

EVALUATION OF PHYTOTOXICITY OF BIOINOCULANTS TREATED ZEA MAYS

Submitted in partial fulfillment of the requirements for the award of Bachelor
of Science degree in Biotechnology

by

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INSTITUTE OF SCIENCE AND TECHNOLOGY

**(DEEMED TO BE UNIVERSITY)
Accredited with Grade "A" by NAAC**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of AZHAGESAN S (38770001) who carried out the project entitled "Evaluation of phytotoxic stress of bio-inoculants treated zeo mays L." under our supervision from October 2020 to March 2021.

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I AZHAGESAN S (38770001) hereby declare that the Project Report entitled “EVALUATION OF PHYTOTOXIC STRESS OF BIO-INOCULANTS TREATED ZEA MAYS” done by me under the guidance of Dr. VALLI NACHIYAR and Dr. KARTHIK RAJA at SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY, CHENNAI is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

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ABSTRACT

Bio-inoculants are living micro-organisms contains strains of specific bacteria, fungi or algae which absorbs nitrogen from the atmosphere and keeps it available to the maize plants resulting in reduced need of nitrogen fertilizers. when applied to seeds they solubilize the inorganic phosphate and micro nutrients to increase seed fertility. Bio-inoculants promotes the plant uptake of phosphorus and zinc. The use of microbial inoculants holds a great promise to improve crop yield without affecting environmental hazard related with chemical fertilizers.

Germination is the process by which a plant grows from a seed under the ideal conditions of light, temperature, moisture, oxygen and nutrients. Bio-inoculants directly affects the seed's vitality, maturation and dormancy. Imbibition is the process of uptake of water by seeds. Generally, maize seeds require darkness to germinate. Phytochrome is a plant pigment found in cytoplasm that senses the presence of red light and induces seed germination. Oxygen in the moisture causes respiration to start metabolism. The germination percent of most seeds will be retarded if the oxygen percent goes below 20 percent. Maintaining the optimal temperature leads to ideal germination of the seed between 24 to 32°C

Therefore, this study is focused on the estimation of total chlorophyll content which quantitate the amount of chlorophyll, a green colored pigment which is responsible for photosynthesis to produce carbohydrate from water and carbon di oxide. Another parameter to analyze is assay of anti-oxidants which helps the plants to tolerate the physicochemical stress. Glutathione, an anti-oxidant present in plants capable of preventing damage to the cellular components caused by reactive oxygen such as free radicals.

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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
OD	Optical Density
GSH	Glutathione
DTNB	5,5'-Dithiobis (2-nitrobenzoic acid)
DMSO	Dimethyl sulfoxide
CDNB	1-chloro-2,4-dinitrobenzene
Chlo-a	Chlorophyll a
Chlo-b	Chlorophyll b
DAP	Diammonium Phosphate
CM	Concentrated Metabolites
RPM	Revolutions Per Minute
PLFA	Phospholipid Fatty Acid

CHAPTER 1

INTRODUCTION

1.1 BIO-FERTILIZERS

Biofertilizers are ready to use live formulates of such beneficial microorganisms which on application to seed, root or soil mobilize the availability of nutrients by their biological activity in particular, and help build up the micro-flora and in turn the soil health in general. They promote growth by increasing the supply or availability of primary nutrients to the host plants through the natural process of nitrogen fixation, solubilizing phosphorus and stimulating plant growth by synthesis of growth promoting substances. Use of bio-fertilizer is recommended for improving the soil fertility in organic farming.

1.2 NECESSITY OF BIO-FERTILIZERS

Increased usage of chemical fertilizer leads to damage in soil texture and raises other environmental problems. Therefore, the usage of bio fertilizers become economic and environment friendly. The pragmatic approach will be to develop the integrated nutrient supply system involving a combination of the use of chemical fertilizers and bio-fertilizers. Moreover, India is not self-sufficient in fertilizer production. Realizing the importance of biofertilizers in supplementing the use of chemical fertilizers, the Government of India had launched the “National Project on Development and use of biofertilizers” with its one national and six regional centers renamed as National Centre of Organic Farming (NCOF). With the introduction of green revolution technologies, the modern agriculture is getting more and more dependent upon the steady supply of chemical inputs (mainly fertilizers), which are products of fossil fuel (coal+ petroleum). Adverse effects are being noticed due to the excessive and imbalanced use of these synthetic inputs. This situation has led to identifying harmless inputs like biofertilizers. Use of such natural products like biofertilizers in crop cultivation will help in safeguarding the soil health and also the quality of crop products.

1.3 CLASSIFICATION OF BIO-FERTILIZERS

Biofertilizers like Rhizobium, Azotobacter, Azo spirillum, blue green algae (BGA), Phosphate solubilizing micro-organism (PSM) are in use since long. These organisms fix atmospheric nitrogen and supply it to plants. Following are the important type of bio-fertilizers. Symbiotic Nitrogen Fixing Bacteria E.g., Rhizobium. It is one of the vital symbiotic nitrogen fixing bacteria. This bacterium obtains food and shelter from host plants. In return, they help by fixing atmospheric nitrogen to plants. Loose Association of Nitrogen Fixing Bacteria for example, Azospirillum is a nitrogen fixing bacteria that live around the roots of higher plants. It is often termed as rhizosphere association as these bacteria collect plant exudate and the same is used as a food by them. This process is known as associative mutualism. Symbiotic Nitrogen Fixing Cyanobacteria for example lichens are some of the nitrogen-fixing cyanobacteria. Free Living Nitrogen Fixing Bacteria for example Azotobacter and *clostridium beijerinckii*. Phosphate Solubilizing Microorganism for example pseudomonas. Phosphorus is one of the essential nutrients for plants growth and development. They hydrolyze insoluble phosphorus compounds to soluble form for uptake by plants.

1.4 AVAILABLE BIOFERTILIZERS FOR PLANTS

- **For Nitrogen**
 - Rhizobium for legume crops.
 - Azotobacter/AZ spirillum for non-legume crops.
 - Acetobacter for sugarcane only.
 - Blue –Green Algae (BGA) and Azolla for low land paddy.
- **For Phosphorous**
 - Phospha tika for all crops to be applied with Rhizobium, Azotobacter, AZ spirillum and Acetobacter
- **For enriched compost**
 - Cellulolytic fungal culture
 - Phospha tika and Azotobacter culture

1.5 ROLE OF BIO-FERTILIZERS

- Makes availability of nutrients
- Produce the root rhizosphere more lively
- Produce growth promoting substances
- More root proliferation
- Improve quality and quantity of yield
- Better germination of seeds
- Improve soil health
- Residual effect
- Improve fertilizer use efficiency
- Restore natural soil fertility.
- Provide protection against drought and some soil
- Improves water holding capacity of soil

1.6 ADVANTAGES OF BIO-FERTILIZERS

- Cost effective.
- Increase crop yield by 20-30%
- Replace chemical nitrogen and phosphorus by 25%
- Eco-friendly
- Reduces the costs towards fertilizers use, especially regarding nitrogen and phosphorus.

1.7 METHODS TO APPLY THE BIOFERTILIZERS

- **SEED TREATMENT:** 200 g of nitrogenous biofertilizer and 200 g of Phosphatika are suspended in 300-400 ml of water and mixed thoroughly. Ten kg seeds are treated with this paste and dried in shade. The treated seeds have to be sown as soon as possible.
- **SEEDLING ROOT DIP:** For rice crop, a bed is made in the field and filled with water. Recommended biofertilizers are mixed in this water and the roots of seedlings are dipped for 8-10 hrs.

- **SOIL TREATMENT:** 4 kg each of the recommended biofertilizers are mixed in 200 kg of compost and kept overnight. This mixture is incorporated in the soil at the time of sowing or planting.

1.8 COMMONLY PRODUCED BIO-FERTILIZERS IN INDIA

Name	Crops suited	Benefits usually seen	Remarks
Rhizobium strains	Legumes like pulses, groundnut, soybean	10-35% yield increase, 50-200 kg N/ha.	Fodders give better results. Leaves residual N in the soil.
Azotobacter	Soil treatment for non- legume crops including dry land crops	10-15% yield increase- adds 20-25 kg N/ha	Also controls certain diseases.
Azospirillum	Non-legumes like maize, barley, oats, sorghum, millet, Sugarcane, rice etc.	10-20% yield increase	Fodders give higher/enriches fodder response. Produces growth promoting substances. It can be applied to legumes as co-inoculant
Phosphate Solubilizers (there are 2 bacterial and 2 fungal species in this group)	Soil application for all crops	5-30% yield increase	Can be mixed with rock phosphate.
Blue-green algae and Azolla	Rice/wet lands	20 -30 kg N/ha, Azolla can give	Reduces soil alkalinity, can be

		biomass up to 40-50 Tonnes and fix 30-100 kg N/ha	used for fishes as feed. They have growth promoting hormonal effects.
Mycorrhizae (VAM)	Many trees, some crops, and some ornamental plants	30-50% yield increase, enhances uptake of P, Zn, S and Water.	Usually inoculated to seedlings.

1.9 TIPS TO GET GOOD RESPONSE TO BIOFERTILIZER APPLICATION

- Biofertilizer product must contain good effective strain in appropriate population and should be free from contaminating microorganisms.
- Select right combination of biofertilizers and use before expiry date.
- Use suggested method of application and apply at appropriate time as per the information provided on the label.
- For seed treatment adequate adhesive should be used for better results.
- For problematic soils use corrective methods like lime or gypsum pelleting of seeds or correction of soil pH by use of lime.
- Ensure the supply of phosphorus and other nutrients.

1.9.1 PRECAUTIONS TO TAKE WHILE USING BIOFERTILIZERS

- Biofertilizer packets need to be stored in cool and dry place away from direct sunlight and heat.
- Right combinations of biofertilizers have to be used.
- As Rhizobium is crop specific, one should use for the specified crop only.
- Other chemicals should not be mixed with the biofertilizers.

- While purchasing one should ensure that each packet is provided with necessary information like name of the product, name of the crop for which intended, name and address of the manufacturer, date of manufacture, date of expiry, batch number and instructions for use.
- The packet has to be used before its expiry, only for the specified crop and by the recommended method of application.
- Biofertilizers are live product and require care in the storage
- Both nitrogenous and phosphatic biofertilizers are to be used to get the best results.

CHAPTER 2

LITERATURE OF SURVEY

Prasanna et al, (2016), tested interactions between ten maize hybrids (Prakash, HM4, HM8, HM9, HQPM1, HQPM5, PEHM7, PEHM3, PEHM5 and VH27) and five cyanobacteria-based inoculants with a control to identify the effective combinations for developed soil microbial activities. During the phospholipid fatty acid-based analysis of particular soil sample, the concentration of PLFAs were calculated to the range from 163 to 398 nmol g⁻¹. Finally, the concluded that the hybrid of Prakash and Anabaena-Trichoderma biofilm was the effective combination with 30% of higher yields than all other combinations.

According to Hussain et al, (2014), the field trail was conducted on three rhizobacteria, *Bacillus megaterium* (K2), *Bacillus subtilis* (K4) and *Bacillus sp.* Cph6 (K6) varying in phosphate solubilization, ACC deaminase activity and auxin production were determined for shoot and root growth, yield and phosphate intake from different sources (control, rock phosphate and DAP) by maize plant. Phosphate solubilization potential increased from K2 to K6 (from 180 to 698 $\mu\text{g mL}^{-1}$) while auxin production potential decreased from 48.1 to 8.2 $\mu\text{g mL}^{-1}$. In spite of this variations, *Bacillus sp.* Cp-h6 showed higher results than other two *Bacillus sp.* Among these three phosphate sources, rock phosphate was more effective. In pot study, shoot length of maize plants were increased from 89.8 to 125 cm with DAP (39% increase) while it increased from 49 to 97.7 cm (99.9% increase) with rock phosphate. In conclusion, rock phosphate could be effectively used as phosphate source with these bioinoculants, particularly *Bacillus sp.* Cp-h6 for field application to improve yield and quality of maize kernels.

Trivedi et al, (2007), investigated the colonization and survival of the inoculated bacteria in rhizosphere of maize in field and pot experiments under rainfed conditions of Himalayan region for 3 years. *Bacillus megaterium*, *Bacillus subtilis* and *Pseudomonas corrugata* were used for this experiment. All these three bacterial inoculations resulted in improved grain yield of maize up to 122.4%, 135.2% and 194.3% respectively, as compared to respective control in the first year.

So, the *Pseudomonas corrugata* was chosen for next two years of experiments. As expected, *P. corrugata* resulted in increased grain yield up to 147.28% and 149.93% in 2nd and 3rd respective years. As *P. corrugata* improved the colonization, survival and stimulation of indigenous microflora in the rhizosphere, suggested as effective bioinoculant for maize under rainfed conditions.

Phosphate-solubilizing bacteria (PSB) enhance the plant nutrition by solubilizing insoluble phosphorus in soil to induce plant growth of both leguminous and non-leguminous crops. In this study, two promising PSB strains *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 were estimated for their potential to improve plant growth and nutritional status of Mung bean and maize crops. The experiment was conducted in pots under greenhouse. This co-inoculation of bacterial strains also increased optimal plant growth and nutritional value of Mung bean and maize crops by increased nitrogen (N) concentration up to 142 and 18%, phosphorus (P) concentration up to 90 and 43%, and potassium (K) concentration up to 71 and 44%, in shoots of Mung bean and maize crops, respectively. Thus, the tested PSB strains *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 have the potential to be used as promising bio-inoculants to maximize plant growth and nutritional enhancement of the crop for sustainable production. Ahmad et al, (2019)

In this study, the effects of concentrated metabolites (CM) were evaluated from *Rhizobium tropici* CIAT 899T (RT1) at two concentrations of active compounds (10⁻⁸ and 10⁻⁹ M) on the performances of plant-microbe interaction of *Azospirillum brasilense* for maize (*Zea mays* L.). For maize, three field experiments were performed to examine the effects of CM from RT1. The best result was obtained with the addition of CM-RT1 at 10⁻⁹ M, increasing grain yield by an average of 11.4%. The result exhibits the biotechnological potential of using secondary metabolites of rhizobia together with inoculants containing rhizobia to improve the growth and yield of crops. Nogueira et al, (2013)

To investigate the crop yield, soil fertility and economic returns, Kaur et al, (2015) conducted a two-year field study on the effects of two phosphate-solubilizing bacteria (PSB), *Pantoea cyripedii* (PSB-3) and *Pseudomonas plecoglossicida* (PSB-5), inoculated singly or together with rock phosphate (RP) fertilization on maize and wheat cropping cycle by comparing with chemical P fertilizer (diammonium phosphate, DAP). Compared to the other treatments, inoculation of PSB together with RP increased the crop growth in terms of shoot height, shoot and root dry biomass, grain yield and total P uptake in both maize and wheat crops. These results suggested that PSB inoculation along with RP fertilization would be an appropriate substitute for chemical phosphate fertilizer application in sustainable agriculture systems.

Azospirillum are prominent plant growth-promoting rhizobacteria (PGPR) extensively used as Phyto stimulatory crop inoculants. Three prominent *Azospirillum* strains as part of three-component consortia including also the PGPR *Pseudomonas fluorescens* F113 and a mycorrhizal inoculant mix composed of three *Glomus* strains were compared. By quantitative PCR, inoculant colonization of maize was evaluated. Then, transcription analysis of auxin synthesis gene *ipdC* in *Azospirillum* was done by RT-PCR and the effects on maize were examined by secondary metabolic profiling and shoot biomass measurements. Unexpectedly, the presence of *Azospirillum* in the inoculum resulted in lower Phyto stimulation in comparison with the *Pseudomonas*–*Glomus* two-component consortium, but this effect was transient. Results showed that there is no negative impact on the prevalence of mycorrhizal taxa in roots. In conclusion, *Azospirillum*–*Pseudomonas*–*Glomus* three-component inoculants may be useful in agricultural biotechnology for enhancing maize growth. Olivier et al, (2013)

Feng et al, (2019) conducted a study to determine the positive effects of potassium-solubilizing bacteria and photosynthetic bacteria on the salt tolerance of maize. They selected three maize inbred lines USTB-265 (salt-sensitive), USTB-109 (moderately salt-tolerant) and USTB-297 (salt-tolerant) to investigate their growth characteristics, enzyme activity and gene expression in response to inoculation with photosynthetic bacteria and potassium-solubilizing bacteria under salt-stress conditions. These bacterial inoculations significantly enhanced the

expression of antioxidant enzyme-related genes and increased the activities of the antioxidant enzymes superoxide dismutase, catalase and ascorbate peroxidase. While the effects of these bacteria differed among the three maize lines, they concluded that the both photosynthetic bacteria and potassium-solubilizing bacteria can enhance salt tolerance in maize.

Maize was co-inoculated with *Herbaspirillum seropedicae* and humic acid resulted in the increased sizes of plant-associated bacterial populations and improved grain yields under invitro and in vivo conditions. While plant-bacterial interactions in the rhizosphere, Root exudation is the prominent mechanism. Humic acid exudate the H⁺ ions and organic acids from maize rhizosphere. Analysis of root exudates was done by gas chromatography-mass spectrometry (GC-MS) and proton nuclear magnetic resonance (¹H NMR). Finally, they concluded that the seedlings treated only with humic acids has increased fatty acid exudation and the seedlings only treated with *H. seropedicae* has increased exudations of nitrogenated compounds and terpenes which improves bioinoculant formulations to achieve high crop yield. Livia da silva et al, (2014)

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

The aim of the study is to evaluate the phytotoxic stress of bio-inoculants treated zeamays.

3.2 OBJECTIVES

- To inoculate the bacterialized maize seed
- To observe the plant growth promotion
- To evaluate the total chlorophyll content
- To quantitate the anti-oxidative enzymes

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 EQUIPMENTS REQUIRED

- Maize seeds
- Conical flask
- Tap water
- Selected bio fertilizer (*Azospirillum brasillense*)
- Urea
- Tissue papers
- Freezer
- Blender
- Water bath
- Centrifuge
- Cuvette tubes
- Test tubes
- Uv spectrometer
- Shaker incubator
- Buffer solutions

4.2 METHODOLOGY

4.2.1 SEED TREATMENT

The healthy maize seeds were taken for seed treatment. Maize seeds were washed with tap water and air dried. Then, three groups of seeds were taken as S1= biofertilizer used seeds, S2= chemical fertilizer used seeds, S3= control (normal seeds). S1 seeds were imbibed in azospirillum solution at the ratio of 10g ml⁻¹. S2 seeds were imbibed in urea solution at the ratio of 10g ml⁻¹. S3 seeds were imbibed in normal water at the ratio of 10g ml⁻¹. These soaked seeds were kept in a dark place for 16 hours. After 16 hours, the seeds were air dried and sown in the field. The growth of the plants was monitored at the regular interval of time.

4.3 SAMPLE PREPARATION

Leaves were collected from 30 days old S1, S2, S3 plants. Then they were washed with distilled water and wiped with cotton tissue paper to avoid excess moisture. Sample was packed in a polythene cover and finally it was kept in an ice cooler box.



Fig 4.1

4.4 ESTIMATION OF PHOTOSYNTHETIC PIGMENT

Chlorophyll a and b contents were extracted from respective dose of leaves and estimated according to the method of Arnon (1949). Two hundred mg of fresh young leaves were ground with 10ml of 80 per cent acetone Pestle mortar and spun at 2500 rpm for 10 min at 20⁰ c in centrifuge. The homogenate was re-extracted with 80 per cent acetone until the green color was disappears in the residue and the extract was pooled and made up to 20 ml with 80 per cent acetone. Two ml of extract was transferred into a cuvette and the absorbance/ optical density (OD) was read at 663, 645 in a spectrometer (Elico Made) against 80 per cent acetone as a blank, to determination chlorophyll a and band carotenoids content.

Chlorophyll content was calculated using the formula of Arnon (1949).

Chlorophyll 'a' (mg/g f wt) = $(1.27) \times (A.663) - (0.269) \times (A.645)$

Chlorophyll 'b' (mg/g f wt) = $(2.29) \times (A.645) - (0.468) \times (A.663)$

Total chlorophyll (mg/g f wt) = $(2.02) \times (A.645) + (0.802) \times (A.663)$

The spectrum graph for chlorophyll Pigment observed from 200nm to 800nm with blank 80% acetone.

4.5 ANTI OXIDANT ASSAY

4.5.1 ESTIMATION OF REDUCED GLUTATHIONE (GSH)

Estimation of reduced glutathione was done according to method of Jallow et al. With slight modification plant sample homogenate (500μL of 0.1M potassium phosphate buffer (pH7.4)) was precipitated by the inclusion of 4% sulfosalicylic acid (500μL). After keeping for 1hr at 4°C, the samples were centrifuged for 20min at 1200 rpm. The supernatant (33μL) was gathered and added to aliquots consisting of 900μl of 0.1M potassium phosphate buffer (pH7.4) and 66μl of 10M (Dithiobis 2-nitrobenzoic acid) (DTNB). GSH reacts with DTNB and produces a yellow-colored complex. Absorbance was taken at 412 nm within 10min 100μl of extract was treated as above and the absorbance was taken at 412 nm. Blank tubes having all the reagents except glutathione solution and the extract were also included.

4.5.2 GLUTATHIONE S-TRANSFERASE ASSAY

30 mg of each sample was weighed and dissolved in 3ml of DMSO solution and mixed well. This extract was further used. A 2ml of phosphate buffer (pH7.4) and 2ml of plant extract were added to blank and sample wells, respectively. Reaction was initiated by adding 1ml of CDNB (1mM/ml) to both the wells and mixed well. The absorbance was read at 340nm. The extinction coefficient for CDNB is $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ at 340nm. Protein was estimated using Bradford's assay.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 LAB ANALYSIS OF MAIZE PLANT

The maize plant was grown in Madathukulam and was processed at VIRTIS BIOLABS, Salem.



Fig 5.1



Fig 5.2



Fig 5.3

5.2 ESTIMATION OF PHOTOSYNTHETIC PIGMENT

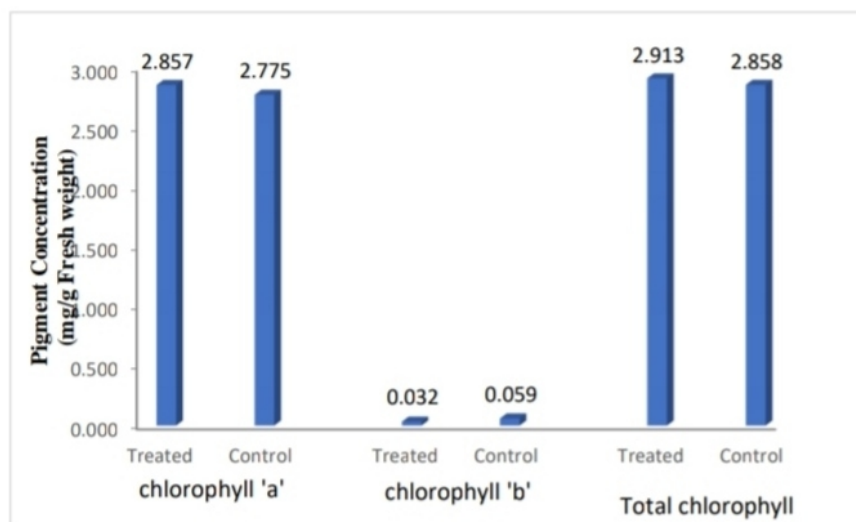


Fig 5.4

The photosynthetic pigment such as chlorophyll a, b and total chlorophyll content were varied in treated plant compared to control plant. The total chlorophyll and chlorophyll a were increased in treated plant compared to control plant, whereas chlorophyll b was decreased in treated plant compared to control plant.

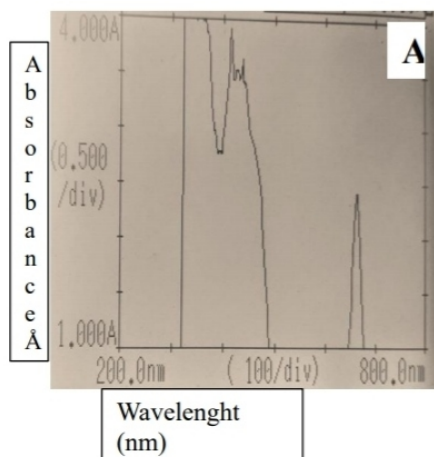


Fig 5.5 Control

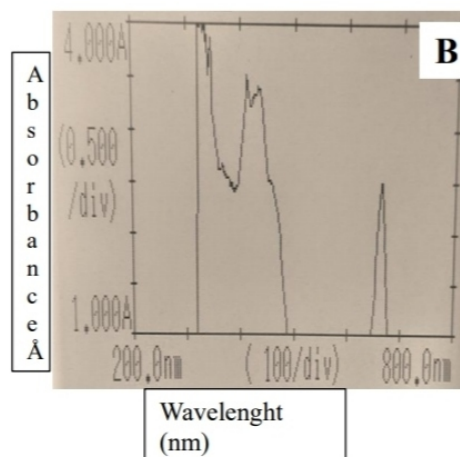


Fig 5.6 Treated

The first absorption peak was observed at 324nm for control plant sample and at 340nm for treated plant sample, whereas the highest absorption peak was observed at 324nm for control plant sample and 340nm for treated plant sample. The lowest absorption was peak was observed at 618nm for both control plant sample and treated plant sample.

5.3 ANTI-OXIDANT ASSAY

5.3.1 ESTIMATION OF REDUCED GLUTATHIONE

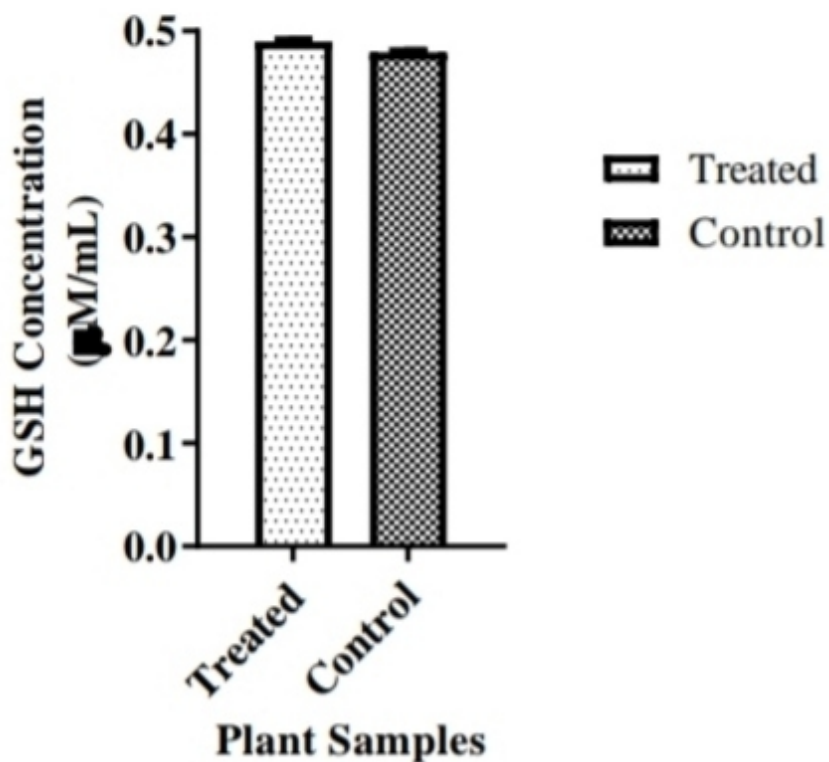


Fig 5.7

The content of glutathione among treated and untreated maize plant's values are expressed as mean ($\pm$ SD). The graphical representation shows the different GSH content in treated group compared to control group, these clearly indicate that there were increased GSH content in the treated group compared to control group.

5.3.2 GLUTATHINE S-TRANSFERASE ASSAY

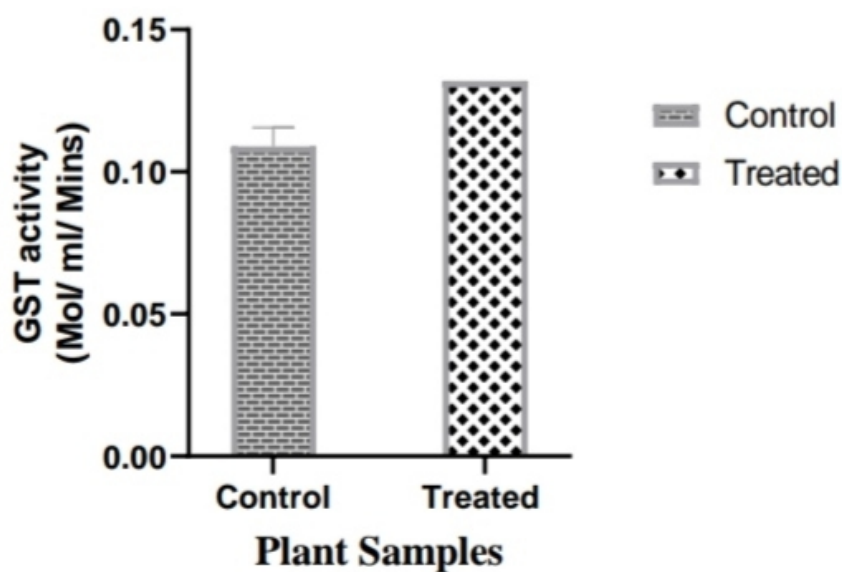


Fig 5.8

The total content of GST activity was performed amid the treated and untreated plant sample values are expressed as mean ($\pm$ SD) analyzed. The graphical representation shows the different GST activity in treated group compared to control group, these clearly indicate that there was increased GST activity in the treated group compared to control group.

DISCUSSION

Bio-inoculants are an effective alternative of synthetic fertilizers due to their environmental and agricultural benefits. Among the various bio-inoculants, Azospirillum is an important biofertilizer used in agriculture for its significant nitrogen fixation efficacy. In this study, seed inoculation of Azospirillum on maize and its impact on various growth promoting efficacy or stress induction was investigated. Evaluation of total chlorophyll content of Azospirillum inoculated plants was not reduced. Anti-oxidative enzymes of the treated plants were not decreased which implies that the bioinoculant was not inducing any sign of toxicity or stress.

CHAPTER 6

SUMMARY AND CONCLUSION

Plants are highly susceptible to various stresses induced by various physical, chemical and biological agents. Synthetic fertilizers have been replaced with bio-fertilizers for last few decades due to their diverse environmental and health concerns. Biofertilizers or bio-inoculants based on *azospirillum* spp. have been utilized in agriculture sector for nitrogen fixation. Inoculation of *Azospirillum* on maize plant may elicit or trigger some specific biochemical changes. In this study, *Azospirillum* mediated inoculation maize seedlings was evaluated by determining the total chlorophyll content and anti-oxidative enzymes. The present study clearly indicates that the bio-fertilizer inoculated plants were not shown any undesirable toxic effect or stress. This finding clearly indicates that the bio-inoculants can be used as an effective and safe plant growth promoting efficacy.

REFERENCES

1. Vessey, J. Kevin (2003). "Plant growth promoting rhizobacteria as biofertilizers". *Plant and Soil*. **255** (2): 571–586. doi:[10.1023/A:1026037216893](https://doi.org/10.1023/A:1026037216893). S2CID [37031212](https://pubmed.ncbi.nlm.nih.gov/37031212/).
2. ^ ["Listing 17 bio-fertilizer microbes and their effects on the soil and plant health functions"](#). Explogrow. 15 June 2016.
3. ^ ["Archived copy"](#) (PDF). Archived from [the original](#) (PDF) on 2011-07-18. Retrieved 2010-05-03.
4. ^ Malboobi, Mohammad Ali; Behbahani, Mandana; Madani, Hamid; Owlia, Parviz; Deljou, Ali; Yakhchali, Bagher; Moradi, Masoud; Hassanabadi, Hassan (2009). "Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere". *World Journal of Microbiology and Biotechnology*. **25** (8): 1479. doi:[10.1007/s11274-009-0038-y](https://doi.org/10.1007/s11274-009-0038-y). S2CID [58889613](https://pubmed.ncbi.nlm.nih.gov/58889613/).
5. ^ Pandey, Anita; Trivedi, Pankaj; Kumar, Bhavesh; Palni, Lok Man S (2006). "Characterization of a Phosphate Solubilizing and Antagonistic Strain of *Pseudomonas putida* (B0) Isolated from a Sub-Alpine Location in the Indian Central Himalaya". *Current Microbiology*. **53** (2): 102–7. doi:[10.1007/s00284-006-4590-5](https://doi.org/10.1007/s00284-006-4590-5). PMID [16832725](https://pubmed.ncbi.nlm.nih.gov/16832725/). S2CID [23129843](https://pubmed.ncbi.nlm.nih.gov/23129843/).
6. ^ [Jump up to:](#) ^ Schütz, Lukas; Gättinger, Andreas; Meier, Matthias; Müller, Adrian; Boller, Thomas; Mäder, Paul; Mathimaran, Natarajan (2018-01-12). ["Improving Crop Yield and Nutrient Use Efficiency via Biofertilization—A Global Meta-analysis"](#). *Frontiers in Plant Science*. **8**: 2204. doi:[10.3389/fpls.2017.02204](https://doi.org/10.3389/fpls.2017.02204). ISSN [1664-462X](https://pubmed.ncbi.nlm.nih.gov/1664-462X/). PMC [5770357](https://pubmed.ncbi.nlm.nih.gov/5770357/). PMID [29375594](https://pubmed.ncbi.nlm.nih.gov/29375594/).
7. ^ Htwe, Aung Zaw; Moh, Seinn Moh; Soe, Khin Myat; Moe, Kyi; Yamakawa, Takeo (February 2019). ["Effects of Biofertilizer Produced from *Bradyrhizobium* and *Streptomyces griseoflavus* on Plant Growth, Nodulation, Nitrogen Fixation, Nutrient Uptake, and Seed Yield of Mung Bean, Cowpea, and Soybean"](#). *Agronomy*. **9** (2): 77. doi:[10.3390/agronomy9020077](https://doi.org/10.3390/agronomy9020077).
8. ^ Soe, Khin Myat; Yamakawa, Takeo (2013-06-01). "Evaluation of effective Myanmar *Bradyrhizobium* strains isolated from Myanmar soybean and effects of coinoculation with *Streptomyces griseoflavus* P4 for nitrogen fixation". *Soil Science and Plant Nutrition*. **59** (3): 361–370. doi:[10.1080/00380768.2013.794437](https://doi.org/10.1080/00380768.2013.794437). ISSN [0038-0768](https://pubmed.ncbi.nlm.nih.gov/0038-0768/). S2CID [85207082](https://pubmed.ncbi.nlm.nih.gov/85207082/).
9. ^ John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D (September 2011). "Bio-encapsulation of microbial cells for targeted agricultural delivery". *Critical Reviews in Biotechnology*. **31** (3): 211–226. doi:[10.3109/07388551.2010.513327](https://doi.org/10.3109/07388551.2010.513327). PMID [20879835](https://pubmed.ncbi.nlm.nih.gov/20879835/). S2CID [207467630](https://pubmed.ncbi.nlm.nih.gov/207467630/).
10. ^ Ahmed, Sohail; Hassan, Babar; Farooq, Muhammad Umer (December 2018). ["Effect of biofertilizers and diatomaceous earth on life and movement of subterranean termites under laboratory conditions"](#). *International Journal of Tropical Insect Science*. **38** (4): 348–352. doi:[10.1017/S1742758418000103](https://doi.org/10.1017/S1742758418000103). ISSN [1742-7584](https://pubmed.ncbi.nlm.nih.gov/1742-7584/). S2CID [91596645](https://pubmed.ncbi.nlm.nih.gov/91596645/).
11. ^ ["Unigrow"](#). Retrieved 2020-05-05.

12. [^] Naveed, Muhammad; Mehboob, Ijaz; A. Shaker, Masood; Hussain, M. Baqir; Farooq, Muhammad (2015-04-01). ["Biofertilizers in Pakistan: Initiatives and Limitations" \(PDF\)](#).
13. *International Journal of Agriculture and Biology*. **17** (3): 411–420. doi:[10.17957/IJAB/17.3.14.672](#).
14. [^] Brookshire, E. N. J.; Wurzbarger, Nina; Currey, Bryce; Menge, Duncan N. L.; Oatham, Michael P.; Roberts, Carlton (20 May 2019). ["Symbiotic N fixation is sufficient to support net aboveground biomass accumulation in a humid tropical forest"](#). *Scientific Reports*. **9** (1): 7571. Bibcode:2019NatSR...9.7571B. doi:[10.1038/s41598-019-43962-5](#). PMC [6527854](#). PMID [31110241](#).
15. [1]. Clark, M. 1997. Microorganisms Standard Isolation Techniques, Academic Press, London. P 271-275
16. [2]. Nakkeeran, S., Kavitha, K., Mathiyazhagan, S., Fernando, W. G. D., Chandrasekar, G., and Renukadevi, P., 2004, Induced systemic resistance and plant growth promotion by *Pseudomonas chlororaphis* strain PA-23 and *Bacillus subtilis* strain CBE4 against rhizome rot of turmeric (*Curcuma longa* L.). *Can. J. Plant Pathol.* 26: 417-418
17. [3]. Bateman RP. Method of application of microbiological pesticide formulations for the control of grasshoppers and locust. *Mem Entomol Soc Can* 1997; 171: 69–81.
18. [4]. Harmann, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorto, M. 2004. Trichoderma species– opportunistic, avirulent and plant symbionts. *Nature Review of Microbiology*, 2: 43–56.
19. [5]. Abdollahzadeh, J., Goltapeh, E.M. and Rouhani, H. 2003. Evaluation of antagonistic effect of Trichoderma species in biological control of causal agents of crown and root rot of sunflower (*Sclerotinia minor*) in vitro. *Agricultural Sciences Tabriz* 13: 13-23.
20. [6]. Saba Banday, Dar, G.H., Ghani, M.Y., Sagar, V. and Nasreen, F. 2008. In vitro interaction of bioagents against *Dematophora necatrix* and *Pythium ultimum* causing apple root rot in Jammu and Kashmir *SKUAST Journal* 10: 341- 350.
21. [7]. Khan, J., Ooka, J. J., Miller, S. A., Madden, L. V. and Hoitink, H. A. J. 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora* crown rot and leaf blight. *Plant Disease*, 88: 280–286.
22. [8]. Booth RH, Bucle TS, Cardenas OS, Gomez G, Hevas E (1976). Changes in quality of cassava roots during storage. *J. Food technol.* 11: 245-264.
23. [9]. Dennis, C. and Webster, J. 1971c. Antagonistic properties of species groups of Trichoderma. *Transactions of British Mycological Society* 57: 25-39.
24. [10]. Dohroo, N.P., Gupta, S.K., Shyam, K.R. and Sharma, K. 1990. Antagonistic studies on causal fungi of wire stem and stalk rot of cauliflower. *Indian Journal of Plant Pathology* 8: 77-78.

25. [11].Jenkins NE. Methods for mass- production of microbial control agents of grasshoppers and locusts. In: Goettel MS, Johnson DL, editors. 1997. Microbial Control of Grasshoppers and Locusts 37-48. Memoirs of the Entomological Society of Canada #171
26. [12].Kanjnamaneesathian, M., Pengnoo, A., Jantharangsri, A., Niratana, L., and Kusonwiriawong, C. 2000. Scanning electron microscopic examination of a pellet formulation of *Bacillus megaterium* and *B. pumilus*, antagonists of *Rhizoctonia solani*, and survival during storage. *World J. Microbiol. Biotechnol.* 16:523-527.
27. [13].Karthick Raja Namasivayam,S, Santhosh M.T and Sankar,A.V.2014.Biocompatible formulation of potential fungal biopesticide *Nomuraea rileyi* (F.) Samson for the improved post treatment persistence and biocontrol potential. *Asian Jr. of Microbiol. Biotech. Env. Sc.* . 16 (4) : 895-899
28. [14].Karthick Raja Namasivayam S, Allen Roy.2013.Enhanced antibiofilm activity of chitosan stabilized chemogenic silver nanoparticles against *Escherichia coli*.*International Journal of Scientific and Research Publications.* 3(4), 1-9.
29. [15].Lei Z, Huaying Z (1998). *Trichoderma* and seed biotreatment, *J. Microbiol.* 18(3): 50-57.
30. Liansheng T, Wweihua W (2000). Control of *Trichoderma* against *Botrytis cinera* of strawberries in greenhouse, *Plant Prot.*, 16(3): 294- 298.
31. [16].Mujeebur, Khan R., Shahana, M. Khan and F.A. Mohiddin, 2004. Biological control of fusarium wilt of chickpea through seed treatment with the commercial formulation of *Trichoderma harzianum* and/or *Pseudomonas fluorescens*. *Phytopathol Mediterr*, 43: 20-25.
32. [17].Robert, R., Ghisellini, L., Pisi, A., Flori, P. and Filippini, G. 1993. Efficacy of two species of *Trichoderma* as a biological control against *R. solani* isolated from bean root rot in Italy. *Advances in Horticulture Science* 7: 19-25.
33. [18].Yedidia I, Benhamou N, Kapulnik Y, Chet I 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T- 203, *Plant. Physiol. Biochem.*, 38(11): 863-873..

