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DISSERTATION REPORT
(AGR- 596)

"ROLE OF POLYAMINES AND MYCORRHIZA FOR THE MITIGATION OF ARSENIC TOXICITY IN PEA PLANT"

Submitted To
Department of Agronomy,
School of Agriculture.

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by
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UNDER THE GUIDANCE OF
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May-2018

SUPERVISORS CERTIFICATE

This is to certify that the work synopsis report in **“Role of polyamines and mycorrhiza for the mitigation of arsenic toxicity in Pea”** submitted by **Panuganti Swaraj kumar, 11715705,** at **Lovely Professional University, Phagwara, India** is a bonafide record of his original work carried out under my supervision. This work has not been submitted elsewhere for any other degree.

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Panuganti Swaraj kumar
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CANDIDATES DECLARATION

We hereby certify that the work which is being presented in the **"Role of polyamines and mycorrhiza for the mitigation of Arsenic toxicity in Pea"** in partial fulfilment of the requirement for the award of the **"Master of Science In Agriculture"** and submitted to the school of Agriculture of Lovely Professional University is a work carried out under the supervision of **Dr.Prasann Kumar,** Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, Punjab.

The work presented in the report has not been submitted by us for the award of any other degree elsewhere.

(Signature of the student)

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Introduction

Currently, the available literature focuses mainly on different features of heavy metal buildup both in the ecosystem and in the food chain. Reports are also available on the occurrence of high concentration of these metals in crop plants, and on consumption of the produce or the plants,animals and human beings are also affected (Gopal et al. 2003). Being sessile organisms,plants are continuously exposed during their life cycle to adverse environmental conditionsthat detrimentally affect growth, development, or compounds, such as heavy metals (HMs), is one important factor that can cause damage to plants by altering most important plant physiological and metabolic processes. Heavy metals are important environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons. The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech Water Treatment and Air Purification 2004). “Heavy metals” in a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm3, or five times or more, are greater than water (Hawkes 1997). *Pisum sativum* also known as the Garden pea belongs to the family Fabaceae. Pea is an annual herbaceous plant with a life cycle of one year. It is believed to have originated from the Mediterrean region, Central and Western Asia and Ethopia. It is the cool season crop which is cultivated throughout the world. Pea is a rich source of protein, fibre, carbohydrate, minerals like iron, magnesium, phosphorus, zinc and vitamin like A and C,etc.There are generally 3 types of pea that are commonly eaten. They are Garden pea or green pea- *Pisum sativum*, snow pea- *Pisumsativum var. macrocarpon*, snap pea-*Pisum sativum var. macrocarpon ser cv*. These peas have rounded pods that are usually slightly curved in shape with a smooth texture and vibrant green colour. Several peas are enclosed inside the pods. Usually snow peas are used as a vegetable in their immature stage. They have tender pods and are consumed fresh, frozen or canned whereas field pea are generally grown to produce dry pea like the split pea shelled from the mature pods. *Pisum sativum* cultivars are either low growing or vining type. The vining type of cultivar grows tendrils that coils around any available support and can climb upto 1-2m. Pea like many other legumes contains symbiotic bacteria called Rhizobia within their root nodules. These bacteria help in fixing the atmospheric nitrogen. Using the symbiotically fixed nitrogen instead of using chemical nitrogen fertilizer will help in the development of sustainable agriculture and also increase the yield production. A polyamine is an organic compound having two or more primary amino groups-NH2. They are also known as a group of natural compounds with an aliphatic nitrogen structure present in almost all living organisms. It plays an important role in many physiological processes such as cell growth and development. Polyamines which are commonly found are putrescine. Spermidine, spermine etc. the diamine putrescine, the triamine spermidine and the tetramine spermine are ubiquitously found in plant cells while other polyamines are of more limited occurrence. In animals, their levels are maintained from both the diet and denovo synthesis. Polyamine metabolism is regulated by the activity of the enzyme ornithine decarboxylase. Polyamine can also be synthesized from the aminoacid arginine and methionine. The first step in the pathway is the production of ornithine from arginine by the mitochondrial enzyme arginase. Ornithine is then decarboxylated by ornithine decarboxylase to produce putrescine. Polyamine is found in high concentration in the mammalian brain. The polyamines declines with the ages in the organism.

Mycorrhiza is a fungus having a symbiotic or mutualistic relationship with the rhizosphere or roots of the plants. They share a mutualistic relationship in which the mycorrhiza forms a network of filaments that associates with the plant roots which helps them in uptaking the mineral nutrients or water while the mycorrhiza are benefitted by getting access to carbohydrates such as glucose and sucrose. The carbohydrates are translocated from their source to root tissues and onto the plant fungal partners. Some plant roots may be unable to uptake nutrients that are chemically or physically immobilized. For example, phosphate ions and micronutrients such as ions. Polyamines including putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and developmental processes (Farooq *et al*., 2009). They are the recent additions to the class of plant growth regulators, and also considered as a secondary messenger in signaling pathways (Kusano *et al*., 2008). Polyamines are involved in abiotic stress tolerance in plants (Nayyer *et al*., 2005). Increased polyamines level in stressed plants are of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss, and stimulation of protein, nucleic acid and protective alkaloids (Sharma, 1999). Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions (Roberts *et al*., 1986). Polyamines also protect membranes from oxidative damages as they as free radical scavengers (Besford *et al*., 1993).

2.HYPOTHESIS

For the present study, I select pea plant, because

[A] the research of the poisoning effect of the heavy metal arsenic(As) on plant mainly focuses on food crops such as rice, wheat and maize, but less on pea plants;

[B] Pea which is often as protein feed sources and quality assurance particular in concern with arsenic is not known that's why evidence of presence of heavy metal in pea is still a point of research;

[C] Level of sensitivity of pea for heavy metal is not known for arsenics; by conducting this type of experiment, I will be able to detect level of sensitivity which will be beneficial for plant breeders for development of arsenic tolerant pea.

3.OBJECTIVES

The objectives of my work are to study:

1. The effect of arsenic(As) stress on various morphological, biochemical and yield attributes in pea.

2. The effect of polyamines (putrescine) and mycorrhiza in ameliorating arsenic(As) induced stress in pea.

3. The scavenging capacity of pea plant for arsenic (As) present in the soil.

4.REVIEW OF LITERATURE

Heavy metal is a naturally occurring element which has a huge deterioration effect on the health of the environment and it does not degrade naturally and heavy metals cannot be destroyed biologically, they can only be transformed from one oxidation state or organic compound to another. Remediation of soil contaminated with heavy metals is more difficult than the remediation of other contaminants [De Jing et al. 2007].

Abedin *et al.* (2002) studied the uptake kinetics of arsenic species, arsenite and arsenate, in rice plants and found that arsenate uptake was strongly suppressed in the presence of arsenite.

Ackerson and Krieg (1977) reported that sorghum stomata are relatively less sensitive to Vapor Pressure Deficit as compared with other C4 species like maize.

Akdeniz *et al*. (2006) reported that there was a relationship among Cr, Zn and P concentrations. In the leaf, there was a significant relationship between Ni and Zn, Pb and Cd, but the relation between Mg and Cu concentration was negative. Similarly, there was a significant and positive correlation for P, K, Mg and Ca concentrations in seed. It can be suggested that sewage biosolids application did not cause any significant increase in heavy metal levels in leaf and seed of grain sorghum. Metals have toxic effects on living organisms when they exceed a certain concentration.

Al-Enezi *et al.* (2005) reported that the propagation of heavy metals in biological food chain is one of the important issues of this behavior, as increasing the amount of several heavy metals in higher stages of the food chain is many times more than initial levels

Al-Jaloud *et al.* (1995) reported that, sorghum crop is important to provide livestock forage, and forage health has a direct effect on human health.

Arduini *et al.* (2004) found that changes in root morphology did not affect cadmium translocation to the above ground parts of the plant. The distribution of the Cd in leaves, bulbs and roots of plants differed with the increasing concentration of the cadmium concentration.

Bavi *et al.* (2006) studied that isolated chloroplast of 21 days old plants grown in nutrient solution containing different concentrations of cadmium did not show a significant changes in the rate of Hill reaction.

Bhardwaj *et al.* (2009) studies that higher concentration of Cd is responsible for the complete inhibition of germination and growth also decreased as concentration of heavy metals was increased as compared to control plants.

Bhardwaj *et al.* (2009) reported that along with the growth of plant under increasing concentration of heavy metals, the entire metabolic activity of the plants under metal stress was affected resulting in recued metabolic activity.

Bi *et al.* (2003) reported that, the metal concentration in plants has been shown to be higher or lower compared to the control (non-bioaugmented soil), depending on the metal (Bi *et al.,* 2003 and/or its concentration in the soil. Nevertheless bio-augmentation almost always increases the rate of metal extracted from plants.

Bjerrum's (1959) definition of heavy metals is based upon the density of the elemental form of the metal, and he classifies heavy metals as those metals with elemental densities above 7 g cm^{-3}

Brooks (1998) reported that the hyperaccumulator can accumulate large amounts of element in their above ground biomass from the surrounding soils and tolerate high soil concentration. Therefore, Hyperaccumulator usually contain over 1000 mg kg^{-1} of elements in their above ground biomass, or the enrichment factor (EF) (the ratio of element concentrations in Hyperaccumulator to those in the surrounding soil) should be above one. The most common method to identify hyperaccumlating plants is to collect plant species that are growing in contaminated sites, and determine the concentrations of elements in the biomass.

Caddel and Weibel (1971) reported that under long day condition the number of leaves increased with the increase in temperature. This happening is not common in all but reported in some genotypes.

Cao *et al.* (2007) reported that microbial performances cannot be compared with each other because the methods used to estimate the bioavailable fractions of metals vary from one experiment to other experiment. Indeed several extractants are used such as water, $MgCl_2$, NH_4NO_3, DTPA, KNO_3 and HCl. Bioavailability of metals is even divided into three different pools: immediately soluble metals (with water), exchangeable metal forms (with KNO_3) and complexes or adsorbed metal form (with EDTA).

Chander and Brookest (1991) reported that the when *Scirpus sp.*, *Phragmites karka* and *Bacopa mannieri* were grown in tannery effluent and sludge containing 2.3µg ml^{-1} and 214 mg kg^{-1} Cr, respectively, there was significant reduction in Cr concentrations. While there was an increase in biomass, no visible phytotoxic symptoms were shown by treated plants.

Cho and Kim (2003) Noticed that cadmium has a great mobility in soil as compared with other heavy metals and is easily taken up by roots and is translocated to different pats of the plants.

Clark *et al*, (1968) reported that seed pericarp and tests may contain the germination inhibitor and which lead to the dormancy in the sorghum and when this pericarp was removed from the seed surface then it is observed that germination rate will enhance. Dormancy in seed is associated with ABA because the hormone can be detected in both developing and mature seeds and is proved to be inhibited when applied exogenously. In general dormancy is defined in a functional sense. Seeds that do not germinate given optimal conditions of temperature, water and oxygen are considered dormant, whereas those that do germinate are considered non-dormant.

Cramer and Quarric (2002) reported that excessive concentrations of the various essential micronutrients had reported to affect initiation of the leaf primordial but did not depict any decline in the leaf number of concerned plants.

Da Lin *et al.* (2011) reported some important changes in growth and physiological characteristics of the sorghum plant under cadmium stress, such as height, chlorophyll content, root activities and MDA content. They concluded that Cd effect was the manifestation of oxidative stress. Low concentration of cadmium can promote the growth of hard wheat, while under a relatively high concentration, the growth of wheat and tillering were both inhibited and the

Delavari *et al.* (2010) reported that the analysis of the variance of photosynthetic pigments is shown in salinity.

Devitt *et al.* (1984) reported that sorghum root growth in saline media was repressed in an exponential relationship to a build up in the root.

Eastein (1993) reported that the physiological maturity of the sorghum grain can be established by the dark placental region at the end of the grain

Elobeid et al. (2012) elucidated that Cd treatment caused significantly growth reductions in leaf formation, shoot height growth, stem radial growth and root elongation compared with controls, which significant decrease in plant biomass. However, the growth reductions differed between the different tissues; shoot elongation and leaf formation almost stopped after one week of Cd exposure, whereas stem radial growth was reduced from two weeks of Cd exposure onwards from the beginning.

Falkowski and Raven (2007) observed that chloroplast contains many different parts that respond to heavy metal stress, therefore any changes in chlorophyll synthesis and activity used as the index of the direct toxic effects of heavy metals.

Galavi et al. (2010) conducted an experiment in order to investigate the effect of treated municipal wastewater on soil chemical properties and heavy metal uptake by sorghum.

Galvez and Clark, (1991) reported that silicon mitigates the aluminum toxicity produced in the plants.

Gisbert et al. (2003) reported that the heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades. These metals unlike other inorganic pollutants are not degraded and converted into harmless compounds via biological processes.

Glass (2000) reported that phytoremediation is eco-friendly approach for the removal of contamination of agricultural soils. After the treatment the soil health going to improve and it allows sustaining crop production. If you have considering the economics incurred in the treatment, then these are supposed to be generally cheaper than physical ones.

Haussmann *et al*. (2000) showed that heterosis in yield is well expressed under a wide range of the stress condition. Scientist after exhaustive research reaches to the conclusion that heterosis in grain sorghum productivity is based on unique development events in the formation of the panicle structure and most likely involved in the stability of some important physiological parameters like photosynthesis, and other under diverse environmental conditions.

Izadiyar and Yargholi, (2010) reported that the maximum cadmium accumulation in sorghum was less than the UN standard set of agricultural and nutritional materials which permits at most a daily use of 35 micro-grams of cadmium by human beings, the standard set by the

American Food Industries which permits a daily use of at most 92 micro-grams of cadmium by human.

Jackson (1993) reported that the root may have a role in affecting shoot senescence. He suggests that soil conditions may be sensed by the shoot via. three major endogenous plant hormones, which are produced in the root, such as ABA, Ethylene and Cytokinins.

Jalil *et al.* (1994) reported that low concentration of cadmium can promote the growth of hard wheat, while under a relatively high concentration, the growth of wheat and tillering were both inhibited and the degree varied among different varieties.

Kayser *et al*. (2001) reported that low availability of the metals in the soil solution is the one of the major significant factors for the lower amount of metals extracted by plants from the soil. In general, scientist reported that, they often account for less than 1% of the total metal in soil and the rate of availability is influenced by the several soil physical and chemical characteristics such as pH, CEC and organic matter.

Kennedy and Filippis (1999) reported that the anthocynin pigments increased with salt stress in both salt tolerant and salt sensitive plant.

Krantev *et al.* (2008) reported that the net photosynthesis is also sensitive to Cd because it directly affects chloroplast biosynthesis and the proper development of the chloroplast ultrastructure.

Ku *et al.* (2001) has successfully transformed rice with maize genes encoding two key enzymes of C4 photosynthesis: PEPC (Phosphoenol Pyruvate Carboxylase) and PPDK (Pyruvate Dikinase). Evidence after the extensive research suggests that both PEPC and PPDK play a key role in organic acid metabolism in the guard cells to regulate stomatal opening.

Kumar and Dwivedi (2014) reported that, the Increasing growth of world population along with the expansion of the agricultural and industrial activities for improving supply of nutritional materials and the occurrence of several consecutive years of drought, are the reason why available water resources in most countries located in the dry-region zone have been used with the highest degree; such a condition may naturally exert a great deal of pressure on water resources.

Kumar *et al* (2011) reported that the cadmium (Cd) is a divalent heavy metal cation and is one of the most toxic heavy metals. It is supplied to soil, air and water mainly by effluent from industries, mining burning, leakage waste, by fertilization with phosphate and sewage sludge. Cd is readily taken up by plants, leading to toxic symptoms such as peroxidation of lipids, decreasing the glutamic acid, protein, potassium ion level & chlorophyll content, damaging cell's photosynthetic machinery, inhibiting stomatal opening, increasing aspartic acid concentration and growth.

Kupier *et al.* (2004) reported that a potential alternative option consists of optimizing the synergistic effect of plants and microorganism by coupling phytoextraction with soil bio-augmentation, also called rhizoremediation.

Kusano *et al.* (2008) polyamines including putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and development processes. This is considered as the recent additions to the class of plant growth regulators, and as a secondary messenger in signaling pathways.

Lewis (1993) suggested that forming soap with fatty acids is an important criterion of "heaviness". Thus, again we have no consistent basis for defining the term. Another group of definitions is based on atomic number. A fourth group of definitions is based on other chemical properties, with little in common, density for radiation screening, density of crystals, and reaction with dithizone.

LIU Da-lin *et al.* (2010) reported that the regulatory mechanism of the soil cadmium (Cd) on antioxidants in sorghum was studied using three sorghum species viz., sweet sorghum and sorghum hybrid sudangrass.

Liu *et al.* (2003) reported that the cadmioum (Cd) pollution in agricultural farmland has become a serious problem in many parts of the world, due to industrial development and the heavy use of the chemical fertilizers, pesticides and herbicides, which produce serious thrust to human health through the food chain.

Major *et al.* (1990) proposed the basic concepts for predicting leaf number on the basis of photoperiodic response.

McBridee (1995) heavy metals represented a portion of important environmental pollutants which cause pollution problems by increasing their use in products in recent decades. In spite of gradual accumulation of heavy metals in the soil, the stability of heavy metals in the environment will lead to pollution since they could not be decomposed like organic pollutants by biological or chemical processes.

Miller *et al*. (1968) established the five groups of sorghum genotype on the basis of their critical photoperiodic requirements.

Molla-hoseini *et al*. (2005) reported that the accumulation of cadmium in different plant parts suggests that with a high degree of movement which this metal has in soil, it can be easily absorbed by the root and then transferred to different organs of the plant. It means accumulation of this element in soil and consequently the undesired increase in the amount of this metal in plants in the long term endangers the health of affected plants and consequently, the health of farm animals and humans.

Monterio *et al.* (2009) reported that membrane permeability was assessed by measuring electrolyte leakage. No significant were observed between control and Cd exposed leaves. Cd induced senescence was not significantly reflected in solute leakage.

Nalini and Chandra (2002) reported that heavy metals by inhibiting cell division or decreased cell expansion in the elongation zone or both of them reduce root length.

Ozdener and Kutbay (2011) reported that the chlorophyll a and b contents were decreased under Cd treatment, chlorophyll a and b contents decreased due to increased Cd concentration.

Pal *et al.* (2002) reported that the phytochelatin (PC) levels increased in the roots after 1 days of Cd treatments. The PC synthase activity decreased in all cases compared to the control in the roots.

Parry and Kelso (1975) reported that sorghum accumulates the silicon in various tissues including the root.

Pescord (1992) reported that, the storage of heavy metals severely threaten human health, but due to their long half-life (e.g. 1460 days for lead and 200 days for cadmium), tendency for storing such elements is dramatic.

Premachandra *et al.* (1995) reported that the main solutes accounting for osmotic adjustment are due to sugars and potassium.

Qin *et al.* (2000) heavy metal caused a reduction in plant photosynthesis, thereby reducing the plant water and nutrient adsorption, which affected the normal growth and development of sorghum.

Qin *et al.* (2004) reported that the heavy metal, poisonous effects caused a reduction in plant photosynthesis, thereby reducing the plant water and nutrient absorption, which affected the normal growth and development of plants.

Quinby (1974) developed the F1 hybrid in sorghum through the use of cytoplasmic genetic male sterility system. He also reported the increasing biomass production with little or no effect on harvest index.

Rainbow (1985), reported that the various industries of electroplating, pigments, paints, stainless steel, mine tailing, battery, coal combustion, etc. are continuously causing soil pollution by adding different heavy metals viz. Cd, Cr, Pb, Ni, Zn, Cu, etc.

Sandalio *et al.* (2001) reported that the chloroplast the major component of photosynthesis organ is highly sensitive to damage exposed Cd toxicity.

Schmidit (2003) reported that elevated heavy metal concentrations in the soil can lead to enhanced crop uptake and negative effect on plant growth.

Senthil *et al,* (2003) reported that the increased chlorophyll content in the green gram plants when sprayed with 40 ppm of NAA at flowering stages.

Serafini-Fracassini and Mossetti (1986) reported that PAs are associated with cell wall polysaccharides or membranous fraction indicating thereby their prospects for a function in formation and anchoring of the plant cell wall polysaccharides, or in coupling membrane proteins in cytoplasmic structural protein. Several reports indicated that the response to abiotic injury and mineral nutrient deficiency is associated with the production of conjugated Pas.

Sharma (1998) increased polyamines level in stressed plants have adaptive significance because of their involvement in the regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss and stimulation of protein, nucleic acid and protective alkaloids.

Sharma *et al.* (2010) reported that the higher the magnitude of reduction in photosynthesis rate in increasing concentration of Cd is caused by inhibition of PSII activities.

Singh *et al.* (2003) showed that both growth and photosynthetic pigmentation system are affected by the presence of heavy metals.

Souza Rauser (2003) reported that plants are seldom exposed in nature to the effect of a single heavy metal since excess metal ions exist mostly in mixtures in various polluted soils and irrigation water worldwide.

Subba Rao (1982) the biofertilizers means the input of plant nutrients of biological origin for the growth of plants. Biofertilizers are also known as 'microbial inoculants' or 'microbial preparations'. They themselves do not increase the soil fertility directly, but instead they exercise their biological effects on improving the nutritional conditions. This has led to the mass production of *Rhizobium*, *Azotobacter*, *Cyanobacteria*, *Azospirillum*, phosphate solublizing bacteria and VAM.

Takeda *et al,* (1983) reported that the polyamines are basic in nature, having positive charge at physiological pH. They have been shown to bind strongly *in vitro* to negatively charged nucleic acids acidic phospholipids and types of enzymatic and non enzymatic proteins whose activity can be affected by the presence of polyamines. These interactions of ions are important for maintenance of the biological function of cells under *in vivo* condition. As in the case of other plant growth regulators, the polyamine pool depends on its [a] synthesis; [b] oxidative damage; [c] conjugation and transport. PAs transport is the energy dependent process and calcium is involved in the corresponding mechanisms.

Tomsett and Thurman (1986) reported that the hyper-accumulation is an eco-physiological adaptation of plants to metalliferous soils. The mechanism of metal accumulation, which involves extra-cellular and intracellular metal chelation, precipitation, compartmentalization and translocation in the vascular system, are poorly understood.

Wilson (1975) reported the negative association between grain number per panicle and weight per gram in sorghum.

Wilson (1988) reported that the effect of the most common environmental factors, such as water, available nutrients, perception of light and ambient CO_2 concentration, root growth can be explained reasonably well by its relationship to shoot growth

Yang *et al.* (2005) researched the effect of sewage irrigation accelerated the decline of wheat seedlings and root, reducing the root number and the root activities significantly.

Yusuf *et al.* (2011) Suggested that a different pattern of response was observed for the marker of oxidative stress, i.e., lipid perioxidation and H_2O_2 content and electrolyte leakage.

Zengin and Munzuroglu (2005) reported that the heavy metals inhibit metabolic processes by inhibiting the action of enzymes and this may be the most important causes of inhibition. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for the chlorophyll biosynthesis. Cadmium was reported to affect chlorophyll biosynthesis and inhibit protochlorohyll reduction.

Zornoza *et al.* (2002) reported that alter the plant's biochemical, metabolic functions, and pathways and also affects their physiological functions such as plant growth, development, cell differentiation, elongation, photosynthesis, nutrition, water transport, respiration, absorption of minerals from the soil.

Van Nostrand's International Encyclopedia of Chemical Science 1964 and in 1987, the editors of Grant and Hackh's Chemical Dictionary included metals with a density greater than 4 g cm^{-3}.

5. METHODOLOGY

The pot experiment will be conducted in the poly house of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab with one genotype of pea Arkel. Pea genotype will be taken from Punjab Agriculture University, Punjab. Pot size for the experiment will be diameter: 30 cm and height 25 cm. Heavy metal stresses will be created in plant by exogenous application of arsenic in soil. One best concentration after initial screening within the range of 1-100 ppm of Arsenic will be finally selected. There is one concentration of heavy metals (after screening), will be applied in soil for creating stress in pea plant. Putrescine will be applied at the rate of 1.50 mM and 3.0 mM through foliar application. The various measurements will be made at three stages such as 30 DAS, 60 DAS and 90 DAS.

5.1 EXPERIMENTAL DETAILS

1. Genotype: Arkel

2. No. of treatment

a. Control

b. Arsenic(As) concentration 1

c. Endomycorrhizal fungi (AMF), *Glomus* species

d. Putrescine 1(1.50 mM)

e. Putrescine 2 (3.0 mM)

f. Arsenic concentration 1 + Putrescine 1

g. Arsenic concentration 1 + Putrescine 2

h. Arsenic concentration1 + Endomycorrhizal fungi (AMF), *Glomus* species

i. Arsenic concentration 1 + Putrescine 1(1.50 mM) + Endomycorrhizal fungi (AMF), Glomus species

j. Arsenics concentration 1 + Putrescine 2 (3.00 mM) + Endomycorrhizal fungi (AMF), *Glomus* species

3. Replication: Three

4. Treatment: 10

5. Total number of pots: 10 x 3= 30

6. Design: CRD

6. OBSERVATIONS TO BE RECORDED

The various measurements will be made at three stages such as 30 DAS, 60 DAS and 90 DAS.

6.1 Morpho-physiological parameters

a. Germination percentage (for screening)

b. Plant Height (cm)

c. Leaf area (cm x cm)

d. Leaf number

e. Days to 50% anthesis

f. Days to maturity

g. Internodal length (cm)

h. Stem girth (cm)

6.2 Biochemical parameters

a. Chlorophyll content (Arnon, 1949)

b. Total soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

c. Total soluble protein (Bradford method, 1976)

d. Lipid peroxidation (Malondialdehyde-MDA) (Hodges *et al*., 1991)

e. Catalase (EC. 1.11.1.6) (Teranish *et al*., 1974)

f. Membrane stability (Sullivan method, 1972)

g. Phenylalanine ammonia lyase (EC. 4.3.1.5) (Brueske, 1980)

h. Superoxide dismutase (SOD) (EC. 1.15.1.1) (Dhindsa *et al*., 1981)

i. Transportable ratio (Using Atomic absorption spectrophotometer in different parts of plant including developing seeds).

6.3 Anatomical features

Transverse section of root to study mycorrhizal colonization (Microscopic conventional method)

6.4 Yield and quality attributes

1. Number of pods

2. Number of seeds per pod

3. Test weight

4. Seed soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

5. Seed total protein content (Bradford method, 1976)

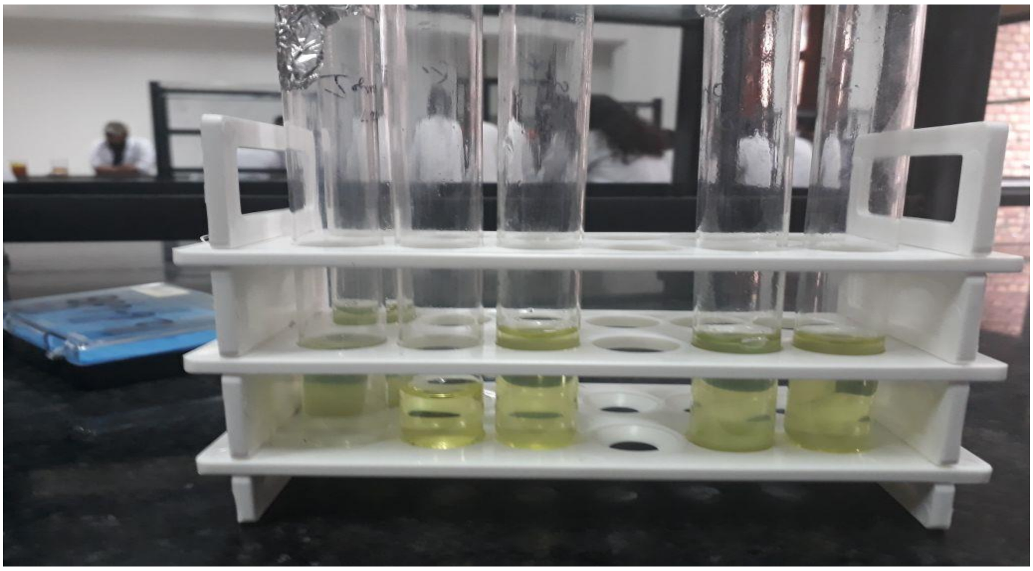
6.5 Screening test

Biochemical observation

a. Estimated the Total Soluble sugar (Anthrone method)(in Seeds)

S.No.	Blank reading	0.000
1	R1	0.517
2	R2	0.482
3	R3	0.280

Figure 1: Total Soluble Sugar



(Source: Photograph by P. Swaraj, 2018, unpublished)

b. Estimated the Total Soluble Protien (Bradford method)

S.No.	Blank reading	0.000
1.	R1	0.507
2.	R2	0.407
3.	R3	0.537

c. Estimated the Total Lipid (Sadashivam and Manickyam)

Weight of Petri plate(X1) -7.56g

Weight along with sample(X2) -11.51g

X2-X1=11.51-7.56=3.96g of fatty acids per 1g of pea seed powder

Since Pea is a *Rabi* season crop during this semester I have done a screening test for determination of lethal concentration of Arsenic in laboratory by using Arsenic trioxide in small cups with different concentrations from 10 ppm to 100 ppm.

d. Seed germination

Number of germinated seeds

S.No.	AsO_3(ppm)	DAS-4	DAS-5	DAS-6	DAS-7
1.	**Control**	3	5	8	9
2.	**10**	1	2	3	3
3.	**20**	1	3	3	5
4.	**30**	1	1	3	4
5.	**40**	2	2	3	4
6.	**50**	1	1	3	5
7.	**60**	1	3	3	3
8.	**70**	1	1	2	5
9.	**80**	1	2	4	5
10.	**90**	0	2	3	5
11.	**100**	0	1	3	4

Figure 2: Germination of Pea seeds

(Source: Photograph by P. Swaraj, 2018, unpublished)

e. Morphological characteristics

Sl. No.	Concentration (ppm)	Plant height(cm)	Leaves number	Branches number
1.	**Control**	15	15	8
2.	**10**	12.3	15	7
3.	**20**	11.3	13	7
4.	**30**	10.6	12	7
5.	**40**	10.5	12	6
6.	**50**	10.2	11	5
7.	**60**	9.8	12	5
8.	**70**	9.5	10	3
9.	**80**	9.3	11	4
10	**90**	9.1	9	3
11.	**100**	8.6	8	4

Figure 3: Morphological characters

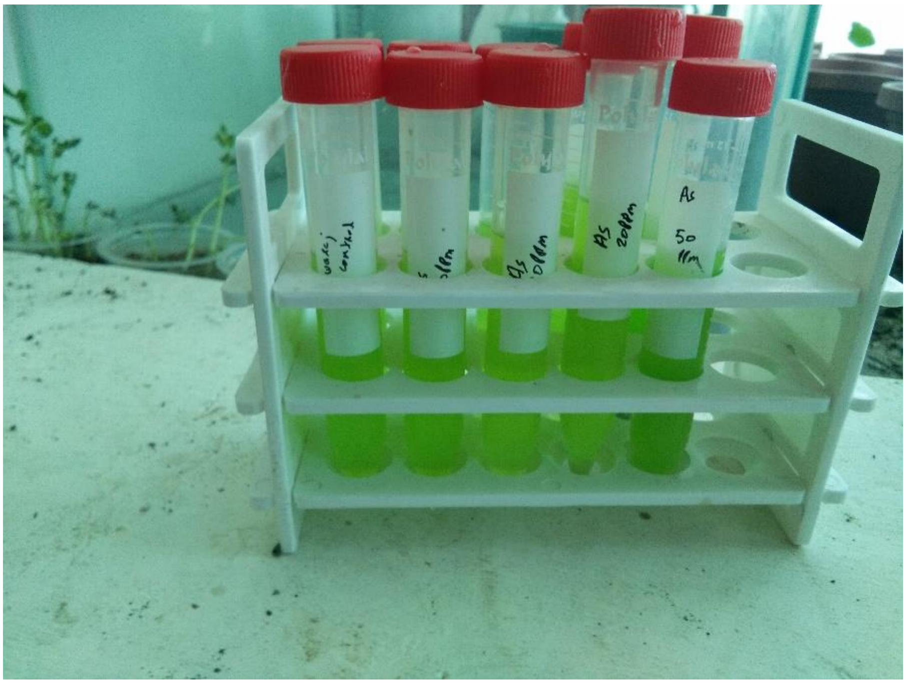
(Source: Photograph by P. Swaraj, 2018, unpublished)

f. Estimated the chlorophyll content by Arnon DI method.

Chlorophyll content (mg/g fresh weight):

TREATMENTS (AsO_3)(ppm)	OPTICAL DENSITY					
	663nm	645nm	410nm	480nm	510nm	590nm
Control	0.644	0.623	0.729	0.375	0.057	0.140
10	0.401	0.406	0.535	0.258	0.044	0.065
20	0.564	0.557	0.727	0.356	0.056	0.087
30	0.487	0.495	0.478	0.423	0.489	0.478
40	0.447	0.504	0.715	0.387	0.046	0.089
50	1.279	1.248	1.489	0.850	0.121	0.195
60	1.110	1.232	1.785	1.452	1.235	1.495
70	0.601	0.590	0.761	0.380	0.049	0.093
80	0.024	0.648	0.089	0.642	0.452	0.015
90	0.533	0.536	0.754	0.367	0.048	0.089
100	0.450	0.477	0.679	0.351	0.041	0.081
Reference	0.000	0.000	0.000	0.000	0.000	0.000

Figure 4: Estimation of Chlorophyll



(Source: Photograph by P. Swaraj, 2018, unpublished)

7. PLAN OF WORK

1st Year

(a) Morpho-physiological parameters

(b) Determination of biochemical parameters

(c) Anatomical features

(d) Yield and quality attributes

2nd Year

The experiment conducted in 1st year will be repeated during the IInd year.

CONCLUSION

Higher the concentration of the Arsenic more is the deteriortation effect it has shown. At100ppm concentration of AsO_3 the plant showed less germination as well as the chlorophyll content measured were seen to be low in 90ppm and 100ppm compared to other AsO_3 concentration to other AsO_3 concentration. So the experiment will be conducted using 100ppm in pot cultivation.

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