ethanethoil (35.83%) and one unidentified 69 (14.15%) were formed. After 7 days only one metabolite was formed 57 ethanethoil (9.5%).

In humic acid treated groups, the isolate PR2 degrades phorate faster than PR1. Seven different metabolites were formed after 3 days at m/z 191(ethoxyphosphonothio) methanethiol(1.28%), 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (2.59%), 149 (M-3) diethyl methylphosphonate (4.7%), 75 methane dithoil (70.34%) , 57 (M+5) ethanethoil (6.12%) and one unidentified metabolites at 121 (0.64%) m/z were formed. After seven days only three metabolites were formed with m/z 175 S-mercaptomethyl O,O-dihydrogen phosphorodithioate (2.36%), 69 (4.9%) and 57 (M+5) ethanethoil (2.99%).

Our findings indicated highest degradation of phorate were different by the two isolates PR1 and PR2 on the incubation of 3 and 7 days showed the formation of same metabolites of different percentages. Effect of Cu^{++} on the degradation of phorate is quite high as compared to the un-supplemented group. The degradation percentage after 7 days was highest in isolate PR2 as compared to PR1. Effect of Fe^{++} on the degradation of phorate was examined under the same circumstances and found that the degradation percentage was highest in PR2 after 7

days as compared to PR1. Effect of humic acid on biodegradation results in the removal of phorate found slower rate after 7 days in both the cases.

The results presented here indicate that phorate can be degraded and detoxified rapidly by the isolated strains. Some species such as *Pseudomonas fulva strain IMBL 5.1, Brevibacterium frigoritolerans strain IMBL 2.1, Bacillus aerophilus strain IMBL 4.1* and some consortium showed degradation of phorate (Jariyal et a;., 2014; Rani and Juwarkar 2012; Rani et al., 2009; Bano and Musarrat 2003; Ortiz-Hernandez et al., 2003; Venkatramesh et al., 1987) in environmental samples Encouragingly, the newly isolated strains endowed with superior bioremediation characteristics up to a concentration of 1000mg/L from environmental samples.

Metal ions and humic acid play an important role in the degradation of phorate. Cu^{++} degrades maximum percentage of the phorate as compared to normal and other samples whereas after 7 days humic acid plays an essential role in the removal of phorate from the samples. Hence, the addition of various metal ions and humic acid increases the efficacy of the strains to degrade the phorate more rapidly. These metal ions and humic acids block the active sites of the phorate and make available to the bacteria to use it as a sole source of carbon and energy.

The isolated strains worked without supplementation of other carbon or nitrogen sources. Such characteristics are desirable in microorganisms to be used for bioremediation purpose.

Therefore, the isolated strains definitely play a possible bioremediation role in the areas contaminated by phorate. Our study confirmed that the isolated strains were capable to remove phorate residues under various conditions by a high percentage.

Table 9: Biodegradation of insecticide phorate by bacterial isolates on day 7 post-inoculation with or without the presence of humic acid and metal ions. Concentration of phorate used in

degradation experiments was 1000 mg/L whereas HA, Fe^{++} and Cu^{++} were included at 100 mg/L concentrations

Treatment	m/z with %age						
	57*	69	75*	121	149**	175	191
3rd Day							
B1+P	9.32	6.26	74.02	----	----	3.34	2.22
B1+P+Cu^{++}	17.53	6.48	60.02	----	9.71	6.27	----
B1+P+Fe^{++}	20.02	15.12	50.86	1.93	----	6.55	
B1+P+HA	15.77	4.92	51.98	----	16.44	3.70	1.97
7th Day							
B1+P	10.46	4.25	72.70	0.71	1.06	2.79	1.18
B1+P+Cu^{++}	32.72	13.20	15.42	----	12.44	7.15	3.49
B1+P+Fe^{++}	18.04	11.73	14.66	----	23.44	7.30	2.70
B1+P+HA	22.47	20.17	31.90	----	18.33	6.29	3.21
3rd Day							
B2+P	21.58	15.28	27.97	1.28	15.52	5.90	4.08
B2+P+Cu^{++}	33.15	22.84	17.52	----	15.09	11.20	6.24
B2+P+Fe^{++}	35.83	14.15	16.90	----	13.62	7.83	3.83
B2+P+HA	6.12	5.64	70.35	0.64	4.70	2.59	1.28
7th Day							
B2+P	18.84	----	----	----	32.92	----	----
B2+P+Cu^{++}	17.03	----	----	----	----	7.52	----
B2+P+Fe^{++}	9.50	----	----	----	----	----	----
B2+P+HA	2.99	4.90	----	----	----	2.36	----

Where B1 = *Pseudomonas* sp. PR_01 and B2= *Pseudomonas* sp. PR_02, P= Phorate

6.3.1 Effect of pesticides, Cu^{++}, Fe^{++} and humic acid on production of Indole acetic acid

In the regulation of plant development, Indole acetic acid acts as signal molecules. The production of Indole acetic acid was influenced by the availability of substrates, culture conditions, etc. and it also varies from species to species (Mirza et al., 2001). The higher production of IAA by some of the *Pseudomonas* species was also been reported (Xie et al., 1996). Some other rhizobacterial species, even pathogenic, free-living bacterial species, etc. were also being reported to synthesize IAA (Ahemad et al., 2014; Tsavkelova et al., 2007).

The addition of precursor L-tryptophan increases the indole acid production were reported and concentration was increased up to ten times than normal (4.94-46.66 mg/L) Yasmin et al., 2010). In a similar study, different villages of district Faridabad, a total of sixty-two isolates were obtained. More than sixty-nine percent of the isolates were found to be producing indole acetic acid (Kumar *et al.*, 2014). The effect of pesticide stress on IAA production was first studied by Ahemad et al., 2012 and suggests the increasing concentration of pesticides alters the production of Indole acetic acid in PGPR strains.

The indole acid production by the 19 isolated strains and two standard strains varies considerably. Highest production of IAA was observed in Strains GP3, MC3 and RK4 respectively, with production concentration up to 95, 94.5 and 89 µg/mL as compared to standard strains RL of 48.1 and *E. coli* 10 µg/mL. With the addition of 1x and 2x concentration of pesticide, significant changes were noticed in all the treated groups at significant level $p < 0.05$. Although metal ions and humic acid have relatively less effect on production as compared to the control groups. It observed that the indole acetic acid production was significantly decreased with the increase of concentration levels of pesticides. The consequence of two different concentrations of each pesticide on the production of IAA by the 19 isolated decreased progressively. On addition of 100 mg/L of metal ions (Cu^{++} and Fe^{++}) and humic acid, the Cu^{++} had the most toxic effect on its production and rate of production was significant decreases at $p < 0.05$. On addition of Fe^{++} and humic acid, the production was relatively increased when amended with 1x concentration of each pesticide at significant level $p < 0.05$. On addition of 2x concentration of each pesticide, the IAA production was decreased in almost all the cases. The production of IAA by the isolated strains along with standard strains, effects of pesticides, Cu^{++} and Fe^{++} and humic acid was summarized in table 10 and 11.

While comparing the type of pesticides, their concentrations, effect of Cu^{++} and Fe^{++} and humic acid, Cu^{++} in all the cases posed a negative effect on its production.

It was observed that IAA production was concentration dependent, as the concentration (0X, 1X and 2X) increased the IAA production decreased. And the exact order of IAA production with the three strains was ACP1 > ACP2 > ACP3. For strain ACP1, at concentration level 0X and 1X, the order of IAA production was, ACP1+HA > ACP1 > ACP1+Fe^{++}> *Rhizobium leguminosarium* > ACP1 + Cu^{++}> *E. coli.* At concentration level 2X, the order of IAA production was, ACP1 + HA > *Rhizobium leguminosarium* > ACP1+Fe^{++}> ACP1 > ACP1+Cu^{++}> *E. coli*. For strain ACP2, at concentration level 0X, the order of IAA production was, ACP2 + HA > *Rhizobium leguminosarium* > ACP2 > ACP2+ Fe^{++}> ACP1 + Cu^{++}> E. Coli, at concentration level 1X, the order of IAA production was, ACP2 + HA > *Rhizobium leguminosarium* > ACP2+Fe^{++}> ACP2 > ACP2+ Cu^{++}> *E. Coli*, and at concentration level 2X, the order of IAA production was, *Rhizobium leguminosarium* > ACP2+HA > ACP2+Fe^{++}> ACP2 > ACP2+Cu^{++}> *E. Coli*. For strain ACP3, at concentration level 0X, 1X and 2X, the order of IAA production was, *Rhizobium leguminosarium* > ACP3+HA > ACP3+Fe^{++}> ACP3 > ACP3+Cu^{++}> E. Coli. Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level $p = 0.05$) with an order control ≥ HA > Fe^{++}>

Cu^{++}. In case of glyphosate, at the significant level (p = 0.05) comprehensive comparison of three stains (GP1, GP2 and GP3), at three concentrations level (0X, 1X and 2X), with the addition of Fe^{++}, Cu^{++}, and HA have shown that the statistical means were significantly different. It was observed that IAA production was concentration dependent, as the concentration (0X, 1X and 2X) increased the IAA production decreased. And the exact order of IAA production with the three strains was GP3 > GP2 > GP1. For strain GP1, at concentration level 0X, the order of IAA production was, GP1+HA > *Rhizobium leguminosarium* > GP1 > GP1+Fe^{++}> GP1+Cu^{++}> *E. Coli*, at concentration level 1X, the order of IAA production was, GP1+HA > *Rhizobium leguminosarium* > GP1+Fe^{++} > GP1 > E. Coli > ACP1+Cu^{++}, and at concentration level 2X, the order of IAA production was *Rhizobium leguminosarium* > GP1+HA > GP1+Fe^{++} > GP1 > *E. Coli* > GP1+Cu^{++}. For strain GP2, at concentration level 0X, the order of IAA production was, GP2+HA > GP2+Fe^{++}> GP2 > *Rhizobium leguminosarium* > *E. Coli* > GP2+Cu^{++}, at concentration level 1X, the order of IAA production was, GP2+HA > GP2+Fe^{++}> GP2 > *Rhizobium leguminosarium* > GP2+Cu^{++}> *E. Coli*, and at concentration level 2X, the order of IAA production was, GP2+HA > GP2+Fe^{++}> *Rhizobium leguminosarium* > GP2 > *E. Coli* > GP2+Cu^{++}. For strain GP3, at concentration level 0X, the order of IAA production was, GP3+HA > GP3 > GP3+Fe^{++}> *Rhizobium leguminosarium* > GP3+Cu^{++}> *E. Coli*, at concentration level 1X, the order of IAA production was, GP3+HA > GP3+Fe^{++}> GP3 > *Rhizobium leguminosarium* > GP3+Cu^{++}> *E. Coli*, and at concentration level 2X, the order of IAA production was, GP3+Fe^{++}> GP3+HA > GP3 > *Rhizobium leguminosarium* > GP3+Cu^{++}> *E. Coli.* Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level p = 0.05) with an order HA ≥ control > Fe^{++}> Cu^{++}, expect GP3+Fe^{++} at 2X concentration level. In case of monocrotophos, at the significant level (p = 0.05) comprehensive comparison of three stains (MC1, MC2 and MC3), at three concentrations level (0X, 1X and 2X), with the addition of Fe^{++}, Cu^{++}, and HA have shown that the statistical means were significantly different. It was observed that IAA production was concentration dependent, as the concentration (0X, 1X and 2X) increased the IAA production decreased. And the exact order of IAA production with the three strains was MC3 > MC2 > MC1. For strain MC1, at concentration level 0X, 1X and 2X, the order of IAA production was, *Rhizobium leguminosarium* > MC1+HA > MC1+Fe^{++}> MC1 > MC1+Cu^{++} > *E. Coli*. For strain MC2, at concentration level 0X, the order of IAA production was, MC2+HA > MC2+Fe^{++}> MC2 > *Rhizobium leguminosarium* > MC2+Cu^{++}> *E. Coli*, at concentration level 1X and 2X, the order of IAA production was, MC2+HA > MC2+Fe^{++}> MC2 >

Rhizobium leguminosarium > MC2+Cu^{++}> *E. Coli*. For strain MC3, at concentration level 0X, the order of IAA production was, MC3+HA > MC3+Fe^{++}> MC3 > MC3+Cu^{++}> *Rhizobium leguminosarium* > *E. Coli*, at concentration level 1X, the order of IAA production was, MC3+HA > MC3+Fe^{++}> MC3 > *Rhizobium leguminosarium* > MC3+Cu^{++}> *E. Coli*, and at concentration level 2X, the order of IAA production was, MC3+Fe^{++}> MC3+HA > MC3 > *Rhizobium leguminosarium* > MC3+Cu^{++}> *E. Coli*. Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level p = 0.05) with an order HA ≥ Fe^{++} > control > Cu^{++}, expect MC2+Fe^{++} at 1X concentration level and MC2+Fe^{++} and MC3+Fe^{++} at 3X concentration level. Production of IAA for phorate, at the significant level (p = 0.05) comprehensive comparison of two stains (PR1 and PR2), at three concentrations level (0X, 1X and 2X), with the addition of Fe^{++}, Cu^{++}, and HA have shown that the statistical means were significantly different. It was observed that IAA production was concentration dependent, as the concentration (0X, 1X and 2X) increased the IAA production decreased. Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level p = 0.05) with an order Fe^{++} > HA > control > Cu^{++}. For strains PR1 and PR2 at 0X and 1X concentrations level, the order of IAA production was, PR+Fe^{++} > PR+HA > PR > *Rhizobium leguminosarium* > PR+Cu^{++}> *E. Coli*. For strains PR1 and PR2 at 2X concentration level, the order of IAA production was, PR+Fe^{++}> PR+HA > *Rhizobium leguminosarium* > PR > *E. Coli* > PR+Cu^{++}.

Table 10: Production of IAA by bacterial isolates inoculated singly and co-supplemented with each pesticide (Acephate, Atrazine and Carbendazim), humic acid (HA), Cu^{++} and Fe^{++} (in µg/mL) at significant level $p < 0.05$

Conc. ↓	Pesticide →	Acephate			Atrazine				Carbendazim			
	Treatment ↓	**ACP1**	**ACP2**	**ACP3**	**RK1**	**RK2**	**RK3**	**RK4**	**CB1**	**CB2**	**CB3**	**CB4**
0x	**Control**	68.5 ±0.7	46.73±1.40	37.22±0.87	32.40±0.65	49.43±0.70	73.53±0.75	89.3±0.98	31.46±0.58	44.3±0.55	50.46±0.70	72.5±0.88
	100 mg/L Cu^{++}	43.5 ±0.98	26.56±0.58	22.63±0.75	26.76±0.80	40.2±0.45	47.13±0.55	60.1±0.79	10.5±0.62	22.33±0.47	26.43±0.77	35.23±0.51
	100 mg/L Fe^{++}	56.4 ±0.83	44.13±0.66	40.33±0.70	25.80±1.10	38.13±0.75	61.03±0.72	74.56±0.90	23.63±0.47	26.6±0.62	42.76±0.70	57.43±0.47
	100 mg/L HA	69.4 ±0.62	51.6±0.81	43.70±0.81	42.60±0.98	53.33±0.75	80.63±0.75	92.43±0.55	32.53±0.70	47.03±0.85	56.73±0.41	78.03±0.60
1x	**Control**	56.7 ±0.65	32.5±0.72	27.50±0.72	28.43±0.85	36.23±0.37	54.16±0.75	70.9±0.62	27.76±0.90	36.46±0.75	41.5±0.65	56.6±0.65
	100 mg/L Cu^{++}	23.3 ±0.45	17.6±1.11	16.23±0.51	21.16±0.70	34.23±0.51	47.2±0.88	51.53±0.94	8.26±0.32	14.5±0.79	16.7±0.8	27.23±0.40
	100 mg/L Fe^{++}	51.3 ±0.55	39.56±0.61	36.36±0.87	18.73±0.96	26.56±1.06	46.3±0.62	62.16±0.76	37.96±0.86	35.5±0.88	42.26±0.56	56.56±0.65
	100 mg/L HA	70.8 ±1.24	57.43±1.10	47.52±0.74	50.93±0.41	65.63±0.66	85.56±0.65	96.7±0.52	38.23±0.40	53.03±0.25	62.06±0.65	83.8±0.75
2x	**Control**	44.1 ±0.47	28.2±0.75	21.04±0.62	22.46±0.60	28.93±0.20	38.36±0.50	54.16±0.65	16.3±0.45	30.93±0.75	34.26±0.47	44.93±0.80
	100 mg/L Cu^{++}	18.1±0.7	11.1±0.7	08.36±0.45	17.63±0.75	29.1±0.65	36.4±0.65	40.36±0.60	3. 60±0.40	9.16±0.41	11.2±0.55	14.4±0.45
	100 mg/L Fe^{++}	46.3 ±0.62	33.06±0.60	28.63±0.74	10.53±0.76	22. ±0.75	38.1±0.5	46.46±0.49	35.06±0.73	31.33±0.81	38.46±0.80	52.33±0.45
	100 mg/L HA	59.4 ±0.87	38.13±0.86	31.13±0.72	38.56±0.80	46±0.81	63±0.36	65.3±0.55	35.66±0.98	46.33±0.70	58.6±0.72	77.13±0.65

where 0 x = no pesticides, 1x = 500mg/L of pesticides and 2x = 1000 mg/L of pesticides, Cu^{++} = copper chloride, Fe^{++} = ferrous chloride and HA = humic acid.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values) at significant level $p < 0.05$

ACP1 = *Pseudomonas* sp. ACP1, ACP2 = *Pseudomonas sp* ACP2, ACP3 = *Pseudomonas sp* ACP3, RK1 =*Streptomycetaceae bacterium* RK1, RK2 = *Pseudomonas fluorescens* strain RK2, RK3 = *Azotobacter chroococcum* strain RK3, RK4 = *Rhizobium leguminosarum* strain RK4, CB1 = *Actinomyces* sp. CB1, CB2 = *Bacillus subtilis* CB2, CB3 = *Pseudomonas aeruginosa* CB3, CB4 = *Rhizobium leguminosarum* CB4.

Table 11: Production of IAA by bacterial isolates inoculated singly and co-supplemented with each pesticide (Glyphosate, Monocrotophos and Phorate) humic acid (HA), Cu^{++} and Fe^{++} (in µg/mL) at significant level $p < 0.05$

		Glyphosate			Monocrotophos			Phorate		Standard Microorganisms	
	Treatment ↓	**GP1**	**GP2**	**GP3**	**MC1**	**MC2**	**MC3**	**PR1**	**PR2**	**R.L**	***E. coli***
0x	**Control**	44.26±0.45	52.66±0.90	95.5±1.13	18.13±1.02	64.36±0.61	94.16±0.61	65.4±0.7	78.56±1.38	48.3±0.91	10.33±1.52
	100 mg/L Cu^{++}	18.5±0.55	2.65±0.47	29.56±0.90	8.6±0.81	23.8±0.78	52.3±0.35	25.36±0.55	27.76±1.81		
	100 mg/L Fe^{++}	26.93±0.77	52±0.81	89.43±0.85	22.66±0.80	73.4±0.62	96.16±0.47	79.16±0.76	81.1±1.15		
	100 mg/L HA	59.36±0.73	62.73±0.58	96.33±0.55	26.16±0.80	72.8±0.7	98.66±0.45	74.26±1.55	79.4±0.7		
1x	**Control**	34.03±0.45	49.53±0.75	67.56±0.81	14.1±0.7	48.13±1.00	56.73±0.60	57.36±0.70	59.46±0.75		
	100 mg/L Cu^{++}	9.03±0.35	14.26±0.77	20.43±0.87	5.33±0.45	14.2±0.88	20.43±0.80	10.8±0.7	11.13±0.65		
	100 mg/L Fe^{++}	36.7±0.45	67.06±0.75	91.53±0.75	32.7±0.85	83.46±0.75	97.06±0.80	82.93±0.58	88.13±0.90		
	100 mg/L HA	61.23±0.55	75.56±0.65	99.33±0.70	34.5±0.7	74.5±1.24	98.36±0.56	82.53±0.32	92.2±0.45		
2x	**Control**	22.23±0.51	33.66±0.41	58.16±0.70	11.16±0.56	31.5±1.05	43.36±0.51	44.86±1.15	48.6±0.62		
	100 mg/L Cu^{++}	3.36±0.45	8.33±0.47	11.3±0.45	3.133±0.25	9.53±0.77	16.96±0.25	8.26±0.40	9.03±0.35		
	100 mg/L Fe^{++}	27.43±0.47	51.43±0.65	81.16±0.41	13.46±0.61	64.13±0.65	84.2±0.96	71.73±0.97	83.26±1.12		
	100 mg/L HA	46.56±0.77	66.4±0.81	72.23±0.70	29.36±0.87	51.16±0.75	62.4±0.6	68.43±0.60	70.83±1.36		

where 0 x = no pesticides, 1x = 500mg/L of pesticides and 2x = 1000 mg/L of pesticides, Cu^{++} = copper chloride, Fe^{++} = ferrous chloride and HA = humic acid.

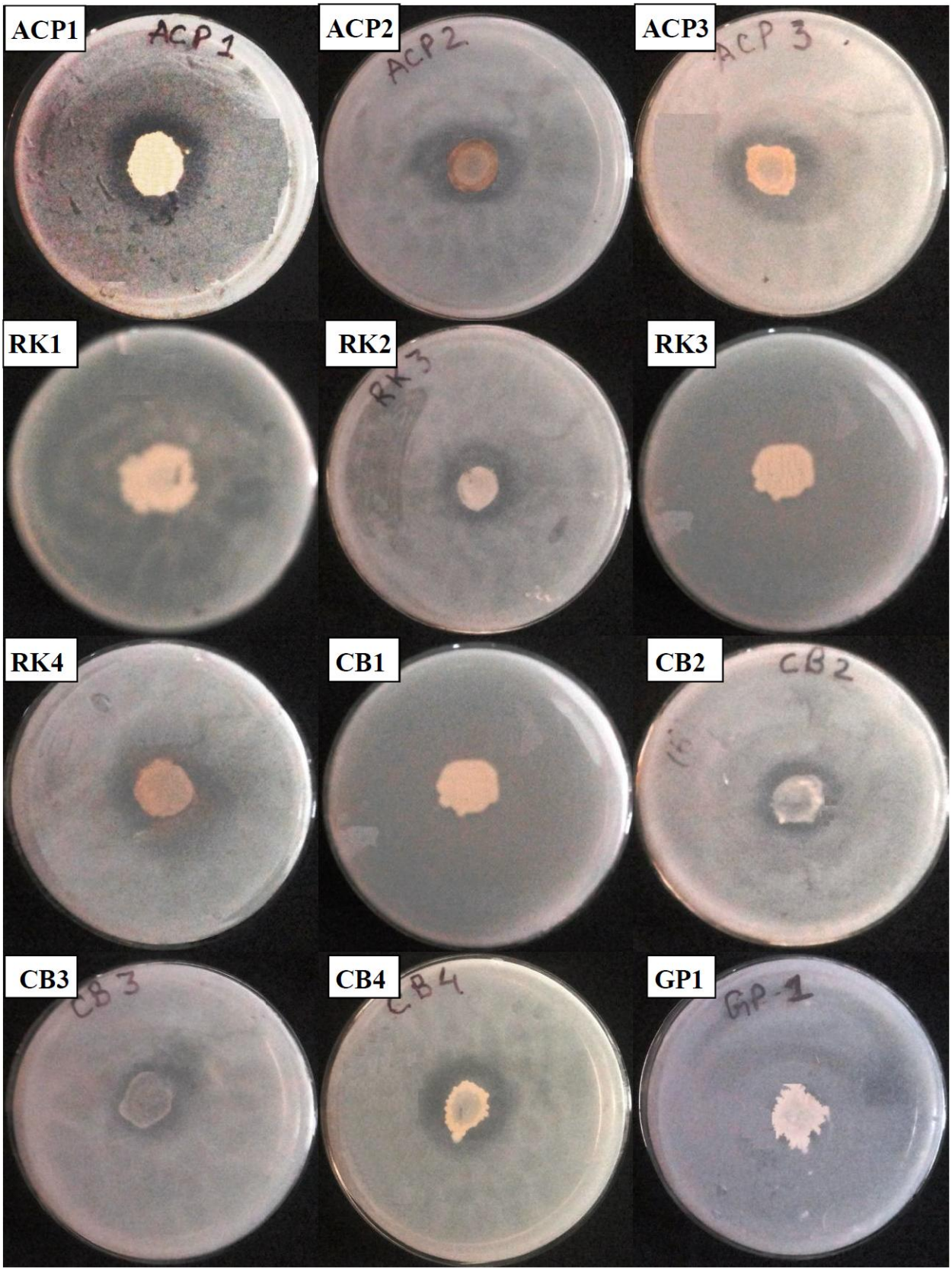
± Standard deviation (values indicate change decrease (-) or increase (+) over the values) at significant level $p < 0.05$

GP1 = *Actinomyces* sp. GP1, GP2 = *Bacillus subtilis* GP2, GP3 =*Rhizobium leguminosarum* GP3, MC1 = *Actinomyces* sp. MC1, MC2 = *Bacillus subtilis* MC2, MC3 = *Rhizobium leguminosarum* MC3, PR1 = *Pseudomonas* sp. PR_01 and PR2, *Pseudomonas* sp. PR_2, RL = *Rhizobium leguminosarum*.

6.3.2 Effect of pesticides, metal ions and humic acid on qualitative and quantitative production on phosphate solubilisation

It was reported that the main mechanism used by bacteria for solubilization of inorganic phosphorous is based on the production of small molecular weight organic acids such as a citric and gluconic acid (Ahemad et al., 2014; Rodriguez *et al.*, 2004; Bnayahu, 1991). The production of chelating cations by bacteria converts these organic acids into soluble phosphate by binding phosphate with their carboxyl and hydroxyl groups thereby chelating cations (Rodriguez *et al.*, 2004; Kpomblekou and Tabatabai, 1994; Bnayahu, 1991). In a similar study, the concentrations of phosphate solubilizing bacteria are commonly found in rhizospheric soil as compared to non-rhizospheric soils were also being reported (Raja et al., 2006). Under pesticide stress, the PSB activity of the strains was affected as earlier reported by Ahemad (2012). In our study, Out of 19 isolates, only 15 strains were capable to solubilise phosphorous both qualitatively and quantitatively. Highest solubilisation efficacy was observed in most of the species of *Rhizobium* and *Pseudomonas*. With the addition of 1x and 2x concentration of pesticides, significant changes were noticed in all the treated groups at significant level $p < 0.05$. Metal ions and humic acid have relatively less effect on production as compared to the control groups. It observed that the phosphate solubilization production was significantly decreased with the increase of concentration levels of pesticides. On addition of 100 mg/L of metal ions (Cu^{++} and Fe^{++}) and humic acid, the Cu^{++} had the most toxic effect on its production. On addition of Fe^{++} and humic acid, the solubilisation efficacy and concentration of phosphate solubilised were relatively increased when amended with 1x concentration of each pesticide at significant level $p < 0.05$. On addition of 2x concentration of each pesticide, the phosphate solubilisation (both qualitatively and quantitatively) was decreased in almost all the cases. The solubilisation efficacy (qualitative) and concentration of phosphorous solubilised (quantitative) by the isolated strains along with standard strains, effects of pesticides, Cu^{++}, Fe^{++} and humic acid were summarized in table 12-15 and fig 22.

Phosphate solubilization was analysed by zone inhibition method and UV-visible method. It was noticed that, at the significant level ($p = 0.05$), comprehensive at three concentrations level (0X, 1X and 2X), with the addition of Fe^{++}, Cu^{++}, and HA the statistical means were significantly different. It was observed that Siderophore production was concentration dependent, as the concentration (0X, 1X and 2X) increased the IAA production decreased. Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level $p = 0.05$) with an order HA > control > Fe^{++}> Cu^{++}.


ACP1
ACP 1
ACP2
ACP 2
ACP3
ACP 3
RK1
RK2
RK 3
RK3
RK4
CB1
CB2
CB 2
CB3
CB 3
CB4
CB 4
GP1
GP 1

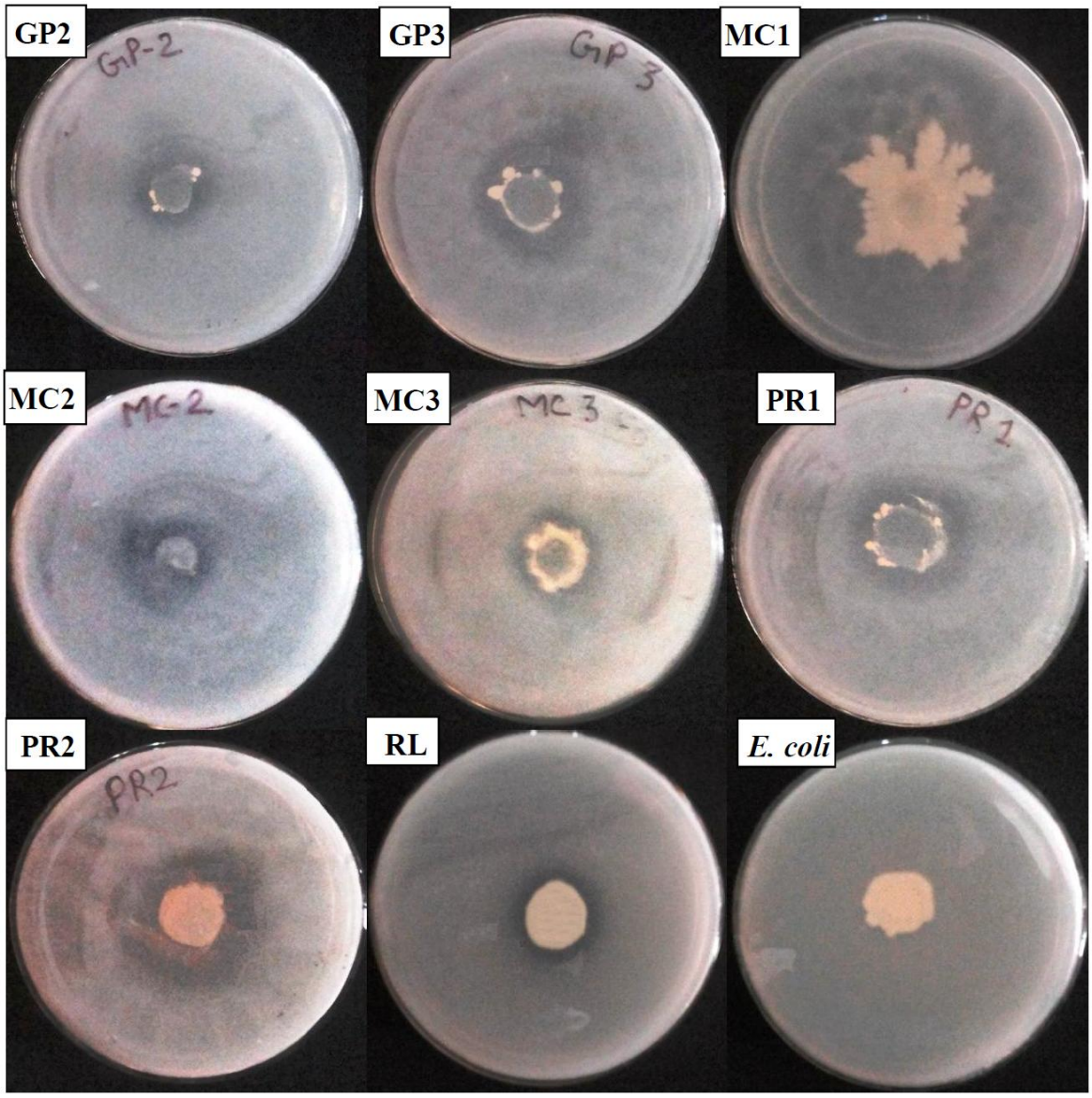


Figure 22: Phosphate solubilization activities of the strains isolated for the study from various pesticides contaminated sites. where ACP1 = *Pseudomonas* sp. ACP1, ACP2 = *Pseudomonas* sp. ACP2, ACP3 = *Pseudomonas* sp. ACP3,RK1 = *Streptomycetaceae bacterium* RK1, RK2 = *Pseudomonas fluorescens* strain RK2, RK3 = *Azotobacter chroococcum* strain RK3, RK4 = *Rhizobium leguminosarum* strain RK4, CB1 = *Actinomyces* sp. CB1, CB2 = *Bacillus subtilis* CB2, CB3 = *Pseudomonas aeruginosa* CB3, CB4 = *Rhizobium leguminosarum* CB4, GP1 = *Actinomyces* sp. GP1, GP2 = *Bacillus subtilis* GP2, GP3 = *Rhizobium leguminosarum* GP3, MC1 = *Actinomyces* sp. MC1, MC2 = *Bacillus subtilis* MC2, MC3 = *Rhizobium leguminosarum* MC3, PR1 = *Pseudomonas* sp. PR_01 and PR2 = *Pseudomonas* sp. PR_2, RL = *Rhizobium leguminosarum* MTCC 10096, RL = *E. coli* MTCC 1696

Table 12: Qualitative solubilisation of phosphate by bacterial isolates inoculated singly and co-supplemented with pesticides (Acephate, Atrazine and Carbendazim), humic acid (HA), Cu^{++} and Fe^{++} (Solubilization Efficiency in %) at significant level $p < 0.05$

Conc ↓.	**Pesticides →**	**Acephate**			**Atrazine**		**Carbendazim**		
	Treatment ↓	ACP1	ACP2	ACP3	RK3	RK4	CB2	CB3	CB4
0x	**Control**	89.96±0.45	116.80±0.6	89.22±0.54	107.20±0.4	105.13±0.7	39.15±0.51	76.36±0.46	140.16±0.6
	100 mg/L Cu^{++}	83.46±0.73	98.33±0.39	96.97±0.69	94.33±0.55	87.34±0.63	53.07±0.64	68.56±0.62	97.12±0.72
	100 mg/L Fe^{++}	87.57±0.76	103.36±0.5	117.26±0.3	111.26±0.5	94.26±0.45	57.22±0.54	80.61±0.72	143.42±0.5
	100 mg/L HA	92.53±0.97	124.33±0.9	86.46±0.51	124.35±0.6	117.53±0.6	34.92±0.68	89.47±0.63	149.87±0.6
1x	**Control**	79.18±0.90	98.37±0.81	76.48±0.45	95.25±0.54	84.5±0.75	35.66±0.73	59.28±0.65	113.38±0.5
	100 mg/L Cu^{++}	72.72±0.65	62.62±0.61	54.17±0.4	72.3±0.62	69.57±0.59	58.63±0.48	41.84±0.91	79.38±0.89
	100 mg/L Fe^{++}	89.96±0.45	113.15±0.2	123.15±0.5	117.52±0.7	99.21±0.64	73.3±0.45	93.48±0.71	151.27±0.6
	100 mg/L HA	106.4±0.73	137.30±0.4	144.7±0.49	144.38±0.5	131.31±0.6	122.3±0.46	102.59±0.7	163.32±0.4
2x	**Control**	87.57±0.76	77.05±0.63	59.22±0.51	78.12±0.62	56.71±0.61	26.75±0.64	35.3±0.65	93.14±0.65
	100 mg/L Cu^{++}	62.53±0.97	68.27±0.35	72.33±0.93	57.32±0.64	46.79±0.82	43.97±0.70	36.37±0.74	47.21±0.79
	100 mg/L Fe^{++}	79.18±0.90	84.35±0.40	104.26±0.5	81.13±0.39	74.83±0.75	56.57±0.58	79.74±0.66	102.45±0.5
	100 mg/L HA	72.72±0.65	98.26±0.40	105.39±0.7	108.37±0.6	97.28±0.64	73.28±0.41	79.96±0.73	104.48±0.8

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values) at significant level $p < 0.05$.

Table 13: Qualitative solubilisation of phosphate by bacterial isolates inoculated singly and co-supplemented with pesticides (Glyphosate, Mono and Phorate), humic acid (HA), Cu^{++} and Fe^{++} (Solubilization Efficiency in %) at significant level $p < 0.05$.

Conc. ↓	**Pesticide →**	**Glyphosate**		**Monocrotophos**		**Phorate**		
	Treatment ↓	**GP 2**	**GP 3**	**MC2**	**MC3**	**PR1**	**PR2**	**RL**
0x	**Control**	65.3±0.55	127.47±0.43	125.03±0.75	49.85±0.58	79.93±0.70	149.98±0.77	83.74±0.62
	100 mg/L Cu^{++}	104.04±0.5	86.35±0.49	90.06±0.60	39.44±0.56	65.97±0.57	93.36±0.51	-
	100 mg/L Fe^{++}	118.06±0.7	133.28±0.70	129.42±0.45	62.31±0.51	85.34±0.69	146.04±0.86	-
	100 mg/L HA	75.83±0.75	141.08±0.67	137.36±0.49	75.06±0.45	94.16±0.79	163.29±0.42	-
1x	**Control**	48.44±0.76	82.14±0.64	111.31±0.62	34.05±0.77	68.34±0.50	127.47±0.92	-
	100 mg/L Cu^{++}	39.61±0.8	54.77±0.57	61.16±0.70	26.37±0.45	46.48±0.45	83.15±0.66	-
	100 mg/L Fe^{++}	129.15±0.4	141.2±0.55	136.40±0.58	74.43±0.73	92.99±0.48	151.41±0.61	-
	100 mg/L HA	146.7±0.78	156.32±0.51	144.03±0.77	85.17±0.56	102.5±0.80	177.27±0.76	-
2x	**Control**	31.36±0.60	61.4±0.60	74.28±0.47	19.53±0.85	47.41±0.52	89.31±0.90	-
	100 mg/L Cu^{++}	23.55±0.67	38.51±0.48	38.06±0.61	23.29±0.58	26.26±0.47	75.27±0.61	-
	100 mg/L Fe^{++}	92.39±0.56	114.71±0.40	107.47±0.42	45.22±0.80	74.22±0.64	119.20±0.57	-
	100 mg/L HA	100.51±0.8	105.61±0.61	122.22±0.55	64.67±0.78	90.14±0.54	129.33±0.60	-

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values) at significant level $p < 0.05$

Table 14: Quantitative Solubilisation of phosphate by bacterial isolates inoculated singly and co-supplemented with pesticides (Acephate, Atrazine and Carbendazim), humic acid (HA), Cu^{++} and Fe^{++} (in µgP/mL) at significant level $p < 0.05$

Conc. ↓	**Pesticide →**	**Acephate**			**Atrazine**		**Carbendazim**		
	Treatment ↓	ACP1	ACP2	ACP3	RK3	RK4	CB2	CB3	CB4
0x	**Control**	0.73±0.04	1.10±0.07	0.91±0.04	0.85±0.13	0.93±0.04	0.85±0.05	0.91±0.04	1.08±0.07
	100 mg/L Cu^{++}	0.34±0.04	0.47±0.04	0.31±0.02	0.86±0.05	0.71±0.07	0.46±0.15	0.64±0.02	0.80±0.04
	100 mg/L Fe^{++}	0.82±0.04	1.23±0.07	1.12±0.11	0.95±0.05	1.13±0.10	0.91±0.05	0.98±0.1	1.13±0.10
	100 mg/L HA	0.8±0.1	1.3±0.03	1.18±0.10	1.02±0.03	0.86±0.15	0.92±0.06	1.04±0.08	1.21±0.12
1x	**Control**	0.57±0.05	0.90±0.03	0.33±0.03	0.85±0.06	0.79±0.02	0.72±0.03	0.78±0.03	0.85±0.03
	100 mg/L Cu^{++}	0.44±0.01	0.40±0.04	0.25±0.02	0.72±0.04	0.63±0.04	0.3±0.01	0.46±0.07	0.55±0.06
	100 mg/L Fe^{++}	0.99±0.05	1.31±0.03	1.16±0.08	1.12±0.16	1.13±0.06	1.05±0.09	1.07±0.07	1.09±0.09
	100 mg/L HA	1.04±0.04	1.63±0.05	1.45±0.07	1.10±0.06	1.15±0.06	1.07±0.08	1.17±0.08	1.44±0.05
2x	**Control**	0.37±0.03	0.74±0.03	0.45±0.03	0.55±0.05	0.36±0.03	0.16±0.04	0.18±0.07	0.25±0.03
	100 mg/L Cu^{++}	0.07±0.01	0.15±0.02	0.18±0.01	0.38±0.01	0.33±0.03	0.27±0.02	0.31±0.03	0.36±0.02
	100 mg/L Fe^{++}	0.67±0.04	1.01±0.07	0.07±0.02	0.67±0.02	0.97±0.04	0.55±0.02	0.71±0.04	0.85±0.01
	100 mg/L HA	0.72±0.04	1.23±0.18	1.13±0.11	0.97±0.07	0.83±0.04	0.63±0.04	0.64±0.04	1.15±0.14

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values) at significant level $p < 0.05$

Table 15: Quantitative Solubilisation of phosphate by bacterial isolates inoculated singly and co-supplemented with pesticides (Glyphosate, Mono and Phorate), humic acid (HA), Cu^{++} and Fe^{++} (in µgP/mL**)** at significant level $p < 0.05$

Conc. ↓	**Pesticide →**	**Glyphosate**		**Monocrotophos**		**Phorate**		
	Treatment ↓	**GP 2**	**GP 3**	**MC2**	**MC3**	**PR1**	**PR2**	**RL**
0x	**Control**	0.84±0.04	1.06±0.05	1.03±0.05	0.66±0.05	1.17±0.08	1.42±0.04	0.63±0.04
	100 mg/L Cu^{++}	0.41±0.02	0.46±0.04	0.46±0.05	0.29±0.05	0.86±0.07	1.01±0.03	-
	100 mg/L Fe^{++}	0.76±0.15	1.12±0.08	1.17±0.07	0.77±0.06	1.08±0.08	1.50±0.07	-
	100 mg/L HA	0.92±0.03	1.19±0.06	1.16±0.06	0.79±0.05	1.23±0.05	1.51±0.08	-
1x	**Control**	0.71±0.02	0.85±0.04	0.73±0.03	0.47±0.03	0.81±0.02	1.14±0.08	-
	100 mg/L Cu^{++}	0.29±0.02	0.32±0.02	0.34±0.03	0.24±0.04	0.56±0.06	0.94±0.03	-
	100 mg/L Fe^{++}	1.07±0.06	1.12±0.09	1.33±0.04	0.97±0.06	1.18±0.07	1.65±0.04	-
	100 mg/L HA	1.14±0.06	1.28±0.07	1.33±0.05	1.03±0.04	1.32±0.04	1.65±0.06	-
2x	**Control**	0.41±0.03	0.56±0.05	0.41±0.02	0.15±0.03	0.72±0.05	0.8±0.04	-
	100 mg/L Cu^{++}	0.15±0.02	0.14±0.03	0.3±0.04	0.12±0.03	0.31±0.03	0.44±0.06	-
	100 mg/L Fe^{++}	0.64±0.04	0.72±0.04	1.05±0.05	0.45±0.04	0.91±0.06	1.34±0.08	-
	100 mg/L HA	0.77±0.02	0.90±0.04	1.14±0.08	0.56±0.04	0.99±0.07	1.45±0.08	-

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values) at significant level $p < 0.05$

6.3.3 Effect of six different pesticides, Cu^{++}, Fe^{++} and humic acid on qualitative and quantitative production on Sidherophoric Production

Siderophores ("metal ions carrier") are chelating agents with low molecular weight (200–2000 Da), high-affinity iron chelating compounds secreted mostly by microorganisms such as *Pseudomonas, Bacillus, Rhizobium, Actinomyces* etc. Usually, siderophores act as solubilizing agents for metal ions from minerals or organic compounds under conditions of metal ions limitation.

The effect of pesticides on PGPR, maximum investigations highlighting the dose-dependent effects of pesticides on PGPR (Ahemad et al., 2014), i.e. with the increase in dose adverse effect of pesticides on PGPR increases. The perilous effect of pesticides on PGPR is directly proportional to the perseverance and dose level. Continuous and repeated use of pesticides having a high persistency level in the soils may further increase the menace.

Moreover, acephate, atrazine, carbendazim, glyphosate monocrotophos and phorate are widely used pesticides in agricultural applications worldwide.

PGPR are reported to exude some extracellular metabolites called siderophores. The presence of ligand (Siderophore) producing PGPR in rhizosphere increases the rate of Iron supply to plants and consequently enhances the plant growth and productivity of the crop. Additional, this compound after chelating Fe^{3+} makes the soil Fe^{3+} scarce for other soil microbes and thus inhibits the activity of competitive microbes. Ahemad et al 2014 illustrate the under pesticide stress relatively decrease the functioning of the rhizobacteria to produce Siderophores. Our findings conclude that, out of 19 isolates, only 14 strains were able to produce Siderophores both qualitatively and quantitatively. Highest production was observed in most of the species of *Rhizobium* and *Pseudomonas*. Each of the pesticides, when used in increasing concentrations, shows an adverse effect on Siderophore production. On addition of 100 mg/L of Cu^{++} and Fe^{++} and humic acid, the Cu^{++} had the most toxic effect on its production. On addition of Fe^{++} and humic acid, the Siderophore production (both qualitative and quantitative) was relatively increased when amended with 1x concentration of each pesticide at significant level $p < 0.05$. On addition of 2x concentration of each six different pesticides, the adverse effect of pesticides was seen in both qualitatively and quantitatively approaches. The zone of appearance (qualitative) and concentration of sidherophoric units (quantitative) by the isolated strains along with standard strains, the effect of pesticides, Cu^{++}, Fe^{++} and humic acid ws summarized in fig. 23 and in table 16-19.

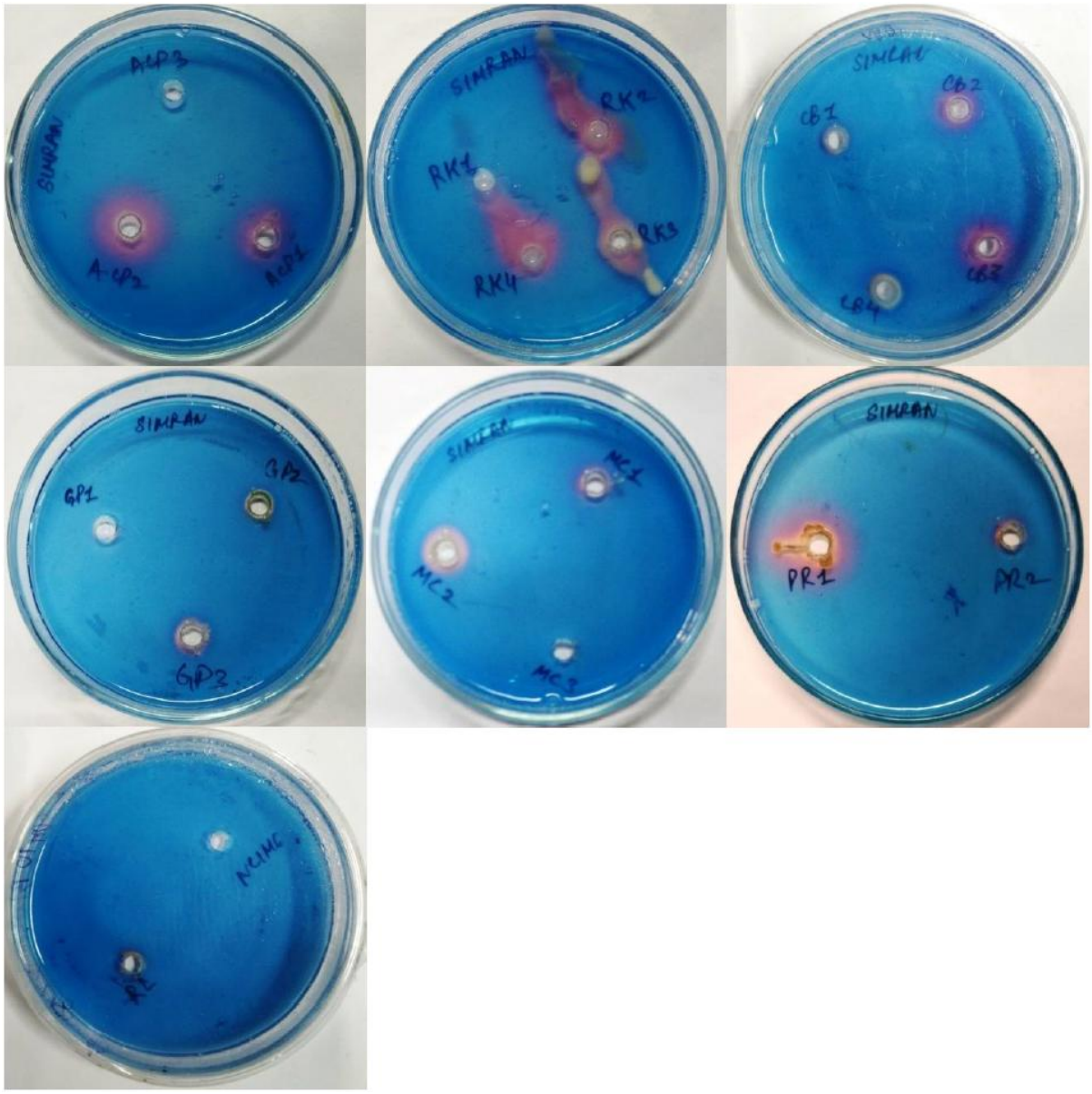


Figure 23: Siderophore Production by the isolated and standard strains from pesticide contaminated soils.

where ACP1 = *Pseudomonas* sp. ACP1, ACP2 = *Pseudomonas sp* ACP2, ACP3 = *Pseudomonas sp* ACP3, RK1 = *Streptomycetaceae bacterium* RK1, RK2 = *Pseudomonas fluorescens* strain RK2, RK3 = *Azotobacter chroococcum* strain RK3, RK4 = *Rhizobium leguminosarum* strain RK4, CB1 = *Actinomyces* sp. CB1, CB2 = *Bacillus subtilis* CB2, CB3 = *Pseudomonas aeruginosa* CB3, CB4 = *Rhizobium leguminosarum* CB4, GP1 = *Actinomyces* sp. GP1, GP2 = *Bacillus subtilis* GP2, GP3 = *Rhizobium leguminosarum* GP3, MC1 = *Actinomyces* sp. MC1, MC2 = *Bacillus subtilis* MC2, MC3 = *Rhizobium leguminosarum* MC3, PR1 = *Pseudomonas* sp. PR_01 and PR2 = *Pseudomonas* sp. PR_2

Table 16: Qualitative estimation of Siderophore production by bacterial isolates inoculated singly and co-supplemented with pesticides (Acephate, Atrazine and Carbendazim), humic acid (HA), Cu^{++} and Fe^{++} (Zone of detection: Halo Orange) at significant level $p < 0.05$

Conc. ↓	**Pesticide →**	**Acephate**		**Atrazine**			**Carbendazim**		
	Treatment ↓	ACP1	ACP2	RK2	RK3	RK4	CB2	CB3	CB4
0x	**Control**	3.66±0.57	6.03±0.55	3.73±0.30	4.96±0.25	7.06±0.70	3.06±0.30	4.06±0.40	2.1±0.36
	100 mg/L Cu^{++}	2.96±0.45	4.1±0.55	2.96±0.15	3.06±0.20	2.16±0.37	2.1±0.36	4.1±0.55	--
	100 mg/L Fe^{++}	4.83±0.20	8.96±0.25	4.03±0.45	6.1±0.26	8.06±0.50	4.23±0.58	5.03±0.35	5.13±0.41
	100 mg/L HA	5.7±0.43	10.9±0.36	7.03±0.35	8.13±0.41	13.16±0.47	6.13±0.51	7.13±0.41	6±0.3
1x	**Control**	1.7±0.36	7±0.2	2.06±0.30	2.06±0.30	2.96±0.55	1.13±0.41	2.1±0.45	--
	100 mg/L Cu^{++}	1.6±0.45	1.03±0.15	--	--	--	--	--	--
	100 mg/L Fe^{++}	5.76±0.25	10.16±0.56	4.96±0.35	6.03±0.25	9.1±0.45	4±0.6	5.13±0.41	2.03±0.35
	100 mg/L HA	7±0.4	12.43±0.55	9.03±0.35	9.03±0.35	15.06±0.30	6.96±0.35	8.16±0.47	3.1±0.26
2x	**Control**	1.03±0.25	3.1±0.36	--	--	1.1±0.36	--	--	--
	100 mg/L Cu^{++}	0	-	--	--	--	--	--	--
	100 mg/L Fe^{++}	2.1±0.26	3.13±0.61	1.13±0.32	2.03±0.35	4.16±0.47	--	2.13±0.41	--
	100 mg/L HA	4.2±0.43	6.06±0.30	4.16±0.47	5.06±0.30	6.06±0.30	4.1±0.75	3.03±0.35	--

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 17: Qualitative estimation of Siderophore production by bacterial isolates inoculated singly and co-supplemented with pesticides (Glyphosate, Mono and Phorate), humic acid (HA), Cu^{++} and Fe^{++} (Zone of detection: Halo Orange) at significant level $p < 0.05$

Conc. ↓	**Pesticide →**	**Glyphosate**		**Monocrotophos**		**Phorate**		
	Treatment ↓	GP 2	GP 3	MC2	MC3	PR1	PR2	*P. fluorescens*
0x	**Control**	1.06±0.40	3.03±0.25	2.03±0.35	4.16±0.47	7.86±0.41	1.8±0.2	6.1±0.55
	100 mg/L Cu^{++}	--	--	--	2.06±0.30	4.5±0.4	--	--
	100 mg/L Fe^{++}	2.13±0.32	4±0.3	2.2±0.43	5.13±0.41	6.93±0.20	3.23±0.49	--
	100 mg/L HA	4.03±0.35	5.03±0.25	4.63±0.32	4.93±0.30	13.03±0.35	3.93±0.40	--
1x	**Control**	--	2.2±0.43	--	2±0.2	3.93±0.30	--	--
	100 mg/L Cu^{++}	--	--	--	--	--	--	--
	100 mg/L Fe^{++}	1.03±0.35	2.06±0.40	3.13±0.41	3.03±0.35	7.7±0.3	1.9±0.36	--
	100 mg/L HA	4.06±0.30	6.06±0.30	5.03±0.25	6.9±0.17	14.43±0.89	2.13±0.32	--
2x	**Control**	--	--	--	--	--	--	--
	100 mg/L Cu^{++}	--	--	--	--	--	--	--
	100 mg/L Fe^{++}	--	--	--	2±0.3	3.13±0.41	--	--
	100 mg/L HA	2.1±0.36	2.06±0.20	2.1±0.55	4±0.3	10.96±0.15	--	--

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

Table 18: Quantitative estimation of Siderophore production by bacterial isolates inoculated singly and co-supplemented with pesticides (Acephate, Atrazine and Carbendazim), humic acid (HA), Cu^{++} and Fe^{++} (S.U %) at significant level $p < 0.05$

Group	**Pesticide →**	**Acephate**		**Atrazine**			**Carbendazim**		
	Treatment ↓	ACP1	ACP2	RK2	RK3	RK4	CB2	CB3	CB4
0x	**Control**	56.76±0.46	64.52±0.85	60.42±0.80	61.37±0.47	64.45±0.41	55.01±0.76	48.36±0.39	20.44±0.55
	100mg/L Cu^{++}	53.47±0.44	57.50±0.68	53.12±0.17	54.28±0.33	56.31±0.52	51.29±0.66	43.26±0.31	14.73±0.39
	100mg/L Fe^{++}	60.42±0.40	71.69±0.41	60.30±0.52	66.09±0.36	69.45±0.54	58.28±0.50	50.69±0.50	35.93±0.60
	100mg/L HA	63.35±0.59	72±0.67	65.92±0.45	69.55±0.56	72.42±0.57	66.25±0.51	57.32±0.39	38.2±0.55
1x	**Control**	49.16±0.53	61.2±0.41	52.64±0.45	57.29±0.43	58.85±0.53	50.05±0.67	44.35±0.51	9.37±0.49
	100mg/L Cu^{++}	33.63±0.61	36.14±0.38	38.29±0.40	43.60±0.45	49.36±0.55	47.54±0.63	30.69±0.42	12.16±0.56
	100mg/L Fe^{++}	63.82±0.64	73.12±0.53	63.37±0.52	68.39±0.57	71.08±0.63	66.12±0.68	53.29±0.46	40.19±0.40
	100mg/L HA	70.57±0.77	75.80±0.43	70.06±0.30	75.09±0.70	79.08±0.58	70.13±0.57	60.30±0.50	43.33±0.33
2x	**Control**	40.70±0.35	45.86±0.33	44.27±0.56	47.83±0.65	50.81±0.44	43.29±0.42	36.34±0.45	2.16±0.27
	100mg/L Cu^{++}	19.4±0.53	25.87±0.58	31.29±0.48	37.61±0.44	40.15±0.45	42.41±0.51	24.41±0.54	10.49±0.34
	100mg/L Fe^{++}	44.35±0.53	52.68±0.49	48.42±0.50	51.32±0.64	62.11±0.57	59.60±0.52	46.33±0.65	26.44±0.60
	100mg/L HA	55.32±0.62	60.28±0.55	59.66±0.44	61.19±0.39	71.28±0.47	66.12±0.49	46.34±0.58	26.30±0.47

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 19: Quantitative estimation of Siderophore production by bacterial isolates inoculated singly and co-supplemented with pesticides (Glyphosate, Mono and Phorate), humic acid (HA), Cu^{++} and Fe^{++} (S.U %) at significant level $p < 0.05$

Conc. ↓	**Pesticide →**	**Glyphosate**		**Monocrotophos**		**Phorate**		
	Treatment ↓	**GP 2**	**GP 3**	**MC2**	**MC3**	**PR1**	**PR2**	***P. fluorescens***
0x	**Control**	15.35±0.37	52.74±0.53	25.35±0.51	53.77±0.53	75.34±0.65	25.28±0.59	43.31±0.65
	100 mg/L Cu^{++}	13.42±0.43	47.39±0.54	22.29±0.42	37.76±0.55	69.27±0.62	22.08±0.42	--
	100 mg/L Fe^{++}	28.41±0.44	52.19±0.73	32.2±0.59	61.06±0.51	77.35±0.44	31.37±0.46	--
	100 mg/L HA	33.06±0.42	54.81±0.55	39.32±0.58	63.34±0.57	81.28±0.33	39.14±0.51	--
1x	**Control**	4.37±0.53	45.49±0.48	21.23±0.35	48.21±0.43	61.33±0.50	19.12±0.50	--
	100 mg/L Cu^{++}	7.36±0.44	40.23±0.53	17.60±0.42	29.26±0.45	61.3±0.47	14.33±0.55	--
	100 mg/L Fe^{++}	38.13±0.30	57.28±0.45	40.68±0.77	64.54±0.47	80.14±0.51	40.98±0.41	--
	100 mg/L HA	40.24±0.48	59.30±0.39	53.38±0.53	67.11±0.33	84.23±0.45	47.21±0.57	--
2x	**Control**	1.53±0.09	35.11±0.40	10.06±0.19	40.19±0.55	48.18±0.48	12.46±0.49	--
	100 mg/L Cu^{++}	3.10±0.18	32.16±0.37	14.31±0.61	21.88±0.66	52.22±0.56	4.17±0.43	--
	100 mg/L Fe^{++}	20.7±0.63	48.28±0.34	31.51±0.58	56.38±0.54	74.33±0.63	20.23±0.46	--
	100 mg/L HA	30.08±0.43	36.22±0.40	44.85±0.56	56.08±0.40	76.12±0.44	36.62±0.40	--

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

6.3.4 Effect of pesticides, Cu^{++} and Fe^{++} and humic acid on qualitative production on Hydrogen Cyanide production

Cyanide production by numerous rhizobacteria and is postulated to play a function in the control of biological harmful pathogens (Defago et al., 1990). The suppression of soil-borne pathogens by the production of these hormones by fluorescent *Pseudomonas* has also been reported worldwide (Ahemad et al., 2014; Chitra et al., 2002; Voisard et al., 1989). Various diseases in tobacco and wheat were also being suppressed by Cyanide production were reported by Stutz et al., 1986; Defago et al., 1990. HCN production also improves root length and seed germination in some plants (Saxena et al., 1996). Ahemad et al (2012) reported the effect of HCN production under pesticide stress. Out of 19 isolates, only 3 strains (ACP3, CB1 and GP1) were able to synthesize HCN production and the results were summarized in table 19. Pesticides at high concentrations posed a negative effect on its production while the addition of Fe^{++} and humic acid along with pesticides has no effect on its production.

Table 20: Qualitative assay for hydrogen Cyanide production by bacterial species isolated from rhizospheric soils

Conc. ↓	**Pesticide →**	**Acephate**	**Carbendazim**	**Glyphosate**	**Control**
	Treatment ↓	*Pseudomonas* sp. ACP3	*Actinomyces* sp. CB1	*Actinomyces* sp. GP1	*P fluorescens*
0x	**Control**	+++	++	++	++
	100 mg/L Cu^{++}	+	+	+	--
	100 mg/L Fe^{++}	+++	+++	+++	--
	100 mg/L HA	+++	+++	+++	--
1x	**Control**	+++	++	++	--
	100 mg/L Cu^{++}	+	+	+	--
	100 mg/L Fe^{++}	++	++	++	--
	100 mg/L HA	+++	+++	+++	--
2x	**Control**	+	++	++	--
	100 mg/L Cu^{++}	---	---	---	--
	100 mg/L Fe^{++}	+	++	++	--
	100 mg/L HA	++	++	+	--

+++ = High Detection, ++ = Moderate Detection, + = Low Detection, --- = No Change in Colour

6.4 Effect of pesticides, Cu^{++}, Fe^{++} and humic acid on plant growth

6.4.1 Biocompatibility test

The compatible interaction was considered by observing the absence of inhibition zone around the spotted colony. Four petriplates were impregnated with four discs of different cultures, three petriplates were impregnated with three cultures and three petriplates were impregnated with two cultures with one control plate without any culture, respectively. After placing these discs, cultures were again allowed to grow for 2 h at 37°C. Finally, the growth was observed which shows that there was no zone of inhibition in any of the inoculated cultures; hence all the bacterial cultures are not a competitor but are compatible with each other and can be used as a good consortium (Fig 24-29). Our results were found similar with the findings of Anandaraj et al., (2010) in which the species of *Rhizobium sp.*, *Bacillus megaterium* and *P. fluorescens* were compatible with each other in cross streak plate assay. Thus, treatment of seeds with effective strains of *Rhizobium, Bacillus* alone or in combination with other beneficial microorganisms may be preferred over the pesticides, because of their multiple potentials to control disease, fix nitrogen, increase crop productivity, improve of soil fertility besides reducing the negative environmental impact associated with chemical use (Jensen et al., 2002; Huang and Erickson, 2007).

Figure 24: Test of biocompatibility between rhizobacterial strains isolated from acephate contaminated soils

Figure 25: Test of biocompatibility between rhizobacterial strains isolated from atrazine contaminated soils

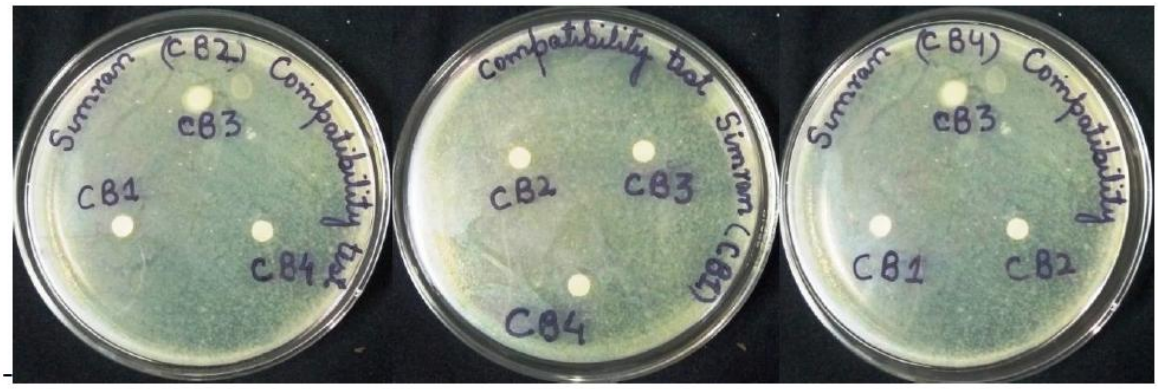


Figure 26: Test of biocompatibility between rhizobacterial strains isolated from carbendazim contaminated soils

Figure 27: Test of biocompatibility between rhizobacterial strains isolated from monocrotophos contaminated soils

Figure 28: Test of biocompatibility between rhizobacterial strains isolated from glyphosate contaminated soils

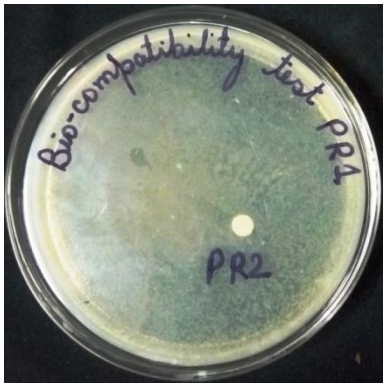


Figure 29: Test of biocompatibility between rhizobacterial strains isolated from phorate contaminated soils.

6.4.2 Effect of pesticides on seed germination

6.4.2.1 Effect of metal ions (100mg/L Cu^{++} and 100mg/L Fe^{++}) and humic acid on untreated group of pesticides on seed germination

In the untreated group, the seeds inoculated in humic acid shows high germination rate as compared to other three different treatments. The germination rate was increased significantly at significant level $p < 0.05$. The germination rate is highest in humic acid, Fe^{++} and same in case of Cu^{++} and untreated. The exact order of seed germination was seed inoculated with humic acid > Fe^{++} > control > Cu. Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level $p = 0.05$) with an order HA > Fe^{++} > control ≥ Cu^{++}. Fe^{++} is considered as most important part of plant growth including crops and having less toxicity (LD_{50} 230 mg/kg in rats) than Cu. Fe can increase the growth of the wheat plant and seeds also (Celletti et al., 2016). In bio-inorganic viewpoint, Cu is a toxic heavy metal ion (LD_{50} 30 mg/kg in rats) which has a variable effect on different organisms. Also, Cu has a negative effect on all the plant parameters due to its toxic nature (Lu et al., 2016: Adrees et al., 2015). Humic acid is considered to be always good for crop growth when applied in singular form (Vaccaro et al., 2015). Once it is added with pesticides or metal ions or in the presence of both, the things may vary due to different mechanisms. (1) HA decomposed the pesticides and decomposition reaction rate increase once metal ions like Fe and Cu are applied. (2) Sometimes there is association and dissociation mechanism between HA and pesticides and decomposition becomes slow because active sites of HA get blocked by pesticides. Due to this, free HA is not available for the growth of crops (wheat) hence growth affected adversely.

6.4.2.2 Effect of Acephate, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions on seed germination

In un-inoculated groups, on the addition of 1x concentration of Acephate to the wheat plant, germination rate was found to be inhibited. The inhibition was found dose-dependent i.e. inhibition in germination rate was found more when 2x acephate was applied on to the crop compared to a 1x dose level of Acephate. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of Acephate, the germination rate in inoculated groups was higher as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate was almost similar. In case of 2x treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases at significant level $p < 0.05$. In the presence of metal ions and humic acid, a slight increment was been observed (Table 21).

Table 21: Germination rate of acephate treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Acephate (G.R. %)		2x Acephate (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
	60%	80%	50%	60%
Cu^{++}	70%	80%	50%	60%
Fe^{++}	70%	80%	50%	70%
Humic acid	70%	80%	70%	70%

6.4.2.3 Effect of Atrazine, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions on seed germination

In Un-inoculated groups, on the addition of 1x concentration of Atrazine to the wheat plant, germination rate was found to be inhibited. The inhibition was found dose-dependent i.e. inhibition in germination rate was found more when 2x atrazine was applied on to the crop compared to a 1x dose level of atrazine. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of atrazine, the germination rate in inoculated groups was higher as compared to un-inoculated groups. In addition of metal ions

and humic acid, the growth rate was almost similar. In case of 2x treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases. In the presence of metal ions and humic acid, a slight increment of was been observed in case of inoculated groups (Table 22)

Table 22: Germination rate of atrazine treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Atrazine (G.R. %)		2x Atrazine (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
	60%	70%	30%	60%
Cu^{++}	60%	70%	50%	60%
Fe^{++}	70%	80%	50%	70%
Humic acid	90%	90%	60%	70%

6.4.2.4 Effect of Carbendazim, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In Un-inoculated groups, on the addition of 1x concentration of carbendazim to the wheat plant, germination rate was found to be inhibited. The inhibition was found dose-dependent i.e. inhibition in germination rate was found more when 2x carbendazim was applied on to the crop compared to a 1x dose level of carbendazim. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth. However, in case of Cu^{++} treated groups, there is a decrease in seed germination in both the 1x and 2x concentrations.

In inoculated groups, on the addition of 1x concentration of carbendazim, the germination rate in inoculated groups was higher as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate was almost similar. In case of 2x treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases. In the presence of metal ions and humic acid, a slight increment of Germination rate has been observed in both the inoculated groups of 1x and 2x groups (Table 23).

Table 23: Germination rate of carbendazim treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Carbendazim (G.R. %)		2x Carbendazim (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated

	60%	60%	40%	50%
Cu^{++}	40%	60%	30%	50%
Fe^{++}	60%	80%	50%	70%
Humic acid	80%	80%	80%	70%

6.4.2.5 Effect of Glyphosate, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In addition of Glyphosate, the germination rate in the un-inoculated is inhibited. Not even a single seed showed germination rate, but in the addition of metal ions, the germination rate was increased up to 20-40%. In inoculated groups, germination rate increased significantly with the addition of metal ions. In 1x concentration, the germination rate was higher than the un-inoculated groups and in 2x only small percentage germination rate was observed (Table 24).

Table 24: Germination rate of glyphosate-treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	**1x Glyphosate (G.R. %)**		**2x Glyphosate (G.R. %)**	
	Un-inoculated	**Inoculated**	**Un-inoculated**	**Inoculated**
	0%	50%	0%	10%
Cu^{++}	20%	40%	10%	20%
Fe^{++}	30%	60%	10%	20%
Humic acid	40%	70%	20%	30%

6.4.2.6 Effect of Monocrotophos, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In Un-inoculated groups, on the addition of 1x concentration (recommended) of Monocrotophos to the wheat plant, germination rate was found to be non-affected. Inhibition of germination rate was found more when 2x Monocrotophos was applied on to the crop compared to a 1x dose level of Monocrotophos. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of Monocrotophos, the germination rate in inoculated groups was similar as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate increased in 1x treated samples. In case of 2x

treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases. In the presence of metal ions and humic acid, a slight increment was been observed (Table 25).

Table 25: Germination rate of monocrotophos treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Monocrotophos (G.R. %)		2x Monocrotophos (G.R. %)	
	Un-inoculated	**Inoculated**	**Un-inoculated**	**Inoculated**
	60%	60%	20%	30%
Cu^{++}	60%	70%	20%	30%
Fe^{++}	70%	80%	30%	40%
Humic acid	70%	80%	40%	50%

6.4.2.7 Effect of Phorate, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In Un-inoculated groups, on the addition of 1x concentration of Phorate to the wheat plant, germination rate was found to be highly affected. Inhibition of germination rate was found more when 2x Phorate was applied on to the crop compared to a 1x dose level of Phorate. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of Phorate, the germination rate in inoculated groups was different as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate increased in 1x treated samples. In case of 2x treated groups, the germination rate in the inoculated group decreases. In the presence of metal ions and humic acid, a slight increment in growth has been observed (Table 26)

Table 26: Germination rate of phorate treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Phorate (G.R. %)		2x Phorate (G.R. %)	
	Un-inoculated	**Inoculated**	**Un-inoculated**	**Inoculated**
	50%	60%	30%	30%
Cu^{++}	60%	60%	40%	30%
Fe^{++}	60%	70%	40%	40%
Humic acid	70%	80%	50%	50%

6.4.3 Effect of pesticides on length of radical and plumule treated with and without metal ions and humic acid

The length of radical and plumule was measured after 3 days of seed germination. It was observed in all the cases, that 1x and 2x concentrations of pesticides inhibit all three parameters. But with the addition of metal ions and humic acid with a different concentration of pesticides, stimulation in all the three parameters was observed at significant level $p < 0.05$. Glyphosate was found more toxic showing complete inhibition of radical and plumule growth in untreated groups at both the concentrations than rest of the pesticides (Table 27).

Table 27: Effect of different pesticides on growth of radical and plumule germination supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions at significant level $p < 0.05$

Treatment	**Radicle (in cm)**				**Plumule (in cm)**			
Control	1.94±0.09				2.85±0.04			
100 mg/L Cu^{++}	0.98±0.12				1.86±0.07			
100 mg/L Fe^{++}	1.62±0.09				2.58±0.04			
100 mg/L Humic acid	3.03 ±1.01				3.84±0.05			
	1x PC	**BS + 1x PC**	**2x PC**	**BS + 2x PC**	**1x PC**	**BS + 1x PC**	**2x PC**	**BS + 2x PC**
Acephate (ACP)	1.8±0.07	2.04±0.03	1.07±0.04	1.80±0.04	1.42±0.06	1.65±0.04	1.12±0.06	1.08±0.04
ACP + 100 mg/L Cu^{++}	1.6±0.05	1.86±0.04	0.96±0.04	1.17±0.06	1.04±0.04	1.08±0.09	0.88±0.08	0.83±0.05
ACP + 100 mg/L Fe^{++}	2.10±0.09	2.5±0.06	1.79±0.02	2.07±0.05	1.85±0.09	1.93±0.06	0.95±0.04	1.33±0.03
ACP+ 100 mg/L HA	2.49±0.01	3.17±0.04	1.16±0.04	3.03±0.04	1.87±0.05	2.2±0.1	0.94±0.06	2.73±0.11
Atrazine (ATR)	1.52±0.09	1.9±0.05	1.08±0.07	1.60±0.07	1.26±0.06	1.73±0.08	1.09±0.09	1.48±0.03
ATR + 100 mg/L Cu^{++}	1.83±0.09	2.12±0.07	1.23±0.09	1.66±0.09	1.61±0.04	2.05±0.05	0.94±0.06	1.04±0.05
ATR + 100 mg/L Fe^{++}	2.05±0.11	2.28±0.06	1.51±0.05	1.81±0.04	1.12±0.05	1.48±0.07	0.95±0.06	1.08±0.07
ATR + 100 mg/L HA	2.55±0.09	2.86±0.05	1.75±0.07	1.88±0.09	2.51±0.06	2.7±0.1	1.26±0.05	1.25±0.22
Carbendazim (CBZ)	3.06±0.15	3.88±0.05	2.44±0.04	2.69±0.08	1.81±0.07	2.39±0.09	1.47±0.03	1.73±0.06
CBZ + 100 mg/L Cu^{++}	1.15±0.04	1.80±0.03	1.68±0.03	1.89±0.08	1.38±0.06	1.56±0.07	1.11±0.02	1.43±0.10
CBZ + 100 mg/L Fe^{++}	3.96±0.01	4.63±0.02	3.84±0.05	4.46±0.05	3.16±0.05	3.42±0.09	2.48±0.08	3.27±0.07
CBZ + 100 mg/L HA	4.29±0.07	4.68±0.05	4.13±0.03	4.49±0.08	3.19±0.07	3.76±0.06	2.81±0.03	3.20±0.030

Glyphosate (GP)	0	0.30±0.03	0	0	0	0.35±0.05	0	0
GP + 100 mg/L Cu^{++}	0	0.35±0.04	0	0	0	0.35±0.04	0	0
GP + 100 mg/L Fe^{++}	0.53±0.02	0.66±0.04	0.23±0.05	0.39±0.07	0.20±0.03	0.37±0.03	0.11±0.03	0.24±0.05
GP + 100 mg/L HA	0.89±0.01	1.30±0.04	0.61±0.03	0.8±0.06	0.8±0.04	0.94±0.05	0.25±0.05	0.62±0.07
Monocrotophos (MC)	2.43±0.07	3.06±0.06	2.23±0.06	3.10±0.06	1.89±0.08	2.21±0.07	1.85±0.07	1.20±0.06
MC + 100 mg/L Cu^{++}	1.75±0.04	2.45±0.03	1.42±0.03	1.97±0.06	1.51±0.06	2.06±0.06	1.06±0.04	1.20±0.07
MC + 100 mg/L Fe^{++}	2.91±0.03	3.39±0.05	2.17±0.06	2.50±0.09	2.73±0.05	3.16±0.04	1.7±0.08	2.38±0.02
MC + 100 mg/L HA	3.08±0.04	3.75±0.04	2.87±0.07	3.45±0.05	2.82±0.04	3.68±0.06	2.39±0.06	3.10±0.03
Phorate (PR)	2.14±0.07	2.53±0.07	0.99±0.07	1.29±0.06	1.87±0.06	2.1±0.08	0.63±0.05	0.81±0.04
PR + 100 mg/L Cu^{++}	1.64±0.05	1.94±0.06	0.85±0.06	1.11±0.04	0.83±0.08	1.27±0.09	0.47±0.09	0.65±0.09
PR + 100 mg/L Fe^{++}	2.23±0.04	2.92±0.05	1.85±0.04	2.29±0.09	1.58±0.04	2.47±0.07	1.24±0.10	1.81±0.07
PR + 100 mg/L HA	3.08±0.05	3.84±0.03	2.20±0.02	2.85±0.04	3.51±0.08	3.89±0.11	2.09±0.08	2.50±0.11

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

Where 1x PC = recommended concentration of Pesticide; BS + 1x PC = Bacterial Suspension + recommended concentration of Pesticide; 2x PC = Twice recommended concentration of pesticide; BS + 2x PC = Bacterial Suspension + Twice recommended concentration of Pesticide; Cu^{++} = Copper Chloride; Fe^{++} = Ferrous Chloride and HA = Humic Acid.

6.4.4 Soils collection and characterization for pot experimentation

This work is carried out in the Department of Biotechnology, under the domain of School of bio-engineering and biosciences in the month of November to March 2016. The physiochemical characteristics of the soil are in table 28.

Table 28: Physiochemical characterization of soil

Particulars	Unit	Soil test value	Status	Soil test Rating		
				Acidic	**Neutral**	**Alkaline**
pH		7.6	Alkaline	<6.5	6.5-7.5	>7.5
				Non-Saline	**Increasingly Saline**	
Electric Conductivity	ds/m	0.06	Non-Saline	<1.0	1.0-2.0	>2.0
				Low	**Medium**	**High**
Organic Carbon	kg/ha	0.30	Low	<0.50	0.50-0.75	>0.75
Avail. Nitrogen	kg/ha	62.8	Low	<250	250-500	>500
Avail. Phosphorous	kg/ha	11.8	Medium	<10	10-25	>25
Avail. Potassium	kg/ha	87.2	Low	<125	125-250	>250
Exch. Calcium	mg/L	487	Low	<500	500-1000	>1000
Exch. Magnesium	mg/L	363.4	High	<125	125-250	>250
Avail. Sulphur	mg/L	16.3	Medium	<10	10-50	>50
Avail. Zinc	mg/L	1.76	Medium	<1	1.0-5.0	>5.0
Avail. Copper	mg/L	0.78	Medium	<0.5	0.5-2.5	>2.5
Avail. Iron	mg/L	42.6	High	<2.5	2.5-10.0	>10.0
Avail. Manganese	mg/L	11.3	Medium	<5.0	5.0-20.0	>20.0
Boron	mg/L	0.19	Low	<0.5	0.5-1.0	>1.0

6.4.5 Effect of pesticides Cu^{++}, Fe^{++} and humic acid on plant growth parameters, chlorophyll and carotenoid content

6.4.5.1 Effect of Acephate on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

The length of the wheat plant was measured after 60 days of seed germination. It was observed in all the cases, that 1x and 2x concentrations of pesticides inhibit all four parameters. But with the addition of Fe^{++} and humic acid witha different concentration of pesticides, stimulation in the three parameters was observed at significant level $p < 0.05$

except Cu^{++}. The length of the plant (Table 29), chlorophyll a content (Table 30), chlorophyll b content (Table 31) and carotenoids (Table 32) summarizes the pessimistic effect of acephate on plant growth. Similar effects were observed on the concentrations of photosynthetic pigmentation production by the plant. Another similar study by Rajashekar, et al. (2012) in which the abiotic stress caused by pendamethalin among *Zea mays* L. cv NAAC- 6002 and illustrated that germination under control condition was maximum about 95%, whereas drastic declination in germination percentage was observed among the seeds sets which were treated with high amount of pendimethalin. An acute declination of 69% was visualized on using the 10 ppm solution of pendimethalin which contributed its role in initiating the consequences of the herbicide in eradication and mobilization process during seed reserves. The study conducted by Moore and Kroger (2010) also highlights the effect of insecticides and herbicides (individually as well as in combination) on a seedling of rice germination conditions.

Table 29: Effect of Acephate on physical parameters (height) of plant (cm) at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	26.4±0.56	22.2±0.40	29.33±0.56	27.67±0.42
No bacteria	1x Acephate	22.4±0.46	27.3±0.47	21.2±0.65	27.6±0.53
No bacteria	2x Acephate	19.97±0.75	24.1±0.61	18.4±0.45	25.93±0.45
Pseudomonas sp. ACP_01	Control	32.3±0.46	29.8±0.62	33.07±0.40	30.23±0.47
Pseudomonas sp. ACP_01	1x Acephate	27.97±0.65	25.1±0.55	27.4±0.55	25.97±0.35
Pseudomonas sp. ACP_01	2x Acephate	20.2±0.36	18.4±0.55	22.3±0.43	23.17±0.47
Pseudomonas sp. ACP_02	Control	30.33±0.5	25.3±0.60	27.9±0.45	28.37±0.5
Pseudomonas sp. ACP_02	1x Acephate	27.07±0.7	22.6±0.45	26.33±0.47	27.33±0.57
Pseudomonas sp. ACP_02	2x Acephate	22.27±0.49	24±0.65	26.87±0.41	27.03±0.42
Pseudomonas sp. ACP_03	Control	28.33±0.4	21.5±0.66	27.9±0.75	28.8±0.56
Pseudomonas sp. ACP_03	1x Acephate	26.33±0.47	23±0.4	27.07±0.65	25.97±0.45
Pseudomonas sp. ACP_03	2x Acephate	24.13±0.61	29.9±0.70	24.23±0.61	23.17±0.6
Bacterial Suspension	Control	37.07±0.45	32.1±0.56	40.23±0.56	33.53±0.45
Bacterial Suspension	1x Acephate	31.2±0.56	27.4±0.45	37.13±0.70	28.27±0.57
Bacterial Suspension	2x Acephate	28.17±0.4	24.3±0.55	31.23±0.55	21.83±0.55

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 30: Content of chlorophyll A in wheat (ug/mL) as mono-cropping system in Acephate contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.84±0.04	0.52±0.04	1.04±0.07	0.93±0.06
No bacteria	1x Acephate	0.94±0.05	0.58±0.04	0.92±0.03	0.87±0.06
No bacteria	2x Acephate	0.66±0.03	0.56±0.03	0.72±0.05	0.67±0.05
Pseudomonas sp. ACP_01	Control	1.14±0.08	1.05±0.06	1.72±0.1	1.23±0.08
Pseudomonas sp. ACP_01	1x Acephate	1.03±0.06	0.93±0.04	1.18±0.05	1.04±0.06
Pseudomonas sp. ACP_01	2x Acephate	0.94±0.02	0.83±0.03	1.07±0.13	0.92±0.06
Pseudomonas sp. ACP_02	Control	1.19±0.16	1.12±0.11	1.75±0.16	1.44±0.08
Pseudomonas sp. ACP_02	1x Acephate	1.05±0.06	0.92±0.05	1.34±0.06	1.13±0.11
Pseudomonas sp. ACP_02	2x Acephate	0.84±0.1	0.73±0.05	1.25±0.11	1.05±0.09
Pseudomonas sp. ACP_03	Control	1.12±0.09	0.79±0.1	1.48±0.1	1.19±0.09
Pseudomonas sp. ACP_03	1x Acephate	0.92±0.03	0.83±0.06	1.24±0.11	1.14±0.07
Pseudomonas sp. ACP_03	2x Acephate	0.84±0.04	0.65±0.06	1.09±0.06	0.84±0.05
Bacterial Suspension	Control	1.65±0.04	1.43±0.1	2.14±0.1	1.95±0.1
Bacterial Suspension	1x Acephate	1.4±0.02	1.22±0.08	1.91±0.03	1.45±0.07
Bacterial Suspension	2x Acephate	1.3±0.26	1.04±0.07	1.75±0.07	1.22±0.07

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 31: Content of chlorophyll b in wheat (ug/mL) as mono-cropping system in Acephate contaminated soil supplemented with Cu^{2+}, Fe^{2+} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 ppm Cu^{++}**	**100 ppm Fe^{++}**	**100 ppm Humic acid**
No bacteria	Control	0.6±0.04	0.43±0.07	0.85±0.05	0.7±0.06
No bacteria	1x Acephate	0.6±0.04	0.5±0.08	0.88±0.04	0.77±0.06
No bacteria	2x Acephate	0.4±0.06	0.33±0.04	0.62±0.05	0.54±0.05
Pseudomonas sp. ACP_01	Control	1±0.06	0.88±0.04	1.31±0.08	1.20±0.08
Pseudomonas sp. ACP_01	1x Acephate	0.8±0.04	0.79±0.06	1.07±0.06	0.94±0.06
Pseudomonas sp. ACP_01	2x Acephate	0.7±0.04	0.62±0.04	0.79±0.04	0.81±0.07
Pseudomonas sp. ACP_02	Control	1.1±0.07	0.94±0.04	1.57±0.05	1.35±0.07
Pseudomonas sp. ACP_02	1x Acephate	0.8±0.15	0.83±0.05	1.23±0.05	1.16±0.06
Pseudomonas sp. ACP_02	2x Acephate	0.7±0.05	0.7±0.06	0.96±0.05	0.82±0.04
Pseudomonas sp. ACP_03	Control	0.9±0.06	0.8±0.06	1.14±0.09	1.13±0.07
Pseudomonas sp. ACP_03	1x Acephate	0.8±0.07	0.73±0.04	0.97±0.06	0.9±0.08
Pseudomonas sp. ACP_03	2x Acephate	0.7±0.05	0.62±0.04	0.82±0.06	0.71±0.08
Bacterial Suspension	Control	1.4±0.08	1.34±0.07	2.05±0.07	1.82±0.05
Bacterial Suspension	1x Acephate	1±0.05	1.1±0.06	1.43±0.05	1.36±0.05
Bacterial Suspension	2x Acephate	0.8±0.05	0.92±0.05	1.23±0.04	1.03±0.04

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 32: Content of carotenoids in wheat (ug/mL) as mono-cropping system in Acephate contaminated soil supplemented with Cu^{2+}, Fe^{2+} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 ppm Cu^{++}**	**100 ppm Fe^{++}**	**100 ppm Humic acid**
No bacteria	Control	0.10±0.005	0.07±0.006	0.133±0.005	0.112±0.010
No bacteria	1x Acephate	0.11±0.006	0.096±0.007	0.144±0.007	0.124±0.006
No bacteria	2x Acephate	0.09±0.006	0.083±0.006	0.112±0.010	0.097±0.005
Pseudomonas sp. ACP_01	Control	0.32±0.009	0.248±0.007	0.367±0.008	0.318±0.006
Pseudomonas sp. ACP_01	1x Acephate	0.29±0.007	0.185±0.007	0.323±0.007	0.303±0.005
Pseudomonas sp. ACP_01	2x Acephate	0.21±0.006	0.163±0.007	0.284±0.008	0.265±0.006
Pseudomonas sp. ACP_02	Control	0.34±0.007	0.315±0.007	0.379±0.006	0.344±0.007
Pseudomonas sp. ACP_02	1x Acephate	0.30±0.009	0.194±0.006	0.354±0.004	0.337±0.004
Pseudomonas sp. ACP_02	2x Acephate	0.25±0.006	0.176±0.006	0.311±0.009	0.293±0.006
Pseudomonas sp. ACP_03	Control	0.21±0.008	0.192±0.005	0.295±0.006	0.244±0.004
Pseudomonas sp. ACP_03	1x Acephate	0.16±0.006	0.442±0.016	0.212±0.009	0.174±0.007
Pseudomonas sp. ACP_03	2x Acephate	0.14±0.006	0.093±0.006	0.114±0.009	0.13±0.005
Bacterial Suspension	Control	0.36±0.009	0.282±0.006	0.408±0.007	0.394±0.005
Bacterial Suspension	1x Acephate	0.34±0.007	0.215±0.007	0.388±0.006	0.365±0.007
Bacterial Suspension	2x Acephate	0.29±0.065	0.165±0.007	0.365±0.017	0.346±0.006

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

6.4.5.2 Effect of Atrazine on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

Impacts of two dose levels (1x and 2x) of Atrazine on the physical parameters (height) of growing wheat crops were studied and were found significantly affected by the applications of atrazine. When plants were at anthesis stage, the height of stem and head of each plant were measured. At a 1x dose level of atrazine, the height of stem and head was found to be reduced, while, on the application of 2x of Atrazine the height of stem and head was also reduced as compared to control (Table 33).

To check the effect of Cu^{++}, Fe^{++} and humic acid on wheat growth experiment were also carried out in presence of Atrazine (1x and 2x). A significant decrease in the weight of chlorophyll content/ carotenoid and height of stem was observed and it was found that with

the additional supply of Fe^{++} and humic acid, plant growth was found to be increased at significant level $p < 0.05$. Consequently, the chlorophyll A content (Table 34), chlorophyll b content (Table 35) carotenoid (Table 36) as well as in height of stem and head was observed. A similar experiment was carried out to investigate the effect of two herbicides and three insecticides on coleoptiles, radical and germination of rice seeds. The effect of pesticides on shoot germination was significantly decreased in lambda cyhalothrin, diazinon, metachlor and atrazine mixture treated groups. From the pesticides that were used, fipronil indicated the least percent of seed germination i.e. 76% in the examination with a control which was 80%, in as much as on the different side diazinon indicated 85% in the examination to control (Moore and Kroger 2010). The effect declination around 85% plant density, 67% plant height and 91% dry weight was observed for germination of Hemp sesbania which was treated with naproanilide and 2-naphthyloxy propionic acid at 2.25kg/Ha as documented by Hirase and Molin in the year 2002. The reaction of herbicide named pendimethalin and trifluralin, which inhibits the early growth and seed germination in *Zea mays* L. Crops (Nehru et al., 1999). Moreover, it has been found that atrazine and metribuzin have the toxic effect, which reduces the amount of photosynthate transferred to the radicle and the in vitro study conducted on the *Bradyrhizobium* for evaluation of these two herbicides on consequences of the functionality of the plant. Data from the studies strengthen the concept that the atrazine and metribuzin are harmful and affect this plant in association with a bacterium which indirectly affects the nodulation and yield of crops (Alonge, 2000).

Kaushik and Inderjit (2006) established the fact that beans grown in soil treated with herbicides, illustrated the consistent decrease in the level of chlorophyll (chl) when the concentration of the herbicide increased. They concluded that almost all the symptoms of biochemical origin are related to toxicity level of pesticides, which results in declination of chlorophyll content and oxidation process activation.

Table 33: Effect of Atrazine on physical parameters (height) of plant or plant growth (in cm) at significant level $p < 0.05$

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

Table 34: Content of chlorophyll A in wheat (ug/mL) as mono-cropping system in Atrazine contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L HA**
No bacteria	Control	0.86±0.04	0.54±0.05	1.14±0.06	0.91±0.07
No bacteria	1x Atrazine	1.15±0.08	0.93±0.05	1.44±0.09	1.31±0.05
No bacteria	2x Atrazine	1.04±0.08	0.83±0.04	1.17±0.08	1.05±0.07
Streptomycetaceae bacterium RK1	Control	1.75±0.08	1.53±0.04	1.9±0.07	1.72±0.08
Streptomycetaceae bacterium RK1	1x Atrazine	1.54±0.09	1.34±0.05	1.62±0.04	1.55±0.05
Streptomycetaceae bacterium RK1	2x Atrazine	1.31±0.06	1.14±0.07	1.33±0.05	1.23±0.05
P. fluorescens strain RK2	Control	1.33±0.04	1.02±0.04	1.69±0.07	1.58±0.06
P. fluorescens strain RK2	1x Atrazine	1.22±0.05	0.92±0.05	1.55±0.06	1.33±0.08
P. fluorescens strain RK2	2x Atrazine	0.94±0.03	0.84±0.07	1.23±0.05	1.03±0.08
A. chroococcum strain RK3	Control	1.17±0.08	0.86±0.05	1.54±0.05	1.32±0.09
A. chroococcum strain RK3	1x Atrazine	0.97±0.05	0.83±0.05	1.33±0.06	1.07±0.09
A. chroococcum strain RK3	2x Atrazine	0.80±0.08	0.69±0.07	1.05±0.07	0.94±0.06
R. leguminosarum strain RK4	Control	1.44±0.03	1.04±0.01	1.66±0.09	1.54±0.19
R. leguminosarum strain RK4	1x Atrazine	1.16±0.02	0.99±0.06	1.38±0.11	1.34±0.22
R. leguminosarum strain RK4	2x Atrazine	0.93±0.05	0.81±0.04	1.22±0.14	1.11±0.14
Bacterial Suspension	Control	1.82±0.06	1.63±0.09	2.05±0.07	1.88±0.07
Bacteria suspension	1x Atrazine	1.43±0.05	1.25±0.07	1.87±0.07	1.54±0.09
Bacteria suspension	2x Atrazine	1.22±0.06	1.066±0.06	1.61±0.05	1.05±0.06

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 35: Content of chlorophyll b in wheat (ug/mL) as mono-cropping system in Atrazine contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.65±0.06	0.39±0.02	0.82±0.06	0.70±0.07
No bacteria	1x Atrazine	1.04±0.07	0.84±0.05	1.27±0.11	1.14±0.08
No bacteria	2x Atrazine	0.86±0.09	0.65±0.05	1.04±0.06	0.92±0.06
Streptomycetaceae bacterium RK1	Control	1.41±0.09	1.08±0.10	1.68±0.10	1.55±0.08
Streptomycetaceae bacterium RK1	1x Atrazine	1.27±0.06	0.91±0.05	1.43±0.06	1.30±0.08
Streptomycetaceae bacterium RK1	2x Atrazine	1.03±0.09	0.72±0.03	1.15±0.07	1.05±0.09
P. fluorescens strain RK2	Control	1.16±0.07	1.32±0.07	1.88±0.06	1.61±0.07
P. fluorescens strain RK2	1x Atrazine	0.93±0.05	1±0.08	1.41±0.06	1.35±0.05
P. fluorescens strain RK2	2x Atrazine	0.73±0.07	0.83±0.04	1.13±0.05	1.05±0.11
A. chroococcum strain RK3	Control	0.92±0.05	0.73±0.05	1.23±0.04	1.04±0.13
A. chroococcum strain RK3	1x Atrazine	0.81±0.05	0.62±0.06	0.90±0.04	0.82±0.06
A. chroococcum strain RK3	2x Atrazine	0.61±0.05	0.44±0.07	0.76±0.06	0.71±0.07
R. leguminosarum strain RK4	Control	1.56±0.09	1.24±0.17	1.6±0.09	1.68±0.08
R. leguminosarum strain RK4	1x Atrazine	1.34±0.11	1.09±0.07	1.44±0.10	1.52±0.06
R. leguminosarum strain RK4	2x Atrazine	1.19±0.07	0.98±0.05	1.23±0.13	1.34±0.05
Bacterial Suspension	Control	1.69±0.06	1.14±0.07	1.87±0.05	1.7±0.06
Bacteria suspension	1x Atrazine	1.31±0.04	0.89±0.07	1.55±0.06	1.48±0.06
Bacteria suspension	2x Atrazine	1.03±0.06	0.67±0.06	1.17±0.06	1.02±0.08

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 36: Content of carotenoids in wheat (ug/mL) as mono-cropping system in Atrazine contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.401±0.050	0.070±0.007	0.133±0.006	0.114±0.008
No bacteria	1x Atrazine	0.204±0.007	0.187±0.006	0.220±0.009	0.205±0.008
No bacteria	2x Atrazine	0.172±0.008	0.142±0.006	0.187±0.004	0.186±0.007
Streptomycetaceae bacterium RK1	Control	0.351±0.008	0.310±0.009	0.372±0.004	0.325±0.007
Streptomycetaceae bacterium RK1	1x Atrazine	0.322±0.010	0.291±0.007	0.337±0.008	0.312±0.012
Streptomycetaceae bacterium RK1	2x Atrazine	0.284±0.015	0.262±0.009	0.315±0.005	0.285±0.006
P. fluorescens strain RK2	Control	0.326±0.008	0.287±0.009	0.331±0.004	0.315±0.005
P. fluorescens strain RK2	1x Atrazine	0.277±0.009	0.245±0.008	0.287±0.007	0.264±0.007
P. fluorescens strain RK2	2x Atrazine	0.246±0.007	0.215±0.013	0.261±0.007	0.235±0.012
A. chroococcum strain RK3	Control	0.265±0.006	0.206±0.009	0.245±0.008	0.203±0.015
A. chroococcum strain RK3	1x Atrazine	0.193±0.012	0.165±0.007	0.205±0.007	0.191±0.006
A. chroococcum strain RK3	2x Atrazine	0.166±0.009	0.114±0.006	0.185±0.009	0.167±0.006
R. leguminosarum strain RK4	Control	0.282±0.11	0.240±0.024	0.294±0.011	0.267±0.022
R. leguminosarum strain RK4	1x Atrazine	0.204±0.09	0.194±0.017	0.255±0.019	0.222±0.017
R. leguminosarum strain RK4	2x Atrazine	0.187±0.061	0.166±0.004	0.211±0.007	0.188±0.02
Bacterial Suspension	Control	0.361±0.008	0.355±0.007	0.387±0.009	0.375±0.007
Bacteria suspension	1x Atrazine	0.338±0.007	0.315±0.009	0.356±0.008	0.341±0.007
Bacteria suspension	2x Atrazine	0.282±0.006	0.266±0.007	0.286±0.006	0.262±0.009

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

6.4.5.3 Effect of Carbendazim on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

Impacts of two dose levels (1x and 2x) of carbendazim on the physical parameters (height) of growing wheat crops were studied and were found significantly affected with the applications of carbendazim (Table 37). When plants were at anthesis stage, the height of stem and head of each plant were measured. At a 1x dose level of carbendazim, the height of stem and head

was found to be normal, while, on the application of 2x of Carbendazim the height of stem and head was also same as compared to control.

To check the effect of Cu^{++}, Fe^{++} and humic acid on wheat growth experiment were also carried out in presence of Carbendazim (1x and 2x). Significant gain in the weight of chlorophyll content/ carotenoid and height of stem were observed at significant level $p < 0.05$ and it was found that with the additional supply of Fe^{++} and humic acid, plant growth was found to be increased, consequently, the chlorophyll A content (Table 38), chlorophyll b content (Table 39) and carotenoid (Table 40) as well as in height of stem and head was observed. Development in crops doesn't take place at the persistent rate. Development process continues throughout the life of a plant, which involves the enlargement of organs and gathering of the biomass (dry), predominantly in the form of sugar at the different parts of the plant (Durbak et al., 2012). Assessment of plant growth is the first step to acquire knowledge about the performances and productivity of the plant which is observed at the different level of plant growth for its survival in adverse conditions in which rate limiting factors are incorporated. In our study, we tried to explore the role of pesticide concentration in declining the growth and development of wheat and we found that carbendazim has no or little effect on the plant growth and was found to be increased, consequently, the chlorophyll A content.

Table 37: Effect of carbendazim on physical parameters (height) of plant or plant growth (in cm) at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	26.46±0.75	22.6±0.5	29.20±0.46	27.93±0.40
No bacteria	1x Carbendazim	26.56±1.00	26.86±0.60	27.43±0.54	24.86±0.55
No bacteria	2x Carbendazim	24.4±0.75	27.23±0.55	27.56±0.75	24.56±0.50
Actinomyces sp. CB1	Control	33.1±0.45	28.15±0.47	35.16±0.66	29.26±0.58
Actinomyces sp. CB1	1x Carbendazim	29.03±0.35	24.14±0.58	38.16±0.65	27.33±0.45
Actinomyces sp. CB1	2x Carbendazim	24.36±0.61	23.39±0.44	32.16±0.55	25.9±0.45
B. subtilis strain CB2	Control	27.63±0.55	27.38±0.53	24.26±0.65	33.13±0.51
B. subtilis strain CB2	1x Carbendazim	32.1±0.45	29.17±0.32	25.8±0.7	29.45±0.67
B. subtilis strain CB2	2x Carbendazim	24.96±0.56	27.13±0.41	24.96±0.35	26.96±0.55
P. aeruginosa CB3	Control	26.33±0.60	23.08±0.65	25.36±0.55	27.96±0.65

P. aeruginosa CB3	1x Carbendazim	24.93±0.30	23.26±0.58	26.9±0.45	26.56±0.73
P. aeruginosa CB3	2x Carbendazim	24.4±0.45	21.14±0.47	25.4±0.45	24.46±0.65
R.leguminosarum CB4	Control	28.41±0.86	26.5±1.12	30.86±1.84	33.84±0.54
R.leguminosarum CB4	1x Carbendazim	26.24±0.74	24.86±1.66	28.42±1.71	30.4±0.68
R.leguminosarum CB4	2x Carbendazim	25.63±0.91	23.23±0.97	26.53±1.12	29.34±0.47
Bacterial Suspension	Control	32.06±0.30	29.20±0.52	29.9±0.65	35.1±0.55
Bacterial Suspension	1x Carbendazim	31.36±0.41	27.18±0.54	33.73±0.55	34.3±0.60
Bacterial Suspension	2x Carbendazim	29.13±0.3	28.44±0.59	30.23±0.60	29.36±0.65

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 38: Content of chlorophyll A in wheat (ug/mL) as a mono-cropping system in Carbendazim contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid. at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.826±0.045	0.543±0.047	1.136±0.086	0.93±0.045
No bacteria	1x Carbendazim	1.436±0.060	1.136±0.090	1.733±0.056	1.553±0.065
No bacteria	2x Carbendazim	1.313±0.080	1.06±0.0866	1.596±0.080	1.453±0.094
Actinomyces sp. CB1	Control	1.523±0.055	1.176±0.080	1.926±0.065	1.846±0.095
Actinomyces sp. CB1	1x Carbendazim	1.473±0.070	1.136±0.075	1.906±0.060	1.813±0.041
Actinomyces sp. CB1	2x Carbendazim	1.433±0.037	1.093±0.100	1.833±0.050	1.733±0.045
B. subtilis strain CB2	Control	1.756±0.065	1.336±0.055	1.886±0.075	1.876±0.050
B. subtilis strain CB2	1x Carbendazim	1.696±0.065	1.286±0.061	1.893±0.045	1.793±0.041
B. subtilis strain CB2	2x Carbendazim	1.643±0.085	1.283±0.040	1.863±0.065	1.753±0.041
P. aeruginosa CB3	Control	1.573±0.060	1.296±0.061	1.623±0.060	1.61±0.065
P. aeruginosa CB3	1x Carbendazim	1.543±0.087	1.276±0.060	1.616±0.05	1.553±0. 070
P. aeruginosa CB3	2x Carbendazim	1.456±0.068	1.226±0.040	1.546±0.061	1.483±0.075
R.leguminosarum CB4	Control	1.463±0.044	1.375±0.097	1.614±0.14	1.55±0.097
R.leguminosarum CB4	1x Carbendazim	1.392±0.056	1.345±0.083	1.587±0.11	1.447±0.091
R.leguminosarum CB4	2x Carbendazim	1.373±0.068	1.304±0.096	1.515±0.10	1.422±0.087
Bacterial Suspension	Control	1.913±0.055	1.763±0.070	2.103±0.070	1.976±0.055

-Bacterial Suspension	1x Carbendazim	1.866±0.040	1.686±0.040	2.026±0.086	1.843±0.080
Bacterial Suspension	2x Carbendazim	1.806±0.041	1.613±0.061	1.866±0.077	1.796±0.075

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 39: Content of chlorophyll b in wheat (ug/mL) as mono-cropping system in Carbendazim contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.64±0.045	0.406±0.070	0.873±0.080	0.706±0.065
No bacteria	1x Carbendazim	1.143±0.098	0.856±0.058	1.246±0.083	1.083±0.075
No bacteria	2x Carbendazim	1.143±0.065	0.86±0.055	1.196±0.065	1.006±0.070
Actinomyces sp. CB1	Control	1.317±0.070	1.036±0.076	1.33±0.065	1.173±0.085
Actinomyces sp. CB1	1x Carbendazim	1.29±0.075	0.993±0.045	1.343±0.055	1.133±0.087
Actinomyces sp. CB1	2x Carbendazim	1.273±0.075	0.933±0.060	1.293±0.060	1.046±0.070
B. subtilis strain CB2	Control	1.423±0.040	1.116±0.110	1.546±0.055	1.446±0.080
B. subtilis strain CB2	1x Carbendazim	1.42±0.062	1.063±0.076	1.53±0.055	1.333±0.050
B. subtilis strain CB2	2x Carbendazim	1.38±0.065	1.066±0.102	1.486±0.060	1.34±0.055
P. aeruginosa CB3	Control	1.393±0.061	1.07±0.111	1.406±0.065	1.356±0.065
P. aeruginosa CB3	1x Carbendazim	1.326±0.045	1.123±0.087	1.343±0.100	1.383±0.061
P. aeruginosa CB3	2x Carbendazim	1.323±0.058	1.05±0.098	1.376±0.065	1.293±0.075
R.leguminosarum CB4	Control	1.372±0.057	1.240±0.076	1.465±0.084	1.422±0.066
R.leguminosarum CB4	1x Carbendazim	1.324±0.054	1.204±0.089	1.385±0.096	1.377±0.1
R.leguminosarum CB4	2x Carbendazim	1.288±0.085	1.194±0.12	1.36±0.14	1.14±0.16
Bacterial Suspension	Control	1.756±0.070	1.426±0.060	1.723±0.040	1.556±0.087
Bacterial Suspension	1x Carbendazim	1.683±0.060	1.383±0.050	1.686±0.051	1.6±0.081
Bacterial Suspension	2x Carbendazim	1.653±0.075	1.353±0.070	1.686±0.050	1.536±0.045

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 40: Content of carotenoids in wheat (ug/mL) as mono-cropping system in Carbendazim contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.107±0.010	0.070±0.007	0.135±0.006	0.113±0.010
No bacteria	1x Carbendazim	0.29±0.055	0.244±0.008	0.327±0.011	0.291±0.006
No bacteria	2x Carbendazim	0.275±0.007	0.254±0.006	0.317±0.009	0.291±0.004
Actinomyces sp. CB1	Control	0.318±0.007	0.296±0.009	0.343±0.005	0.344±0.005
Actinomyces sp. CB1	1x Carbendazim	0.305±0.008	0.278±0.007	0.345±0.007	0.316±0.007
Actinomyces sp. CB1	2x Carbendazim	0.303±0.045	0.266±0.008	0.350±0.008	0.315±0.008
B. subtilis strain CB2	Control	0.413±0.020	0.368±0.007	0.444±0.010	0.427±0.006
B. subtilis strain CB2	1x Carbendazim	0.397±0.009	0.347±0.009	0.437±0.009	0.412±0.006
B. subtilis strain CB2	2x Carbendazim	0.403±0.061	0.354±0.007	0.433±0.007	0.411±0.006
P. aeruginosa CB3	Control	0.383±0.045	0.337±0.009	0.403±0.007	0.426±0.009
P. aeruginosa CB3	1x Carbendazim	0.378±0.007	0.323±0.008	0.412±0.008	0.412±0.015
P. aeruginosa CB3	2x Carbendazim	0.373±0.009	0.316±0.021	0.399±0.031	0.393±0.006
R.leguminosarum CB4	Control	0.394±0.014	0.349±0.01	0.430±0.028	0.426±0.007
R.leguminosarum CB4	1x Carbendazim	0.387±0.012	0.338±0.006	0.411±0.022	0.421±0.011
R.leguminosarum CB4	2x Carbendazim	0.379±0.009	0.314±0.008	0.386±0.041	0.396±0.02
Bacterial Suspension	Control	0.433±0.012	0.405±0.013	0.466±0.060	0.453±0.005
Bacterial Suspension	1x Carbendazim	0.447±0.005	0.391±0.005	0.453±0.009	0.445±0.007
Bacterial Suspension	2x Carbendazim	0.425±0.006	0.391±0.010	0.451±0.004	0.444±0.008

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

6.4.5.4 Effect of Glyphosate on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

Impacts of two dose levels (1x and 2x) of glyphosate on the physical parameters (height) of growing wheat crops were studied and were found significantly affected and kills all the plants with the applications of glyphosate in all the treated and untreated groups. Similar studies were conducted by Stevens et al. (2008) in which the impact of imidacloprid on the seed of wheat and barley without taking into consideration its phytotoxic and severe effect on the plant. The illustrated result provides the significant stimulation instead of the impairment by the imidacloprid. Enhancement in growth was observed after the exposure of imidacloprid in varing concentrations ranging from 500-1000mg/L. In another study, the limited exposure of imidacloprid was given during initial stage of seed wetting prior to sowing/germination, showed no effect on the crop. The work conducted by Mishra, et al., (2008) revealed the effect of insecticide dimethoate which in high concentration results declination in the length of both root and shoot.

6.4.5.5 Effect of Monocrotophos on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

Impacts of two dose levels (1x and 2x) of monocrotophos on the physical parameters (height) of growing wheat crops were studied and were found significantly affected at significant level $p < 0.05$ (Table 41). When plants were at anthesis stage, the height of stem and head of each plant were measured. At a 1x dose level of monocrotophos, the height of stem was found to be quite low, while, on the application of 2x of Monocrotophos the height of stem and head was also low as compared to control.

To check the effect of Cu^{++}, Fe^{++} and humic acid on wheat growth experiment were also carried out in presence of Monocrotophos (1x and 2x). Significant gain in the weight of chlorophyll content/ carotenoid and height of stem was observed and it was found that with the additional supply of Fe^{++} and humic acid, plant growth was found to be increased, consequently, the chlorophyll A (Table 42), chlorophyll b content (Table 43) and carotenoid (Table 44) as well as in height of stem and head was observed. A similar observation was noticed by Murthy, et al., (2005) for *Glycine max* L. Almost complete inhibition of growth was seen by monocrotophos as documented by Saraf and Sood (2002). For treating the maize, there are prescribed a dosage of atrazine, alachlor, imazethapyr, fluometuron and rimsulfuron, which notably reduce the size of shoot, fresh and dry weight after 10 days in old

maize seedlings during 12 days. The trend of fluometuron, atrazine and alachlor was constant during the time of the study; it means that it got nullified after the 5th day when the rimsulfuron and imazethapyr treatment was given (Scarponi et al., 1995). Basantani et al., (2011), provided the information about the reduction of fresh weight, germination and length of the roots of the two *V. radiata* varieties, after treating with the 10mM glyphosate. Earlier it was reported that use of glyphosate addition during the preharvest time of the pea, cause the declination in the shoot, fresh seedling emergence and seed germination (Baig, et al., 2003).

Table 41: Effect of Monocrotophos on physical parameters (height) of plant (in cm) at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	26.4±0.45	22.43±0.47	29.6±0.55	27.9±0.45
No bacteria	1x Monocrotophos	24.06±0.40	19.96±0.65	19.9±0.45	24.06±0.40
No bacteria	2x Monocrotophos	22.43±0.60	18.2±0.65	21.06±0.40	21.93±0.50
Actinomyces sp. MC1	Control	26.16±0.47	25.33±0.66	28.13±0.61	29.13±0.51
Actinomyces sp. MC1	1x Monocrotophos	23.56±0.77	24.36±0.61	26.2±0.79	26.93±0.60
Actinomyces sp. MC1	2x Monocrotophos	20.4±0.45	20.46±0.75	24.33±0.66	22.36±0.55
B. subtilis strain MC2	Control	26.43±0.50	25.23±0.58	30.6±0.81	27.8±0.43
B. subtilis strain MC2	1x Monocrotophos	23.96±0.35	23.2±0.65	26.93±0.60	25.23±0.55
B.subtilis strain MC2	2x Monocrotophos	21.3±0.51	21.9±0.55	24.06±0.50	21.03±0.45
R.leguminosarum MC3	Control	26.13±0.51	24.26±0.56	25.1±0.45	26.360.66
R.leguminosarum MC3	1x Monocrotophos	22.26±0.56	21.73±0.75	23.36±0.61	23.1±0.45
R.leguminosarum MC3	2x Monocrotophos	18.2±0.55	18.1±0.45	21.33±0.56	22.58±0.72
Bacterial Suspension	Control	28.03±0.45	27.3±0.72	32.03±0.45	34.4±0.62
Bacterial Suspension	1x Monocrotophos	24.2±0.55	25.26±0.55	28.3±0.65	31.83±0.65
Bacterial Suspension	2x Monocrotophos	22±0.4	22.13±0.45	26.23±0.50	27.03±0.75

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 42: Content of chlorophyll A in wheat (ug/mL) as mono-cropping system in Monocrotophos contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.883±0.061	0.553±0.070	1.15±0.088	0.956±0.096
No bacteria	1x Monocrotophos	1.043±0.070	0.913±0.041	1.413±0.070	1.253±0.085
No bacteria	2x Monocrotophos	0.803±0.040	0.726±0.055	1.193±0.077	0.926±0.055
Actinomyces sp. MC1	Control	1.236±0.065	0.926±0.060	1.883±0.070	1.733±0.060
Actinomyces sp. MC1	1x Monocrotophos	1.036±0.050	0.853±0.090	1.696±0.075	1.336±0.050
Actinomyces sp. MC1	2x Monocrotophos	0.69±0.055	0.716±0.060	1.15±0.072	1.08±0.081
B. subtilis strain MC2	Control	1.093±0.090	0.826±0.077	1.556±0.077	1.276±0.056
B. subtilis strain MC2	1x Monocrotophos	0.916±0.055	0.653±0.065	1.353±0.070	1.143±0.085
B.subtilis strain MC2	2x Monocrotophos	0.65±0.081	0.426±0.066	1.053±0.065	0.863±0.073
R.leguminosarum MC3	Control	1.47±0.075	1.153±0.087	2.01±0.126	1.82±0.065
R.leguminosarum MC3	1x Monocrotophos	1.183±0.068	0.826±0.060	1.83±0.088	1.39±0.065
R.leguminosarum MC3	2x Monocrotophos	0.956±0.075	0.713±0.055	1.156±0.090	0.97±0.055
Bacterial Suspension	Control	1.683±0.080	1.253±0.085	2.236±0.116	1.943±0.075
Bacterial Suspension	1x Monocrotophos	1.433±0.060	1.04±0.062	1.94±0.07	1.353±0.080
Bacterial Suspension	2x Monocrotophos	1.373±0.055	0.806±0.050	1.35±0.080	0.98±0.095

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

Table 43: Content of chlorophyll b in wheat (ug/mL) as mono-cropping system in Monocrotophos contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.653±0.065	0.406±0.070	0.873±0.060	0.696±0.070

No bacteria	1x Monocrotophos	0.863±0.087	0.706±0.065	1.286±0.056	1.073±0.085
No bacteria	2x Monocrotophos	0.75±0.062	0.523±0.055	0.926±0.055	0.793±0.070
Actinomyces sp. MC1	Control	1.12±0.055	0.82±0.055	1.42±0.055	1.28±0.06
Actinomyces sp. MC1	1x Monocrotophos	0.833±0.045	0.693±0.040	0.933±0.050	1.076±0.070
Actinomyces sp. MC1	2x Monocrotophos	0.706±0.055	0.55±0.06	0.756±0.065	0.853±0.049
B. subtilis strain MC2	Control	0.926±0.070	0.783±0.075	1.326±0.045	1.196±0.085
B. subtilis strain MC2	1x Monocrotophos	0.7±0.045	0.56±0.065	0.94±0.05	0.916±0.055
B.subtilis strain MC2	2x Monocrotophos	0.573±0.051	0.45±0.075	0.783±0.070	0.723±0.050
R.leguminosarum MC3	Control	0.856±0.639	0.986±0.075	1.553±0.070	1.363±0.047
R.leguminosarum MC3	1x Monocrotophos	0.81±0.05	0.8±0.08	1.143±0.085	1.023±0.061
R.leguminosarum MC3	2x Monocrotophos	0.703±0.075	0.656±0.061	0.85±0.065	0.816±0.066
Bacterial Suspension	Control	1.353±0.092	1.2±0.075	1.863±0.087	1.673±0.077
Bacterial Suspension	1x Monocrotophos	0.97±0.091	0.936±0.085	1.403±0.075	1.203±0.045
Bacterial Suspension	2x Monocrotophos	0.71±0.062	0.76±0.045	1.086±0.080	0.96±0.055

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 44: Content of carotenoids in wheat (ug/mL) as a mono-cropping system in Monocrotophos contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$.

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.108±0.006	0.070±0.008	0.133±0.006	0.113±0.007
No bacteria	1x Monocrotophos	0.187±0.005	0.148±0.005	0.216±0.006	0.168±0.006
No bacteria	2x Monocrotophos	0.165±0.006	0.094±0.006	0.186±0.007	0.133±0.005

Actinomyces sp. MC1	Control	0.263±0.005	0.215±0.007	0.281±0.007	0.254±0.007
Actinomyces sp. MC1	1x Monocrotophos	0.213±0.055	0.162±0.007	0.239±0.006	0.186±0.008
Actinomyces sp. MC1	2x Monocrotophos	0.167±0.009	0.098±0.007	0.143±0.005	0.122±0.006
B. subtilis strain MC2	Control	0.246±0.005	0.207±0.006	0.276±0.008	0.295±0.006
B. subtilis strain MC2	1x Monocrotophos	0.197±0.006	0.153±0.009	0.233±0.009	0.175±0.005
B.subtilis strain MC2	2x Monocrotophos	0.167±0.007	0.095±0.008	0.192±0.007	0.116±0.004
R.leguminosarum MC3	Control	0.265±0.008	0.223±0.007	0.29±0.005	0.248±0.005
R.leguminosarum MC3	1x Monocrotophos	0.238±0.009	0.178±0.007	0.256±0.007	0.191±0.005
R.leguminosarum MC3	2x Monocrotophos	0.187±0.007	0.121±0.005	0.215±0.008	0.117±0.006
Bacterial Suspension	Control	0.345±0.007	0.317±0.006	0.377±0.006	0.361±0.006
Bacterial Suspension	1x Monocrotophos	0.265±0.010	0.205±0.008	0.282±0.005	0.248±0.006
Bacterial Suspension	2x Monocrotophos	0.199±0.007	0.164±0.005	0.233±0.015	0.218±0.004

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

6.4.5.6 Effect of Phorate on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

Impacts of two dose levels (1x and 2x) of phorate on the physical parameters (height) of growing wheat crops were studied and were found significantly affected with the applications of phorate (Table 45). When plants were at anthesis stage, the height of stem and head of each plant were measured. At a 1x dose level of phorate, the height of stem was found to be very very low, while, on the application of 2x of Phorate the height of stem and head was too low as compared to control.

To check the effect of Cu^{++}, Fe^{++} and humic acid on wheat growth experiment were also carried out in presence of Phorate (1x and 2x). A sgnificant gain in the weight of chlorophyll content/ carotenoid and height of stem were observed at significant level $p < 0.05$. and it was found with the additional supply of Fe^{++} and humic acid, plant growth was found to be increased, consequently, the the chlorophyll A (Table 46), chlorophyll b content (Table 47) and carotenoid (Table 48) as well as in height of stem and head was observed but in case of Cu^{++} it decreased.

Table 45: Effect of Phorate on physical parameters (height) of plant or plant growth (in cm) at significant level $p < 0.05$

Isolates	Treatment	Control	100 mg/L Cu^{++}	100 mg/L Fe^{++}	100 mg/L Humic acid
No bacteria	Control	26.53±0.65	22.43±0.47	29.4±0.45	28.01±0.57
No bacteria	1x Phorate	18.56±0.50	18.1±0.55	24.16±0.56	27.1±0.45
No bacteria	2x Phorate	17.76±0.60	14.43±0.50	23.5±0.6	26.13±0.51
Pseudomonas sp. PR_01	Control	22.3±0.43	18.26±0.56	26.13±0.51	28.83±0.70
Pseudomonas sp. PR_01	1x Phorate	19.36±0.61	15±0.4	24.13±0.61	26.4±0.55
Pseudomonas sp. PR_01	2x Phorate	15.06±0.50	11.86±0.70	23.06±0.70	19.03±0.65
Pseudomonas sp. PR_02	Control	20.1±0.55	18.16±0.47	26.53±0.75	30.16±0.47
Pseudomonas sp. PR_02	1x Phorate	15.46±0.55	14.36±0.61	22.86±0.41	27.86±0.41
Pseudomonas sp. PR_02	2x Phorate	13.9±0.3	13.1±0.45	19.26±0.60	28.16±0.51
Bacterial Suspension	Control	22.13±0.61	15.86±0.51	26.26±0.65	30.06±0.40
Bacterial Suspension	1x Phorate	20.06±0.70	13.66±0.65	24.26±0.50	27.66±0.60
Bacterial Suspension	2x Phorate	19.33±0.45	10.6±0.75	22.2±0.62	25.13±0.61

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 46: Content of chlorophyll A in wheat (ug/mL) as mono-cropping system in Phorate contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	Treatment	Control	100 mg/L Cu^{++}	100 mg/L Fe^{++}	100 mg/L Humic acid
No bacteria	Control	0.873±0.065	0.556±0.065	1.143±0.068	0.946±0.060
No bacteria	1x Phorate	0.766±0.075	0.53±0.08	0.973±0.066	0.823±0.065
No bacteria	2x Phorate	0.526±0.047	0.336±0.050	0.816±0.060	0.63±0.065
Pseudomonas sp. PR_01	Control	0.95±0.08	0.833±0.055	1.176±0.080	1.093±0.075
Pseudomonas sp. PR_01	1x Phorate	0.78±0.045	0.72±0.065	0.933±0.051	0.96±0.085

Pseudomonas sp. PR_01	2x Phorate	0.56±0.07	0.46±0.079	0.846±0.070	0.75±0.075
Pseudomonas sp. PR_02	Control	0.923±0.070	0.806±0.070	1.543±0.086	1.293±0.050
Pseudomonas sp. PR_02	1x Phorate	0.733±0.060	0.776±0.076	1.263±0.050	1.113±0.086
Pseudomonas sp. PR_02	2x Phorate	0.52±0.065	0.466±0.070	0.966±0.051	0.893±0.070
Bacterial Suspension	Control	1.183±0.055	1.076±0.040	1.66±0.088	1.456±0.075
Bacterial Suspension	1x Phorate	0.833±0.070	0.806±0.055	1.436±0.055	1.293±0.070
Bacterial Suspension	2x Phorate	0.68±0.07	0.646±0.070	1.07±0.05	1.033±0.068

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 47: Content of chlorophyll b in wheat (ug/mL) as mono-cropping system in Phorate contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.64±0.055	0.41±0.045	0.883±0.075	0.713±0.075
No bacteria	1x Phorate	0.566±0.060	0.376±0.065	0.626±0.045	0.533±0.066
No bacteria	2x Phorate	0.303±0.070	0.333±0.055	0.513±0.041	0.32±0.055
Pseudomonas sp. PR_01	Control	0.633±0.045	0.516±0.060	0.730±0.095	0.703±0.070
Pseudomonas sp. PR_01	1x Phorate	0.556±0.065	0.446±0.070	0.616±0.055	0.493±0.085
Pseudomonas sp. PR_01	2x Phorate	0.493±0.055	0.316±0.050	0.553±0.070	0.396±0.055
Pseudomonas sp. PR_02	Control	0.783±0.040	0.486±0.045	0.616±0.060	0.523±0.041
Pseudomonas sp. PR_02	1x Phorate	0.683±0.075	0.39±0.045	0.436±0.055	0.32±0.045
Pseudomonas sp. PR_02	2x Phorate	0.543±0.068	0.333±0.051	0.383±0.050	0.276±0.056
Bacterial Suspension	Control	0.973±0.050	0.87±0.06	1.076±0.090	0.926±0.060
Bacterial Suspension	1x Phorate	0.886±0.066	0.746±0.055	0.853±0.070	0.686±0.061
Bacterial Suspension	2x Phorate	0.643±0.075	0.53±0.055	0.67±0.06	0.58±0.065

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 48: Content of carotenoids in wheat (ug/mL) as mono-cropping system in Phorate contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.107±0.006	0.071±0.004	0.133±0.009	0.123±0.009

No bacteria	1x Phorate	0.098±0.007	0.084±0.008	0.117±0.008	0.113±0.011
No bacteria	2x Phorate	0.049±0.006	0.052±0.005	0.073±0.005	0.073±0.025
Pseudomonas sp. PR_01	Control	0.230±0.006	0.303±0.015	0.323±0.011	0.27±0.055
Pseudomonas sp. PR_01	1x Phorate	0.128±0.007	0.108±0.007	0.143±0.005	0.142±0.009
Pseudomonas sp. PR_01	2x Phorate	0.082±0.005	0.044±0.006	0.096±0.008	0.085±0.008
Pseudomonas sp. PR_02	Control	0.236±0.050	0.19±0.036	0.286±0.025	0.276±0.035
Pseudomonas sp. PR_02	1x Phorate	0.117±0.022	0.093±0.004	0.147±0.005	0.127±0.006
Pseudomonas sp. PR_02	2x Phorate	0.075±0.007	0.056±0.006	0.097±0.006	0.083±0.008
Bacterial Suspension	Control	0.283±0.075	0.27±0.045	0.284±0.009	0.252±0.009
Bacterial Suspension	1x Phorate	0.080±0.006	0.116±0.006	0.162±0.006	0.141±0.007
Bacterial Suspension	2x Phorate	0.063±0.005	0.067±0.005	0.114±0.008	0.099±0.008

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

On addition of 100 mg/L of metal ions (Cu^{++} and Fe^{++}) and humic acid, the Cu^{++} had the most toxic effect on seed germination and other parameters were significantly decreases at $p < 0.05$. On addition of Fe^{++} and humic acid, the seed germination and other parameters were relatively increased when amended with 1x concentration of each pesticide at significant level $p < 0.05$. On addition of 2x concentration of each pesticide, the seed germination and other parameters was decreased in almost all the cases.

Pesticides affect the plant by various mechanisms which involve the cessation of biological functions like cell division, enzyme regulation, photosynthesis, enzyme regulation in growth and development of the leaves, obstruction during pigment synthesis, DNA or protein, cell membrane eradication, or aid in unrestrained growth (Parween 2014; Fantke *et al.*2013; Ozturk et al., 2008). Defreitas and Germida (1992b) also demonstrated the ability of bacterial strains significantly enhanced early plant growth. According to Lazarovits and Norwak (1997), when tested under ideal climatic situations, the bacterial species marginally increased yields. The production of phytohormones by rhizospheric bacteria has also been reported earlier (Ahemad *et al.* 2012; Ahemad *et al.* 2014 Kumar *et al.* 2015).

The effect of six different pesticides on the plant growth parameters is in the order of glyphosate > phorate > monocrotophos > acephate > atrazine > carbendazim. All the pesticides hinder the growth rate when applied in a concentration above the recommended. Similar effects were observed on the concentrations of photosynthetic pigmentation production by the plant at significant level $p < 0.05$. Glyphosate was found most toxic on all

the parameters including germination, chlorophyll content and carotenoid content. With the addition of metal ions and humic acid, there is an increase in germination rate of the wheat in all the cases. The effect of pesticides on radical and plumule germination is almost similar to the seed germination test.

Effects of six pesticides, metal ions and humic acid on wheat growth parameters, chlorophyll content and total carotenoids were also observed. With the addition of metal ions and humic acid, increase in plant growth and chlorophyll content were observed at significant level $p < 0.05$. It also highlighted the negative impact of pesticides on wheat growth germination and on its parameters.

Utilization of pesticide in early stage affects the plant growth during germination which further leads to amendment in physiological and biochemical process with addition to that it also affects the enzymatic as well as non-enzymatic antioxidants which eventually decline the yields and antioxidants are present in trace amount in fruits and vegetables of plants and among non-targeted organism those who consume it.

The use of pesticide illustrates to be an effective measure for controlling the pest but it also makes us aware of the associated threat on the treated as well as surrounding crops. The evaluation of the side effect of pesticides is also required to be considered before using particular pesticide for the agriculture process. The study should focus on the consequences of pesticides on the microbial flora of soil and their retention in the crop, also on positive aspects of soil organic acids. For this secure method can be a synthesis of the cheap bio-pesticide, which show effective results and also confirms the dissolution of residues of pesticide in the grains.

Table 18: Quantitative estimation of Siderophore production by bacterial isolates inoculated singly and co-supplemented with pesticides (Acephate, Atrazine and Carbendazim), humic acid (HA), Cu^{++} and Fe^{++} (S.U %) at significant level $p < 0.05$

Group	**Pesticide →**	**Acephate**		**Atrazine**			**Carbendazim**		
	Treatment ↓	ACP1	ACP2	RK2	RK3	RK4	CB2	CB3	CB4
0x	**Control**	56.76±0.46	64.52±0.85	60.42±0.80	61.37±0.47	64.45±0.41	55.01±0.76	48.36±0.39	20.44±0.55
	100mg/L Cu^{++}	53.47±0.44	57.50±0.68	53.12±0.17	54.28±0.33	56.31±0.52	51.29±0.66	43.26±0.31	14.73±0.39
	100mg/L Fe^{++}	60.42±0.40	71.69±0.41	60.30±0.52	66.09±0.36	69.45±0.54	58.28±0.50	50.69±0.50	35.93±0.60
	100mg/L HA	63.35±0.59	72±0.67	65.92±0.45	69.55±0.56	72.42±0.57	66.25±0.51	57.32±0.39	38.2±0.55
1x	**Control**	49.16±0.53	61.2±0.41	52.64±0.45	57.29±0.43	58.85±0.53	50.05±0.67	44.35±0.51	9.37±0.49
	100mg/L Cu^{++}	33.63±0.61	36.14±0.38	38.29±0.40	43.60±0.45	49.36±0.55	47.54±0.63	30.69±0.42	12.16±0.56
	100mg/L Fe^{++}	63.82±0.64	73.12±0.53	63.37±0.52	68.39±0.57	71.08±0.63	66.12±0.68	53.29±0.46	40.19±0.40
	100mg/L HA	70.57±0.77	75.80±0.43	70.06±0.30	75.09±0.70	79.08±0.58	70.13±0.57	60.30±0.50	43.33±0.33
2x	**Control**	40.70±0.35	45.86±0.33	44.27±0.56	47.83±0.65	50.81±0.44	43.29±0.42	36.34±0.45	2.16±0.27
	100mg/L Cu^{++}	19.4±0.53	25.87±0.58	31.29±0.48	37.61±0.44	40.15±0.45	42.41±0.51	24.41±0.54	10.49±0.34
	100mg/L Fe^{++}	44.35±0.53	52.68±0.49	48.42±0.50	51.32±0.64	62.11±0.57	59.60±0.52	46.33±0.65	26.44±0.60
	100mg/L HA	55.32±0.62	60.28±0.55	59.66±0.44	61.19±0.39	71.28±0.47	66.12±0.49	46.34±0.58	26.30±0.47

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 19: Quantitative estimation of Siderophore production by bacterial isolates inoculated singly and co-supplemented with pesticides (Glyphosate, Mono and Phorate), humic acid (HA), Cu^{++} and Fe^{++} (S.U %) at significant level $p < 0.05$

Conc. ↓	**Pesticide →**	**Glyphosate**		**Monocrotophos**		**Phorate**		
	Treatment ↓	**GP 2**	**GP 3**	**MC2**	**MC3**	**PR1**	**PR2**	***P. fluorescens***
0x	**Control**	15.35±0.37	52.74±0.53	25.35±0.51	53.77±0.53	75.34±0.65	25.28±0.59	43.31±0.65
	100 mg/L Cu^{++}	13.42±0.43	47.39±0.54	22.29±0.42	37.76±0.55	69.27±0.62	22.08±0.42	--
	100 mg/L Fe^{++}	28.41±0.44	52.19±0.73	32.2±0.59	61.06±0.51	77.35±0.44	31.37±0.46	--
	100 mg/L HA	33.06±0.42	54.81±0.55	39.32±0.58	63.34±0.57	81.28±0.33	39.14±0.51	--
1x	**Control**	4.37±0.53	45.49±0.48	21.23±0.35	48.21±0.43	61.33±0.50	19.12±0.50	--
	100 mg/L Cu^{++}	7.36±0.44	40.23±0.53	17.60±0.42	29.26±0.45	61.3±0.47	14.33±0.55	--
	100 mg/L Fe^{++}	38.13±0.30	57.28±0.45	40.68±0.77	64.54±0.47	80.14±0.51	40.98±0.41	--
	100 mg/L HA	40.24±0.48	59.30±0.39	53.38±0.53	67.11±0.33	84.23±0.45	47.21±0.57	--
2x	**Control**	1.53±0.09	35.11±0.40	10.06±0.19	40.19±0.55	48.18±0.48	12.46±0.49	--
	100 mg/L Cu^{++}	3.10±0.18	32.16±0.37	14.31±0.61	21.88±0.66	52.22±0.56	4.17±0.43	--
	100 mg/L Fe^{++}	20.7±0.63	48.28±0.34	31.51±0.58	56.38±0.54	74.33±0.63	20.23±0.46	--
	100 mg/L HA	30.08±0.43	36.22±0.40	44.85±0.56	56.08±0.40	76.12±0.44	36.62±0.40	--

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

6.3.4 Effect of pesticides, Cu^{++} and Fe^{++} and humic acid on qualitative production on Hydrogen Cyanide production

Cyanide production by numerous rhizobacteria and is postulated to play a function in the control of biological harmful pathogens (Defago et al., 1990). The suppression of soil-borne pathogens by the production of these hormones by fluorescent *Pseudomonas* has also been reported worldwide (Ahemad et al., 2014; Chitra et al., 2002; Voisard et al., 1989). Various diseases in tobacco and wheat were also being suppressed by Cyanide production were reported by Stutz et al., 1986; Defago et al., 1990. HCN production also improves root length and seed germination in some plants (Saxena et al., 1996). Ahemad et al (2012) reported the effect of HCN production under pesticide stress. Out of 19 isolates, only 3 strains (ACP3, CB1 and GP1) were able to synthesize HCN production and the results were summarized in table 19. Pesticides at high concentrations posed a negative effect on its production while the addition of Fe^{++} and humic acid along with pesticides has no effect on its production.

Table 20: Qualitative assay for hydrogen Cyanide production by bacterial species isolated from rhizospheric soils

Conc. ↓	**Pesticide →**	**Acephate**	**Carbendazim**	**Glyphosate**	**Control**
	Treatment ↓	*Pseudomonas* sp. ACP3	*Actinomyces* sp. CB1	*Actinomyces* sp. GP1	*P fluorescens*
0x	**Control**	+++	++	++	++
	100 mg/L Cu^{++}	+	+	+	--
	100 mg/L Fe^{++}	+++	+++	+++	--
	100 mg/L HA	+++	+++	+++	--
1x	**Control**	+++	++	++	--
	100 mg/L Cu^{++}	+	+	+	--
	100 mg/L Fe^{++}	++	++	++	--
	100 mg/L HA	+++	+++	+++	--
2x	**Control**	+	++	++	--
	100 mg/L Cu^{++}	---	---	---	--
	100 mg/L Fe^{++}	+	++	++	--
	100 mg/L HA	++	++	+	--

+++ = High Detection, ++ = Moderate Detection, + = Low Detection, --- = No Change in Colour

6.4 Effect of pesticides, Cu^{++}, Fe^{++} and humic acid on plant growth

6.4.1 Biocompatibility test

The compatible interaction was considered by observing the absence of inhibition zone around the spotted colony. Four petriplates were impregnated with four discs of different cultures, three petriplates were impregnated with three cultures and three petriplates were impregnated with two cultures with one control plate without any culture, respectively. After placing these discs, cultures were again allowed to grow for 2 h at 37°C. Finally, the growth was observed which shows that there was no zone of inhibition in any of the inoculated cultures; hence all the bacterial cultures are not a competitor but are compatible with each other and can be used as a good consortium (Fig 24-29). Our results were found similar with the findings of Anandaraj et al., (2010) in which the species of *Rhizobium sp., Bacillus megaterium* and *P. fluorescens* were compatible with each other in cross streak plate assay. Thus, treatment of seeds with effective strains of *Rhizobium, Bacillus* alone or in combination with other beneficial microorganisms may be preferred over the pesticides, because of their multiple potentials to control disease, fix nitrogen, increase crop productivity, improve of soil fertility besides reducing the negative environmental impact associated with chemical use (Jensen et al., 2002; Huang and Erickson, 2007).

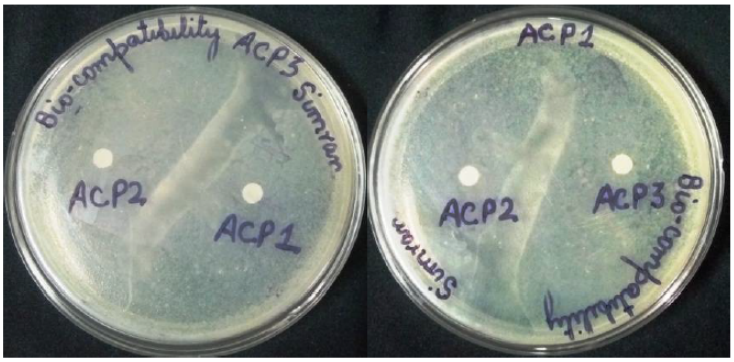


Figure 24: Test of biocompatibility between rhizobacterial strains isolated from acephate contaminated soils

Figure 25: Test of biocompatibility between rhizobacterial strains isolated from atrazine contaminated soils

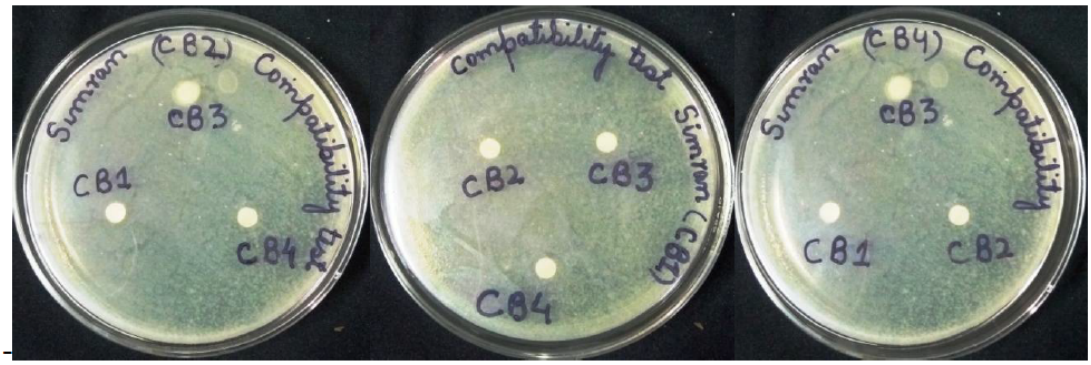


Figure 26: Test of biocompatibility between rhizobacterial strains isolated from carbendazim contaminated soils

Figure 27: Test of biocompatibility between rhizobacterial strains isolated from monocrotophos contaminated soils

Figure 28: Test of biocompatibility between rhizobacterial strains isolated from glyphosate contaminated soils

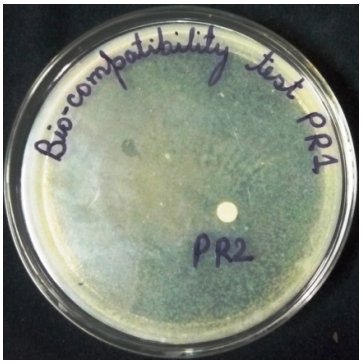


Figure 29: Test of biocompatibility between rhizobacterial strains isolated from phorate contaminated soils.

6.4.2 Effect of pesticides on seed germination

6.4.2.1 Effect of metal ions (100mg/L Cu^{++} and 100mg/L Fe^{++}) and humic acid on untreated group of pesticides on seed germination

In the untreated group, the seeds inoculated in humic acid shows high germination rate as compared to other three different treatments. The germination rate was increased significantly at significant level $p < 0.05$. The germination rate is highest in humic acid, Fe^{++} and same in case of Cu^{++} and untreated. The exact order of seed germination was seed inoculated with humic acid > Fe^{++} > control > Cu. Significant effects of Fe^{++}, Cu^{++}, and HA were observed (at significant level $p = 0.05$) with an order HA > Fe^{++} > control ≥ Cu^{++}. Fe^{++} is considered as most important part of plant growth including crops and having less toxicity (LD_{50} 230 mg/kg in rats) than Cu. Fe can increase the growth of the wheat plant and seeds also (Celletti et al., 2016). In bio-inorganic viewpoint, Cu is a toxic heavy metal ion (LD_{50} 30 mg/kg in rats) which has a variable effect on different organisms. Also, Cu has a negative effect on all the plant parameters due to its toxic nature (Lu et al., 2016: Adrees et al., 2015). Humic acid is considered to be always good for crop growth when applied in singular form (Vaccaro et al., 2015). Once it is added with pesticides or metal ions or in the presence of both, the things may vary due to different mechanisms. (1) HA decomposed the pesticides and decomposition reaction rate increase once metal ions like Fe and Cu are applied. (2) Sometimes there is association and dissociation mechanism between HA and pesticides and decomposition becomes slow because active sites of HA get blocked by pesticides. Due to this, free HA is not available for the growth of crops (wheat) hence growth affected adversely.

6.4.2.2 Effect of Acephate, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions on seed germination

In un-inoculated groups, on the addition of 1x concentration of Acephate to the wheat plant, germination rate was found to be inhibited. The inhibition was found dose-dependent i.e. inhibition in germination rate was found more when 2x acephate was applied on to the crop compared to a 1x dose level of Acephate. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of Acephate, the germination rate in inoculated groups was higher as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate was almost similar. In case of 2x treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases at significant level $p < 0.05$. In the presence of metal ions and humic acid, a slight increment was been observed (Table 21).

Table 21: Germination rate of acephate treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Acephate (G.R. %)		2x Acephate (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
	60%	80%	50%	60%
Cu^{++}	70%	80%	50%	60%
Fe^{++}	70%	80%	50%	70%
Humic acid	70%	80%	70%	70%

6.4.2.3 Effect of Atrazine, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions on seed germination

In Un-inoculated groups, on the addition of 1x concentration of Atrazine to the wheat plant, germination rate was found to be inhibited. The inhibition was found dose-dependent i.e. inhibition in germination rate was found more when 2x atrazine was applied on to the crop compared to a 1x dose level of atrazine. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of atrazine, the germination rate in inoculated groups was higher as compared to un-inoculated groups. In addition of metal ions

and humic acid, the growth rate was almost similar. In case of 2x treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases. In the presence of metal ions and humic acid, a slight increment of was been observed in case of inoculated groups (Table 22)

Table 22: Germination rate of atrazine treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Atrazine (G.R. %)		2x Atrazine (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
	60%	70%	30%	60%
Cu^{++}	60%	70%	50%	60%
Fe^{++}	70%	80%	50%	70%
Humic acid	90%	90%	60%	70%

6.4.2.4 Effect of Carbendazim, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In Un-inoculated groups, on the addition of 1x concentration of carbendazim to the wheat plant, germination rate was found to be inhibited. The inhibition was found dose-dependent i.e. inhibition in germination rate was found more when 2x carbendazim was applied on to the crop compared to a 1x dose level of carbendazim. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth. However, in case of Cu^{++} treated groups, there is a decrease in seed germination in both the 1x and 2x concentrations.

In inoculated groups, on the addition of 1x concentration of carbendazim, the germination rate in inoculated groups was higher as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate was almost similar. In case of 2x treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases. In the presence of metal ions and humic acid, a slight increment of Germination rate has been observed in both the inoculated groups of 1x and 2x groups (Table 23).

Table 23: Germination rate of carbendazim treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Carbendazim (G.R. %)		2x Carbendazim (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated

	60%	60%	40%	50%
Cu^{++}	40%	60%	30%	50%
Fe^{++}	60%	80%	50%	70%
Humic acid	80%	80%	80%	70%

6.4.2.5 Effect of Glyphosate, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In addition of Glyphosate, the germination rate in the un-inoculated is inhibited. Not even a single seed showed germination rate, but in the addition of metal ions, the germination rate was increased up to 20-40%. In inoculated groups, germination rate increased significantly with the addition of metal ions. In 1x concentration, the germination rate was higher than the un-inoculated groups and in 2x only small percentage germination rate was observed (Table 24).

Table 24: Germination rate of glyphosate-treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	**1x Glyphosate (G.R. %)**		**2x Glyphosate (G.R. %)**	
	Un-inoculated	**Inoculated**	**Un-inoculated**	**Inoculated**
	0%	50%	0%	10%
Cu^{++}	20%	40%	10%	20%
Fe^{++}	30%	60%	10%	20%
Humic acid	40%	70%	20%	30%

6.4.2.6 Effect of Monocrotophos, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In Un-inoculated groups, on the addition of 1x concentration (recommended) of Monocrotophos to the wheat plant, germination rate was found to be non-affected. Inhibition of germination rate was found more when 2x Monocrotophos was applied on to the crop compared to a 1x dose level of Monocrotophos. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of Monocrotophos, the germination rate in inoculated groups was similar as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate increased in 1x treated samples. In case of 2x

treated groups, the germination rate in both the groups (inoculated and un-inoculated) decreases. In the presence of metal ions and humic acid, a slight increment was been observed (Table 25).

Table 25: Germination rate of monocrotophos treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Monocrotophos (G.R. %)		2x Monocrotophos (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
	60%	60%	20%	30%
Cu^{++}	60%	70%	20%	30%
Fe^{++}	70%	80%	30%	40%
Humic acid	70%	80%	40%	50%

6.4.2.7 Effect of Phorate, metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

In Un-inoculated groups, on the addition of 1x concentration of Phorate to the wheat plant, germination rate was found to be highly affected. Inhibition of germination rate was found more when 2x Phorate was applied on to the crop compared to a 1x dose level of Phorate. Most interestingly, when the dose of metal ions (100 mg/L of each Fe^{++} and Cu^{++}) and humic acid were added, there was an increase in plant growth.

In inoculated groups, on the addition of 1x concentration of Phorate, the germination rate in inoculated groups was different as compared to un-inoculated groups. In addition of metal ions and humic acid, the growth rate increased in 1x treated samples. In case of 2x treated groups, the germination rate in the inoculated group decreases. In the presence of metal ions and humic acid, a slight increment in growth has been observed (Table 26)

Table 26: Germination rate of phorate treated seeds supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions

Treatment	1x Phorate (G.R. %)		2x Phorate (G.R. %)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
	50%	60%	30%	30%
Cu^{++}	60%	60%	40%	30%
Fe^{++}	60%	70%	40%	40%
Humic acid	70%	80%	50%	50%

6.4.3 Effect of pesticides on length of radical and plumule treated with and without metal ions and humic acid

The length of radical and plumule was measured after 3 days of seed germination. It was observed in all the cases, that 1x and 2x concentrations of pesticides inhibit all three parameters. But with the addition of metal ions and humic acid with a different concentration of pesticides, stimulation in all the three parameters was observed at significant level $p < 0.05$. Glyphosate was found more toxic showing complete inhibition of radical and plumule growth in untreated groups at both the concentrations than rest of the pesticides (Table 27).

Table 27: Effect of different pesticides on growth of radical and plumule germination supplemented with metal ions (Fe^{++} and Cu^{++}) and humic acid on un-inoculated and inoculated bacterial suspensions at significant level $p < 0.05$

Treatment	**Radicle (in cm)**				**Plumule (in cm)**			
Control	1.94±0.09				2.85±0.04			
100 mg/L Cu^{++}	0.98±0.12				1.86±0.07			
100 mg/L Fe^{++}	1.62±0.09				2.58±0.04			
100 mg/L Humic acid	3.03 ±1.01				3.84±0.05			
	1x PC	**BS + 1x PC**	**2x PC**	**BS + 2x PC**	**1x PC**	**BS + 1x PC**	**2x PC**	**BS + 2x PC**
Acephate (ACP)	1.8±0.07	2.04±0.03	1.07±0.04	1.80±0.04	1.42±0.06	1.65±0.04	1.12±0.06	1.08±0.04
ACP + 100 mg/L Cu^{++}	1.6±0.05	1.86±0.04	0.96±0.04	1.17±0.06	1.04±0.04	1.08±0.09	0.88±0.08	0.83±0.05
ACP + 100 mg/L Fe^{++}	2.10±0.09	2.5±0.06	1.79±0.02	2.07±0.05	1.85±0.09	1.93±0.06	0.95±0.04	1.33±0.03
ACP+ 100 mg/L HA	2.49±0.01	3.17±0.04	1.16±0.04	3.03±0.04	1.87±0.05	2.2±0.1	0.94±0.06	2.73±0.11
Atrazine (ATR)	1.52±0.09	1.9±0.05	1.08±0.07	1.60±0.07	1.26±0.06	1.73±0.08	1.09±0.09	1.48±0.03
ATR + 100 mg/L Cu^{++}	1.83±0.09	2.12±0.07	1.23±0.09	1.66±0.09	1.61±0.04	2.05±0.05	0.94±0.06	1.04±0.05
ATR + 100 mg/L Fe^{++}	2.05±0.11	2.28±0.06	1.51±0.05	1.81±0.04	1.12±0.05	1.48±0.07	0.95±0.06	1.08±0.07
ATR + 100 mg/L HA	2.55±0.09	2.86±0.05	1.75±0.07	1.88±0.09	2.51±0.06	2.7±0.1	1.26±0.05	1.25±0.22
Carbendazim (CBZ)	3.06±0.15	3.88±0.05	2.44±0.04	2.69±0.08	1.81±0.07	2.39±0.09	1.47±0.03	1.73±0.06
CBZ + 100 mg/L Cu^{++}	1.15±0.04	1.80±0.03	1.68±0.03	1.89±0.08	1.38±0.06	1.56±0.07	1.11±0.02	1.43±0.10
CBZ + 100 mg/L Fe^{++}	3.96±0.01	4.63±0.02	3.84±0.05	4.46±0.05	3.16±0.05	3.42±0.09	2.48±0.08	3.27±0.07
CBZ + 100 mg/L HA	4.29±0.07	4.68±0.05	4.13±0.03	4.49±0.08	3.19±0.07	3.76±0.06	2.81±0.03	3.20±0.030

Glyphosate (GP)	0	0.30±0.03	0	0	0	0.35±0.05	0	0
GP + 100 mg/L Cu^{++}	0	0.35±0.04	0	0	0	0.35±0.04	0	0
GP + 100 mg/L Fe^{++}	0.53±0.02	0.66±0.04	0.23±0.05	0.39±0.07	0.20±0.03	0.37±0.03	0.11±0.03	0.24±0.05
GP + 100 mg/L HA	0.89±0.01	1.30±0.04	0.61±0.03	0.8±0.06	0.8±0.04	0.94±0.05	0.25±0.05	0.62±0.07
Monocrotophos (MC)	2.43±0.07	3.06±0.06	2.23±0.06	3.10±0.06	1.89±0.08	2.21±0.07	1.85±0.07	1.20±0.06
MC + 100 mg/L Cu^{++}	1.75±0.04	2.45±0.03	1.42±0.03	1.97±0.06	1.51±0.06	2.06±0.06	1.06±0.04	1.20±0.07
MC + 100 mg/L Fe^{++}	2.91±0.03	3.39±0.05	2.17±0.06	2.50±0.09	2.73±0.05	3.16±0.04	1.7±0.08	2.38±0.02
MC + 100 mg/L HA	3.08±0.04	3.75±0.04	2.87±0.07	3.45±0.05	2.82±0.04	3.68±0.06	2.39±0.06	3.10±0.03
Phorate (PR)	2.14±0.07	2.53±0.07	0.99±0.07	1.29±0.06	1.87±0.06	2.1±0.08	0.63±0.05	0.81±0.04
PR + 100 mg/L Cu^{++}	1.64±0.05	1.94±0.06	0.85±0.06	1.11±0.04	0.83±0.08	1.27±0.09	0.47±0.09	0.65±0.09
PR + 100 mg/L Fe^{++}	2.23±0.04	2.92±0.05	1.85±0.04	2.29±0.09	1.58±0.04	2.47±0.07	1.24±0.10	1.81±0.07
PR + 100 mg/L HA	3.08±0.05	3.84±0.03	2.20±0.02	2.85±0.04	3.51±0.08	3.89±0.11	2.09±0.08	2.50±0.11

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

Where 1x PC = recommended concentration of Pesticide; BS + 1x PC = Bacterial Suspension + recommended concentration of Pesticide; 2x PC = Twice recommended concentration of pesticide; BS + 2x PC = Bacterial Suspension + Twice recommended concentration of Pesticide; Cu^{++} = Copper Chloride; Fe^{++} = Ferrous Chloride and HA = Humic Acid.

6.4.4 Soils collection and characterization for pot experimentation

This work is carried out in the Department of Biotechnology, under the domain of School of bio-engineering and biosciences in the month of November to March 2016. The physiochemical characteristics of the soil are in table 28.

Table 28: Physiochemical characterization of soil

Particulars	Unit	Soil test value	Status	Soil test Rating		
				Acidic	**Neutral**	**Alkaline**
pH		7.6	Alkaline	<6.5	6.5-7.5	>7.5
				Non-Saline	**Increasingly Saline**	
Electric Conductivity	ds/m	0.06	Non-Saline	<1.0	1.0-2.0	>2.0
				Low	**Medium**	**High**
Organic Carbon	kg/ha	0.30	Low	<0.50	0.50-0.75	>0.75
Avail. Nitrogen	kg/ha	62.8	Low	<250	250-500	>500
Avail. Phosphorous	kg/ha	11.8	Medium	<10	10-25	>25
Avail. Potassium	kg/ha	87.2	Low	<125	125-250	>250
Exch. Calcium	mg/L	487	Low	<500	500-1000	>1000
Exch. Magnesium	mg/L	363.4	High	<125	125-250	>250
Avail. Sulphur	mg/L	16.3	Medium	<10	10-50	>50
Avail. Zinc	mg/L	1.76	Medium	<1	1.0-5.0	>5.0
Avail. Copper	mg/L	0.78	Medium	<0.5	0.5-2.5	>2.5
Avail. Iron	mg/L	42.6	High	<2.5	2.5-10.0	>10.0
Avail. Manganese	mg/L	11.3	Medium	<5.0	5.0-20.0	>20.0
Boron	mg/L	0.19	Low	<0.5	0.5-1.0	>1.0

6.4.5 Effect of pesticides Cu^{++}, Fe^{++} and humic acid on plant growth parameters, chlorophyll and carotenoid content

6.4.5.1 Effect of Acephate on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

The length of the wheat plant was measured after 60 days of seed germination. It was observed in all the cases, that 1x and 2x concentrations of pesticides inhibit all four parameters. But with the addition of Fe^{++} and humic acid witha different concentration of pesticides, stimulation in the three parameters was observed at significant level $p < 0.05$

except Cu^{++}. The length of the plant (Table 29), chlorophyll a content (Table 30), chlorophyll b content (Table 31) and carotenoids (Table 32) summarizes the pessimistic effect of acephate on plant growth. Similar effects were observed on the concentrations of photosynthetic pigmentation production by the plant. Another similar study by Rajashekar, et al. (2012) in which the abiotic stress caused by pendamethalin among *Zea mays* L. cv NAAC- 6002 and illustrated that germination under control condition was maximum about 95%, whereas drastic declination in germination percentage was observed among the seeds sets which were treated with high amount of pendimethalin. An acute declination of 69% was visualized on using the 10 ppm solution of pendimethalin which contributed its role in initiating the consequences of the herbicide in eradication and mobilization process during seed reserves. The study conducted by Moore and Kroger (2010) also highlights the effect of insecticides and herbicides (individually as well as in combination) on a seedling of rice germination conditions.

Table 29: Effect of Acephate on physical parameters (height) of plant (cm) at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	26.4±0.56	22.2±0.40	29.33±0.56	27.67±0.42
No bacteria	1x Acephate	22.4±0.46	27.3±0.47	21.2±0.65	27.6±0.53
No bacteria	2x Acephate	19.97±0.75	24.1±0.61	18.4±0.45	25.93±0.45
Pseudomonas sp. ACP_01	Control	32.3±0.46	29.8±0.62	33.07±0.40	30.23±0.47
Pseudomonas sp. ACP_01	1x Acephate	27.97±0.65	25.1±0.55	27.4±0.55	25.97±0.35
Pseudomonas sp. ACP_01	2x Acephate	20.2±0.36	18.4±0.55	22.3±0.43	23.17±0.47
Pseudomonas sp. ACP_02	Control	30.33±0.5	25.3±0.60	27.9±0.45	28.37±0.5
Pseudomonas sp. ACP_02	1x Acephate	27.07±0.7	22.6±0.45	26.33±0.47	27.33±0.57
Pseudomonas sp. ACP_02	2x Acephate	22.27±0.49	24±0.65	26.87±0.41	27.03±0.42
Pseudomonas sp. ACP_03	Control	28.33±0.4	21.5±0.66	27.9±0.75	28.8±0.56
Pseudomonas sp. ACP_03	1x Acephate	26.33±0.47	23±0.4	27.07±0.65	25.97±0.45
Pseudomonas sp. ACP_03	2x Acephate	24.13±0.61	29.9±0.70	24.23±0.61	23.17±0.6
Bacterial Suspension	Control	37.07±0.45	32.1±0.56	40.23±0.56	33.53±0.45
Bacterial Suspension	1x Acephate	31.2±0.56	27.4±0.45	37.13±0.70	28.27±0.57
Bacterial Suspension	2x Acephate	28.17±0.4	24.3±0.55	31.23±0.55	21.83±0.55

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 30: Content of chlorophyll A in wheat (ug/mL) as mono-cropping system in Acephate contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L Humic acid**
No bacteria	Control	0.84±0.04	0.52±0.04	1.04±0.07	0.93±0.06
No bacteria	1x Acephate	0.94±0.05	0.58±0.04	0.92±0.03	0.87±0.06
No bacteria	2x Acephate	0.66±0.03	0.56±0.03	0.72±0.05	0.67±0.05
Pseudomonas sp. ACP_01	Control	1.14±0.08	1.05±0.06	1.72±0.1	1.23±0.08
Pseudomonas sp. ACP_01	1x Acephate	1.03±0.06	0.93±0.04	1.18±0.05	1.04±0.06
Pseudomonas sp. ACP_01	2x Acephate	0.94±0.02	0.83±0.03	1.07±0.13	0.92±0.06
Pseudomonas sp. ACP_02	Control	1.19±0.16	1.12±0.11	1.75±0.16	1.44±0.08
Pseudomonas sp. ACP_02	1x Acephate	1.05±0.06	0.92±0.05	1.34±0.06	1.13±0.11
Pseudomonas sp. ACP_02	2x Acephate	0.84±0.1	0.73±0.05	1.25±0.11	1.05±0.09
Pseudomonas sp. ACP_03	Control	1.12±0.09	0.79±0.1	1.48±0.1	1.19±0.09
Pseudomonas sp. ACP_03	1x Acephate	0.92±0.03	0.83±0.06	1.24±0.11	1.14±0.07
Pseudomonas sp. ACP_03	2x Acephate	0.84±0.04	0.65±0.06	1.09±0.06	0.84±0.05
Bacterial Suspension	Control	1.65±0.04	1.43±0.1	2.14±0.1	1.95±0.1
Bacterial Suspension	1x Acephate	1.4±0.02	1.22±0.08	1.91±0.03	1.45±0.07
Bacterial Suspension	2x Acephate	1.3±0.26	1.04±0.07	1.75±0.07	1.22±0.07

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 31: Content of chlorophyll b in wheat (ug/mL) as mono-cropping system in Acephate contaminated soil supplemented with Cu^{2+}, Fe^{2+} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 ppm Cu^{++}**	**100 ppm Fe^{++}**	**100 ppm Humic acid**
No bacteria	Control	0.6±0.04	0.43±0.07	0.85±0.05	0.7±0.06
No bacteria	1x Acephate	0.6±0.04	0.5±0.08	0.88±0.04	0.77±0.06
No bacteria	2x Acephate	0.4±0.06	0.33±0.04	0.62±0.05	0.54±0.05
Pseudomonas sp. ACP_01	Control	1±0.06	0.88±0.04	1.31±0.08	1.20±0.08
Pseudomonas sp. ACP_01	1x Acephate	0.8±0.04	0.79±0.06	1.07±0.06	0.94±0.06
Pseudomonas sp. ACP_01	2x Acephate	0.7±0.04	0.62±0.04	0.79±0.04	0.81±0.07
Pseudomonas sp. ACP_02	Control	1.1±0.07	0.94±0.04	1.57±0.05	1.35±0.07
Pseudomonas sp. ACP_02	1x Acephate	0.8±0.15	0.83±0.05	1.23±0.05	1.16±0.06
Pseudomonas sp. ACP_02	2x Acephate	0.7±0.05	0.7±0.06	0.96±0.05	0.82±0.04
Pseudomonas sp. ACP_03	Control	0.9±0.06	0.8±0.06	1.14±0.09	1.13±0.07
Pseudomonas sp. ACP_03	1x Acephate	0.8±0.07	0.73±0.04	0.97±0.06	0.9±0.08
Pseudomonas sp. ACP_03	2x Acephate	0.7±0.05	0.62±0.04	0.82±0.06	0.71±0.08
Bacterial Suspension	Control	1.4±0.08	1.34±0.07	2.05±0.07	1.82±0.05
Bacterial Suspension	1x Acephate	1±0.05	1.1±0.06	1.43±0.05	1.36±0.05
Bacterial Suspension	2x Acephate	0.8±0.05	0.92±0.05	1.23±0.04	1.03±0.04

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

Table 32: Content of carotenoids in wheat (ug/mL) as mono-cropping system in Acephate contaminated soil supplemented with Cu^{2+}, Fe^{2+} and humic acid at significant level $p < 0.05$

Isolates	Treatment	Control	100 ppm Cu^{++}	100 ppm Fe^{++}	100 ppm Humic acid
No bacteria	Control	0.10±0.005	0.07±0.006	0.133±0.005	0.112±0.010
No bacteria	1x Acephate	0.11±0.006	0.096±0.007	0.144±0.007	0.124±0.006
No bacteria	2x Acephate	0.09±0.006	0.083±0.006	0.112±0.010	0.097±0.005
Pseudomonas sp. ACP_01	Control	0.32±0.009	0.248±0.007	0.367±0.008	0.318±0.006
Pseudomonas sp. ACP_01	1x Acephate	0.29±0.007	0.185±0.007	0.323±0.007	0.303±0.005
Pseudomonas sp. ACP_01	2x Acephate	0.21±0.006	0.163±0.007	0.284±0.008	0.265±0.006
Pseudomonas sp. ACP_02	Control	0.34±0.007	0.315±0.007	0.379±0.006	0.344±0.007
Pseudomonas sp. ACP_02	1x Acephate	0.30±0.009	0.194±0.006	0.354±0.004	0.337±0.004
Pseudomonas sp. ACP_02	2x Acephate	0.25±0.006	0.176±0.006	0.311±0.009	0.293±0.006
Pseudomonas sp. ACP_03	Control	0.21±0.008	0.192±0.005	0.295±0.006	0.244±0.004
Pseudomonas sp. ACP_03	1x Acephate	0.16±0.006	0.442±0.016	0.212±0.009	0.174±0.007
Pseudomonas sp. ACP_03	2x Acephate	0.14±0.006	0.093±0.006	0.114±0.009	0.13±0.005
Bacterial Suspension	Control	0.36±0.009	0.282±0.006	0.408±0.007	0.394±0.005
Bacterial Suspension	1x Acephate	0.34±0.007	0.215±0.007	0.388±0.006	0.365±0.007
Bacterial Suspension	2x Acephate	0.29±0.065	0.165±0.007	0.365±0.017	0.346±0.006

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

6.4.5.2 Effect of Atrazine on wheat growth, Chlorophyll content and total carotenoids on recommended and higher doses in the presence of Cu^{++}, Fe^{++} and humic acid.

Impacts of two dose levels (1x and 2x) of Atrazine on the physical parameters (height) of growing wheat crops were studied and were found significantly affected by the applications of atrazine. When plants were at anthesis stage, the height of stem and head of each plant were measured. At a 1x dose level of atrazine, the height of stem and head was found to be reduced, while, on the application of 2x of Atrazine the height of stem and head was also reduced as compared to control (Table 33).

To check the effect of Cu^{++}, Fe^{++} and humic acid on wheat growth experiment were also carried out in presence of Atrazine (1x and 2x). A significant decrease in the weight of chlorophyll content/ carotenoid and height of stem was observed and it was found that with

the additional supply of Fe^{++} and humic acid, plant growth was found to be increased at significant level $p < 0.05$. Consequently, the chlorophyll A content (Table 34), chlorophyll b content (Table 35) carotenoid (Table 36) as well as in height of stem and head was observed. A similar experiment was carried out to investigate the effect of two herbicides and three insecticides on coleoptiles, radical and germination of rice seeds. The effect of pesticides on shoot germination was significantly decreased in lambda cyhalothrin, diazinon, metachlor and atrazine mixture treated groups. From the pesticides that were used, fipronil indicated the least percent of seed germination i.e. 76% in the examination with a control which was 80%, in as much as on the different side diazinon indicated 85% in the examination to control (Moore and Kroger 2010). The effect declination around 85% plant density, 67% plant height and 91% dry weight was observed for germination of Hemp sesbania which was treated with naproanilide and 2-naphthyloxy propionic acid at 2.25kg/Ha as documented by Hirase and Molin in the year 2002. The reaction of herbicide named pendimethalin and trifluralin, which inhibits the early growth and seed germination in *Zea mays* L. Crops (Nehru et al., 1999). Moreover, it has been found that atrazine and metribuzin have the toxic effect, which reduces the amount of photosynthate transferred to the radicle and the in vitro study conducted on the *Bradyrhizobium* for evaluation of these two herbicides on consequences of the functionality of the plant. Data from the studies strengthen the concept that the atrazine and metribuzin are harmful and affect this plant in association with a bacterium which indirectly affects the nodulation and yield of crops (Alonge, 2000).

Kaushik and Inderjit (2006) established the fact that beans grown in soil treated with herbicides, illustrated the consistent decrease in the level of chlorophyll (chl) when the concentration of the herbicide increased. They concluded that almost all the symptoms of biochemical origin are related to toxicity level of pesticides, which results in declination of chlorophyll content and oxidation process activation.

Table 33: Effect of Atrazine on physical parameters (height) of plant or plant growth (in cm) at significant level $p < 0.05$

Values are the mean of triplicates.
± Standard deviation (values indicate change decrease (-) or increase (+) over the values).

Table 34: Content of chlorophyll A in wheat (ug/mL) as mono-cropping system in Atrazine contaminated soil supplemented with Cu^{++}, Fe^{++} and humic acid at significant level $p < 0.05$

Isolates	**Treatment**	**Control**	**100 mg/L Cu^{++}**	**100 mg/L Fe^{++}**	**100 mg/L HA**
No bacteria	Control	0.86±0.04	0.54±0.05	1.14±0.06	0.91±0.07
No bacteria	1x Atrazine	1.15±0.08	0.93±0.05	1.44±0.09	1.31±0.05
No bacteria	2x Atrazine	1.04±0.08	0.83±0.04	1.17±0.08	1.05±0.07
Streptomycetaceae bacterium RK1	Control	1.75±0.08	1.53±0.04	1.9±0.07	1.72±0.08
Streptomycetaceae bacterium RK1	1x Atrazine	1.54±0.09	1.34±0.05	1.62±0.04	1.55±0.05
Streptomycetaceae bacterium RK1	2x Atrazine	1.31±0.06	1.14±0.07	1.33±0.05	1.23±0.05
P. fluorescens strain RK2	Control	1.33±0.04	1.02±0.04	1.69±0.07	1.58±0.06
P. fluorescens strain RK2	1x Atrazine	1.22±0.05	0.92±0.05	1.55±0.06	1.33±0.08
P. fluorescens strain RK2	2x Atrazine	0.94±0.03	0.84±0.07	1.23±0.05	1.03±0.08
A. chroococcum strain RK3	Control	1.17±0.08	0.86±0.05	1.54±0.05	1.32±0.09
A. chroococcum strain RK3	1x Atrazine	0.97±0.05	0.83±0.05	1.33±0.06	1.07±0.09
A. chroococcum strain RK3	2x Atrazine	0.80±0.08	0.69±0.07	1.05±0.07	0.94±0.06
R. leguminosarum strain RK4	Control	1.44±0.03	1.04±0.01	1.66±0.09	1.54±0.19
R. leguminosarum strain RK4	1x Atrazine	1.16±0.02	0.99±0.06	1.38±0.11	1.34±0.22
R. leguminosarum strain RK4	2x Atrazine	0.93±0.05	0.81±0.04	1.22±0.14	1.11±0.14
Bacterial Suspension	Control	1.82±0.06	1.63±0.09	2.05±0.07	1.88±0.07
Bacteria suspension	1x Atrazine	1.43±0.05	1.25±0.07	1.87±0.07	1.54±0.09
Bacteria suspension	2x Atrazine	1.22±0.06	1.066±0.06	1.61±0.05	1.05±0.06

Values are the mean of triplicates.

± Standard deviation (values indicate change decrease (-) or increase (+) over the values)

List of Publications:

Simranjeet Singh, Vijay Kumar, Arun Chauhan, Shivika Datta, Abdul Basit Wani, Nasib Singh and Joginder Singh (2017). Toxicity, degradation and analysis of the herbicide atrazine. Environmental Chemistry Letters, 1-27. (Publisher:Springer) (Impact factor =3.591).

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Vijay Kumar, **Simranjeet Singh**, Rohit Singh, Niraj Upadhyay, Joginder Singh: Design, synthesis, and characterization of 2,2-bis(2,4-dinitrophenyl)-2-(phosphonatomethylamino)acetate as a herbicidal and biological active agent. Journal of Chemical Biology 07/2017;, DOI:10.1007/s12154-017-0174-z. (Publisher:Springer)

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