AUGMENTATIVE ROLE OF *PIRIFORMOSPORA INDICA* IN MODULATING RESPONSES IN THE MITIGATION OF SALINITY STRESS IN TRIGONELLA-FOENUM GRAECUM

A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE
OF

MASTER OF SCIENCE
IN

BIOTECHNOLOGY

Submitted By:

Sanskriti Bisht

2K19/MSCBIO/28

Under the supervision of

Prof. Jai Gopal Sharma

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi- 110042

MAY, 2021

CANDIDATE'S DECLARATION

I hereby certify that the work which is presented in the Project Work entitled **Augmentative Role of *Piriformospora indica* in Modulating Responses in the Mitigation of Salinity Stress in Trigonella foenum-graecum** is in fulfillment of the requirement for the award of the degree of Master of Science in **Biotechnology** and submitted to the Department of **Biotechnology**, Delhi Technological University, Delhi is an authentic record of my own carried out during a period from **January 2021 -May 2021**, under the supervision of **Prof. Jai Gopal Sharma.**

The matter present in this report/thesis has not been submitted by me for the award of any other degree of this or any other Institute/University.

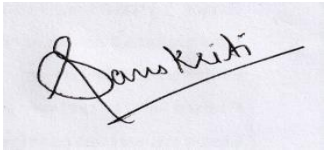
Title of the Paper- Augmentative role of *Piriformospora indica* and Plant growth promoting bacteria (PGPB) in mitigating salinity stress in Trigonella foenum-graecum **Author names**- Sanskriti Bisht, Shatrupa Singh, Jai Gopal Sharma

Name of the journal- Journal of Applied Biology and Biotechnology (JABB)

Status of the paper- Communicated as well as first decision on revising the paper has also been received on 25 May, 2021.

Date of the paper communication- 24 April, 2021 **Date of receiving the Reviewer's comment**- 25 May, 2021

Place : New Delhi **Date: 29.05.2021**



Sanskriti Bisht

2K19/MSCBIO/28

CERTIFICATE

To the best of my knowledge, the above work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere. I, further certify that the publication and indexing information given by the student is correct.

Place: New Delhi

Date: 29-05-2021

Prof. Jaigopal Sharma
(SUPERVISOR)
Professor
Department of Biotechnology
Delhi Technological University

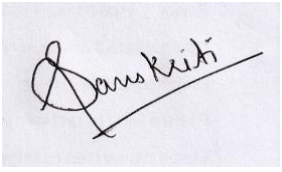
Prof. Pravir Kumar
(Head of the Department)
Department of Biotechnology
Delhi Technological University

ACKNOWLEDGEMENT

I would like to express my gratitude of thanks to lot of people who supported me through this mini journey of dissertation, to all those who provided support, talked things over, read, wrote, offered comments and assisted in the editing and experimental design. I am very thankful for my supervisor **Prof. Jai Gopal Sharma,** the most influential person who have supported me a lot through this journey. I am also grateful to **Mrs. Madhulika Singh,** who felt like a home to me, everytime I encountered any issues while working for my project she was the only one I could talk to and have a solution for it. Literally thank you so much maam.

I appreciate the efforts of my parents for raising me into this responsible person. I took these qualities from them. I really wanted to say that without their support and encouragement, things wouldn't have been possible for me. I wanted to say these kind words to my younger sister Durgma, without you I feel incomplete, you have added colors to my boring life. Also, I wanted to mention my pet Moana, she's so lovely and charming, thank you God for sending a little cute angel into our lives. Word of thanks to my Nani for always helping me out.

Above all I thank almighty for helping me and guiding me in all those times, when I felt low and almost pursuaded myself to quit in the middle of the way, but somehow, I managed to overcome the darkness and stood up. Thank you Almighty for everything.



Sanskriti Bisht

ABSTRACT

Methi (*Trigonella foenum graecum*) plants are rich in nutrients, have strong antioxidant property, aids in the digestion and have multiple health benefits including lowering the blood sugar levels, thereby controlling diabetes. The production of methi plants is negatively affected by increase salinization of soil. In this study, we have used a microbe, *Piriformospora indica* which is a fungus used to alleviate salinity stress in methi. Plants were subjected to different salinity levels (0, 75 and 150 mM NaCl), and various parameters were analyzed viz. growth, physiological and biochemical aspects, in order to understand whether the microbial inoculation can help in mitigation of high salinity stress. The role of *P. indica* has positive effects in mitigation of high salinity stress in methi plants and have elevated various physiological responses like increase in the photosynthetic rate, stomatal conductance, transpiration and internal CO_2. Biochemical aspects like carotenoids, chlorophyll content, nitrogen and protein were also increased in the microbial inoculated plants. *P. indica* was very effective in elevating nitrogen, protein, stomatal conductance, internal CO_2 and transpiration level and various other parameters. Uninoculated plants showed poor results, and were not compatible in mitigating high salinity stress. In conclusion, findings suggest that microbial inoculation of *P. indica* can help the plants to overcome the high salinity stress in methi plants.

Keywords – Photosynthesis, stomatal conductance, transpiration, internal CO_2, carotenoids, chlorophyll a, chlorophyll b, total chlorophyll, protein, nitrogen, biomass.

CONTENTS

Candidate's Declaration **ii**

Certificate Error! Bookmark not defined.

Acknowledgement **iii**

Abstract **v**

Contents **vi**

List of Figures **viii**

List of Tables viii

CHAPTER 1 INTRODUCTION 1

1.1 Effect of salinity in plants 5

1.2 Insights of *Piriformospora indica* 7

CHAPTER 2 MATERIALS AND METHODS 10

2.1 Plant material and experimental design 10

2.2 Microbial and salt treatments 10

2.3 Shoot and Root length measurement 11

2.4 Biomass measurement 11

2.5 Leaf area and Number of leaves measurement 12

2.6 Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2 measurement 13

2.7 Chlorophyll and Carotenoid Estimation 13

2.8 Nitrogen and Protein Estimation 13

2.9 Statistical Analysis 16

CHAPTER 3 RESULTS 17

3.1 Shoot and Root length 17

3.2 Biomass 20

3.3 Number of leaves and Leaf Area 23

3.4 Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2 26

3.5 Carotenoids, Chlorophyll a, Chlorophyll b and Total chlorophyll 29

3.6 Nitrogen and Protein (%) percentage 31

CHAPTER 4 DISCUSSIONS

4.1 Shoot and Root length 34

4.2 Biomass 34

4.3 Number of leaves and Leaf Area 34

4.4 Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2 35

4.5 Carotenoids, Chlorophyll a, Chlorophyll b and Total chlorophyll 35

4.6 Nitrogen and Protein (%) percentage 36

5. CONCLUSIONS 37

REFERENCES 38

LIST OF FIGURES

Figure No.	Titles	Page No.
1	Salt deposition is seen as a white layer on the soil	1
1.1	Effect of salt stress on plants. Salt stress causes physiological drought to plants resulting in hyperosmotic stress. On the other hand, excessive uptake of Na+ and Cl- ions lead to hyperionic stress. These stresses generate a secondary stress "oxidative stress". The combined effect of these stresses ultimately affects plant growth and reduce the yield	6
1.2 (A)	An image of endophytic fungi Piriformospora indica showing its coiled hyphae and pear shape spores	7
1.2 (B)	A diagrammatic representation contrasting the differences of the plants inoculated with microbial inoculation and non- microbial association	8
2.2	Experimental set up showing (A) uninoculated (B) *P. indica* inoculated inoculated plants after 45 days of sowing	11
2.3	Effect of different concentration of NaCl on shoot and root growth in (A) uninoculated (B) *P. indica* inoculated inoculated *T. foenumgraecum* plants.	12
2.8 (A, B)	**(A)** FOSS Kjeldahl block digestion **(B)** FOSS Kjeltec 8200 steam distillation unit	15
2.8 (C, D)	Protein Titration showing blue color protein solution **(D)** Protein Titration showing colorless protein solution endpoint	15
3.1	Effects of different concentration of NaCl on morphological attributes in *T. foenum- graecum* plants inoculated with *P. indica* (A) Shoot Length (B) Root Length	18
3.2	Influence of NaCl at different concentration on morphological characteristics in *T. foenum-graecum* plants inoculated with *P. indica* (A)Shoot Dry Weight (B) Root Dry Weight	21

3.3	Effects of different concentration of NaCl on morphological attributes in *T. foenum-graecum* plants inoculated with *P. indica* (A) Number of leaves (B) Leaf area	24
3.4	Effects of different concentration of NaCl (0mM, 70mM, 150mM) on physiological attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Photosynthesis (B) Stomatal Conductance (C)Transpiration (D) Internal CO_2	27
3.5	Effects of different concentration of NaCl (0mM, 70mM, 150mM) on biochemical attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Carotenoids (B) Chlorophyll a (C) Chlorophyll b (D) Total Chlorophyll	30
3.6	Effects of different concentration of NaCl on (A) Nitrogen (B) Protein in *T. foenum-graecum* plants inoculated with *P. indica*	32

LIST OF TABLES

Table No.	Titles	Page No.
1	Showing various fungi and associated AMF during salinity stress and their responses.	9
3.1	Effects of different concentration of NaCl on morphological attributes in *T. foenum- graecum* plants inoculated with *P. indica* (A) Shoot Length (B) Root Length	19
3.2	Influence of NaCl at different concentration on morphological characteristics in *T. foenum-graecum* plants inoculated with *P. indica* (A)Shoot Dry Weight (B) Root Dry Weight	22
3.3	Effects of different concentration of NaCl on morphological attributes in *T. foenum-graecum* plants inoculated with *P. indica* (A) Number of leaves (B) Leaf area	25
3.4	Effects of different concentration of NaCl (0mM, 70mM, 150mM) on physiological attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Photosynthesis (B) Stomatal Conductance (C)Transpiration (D) Internal CO_2	28
3.5	Effects of different concentration of NaCl (0mM, 70mM, 150mM) on biochemical attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Carotenoids (B) Chlorophyll a (C) Chlorophyll b (D) Total Chlorophyll	31
3.6	Effects of different concentration of NaCl on (A) Nitrogen (B) Protein in *T. foenum-graecum* plants inoculated with *P. indica*	33

1. INTRODUCTION

Fig.1 Salt deposition is seen as a white layer on the soil

World agriculture is facing a crucial challenge of meeting the rising global population's food demand which is currently growing at around 1 percent per year (World Population Prospects, 2019). Several biotic and abiotic stresses have a significant impact on the growth productivity, yield and food quality of plants (Shi-Ying et al., 2018; Singh et al., 2018). Damages or diseases is caused by a variety of pests or pathogens are referred to as biotic stresses whereas salinity, rising temperatures, declining freshwater supplies, heavy metals and other chemical pollutants are example of abiotic stresses which necessitate an integrated solution, collective intervention, and extensive research in order to resolve and adapt (Jogawat et al., 2013).

Fig.1 Salt deposition is seen as a white layer on the soil.

Soil salinity is the most harmful among all the abiotic stresses (Daliakopoulos et al., 2016). Salinization is the existence of various types pf salt ions in soil (chlorides, sulphates, nitrates; cations: sodium, potassium, magnesium, calcium) and is viewed as the most significant constraints on agricultural production and food security since crops react to soil salinity in a variety of ways, and while growing in salinity conditions these factors completely influence their ability to sustain and achieve a sufficient amount of production (Shilev 2020) (Fig.1).

Salinization of agricultural land happen mainly because of the deposition of salt in soil (Bharti et al., 2016). Salinity affects over 20% of agricultural land worldwide and the problem is only getting worse (Gupta and Huang., 2014). About half of the agricultural land would be salinity affected by 2050 as per the estimations. An estimate number of 6.7 million hectares of land in India is

also salt affected with Gujarat having the largest volume of almost 71 percent of the overall salty soils in India. Asia pacific and Australia are two of the world's most salinity influenced countries. In America, there are 12223.41 million hectares of gross agricultural land of which 130.5 million are saline whereas in Europe 17.30 percent land part is affected (FAO, 2019), 6.40 % of Africa's gross agricultural land is impacted by salinity (FAO, 2015) (Kumar et al., 2020).

Since water conductance, soil porosity, and aeration are all hampered by high Na+ levels, NaCl is the most common salt found in soils, and it continues to be a source of concern for scientists (Tavakkoli et al. 2010; Clarke et al.2015). A plant that is under the influence of saltstress goes through series of morphological, physiological, and molecular modifications, eventually obstructing its maturation (Kumar et al., 2020).

Horticultural crops like Tomatoes, potatoes, lettuce, spinach and Cereals (rice, maize, wheat, legumes) are sensitive to salinity and the salinity stress which reduces the yield up to 50–70% (Shilev 2020).Photosynthesis is affected by soil salinity, which results in a reduction in leaf area.With extended salt tension, old leaves begin to experience chlorosis and hence collapse.If the rate at which leaves die outpaces the rate at which they are formed, the plant's photosynthetic ability would be unable to provide the needed carbohydrate to young leaves, resulting in a drastic reduction in their developmental rate (Carillo et al. 2011).

Photosynthesis is effected by salinity in both the short and long term.Short- term salt stress is very quick and happens within a short period of salt exposure so it leads to reduced carbon assimilation by affecting stomatal limitations in photosynthesis whereas the Long lasting affect is that the salt starts to accumulate in young leaves(Parida and Das 2005), this reduces the amount of chlorophyll and carotenoids and changes in the lipid-protein ratio of pigment-protein complexes or even increased chlorophyllase activity may cause a decrease in chlorophyll content (Saravanavel et al. 2011).

The chloroplast's thylakoid forms also become disordered as they are exposed to salt and the number and size of plastoglobuli increases as well. Salt stress is also observed to affect the stomata size and the density of it, leading to stomatal conductance reduction (Hanin et al. 2016). Plants that are subject to high salt concentrations have smaller, faded leaves and are shorter (Shilev 2020). It is also observed that salt stress has an effect on the shoot and also on the

reproductive progress of the plant. Plant growth, root development, stomata closing, flowering, seed germination, and cell death is regulated by Nitric oxide. In the external medium, the presence of Cl salts causes decreased nitrate reductase activity under salt tension which causes reduced nodulation, leghaemoglobin content, and nitrogenase activity. Salinity stress also promotes the formation of reactive oxygen species (ROS), which cause damage to cell membrane, proteins, lipids and nucleic acids (DNA, RNA), as well as programmed cell death (Shi-Ying et al., 2018).

Many studies have produced transgenic plants that mitigate salt stress in plants (Nongpiur et al. 2016), yet these methods happen to be high-priced and take much of the time (Bianco and Defec 2012). Inoculated/Treated plants with microorganisms are quite common now and is therefore less expensive, because they have tremendous stress-relieving capacity (Meena et al., 2017). Microorganisms with unique characteristics are found to boost plant's growth when associated with plants and when exposed to salt stress (Enebe and Babalola 2018). 1-aminocyclopropane1-carboxylate (ACC) deaminase action, Brevibacterium epidermidis RS15, Micrococcus yunnanensis RS222, and Bacillus aryabhattai RS341 were found to increase root elongation (40 percent) and dry weight in canola (Siddikee et al. 2010).

Since, saline ecosystems have insufficient nitrogen, nitrogen input is needed in these conditions (Wang et al. 2018). Chrysanthemum morifolium was helped to uptake nitrogen under salt tension by Funneliformismosseae and Diversisporaversiformis, both single and in mixture. Root length and shoot and root dry weight increased, that aided the plant growth. Bacillus amyloliquefaciens SQR9 has been confirmed to improve chlorophyll content by tolerating salinity in maize seedling (Chen et al., 2016).

Massilia sp.RK4 and Co-culture of Rhizophagusintradices have improved the nitrogen in maize shoots dramatically (Krishnamoorthy et al. 2016). Increased length, chlorophyll content, fresh and dry weight of soybean shoots was reported by H-2-3 Pseudomonas putida in soyabean (Glycine max) (Kang et al 2014). Under high salt conditions, the photosynthetic pigment content [chlorophyll (chl) a, chl b, and carotenoids] was substantially higher in P. indica-inoculated rice seedlings. *Brachybacterium saurashtrense*, *Brevibacterium casei* and *Haererohalobacter* increased total biomass in Peanut *(Arachis hypogaea*L.) (Shukla et al.2012).

Salt tolerance has been identified in host plants provided by plant growth-promoting bacteria and by microbial symbionts which helped the plants' ability to persevere in adverse situations (Bano

and Fatima 2009). Crops grown in saline environments can benefit from mutual symbiosis with beneficial entophytic fungi to alleviate salt stress and yield loss (Hassani, Danial et al., 2019). Growth and biomass in cucuma are improved by *P. indica* (Kumar et al. 2020). In this experiment we analysed the beneficial effect of PGPB and *P. indica* association in methi(fenugreek) plants during high salt situations.

Fenugreek is one of India's most chief cash crops. It's a dicotyledonous annual herb used as vegetable and forage. The seeds (whole, ground, in flour, or roasted) are used as human and animal food, as well as for industrial and medicinal purpose (Petropoulos 2002).

1.1 Detrimental effects of salinity on plants

Soil salinity is one of the most raised issue as it negatively affects the productivity of crops. Salinity stress is one of the most harmful stress for the growth and development of the plants effecting various parameters responsible for the plant's growth including physiological parameter like decrease in the photosynthesis rate, reduction in the stomatal conductance, low transpiration rate and low internal CO_2 in plants which ultimately effects the physiology of plants.

Excessive salts in soil, in particular, Na+ ions alter the basic organization of the soil. The occurrence of Na+ ions in the complex of cation exchange makes the soil more compact and subsequently reduces the soil porosity and hampers the aeration of soil (Manchanda and Garg 2008). Less soil aeration due to high saline concentration directly relates with all major living processes, such as reduction in growth, protein and lipid metabolism (due to salt-induced osmotic imbalance), nutritional disorder and ion toxicity in plants (Porcel et al. 2012) (Fig.1.1).

Soil salinity also effects the biochemical parameters like reduction in the primary pigments like chlorophyll a and chlorophyll b and other accessory pigments like carotenoids and overall total chlorophyll content, reducing these pigments will directly lower down the photosynthetic activity of the plants. Lower photosynthetic rate will correspond to the reduction in the biomass of the plant and ultimately the development of plant is hampered.

In order to reduce the detrimental effects of salinity on plants, various approaches are used like using microbes which can alleviate the high salinity stress on plants. Biological organisms secrete some compounds which helps to neutralize the excess salinity in soil and they establish a symbiotic association with the higher plants shielding them from abrupt environment cues.

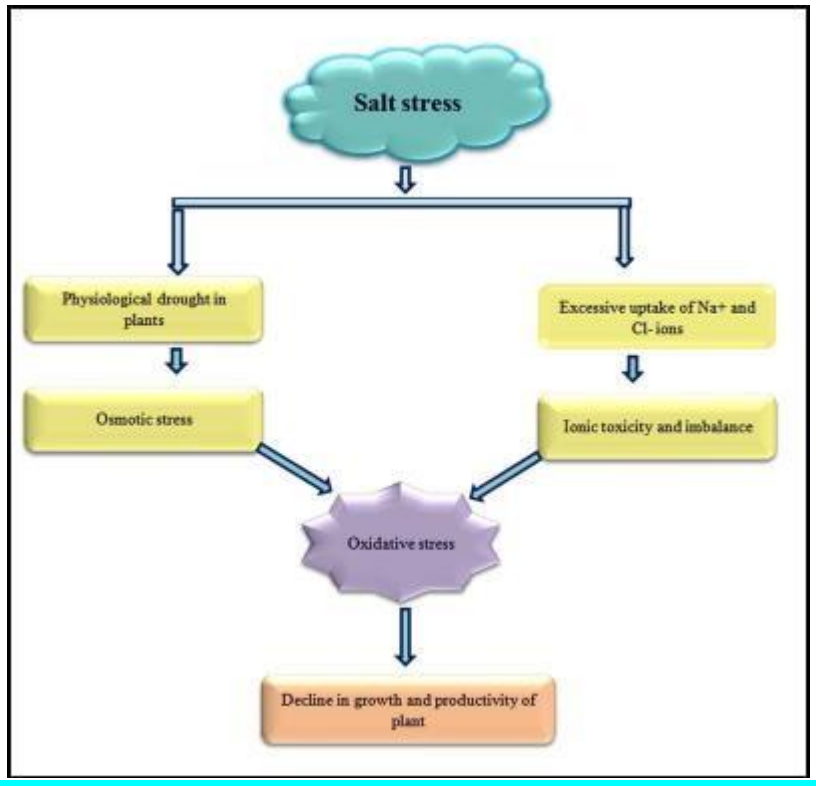


Fig. 1.1 Effect of salt stress on plants. Salt stress causes physiological drought to plants resulting in hyperosmotic stress. On the other hand, excessive uptake of Na+ and Cl- ions lead to hyperionic stress. These stresses generate a secondary stress "oxidative stress". The combined effect of these stresses ultimately affects plant growth and reduce the yield. This image has been taken from Heikham Evelin's paper.

1.2 Insights of Piriformospora indica

Piriformospora indica is a versatile endophytic fungus, which belong to the newly formed order Sebacinales. It belongs to the monotypic genus (Fig.1.2 A). It was discovered by Prof. Ajit Verma and his members in the Thar desert of Rajasthan in orchid plants. *P. indica* establishes relationship with roots of higher plants and linked with the divisions like bryophytes, pteridophytes, and some members of gymnosperms and angiosperms. Experiments have shown that *P. indica* is very beneficial in resisting the pathogens, salinity stress and various other serious environmental cues in colonized plants.

P. indica shows various advantage like increase in the photosynthetic area, enhances the antioxidant system in the plant, generation of higher amount of biomass, homeostasis of ions when colonized with plants (Fig.1.2 B). Various other fungi have beneficial role and helps in alleviation of salinity stress (Table 1.2).

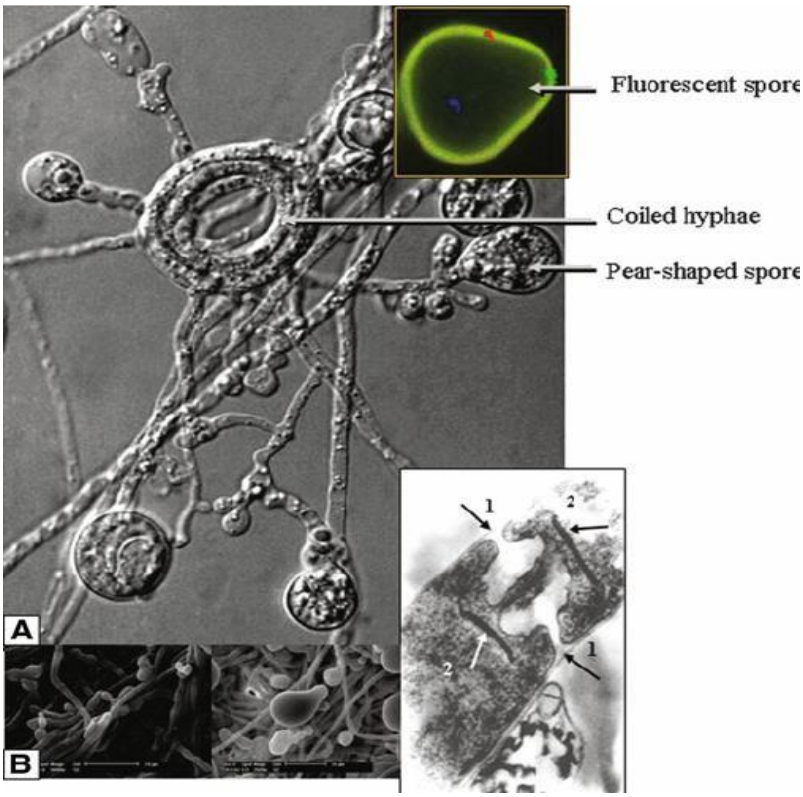


Fig.1.2 A An image of endophytic fungi Piriformospora indica showing its coiled hyphae and pear shape spores.

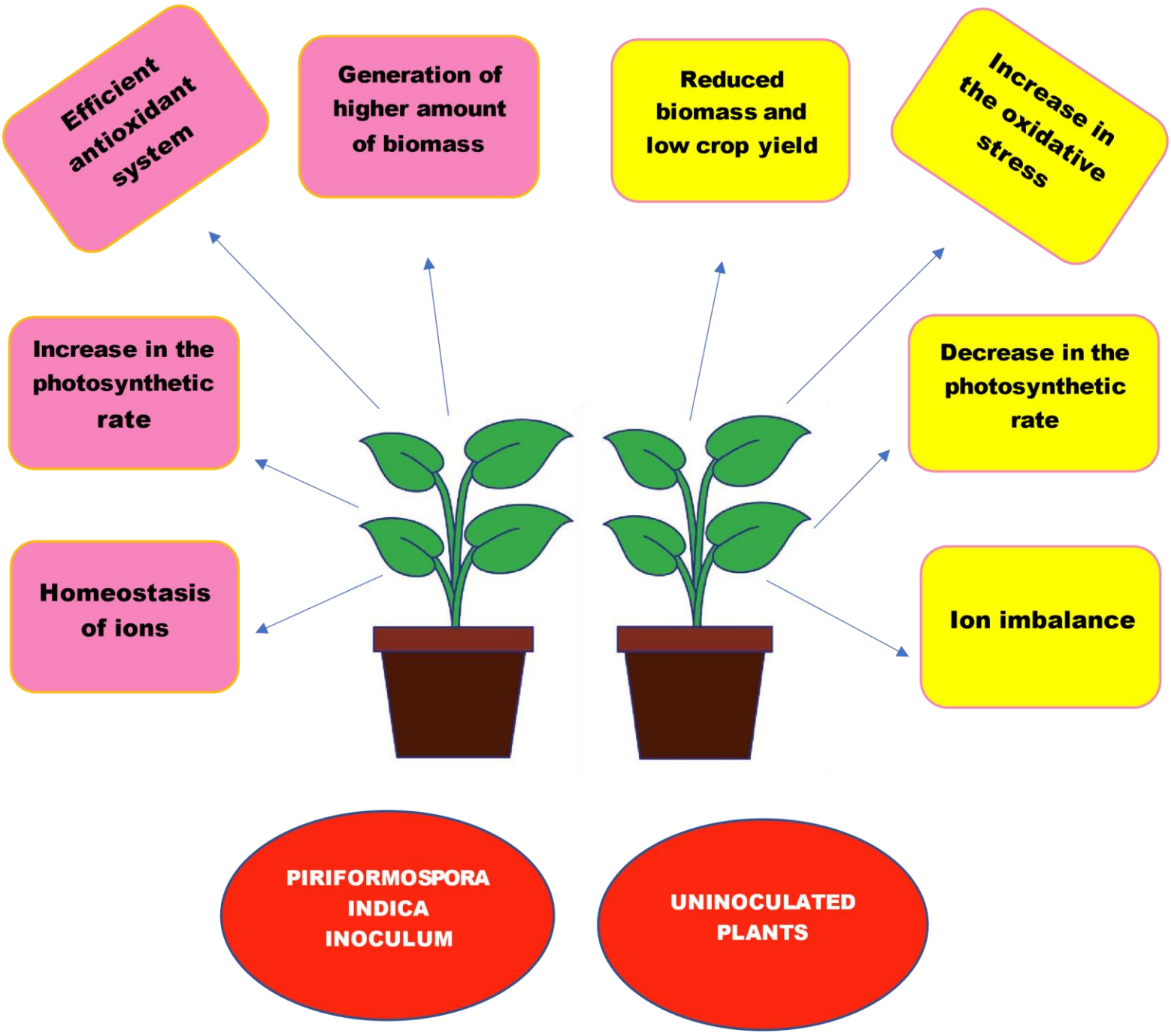


Fig.1.2 B A diagrammatic representation contrasting the differences of the plants inoculated with microbial inoculation and non- microbial association.

Table 1 Showing various fungi and associated AMF during salinity stress and their responses.

HOST PLANTS	FUNGAL SPECIES (AMF AND RELATED STRAINS)	RESPONSES RELATED TO AMF INOCULATION	REFERENCES
Solanum lycopersicum	*Glomus intraradices*	Ion uptake, chlorophyll content, growth parameters and dry matter is improved	Hajiboland et al. (2010)
Solanum lycopersicum	*Rhizophagus irregularis*	Shoot, root, leaf area, leaf number and the growth hormone levels are enhanced	Khalloufi et al. (2017)
Aeluropus littoralis	*Claroideoglomus etunicatum*	Stomatal conductance, root and shoot dry mass, free alpha amino acids, soluble sugars and Na and Cl uptake is increased	Hajiboland et al. (2015)
Acacia nilotica	*Glomus fasciculate*	Shoot and Root biomass are improved along with Zn, Cu and P contents	Giri et al. (2007)
Cucumis sativus	*Glomus mosseae, Glomus etunicatum, Glomus intraradices*	Biomass is increased, Biosynthesis of antioxidant enzymes and photosynthetic pigments	Hashem et al. (2018)

2. MATERIALS AND METHODS

2.1 Plant material and Experimental design

Methi (Fenugreek) Seeds were obtained from National Seeds Corporation, Pusa New Delhi, India. Experimentation was conducted in the horticulture, Delhi Technological University, Delhi, India. During the *Trigonella foenum-graecum* growing season (December-March), under natural light, temperature, and humidity conditions.

The 3 x 6 factorial experiment was designed for *P. indica* with 2 conditions: Treated or nontreated with three Nacl concentrations (0,70, and 150mM). Thus, eighteen combinations were set up in a three-times repeated randomised full block configuration for the fungal microbe (*P. indica*).

2.2 Microbial and salt Treatments

Soil was inoculated with *P. indica* at the time of sowing. In non-inoculated plants same amount of sand was added. Eight sterilized seeds were sown at a depth of 3 cm in each plastic pot having 4 kg of an autoclaved (121°C and 15 psi) sandy loam soil (Fig. 2.2). The soil had a pH: 7.2, organic matter:1.3%, available N: 185 mg g^{-1}, Available P: 49.4 mg g^{-1}, Available K^{+}: 295 mg g^{-1}, Mg^{2+}: 230 mg g^{-1}, Zn^{2+}: 6.8 mg g^{-1}, Fe^{3+}: 11.9 mg g^{-1}, Cu^{2+}: 3.99 mg g^{-1}, Mn^{2+}: 6.98 mg g^{-1}. The plants were grown in greenhouse conditions (Temperature: 23-28 °C; relative humidity: 65 ± 5% and light intensity: 1500 lux).

Salt treatment began following 15 days of plant development. To each pot 50 mL of NaCl solution was added sequentially after 7 days to avoid any osmotic shock to the roots till 45 days after sowing. In control 50 ml of distilled water was added in each pot till 45 days after sowing. Upon addition of NaCl solution, the electrical conductivity (EC) of soil extracts increased to 0.01, 7.67 and 15.50 mS cm^{-1} in the 0, 70 and 150 mM NaCl salinity levels, respectively. The electrical conductivity of the soil was determined by using conductivity meter (HACH analyzer, HQ440d). Autoclaved tap water was used for irrigating the plants twice in a week.
Plants were harvested by uprooting the entire plant manually after 45 days of sowing.

2.3 Shoot and root length Measurement

The plants were harvested 64 days after sowing and detached into roots and shoots. To eliminate any sticking particles, the root and shoot were rinsed thoroughly in tap water and blotted dry. Lengths of root and shoot were measured immediately using a scale (Fig. 2.3).

Fig. 2.2 Experimental set up showing (A) uninoculated (B) *P. indica* inoculated inoculated plants after 45 days of sowing

2.4 Biomass measurement

The fresh plant leaves were wrapped separately in aluminium foil and kept in oven for 72 hours at 70 - 80 °c to record the biomass. Dry weights were measured using weighing balance.

2.5 Leaf area and Number of leaves measurement

The numbers of leaves were counted. Leaf area measurement was performed on portable photosynthesis system Li-6400XT IRGA (Infra-red gas analyser) Department of botany, Delhi University.

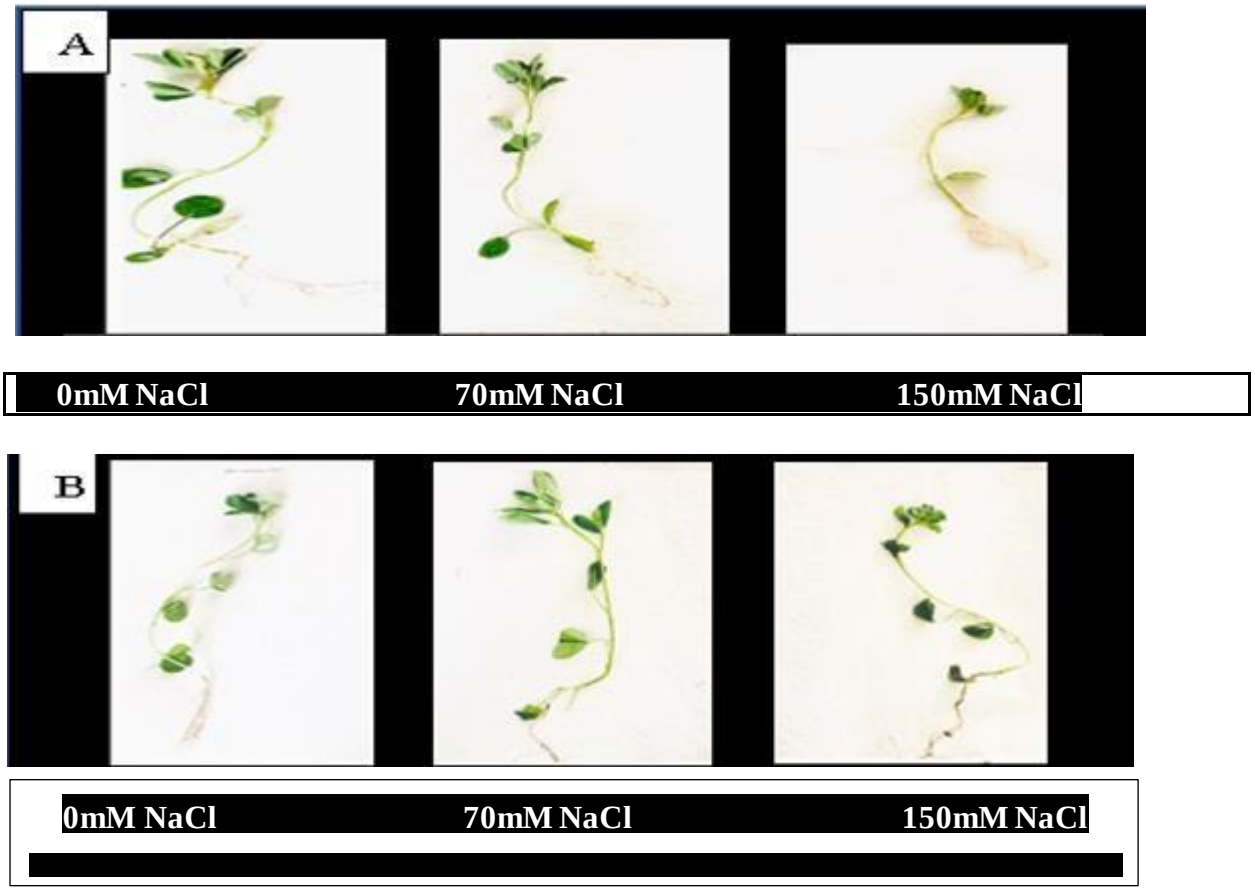


Fig. 2.3 Effect of different concentration of NaCl on shoot and root growth in (A) uninoculated (B) *P. indica* inoculated inoculated *T. foenum-graecum* plants.

2.6 Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2 measurement

Physiological parameters (photosynthesis, stomatal conductance, transpiration, and internal CO2) were also measured by IRGA.

2.7 Chlorophyll and Carotenoid Estimation

Fresh leaflets (0.1 g) were chopped down into small slices and put into a vial of 7 mL DMSO (dimethyl sulfoxide). The leaf tissue in the vials was incubated at 65°C until it turned white. The extracts were transferred to a tube and DMSO was used to make up 10ml of total volume. The extract's absorbance was measured at 645 and 663 nm for chlorophyll content, 645 and 663 nm for carotenoid content and the concentration of chlorophyll and carotenoid was measured using the formulas respectively.

Chlorophyll a (mg/g fresh weight) = $12.7\times D_{663}-2.69D_{645} \times$ Volume/1000× Weight of sample

Chlorophyll b (mg/g fresh weight) = $22.9\times D_{645}-4.68D_{663} \times$ Volume/1000× Weight of sample

Total chlorophyll (mg/g fresh weight) = $20.2\times D_{645}+8.02D_{663} \times$ Volume/1000× Weight of sample

Carotenoid (mg/g fresh weight) = $7.6D_{480}-1.49D_{510} \times$ Volume/1000× Weight of sample

2.8. Nitrogen and Protein Estimation

The determination of nitrogen and protein was done according to the protocol of FOSS Kjeldahl block digestion and steam distillation (AN 300, EN ISO 20483:2006) [29] (Fig. 2.8 A, B). To 0.7g dried leaf sample 7g K_2SO_4, 0.8g $CuSO_4$ and 12 ml concentrated H_2SO_4 were added. Digestion was performed on kjeldahl digester unit for 60 minutes at 420°C.

Distillation was performed using kjeltec 8200 unit.30 ml of 4% Boric acid (receiver solution) was added to receiver flask. 80 ml H_2O and 50 ml 40 % NaOH was added to the digested sample. Distillate was titrated with standardized titrant (0.1N HCL) (Fig. 2.8 C, D). Reagent blank was performed out earlier to every set of samples.

% Nitrogen and % Protein was obtained using the formula

% N = (T-B) ×N×14,007×100/weight sample (mg)

T = Sample titration B = Blank titration N = Normality of titrant

% Protein = N×F

F = 6.25 for Fenugreek

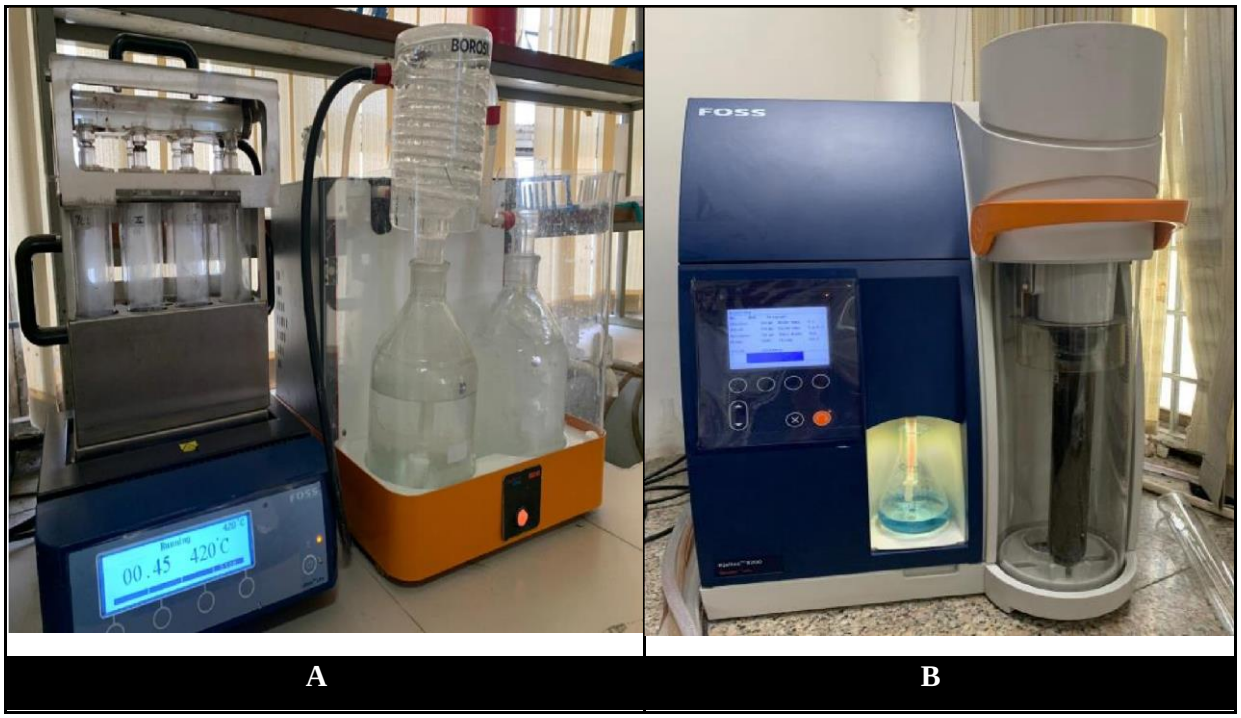


Fig. 2.8 (A) FOSS Kjeldahl block digestion **(B)** FOSS Kjeltec 8200 steam distillation unit

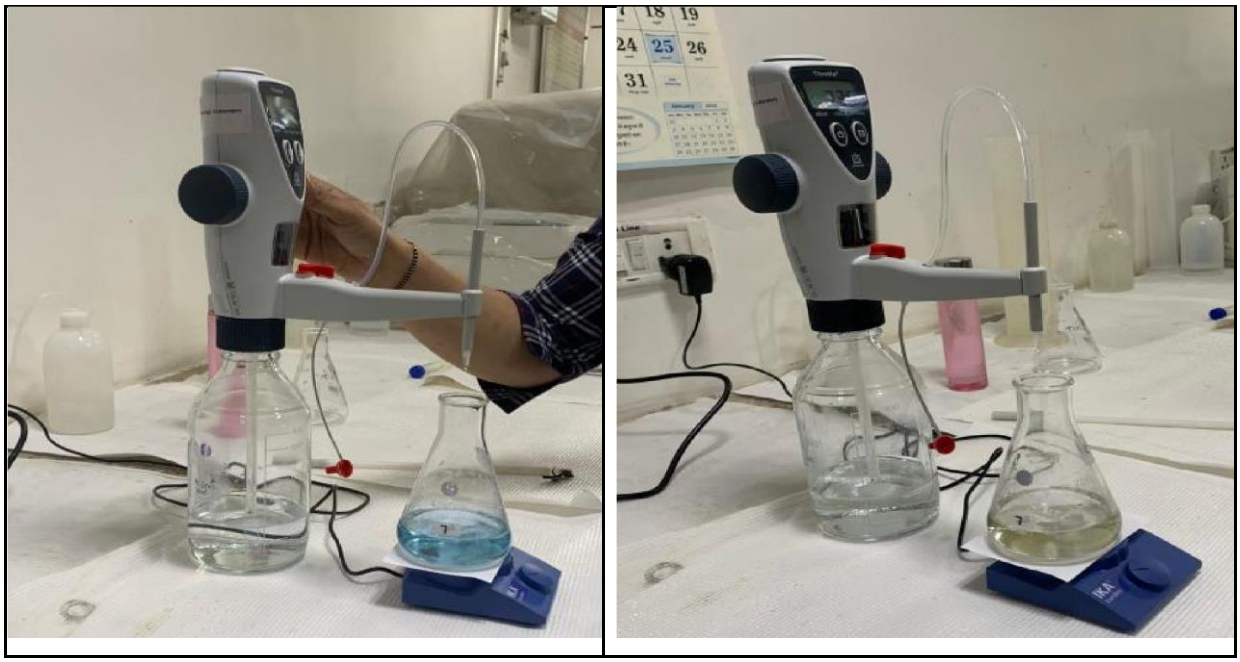


Fig. 2.8 (C) Protein Titration showing blue color protein solution **(D)** Protein Titration showing colorless protein solution endpoint

2.9. Statistical Analysis

The data were analysed using SPSS 21 statistical programme (IBM SPSS Statistics 21) by one way ANOVA with NaCl treatment, microbial inoculation and interactions among them as a source of variation. Comparison of the means were determined by post hoc Duncan's test ($p < 0.05$).

3. RESULTS

3.1. Shoot and Root length

The effects of *P. indica* (fungus) in*Trigonella foenum- graecum* were assessed under saline conditions and non-saline conditions. Salinity stress was given for the duration of 2 months and repeated after every 15th day (0mM NaCl, 70mM NaCl, 150Mm NaCl). Inoculation of microbes have significantly increased shoot and root growth comparing to the uninoculated plants (where no inoculation of microbes was done) at high salinity. *P. indica* showed positive results in improving the growth parameters like shoot and root length as compared to the uninoculated plants (Fig. 3.1A). The results shown by *P. indica* inoculated plants at high salinity or 150 mM NaCl treatment was 47% and at 70 mM NaCl treatment the increase in percentage was only 13%. Remarkable effects were observed under non saline conditions as well where *P. indica* inoculated plants showed increase in the shoot length.

P. indica inoculated plants again showed best positive effects on root length than untreated or controlled plants (Fig.3.1B). *P. indica* has enhanced root length by 22% at 150mM NaCl concentration, and about 9% increase at 70mM NaCl treatment. But overall, the microbial (fungal) inoculation or treatment have helped in the enhancement of root length even under the high salinity stress and non saline conditions as well. Uninoculted or controlled plants showed poor results.

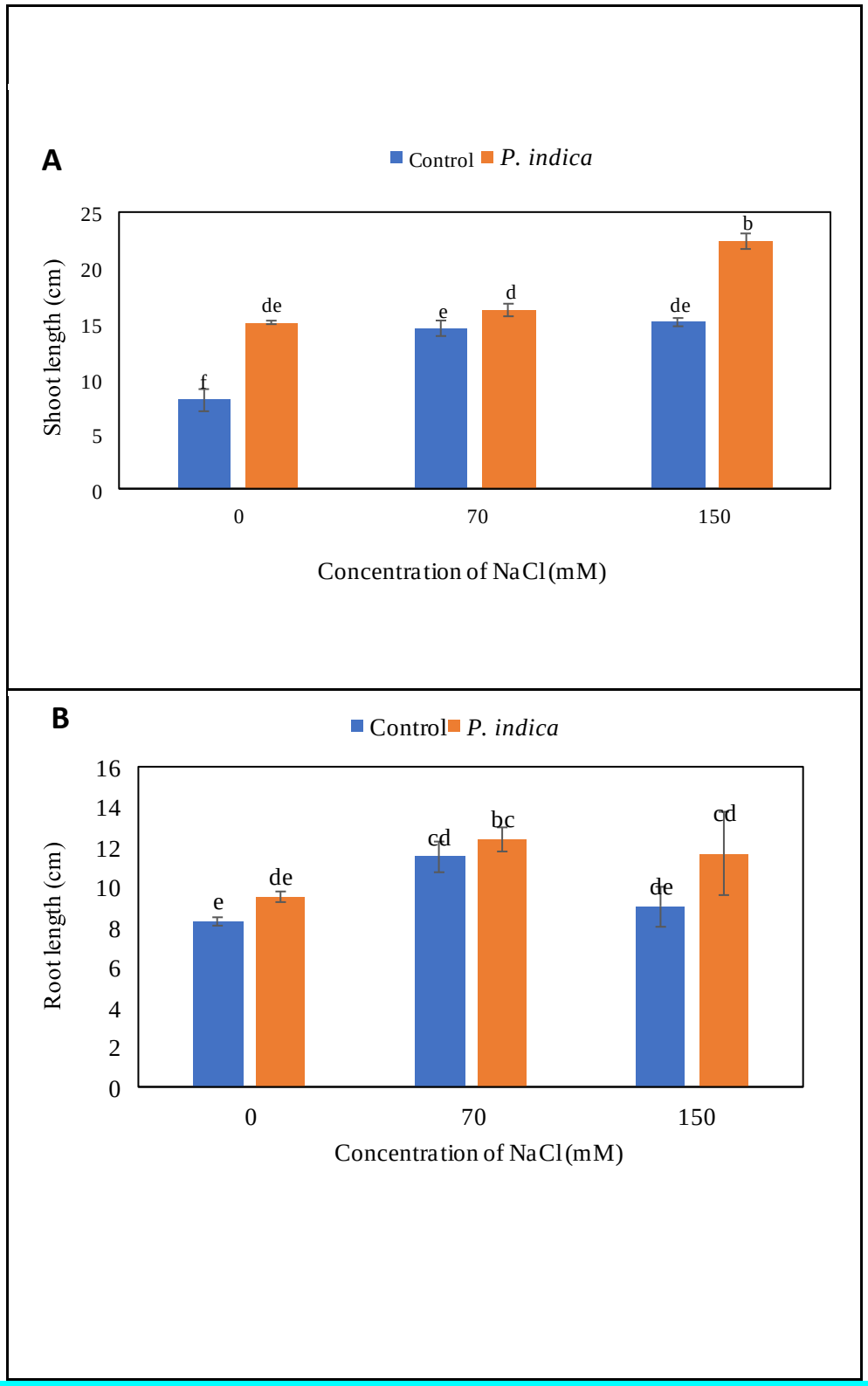


Fig.3.1 Effects of different concentration of NaCl on morphological attributes in *T. foenum-graecum* plants inoculated with *P. indica* (A) Shoot Length (B) Root Length

Table 3.1 Effects of different concentration of NaCl on morphological attributes in *T. foenum- graecum* plants inoculated with *P. indica* (A) Shoot Length (B) Root Length

NaCl (mM) CONTROL	SHOOT LENGTH (cm)	ROOT LENGTH (cm)
0	8 f	8.266667 e
70	14.5 e	11.49333 cd
150	15.05333 de	9 de
NaCl (mM) TREATMENT	**SHOOT LENGTH (cm)**	**ROOT LENGTH (cm)**
0	15.03 de	9.5 de
70	16.15 d	12.36667 bc
150	22.36667 b	11.66667 cd

Values represent mean of replicates in the table mentioned above.

3.2. Biomass

Microbe inoculation has elevated the biomass increase as compared to the uninoculated plants under the high salinity stress. Remarkable positive results were shown by microbial inoculation of *P.indica* in elevating the shoot dry weight (biomass). The results showed that *P.indica* inoculated plants have better impact in elevating the shoot dry weight (biomass). At different NaCl treatment (150Mm and 70mM) the increase in the numbers were up to 50% and 72% respectively in *P.indica* (Fig. 3.2A).

Root Dry weight (biomass) was increased remarkably in *P.indica* (Fig. 3.2B) The responses showed by *P.indica* was effective as compared to the uninoculated plants, under different salinity stress. The best results were found at 70mM NaCl concentration to mitigate the salinity stress in

P.indica. Overall microbe inoculation of fungi contributes in the increase of root biomass as compared to the uninoculated plants.

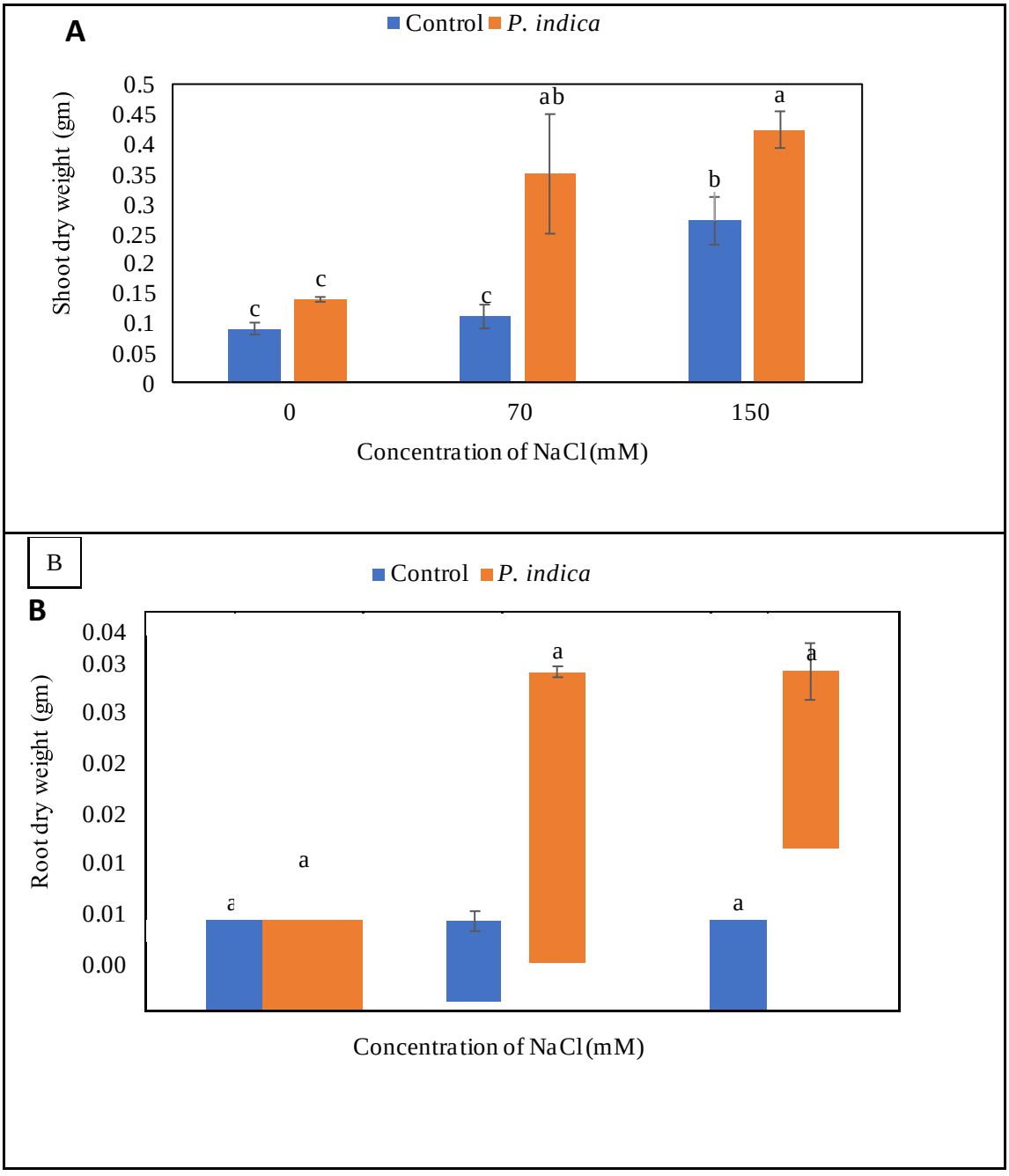


Fig. 3.2 Influence of NaCl at different concentration on morphological characteristics in *T. foenumgraecum* plants inoculated with *P. indica* (A)Shoot Dry Weight (B) Root Dry Weigh

Table 3.2 Influence of NaCl at different concentration on morphological characteristics in *T. foenumgraecum* plants inoculated with *P. indica* (A)Shoot Dry Weight (B) Root Dry Weight

NaCl (mM) CONTROL	SHOOT DRY WEIGHT (gm)	ROOT DRY WEIGHT (gm)
0	0.09 c	0.006597 a
70	0.110233 c	0.008000 a
150	0.270667 b	0.006893 a
NaCl (mM) TREATMENT	SHOOT DRY WEIGHT (gm)	ROOT DRY WEIGHT (gm)
0	0.138933 c	0.011162 a
70	0.349433 ab	0.029087 a
150	0.4236 a	0.017709 a

Values represent mean of replicates in the table mentioned above.

3.3. Number of leaves and Leaf Area

The presenting results of individual microbial inoculation of *P.indica* was about 61% and 16% respectively at 150mM and 70mM NaCl concentration (Fig.3.3A), which thereby shows that high leaf count (number of leaves) was found in *P.indica* inoculated plants as compared to the uninoculated plants. The graph showed the linear increase at varied NaCl concentration on uninoculated, inoculated plants with *P.indica* . Although microbial inoculation has increased the overall leaf count as comparing to the uninoculated plants. However the results proved that at higher salinity stress, the microbial inoculation proves to be the best mediator in increasig the morphological parameters and increases the plant functionality by mitigating salt stress.

Leaf Area was increased upto 66% and 23% in *P.indica* inoculated plants at varied saline conditions of 150mM and 70mM respectively, exhibiting highest increase in leaf area as compared to other uninoculated plants (Fig.3.3B). Overall, microbial inoculation elevated the leaf area under high saline conditions as compared to uninoculated plants.

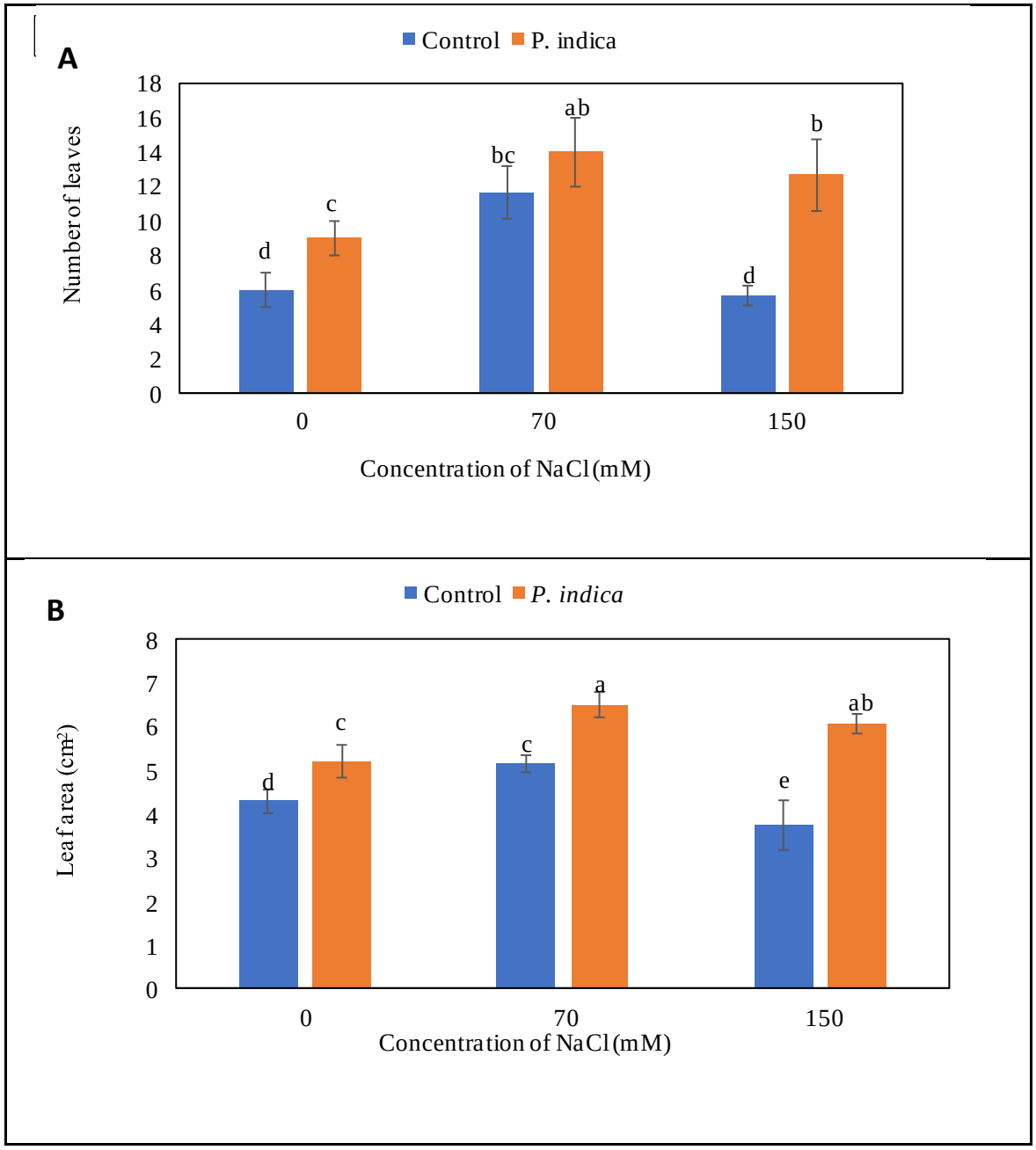


Fig. 3.3 Effects of different concentration of NaCl on morphological attributes in *T. foenum-graecum* plants inoculated with *P. indica* (A) Number of leaves (B) Leaf area

Table 3.3 Effects of different concentration of NaCl on morphological attributes in *T. foenum-graecum* plants inoculated with *P. indica* (A) Number of leaves (B) Leaf area

NaCl (mM) CONTROL	NUMBER OF LEAVES (cm)	LEAF AREA (cm^2)
0	6 d	4.276667 d
70	11.66667 bc	5.14 c
150	5.666667 d	3.733333 e
NaCl (mM) TREATMENT	NUMBER OF LEAVES (cm)	LEAF AREA (cm^2)
0	9 c	5.196667 c
70	14 ab	6.493333 a
150	12.66667 b	6.056667 ab

Values represent mean of replicates in the table mentioned above.

3.4. Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2

Among microbial treatments of *P. indica* and control (where no fungal treatment is given), the photosynthesis rate was positively increased to higher percentage in *P. indica* inoculated plants, it was about 50% and 66% at saline conditions of 150mM and 70mM respectively. The graph showed the linear increase in the photosynthesis rate at different saline condition (Fig. 3.4A). Even at the high salinity stress photosynthesis rate was increased upto higher percentage by the action of microbes, whereas uninoculated plants were not able to increase the rate of photosynthesis at high salinity as compared to the *P. indica* inoculated plants.

The microbial inoculated plants have shown positive effects on stomatal conductance.*P. indica* have shown the rise upto 60% and 57% at (150mM, 70mM) saline conditions respectively (Fig.3.4B). This show that *P. indica* is the best microbe to increase stomatal conductance under

high salinity than untreated (control) plants. Uninoculated plants could not make much higher increase as compared to the microbial inoculation of *P. indica*. Inoculation of *P. indica* was able to increase the stomatal conductance under both saline and non-saline conditions as well.

Salt stress affects the various physiological parameteres but microbes like *P. indica* helps in the mitigation of high salinity stress, also works well under the non saline conditions as compared to the uninoculated plants. Rate of Transpiration was increased upto 79% and 45% respectively (150mM, 70mM) in *P. indica* inoculated plants (Fig.3.4C). But, uninoculated plants again showed poor results and were not effective as *P. indica*, which works well under high salinity stress and increases the transpiration rate. So, even if salt stress occurs these fungal microbial inoculations will help the plants to combat stress.

P. indica again showed the remarkable positive results under high salinity and non saline conditions. *P. indica* has increased Internal CO_2 upto 54% and 16% at (150mM and 70mM) saline condition (Fig.3.4D). *P. indica* was more effective in mitigating high salinity than uninoculated plants. This shows that at higher salinity stress, when plants are inoculated with microbes than uninoculated counterparts, there is a significant increase in physiological parameteres which enhances the plant survival even under the high salinity and promotes other various attributes better for the plant health and survival.

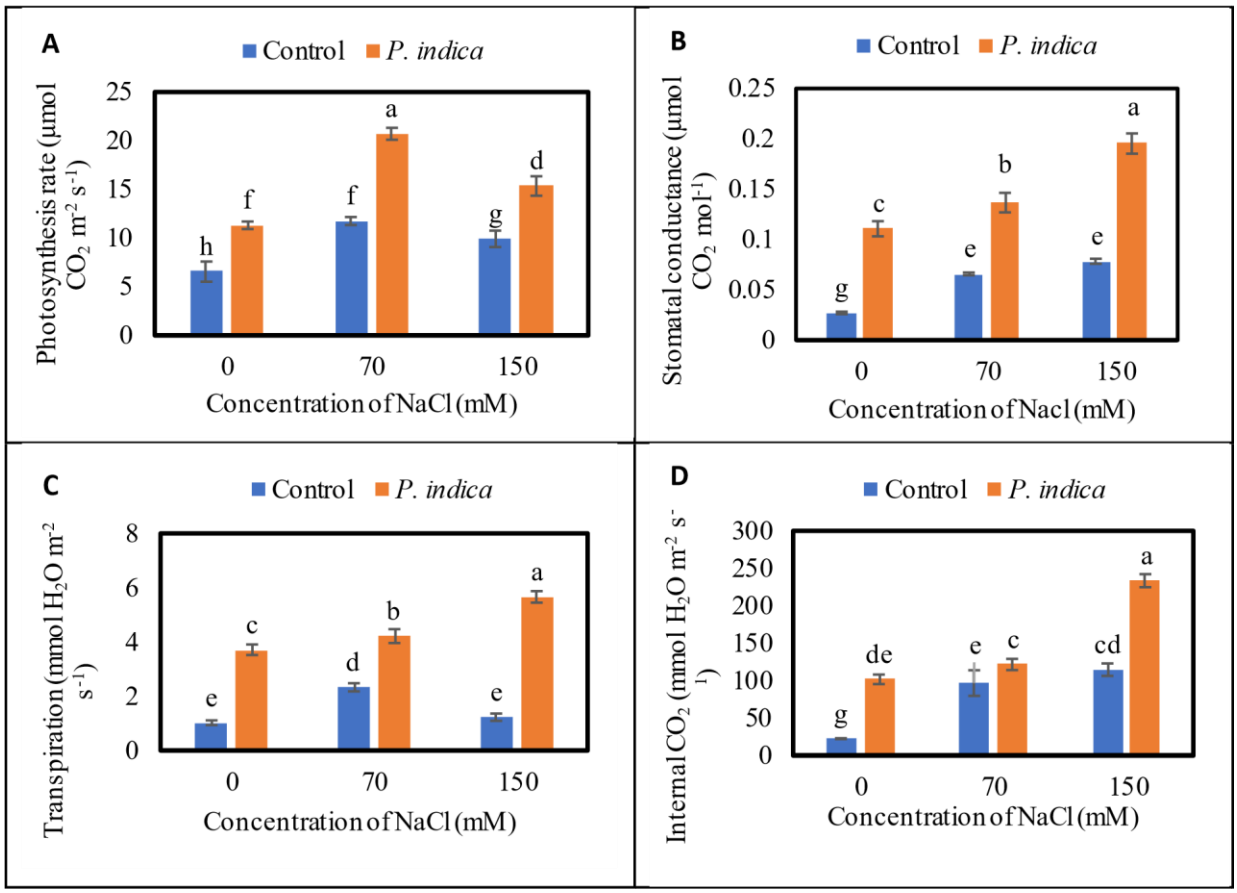


Fig. 3.4 Effects of different concentration of NaCl (0mM, 70mM, 150mM) on physiological attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Photosynthesis (B) Stomatal Conductance (C)Transpiration (D) Internal CO_2

Table 3.4 Effects of different concentration of NaCl (0mM, 70mM, 150mM) on physiological attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Photosynthesis (B) Stomatal Conductance (C)Transpiration (D) Internal CO_2

NaCl (mM) CONTROL	PHOTOSYNTHESIS (μmol CO_2 m^{-2} s^{-1})	STOMATAL CONDUCTANCE (μmol CO_2 mol^{-1})	TRANSPIRATION (m mol H_2O m^{-2} s^{-1})	INTERNAL CO_2 (m mol H_2O m^{-2} s^{-1})
0	6.546667 h	0.027067 g	1.019 e	22.09333 g
70	11.75 f	0.0658 e	2.326667 d	96.45 e
150	9.92 g	0.078333 e	1.223333 e	114.3333 cd
NaCl (mM) TREATMENT	PHOTOSYNTHESIS (μmol CO_2 m^{-2} s^{-1})	STOMATAL CONDUCTANCE (μmol CO_2 mol^{-1})	TRANSPIRATION (m mol H_2O m^{-2} s^{-1})	INTERNAL CO_2 (m mol H_2O m^{-2} s^{-1})
0	11.31333 f	0.110667 c	3.713333 c	101.4667 de
70	20.72 a	0.136667 b	4.216667 b	121.3333 c
150	15.35 d	0.195333 a	5.663333 a	233.3333 a

Values represent mean of replicates in the table mentioned above.

3.5. Carotenoids, Chlorophyll a, Chlorophyll b and Total chlorophyll

P. indica showed upto increase of 30% and 42% carotenoid content at (150mM and 70mM) saline condition respectively (Fig.3.5A). *P. indica* is very effective than uninoculated plants in elevating the carotenoid content at high salinity.

The presenting results about Chlorophyll a show that at high salinity, increase in chlorophyll a content was upto 36% in *P. indica*, the increase was minimal in uninoculated plants at high saline conditions of 150mM NaCl (Fig.3.5B). Uninoculated plants showed reduced results as compared to the microbial inoculation of *P. indica*.

Again, when the results were observed about the chlorophyll b content *P. indica* again showed higher increase in percentage, it was about 58% increase at high salinity,150mM NaCl (Fig.3.5C).

The total chlorophyll content showed the same trend as previous discussed results, *P. indica* again is very effective in elevating the responses associated with photosynthetic pigments, showed a total increase of 39% at high salinity (Fig.3.5D). All the results, showed a linear increase in the percentage at high salinity (150mM NaCl concentration). The plants which were not inoculated with microbes showed poor results and were not effective as fungal treatment of *P. indica.*

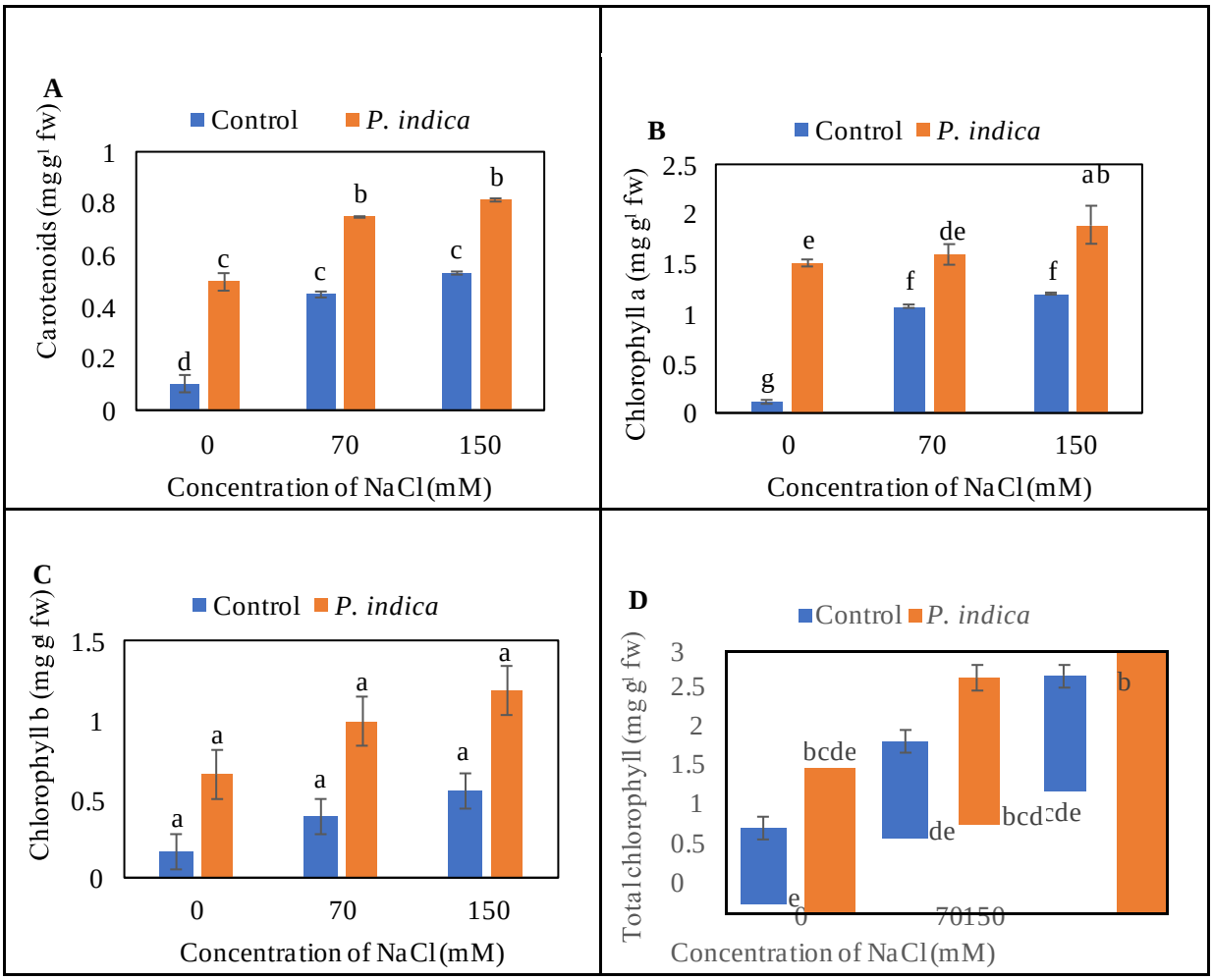


Fig. 3.5 Effects of different concentration of NaCl (0mM, 70mM, 150mM) on biochemical attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Carotenoids (B) Chlorophyll a (C) Chlorophyll b (D) Total Chlorophyll

Table 3.5 Effects of different concentration of NaCl (0mM, 70mM, 150mM) on biochemical attributes in *Trigonella foenum-graecum* plants inoculated with *Piriformospora indica* (A) Carotenoids (B) Chlorophyll a (C) Chlorophyll b (D) Total Chlorophyll

NaCl (mM) CONTROL	CAROTENOID (m g g^{-1} fw)	CHLOROPHYLL a (m g g^{-1} fw)	CHLOROPHYLL b (m g g^{-1} fw)	TOTAL CHLOROPHYLL (m g g^{-1} fw)
0	0.102736 d	0.1114 g	0.1639 a	0.963913 e
70	0.447717 c	1.073688 f	0.386284 a	1.242287 de
150	0.5307 c	1.200046 f	0.548111 a	1.465153 cde
NaCl (mM) TREATMENT	CAROTENOID (m g g^{-1} fw)	CHLOROPHYLL a (m g g^{-1} fw)	CHLOROPHYLL b (m g g^{-1} fw)	TOTAL CHLOROPHYLL (m g g^{-1} fw)
0	0.496431 c	1.508237 e	0.652343 a	1.685357 bcde
70	0.747648 b	1.59384 de	0.989404 a	1.880085 bcd
150	0.813579 b	1.89229 ab	1.1834 a	2.327367 b

Values represent mean of replicates in the table mentioned above.

3.6. Nitrogen and Protein (%)

Nitrogen and Protein content was higher in *P. indica* as compared to the uninoculated plants. Uninoculated plants showed poor results. The presenting results showed that Nitrogen% was upto 34% in *P. indica* at high saline concentration of 150mM (Fig. 3.6A). *P. indica* is very effective in increasing Nitrogen content. Protein % was recorded same as Nitrogen %, *P. indica* showed an increase of 34% whereas uninoculated plants showed the values far less than the inoculated plants with the fungal treatment of *P. indica* (Fig. 3.6B). Microbial inoculation helps in elevating Nitrogen% and Protein % than uninoculated plants.

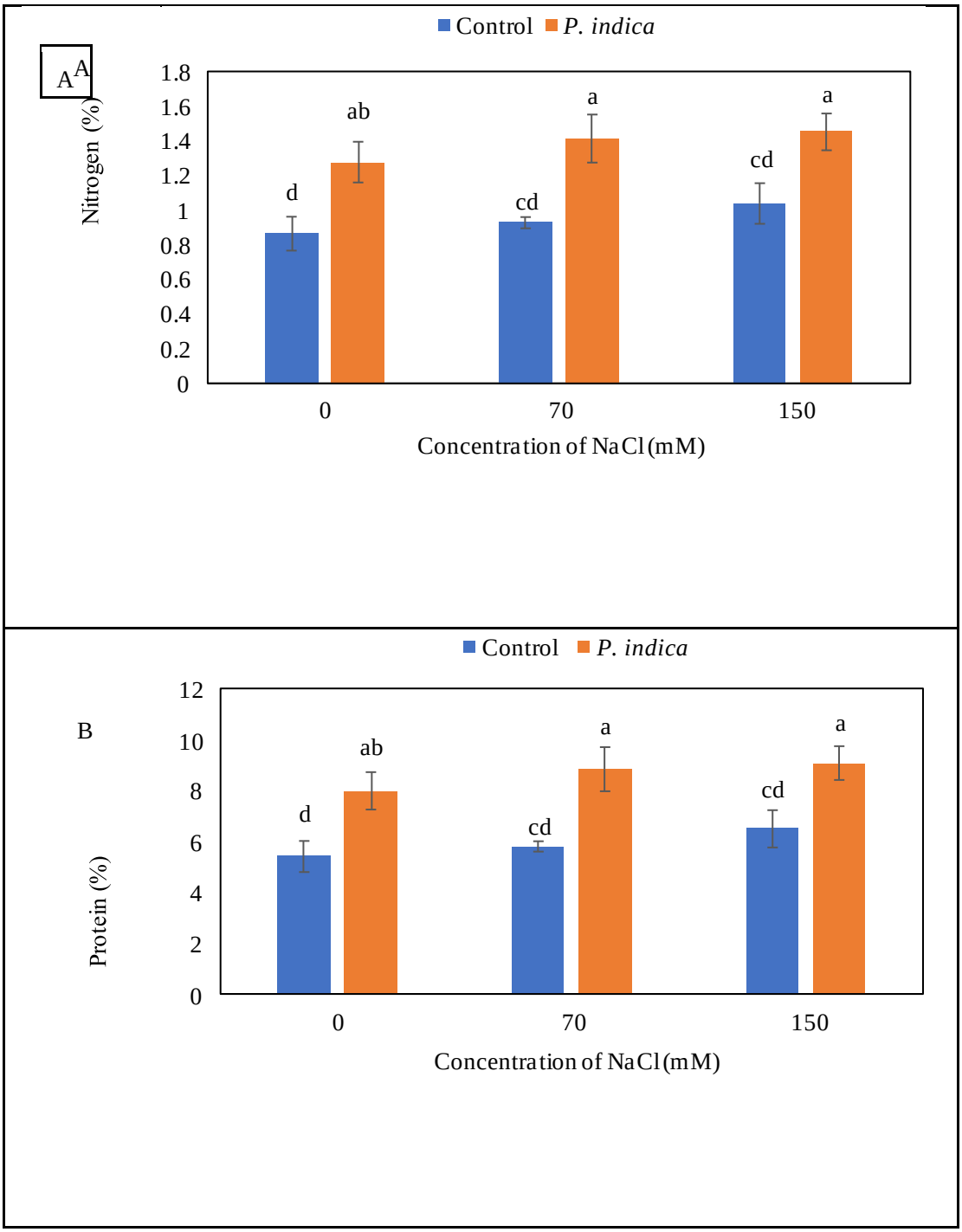


Fig.3.6 Effects of different concentration of NaCl on (A) Nitrogen (B) Protein in *T. foenum-graecum* plants inoculated with *P. indica*

Table 3.6 Effects of different concentration of NaCl on (A) Nitrogen (B) Protein in *T. foenum-graecum* plants inoculated with *P. indica*

NaCl (mM) CONTROL	Nitrogen %	Protein %
0	0.863898	5.399363
70	0.927213	5.795081
150	1.037897	6.486856
NaCl (mM) TREATMENT	Nitrogen %	Protein %
0	1.276618	7.978863
70	1.412628	8.828925
150	1.451086	9.069288

Values represent mean of replicates in the table mentioned above.

4. DISCUSSIONS

4.1 Shoot and Root Length

Inoculation with microorganisms have elevated the morphological responses like increase in the shoot and root length respectively, under high salinity conditions, which was unlike in the uninoculated plants under the high salinity.

Increase in the shoot and root length is caused by the intake of surplus amount of nutrients like nitrogen and many other essential nutrients, when inoculated with beneficial micro-organisms. Gupta and Pandey [30] have also showed increase in shoot and root length in french beans seedlings under salinity stress when inoculated with the strains of PGPB which are ACC02 and ACC06 respectively.

4.2 Biomass

Shoot and Root dry weight (biomass) was increased in the plants under high saline conditions when inoculated with the microorganisms. *P. indica* showed better results in elevating shoot dry mass than uninoculated plants under high salinity stress.

Elevation in the shoot and root biomass in the microbial inoculation is due to the numerous factors, one of them is increase availability of nutrients, proper solar radiation and photoperiod. Uninoculated plants showed poor results. Increase in the shoot and root dry mass was shown by [31] in *Aeluropus littoralis* when inoculated with fungi *Claroideoglomus etunicatum* under salinity stress.

4.3 Number of Leaves and Leaf Area

Number of leaves or leaf count was found to be maximum in the *P. indica* under the high saline conditions whereas uninoculated plants showed least number of leaf count. Uninoculated plants showed poor results under the salt stress. Number of leaves or leaf count increase is due to the division of cells causing change in leaf number. Leaf Area was found to be highest in the *P. indica* inoculation as compared to the uninoculated plants under extreme salinity stress. Leaf area is one of the most important factors which directly co-relates with the photosynthetic active area, elevation in leaf area is caused because of intake of various inorganic and organic nutrients, water availability, proper sunlight, temperature and soil conditions. Khalloufi et al [32] have

showed increase in the number of leaves (leaf count) and leaf area under saline stress when inoculated with fungi *Rhizophagus irregularis* in *Solanum lycopersicum* L. plants.

4.4 Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2 Microbial inoculation is very beneficial as it improves various physio-biochemical parameters like photosynthesis rate, stomatal conductance, transpiration and internal CO_2 even under the high salinity stress. Plants which are not inoculated with micro-organisms under salinity stress shows negative effects on development of plants.

P. indica showed remarkable results in elevating photosynthetic rate, stomatal conductance, transpiration and internal CO_2 than uninoculated plants. Photosynthesis rate is increased in the microbial inoculation even under the high salinity stress because of high leaf area which directly co-relates with the photosynthetic efficiency of plants.

Stomatal Conductance is a measure of the degree of the stomatal opening and acts as an indicator of plant water status, increase in the stomatal conductance is due to various factors like increasing temperature and plant-water relations.

Transpiration, on the other hand, is increased due to the apparent wind movements, light and temperature and also more transpiration means more removal of water vapor, creating suction pressure leading to the surplus intake of nutrients.

Increase in the internal CO_2 leads to the more photosynthesis in plants, causing plant growth and development. Various reasons for the increase in Internal CO_2 are frequent opening of the stomatal pores which leads to the diffusion of CO_2 in the plants causing increasing surge. Increase in the photosynthetic efficiency was shown by [33] in *Ocimum basilicum* L. when inoculated with arbuscular mycorrhizal fungi (*Glomus deserticola*) under high salinity stress.

4.5. Carotenoids, Chlorophyll a, Chlorophyll b and Total chlorophyll

P. indica showed positive remarkable results in elevating the photosynthetic pigments even under the high salinity stress as compared to the other uninoculated plants. Increase in the photosynthetic pigments is due to the increase in the photosynthesis rate. Uninoculated plants showed poor results. Seeds inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens* caused significantly increase in the photosynthetic pigments of radish plants under salinity stress [34].

4.6. Nitrogen and Protein

Beneficial fungi such as *P. indica* helps in the elevating growth, physiological and biochemical responses for the better growth and development of plants, *P. indica* showed remarkable results in elevating nitrogen and protein than PGPB. Nitrogen is a major component of chlorophyll, through which plants uses sunlight and produce sugars and oxygen. Increase in the chlorophyll content marks the increasing surge in Nitrogen content. Also, nitrogen is the building blocks of the amino acids, increase in the nitrogen content co-relates with the increase in protein content in the plants. Nitrogen content was also increased in *Acacia saligna* (Labill.) under high salinity stress when inoculated with arbuscular mycorrhizal fungi [35].

CONCLUSION

P. indica inoculated fenugreek plants showed enhanced morphological attributes (shoot and root length, shoot and root dry mass, leaf count and leaf area) and physiological responses (photosynthesis, stomatal conductance, transpiration, internal CO_2) during salinity stress as compared to uninoculated plants. The results presented in this investigation clearly showed that *P. indica* improved salt stress tolerance potential of fenugreek plants, by enhanced accumulation of carotenoids, chlorophyll a, chlorophyll b, total chlorophyll, nitrogen and protein content in plants during salinity stress. The improved physiological and biochemical responses in *P. indica* inoculated plants under salinity stress, also indicate that plant-microbe interaction is very beneficial in mitigating salinity stress in fenugreek plant.

REFERENCES

1. S. Zhang et al., "Salt-tolerant and plant growth-promoting bacteria isolated from high-yield paddy soil," *Can. J. Microbiol.*, vol. 64, no. 12, pp. 968-978, 2018 [doi:10.1139/cjm-20170571].
2. B. K. Singh et al., "Emerging microbiome technologies for sustainable increase in farm productivity and environmental security," *Microbiol. Aust.*, vol. 39, no. 1, pp. 17-23, 2018 [doi:10.1071/MA18006].
3. A. Jogawat et al., "*Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress," *Plant Signal. Behav.*, vol. 8, no. 10, p. doi: 10.4161/psb.26891, 2013 [doi:10.4161/psb.26891]
4. I. N. Daliakopoulos et al., "The threat of soil salinity: A European scale review," *Sci. Total Environ.*, vol. 573, pp. 727-739, 2016 [doi:10.1016/j.scitotenv.2016.08.177].
5. N. Bharti et al., "Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress" [Sci. rep., p. 34768], *Sci. Rep.*, vol. 6, 34768, 2016 [doi:10.1038/srep34768].
6. S. Shilev, "Plant-growth-promoting bacteria mitigating soil salinity stress in plants," *Appl. Sci.*, vol. 10, no. 20, p. 7326, 2020 [doi:10.3390/app10207326].
7. E. Tavakkoli et al., "High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress," *J. Exp. Bot.*, vol. 61, no. 15, pp. 4449-4459, 2010 [doi:10.1093/jxb/erq251].
8. D. Clarke et al., "Projections of on-farm salinity in coastal Bangladesh," *Environ. Sci. Process Impacts*, vol. 17, no. 6, pp. 1127-1136, 2015 [doi:10.1039/c4em00682h].

9. B. Gupta and B. Huang, "Mechanism of salinity tolerance in plants: Physiological, biochemical and molecular characterization," *Int. J. Genomics*, vol. 2014, p. 701596, 2014 [doi:10.1155/2014/701596].
10. A. Kumar et al., "Plant growth-promoting bacteria: Biological tools for the mitigation of salinity stress in plants," *Front. Microbiol.*, vol. 11, p. 1216, 2020 [doi:10.3389/fmicb.2020.01216].
11. P. Carillo et al., "Salinity stress and salt tolerance" in *Tech*, vol. 1, A. Shanker, Ed.: Abiotic stress in plants – mechanisms and adaptations, 2011, pp. 21-38.
12. A. K. Parida and A. B. Das, "Salt tolerance and salinity effects on plants: A review," *Ecotoxicol. Environ. Saf.*, vol. 60, no. 3, pp. 324-349, 2005 [doi:10.1016/j.ecoenv.2004.06.010].
13. R. Saravanavel et al., "Effect of sodium chloride on photosynthetic pigments and photosynthetic characteristics of *Avicennia officinalis* seedlings," *Recent Res. Sci. Technol.*, vol. 3, pp. 177-180, 2011.
14. M. Hanin et al., "New insights on plant salt tolerance mechanisms and their potential use for breeding," *Front. Plant Sci.*, vol. 7, p. 1787, 2016 [doi:10.3389/fpls.2016.01787].
15. R. C. Nongpiur et al., "Genomics approaches for improving salinity stress tolerance in crop plants," *Curr. Genomics*, vol. 17, no. 4, pp. 343-357, 2016 [doi:10.2174/1389202917666160331202517].
16. B. Carmen and D. Roberto, "Soil bacteria support and protect plants against abiotic stresses" in *Abiotic Stress in Plants Mechanisms and Adaptations*, A. Shaner, Ed., (London: InTech), 2012, pp. 143-170.
17. K. K. Meena et al., "Abiotic stress responses and microbe mediated mitigation in plants: The omics strategies," *Front. Plant Sci.*, vol. 8, p. 172, 2017 [doi:10.3389/fpls.2017.00172].

18. M. C. Enebe and O. O. Babalola, "The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: A survival strategy," *Appl. Microbiol. Biotechnol.*, vol. 102, no. 18, pp. 7821-7835, 2018 [doi:10.1007/s00253-018-9214-z].

19. M. A. Siddikee et al., "Isolation, characterization and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil," *J. Microbiol. Biotechnol.*, vol. 20, no. 11, pp. 1577-1584, 2010 [doi:10.4014/jmb.1007.07011].

20. W. Wang et al., "Plant growth promotion and alleviation of salinity stress in Capsicum annuum L. by Bacillus isolated from saline soil in Xinjiang," *Ecotoxicol. Environ. Saf.*, vol. 164, pp. 520-529, 2018 [doi:10.1016/j.ecoenv.2018.08.070].

21. L. Chen et al., "Veronican NK, Shen," *Physiol. Plant.*, vol. 158, no. 1, pp. 34-44, 2016 [doi:10.1111/ppl.12441].

22. R. Krishnamoorthy et al., "Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil," *Agric. Ecosyst. Environ.*, vol. 231, pp. 233-239, 2016 [doi:10.1016/j.agee.2016.05.037].

23. S. M. Kang et al., "Gibberellin secreting rhizobacterium, Pseudomonas putida H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions," *Plant Physiol. Biochem.*, vol. 84, pp. 115-124, 2014 [doi:10.1016/j.plaphy.2014.09.001].

24. P. S. Shukla et al., "Improved salinity tolerance of Arachis hypogaea (L.) by the interaction of halotolerant plant-growthpromoting rhizobacteria," *J. Plant Growth Regul.*, vol. 31, no. 2, pp. 195-206, 2012 [doi:10.1007/s00344-011-9231-y].

25. A. Bano and M. Fatima, "Salt tolerance in Zea mays (L.) following inoculation with Rhizobium and Pseudomonas," *Biol. Fertil. Soils*, vol. 45, no. 4, pp. 405-413, 2009

[doi:10.1007/s00374-008-0344-9].

26. D. Hassani et al., "Morphophysiological and molecular evidence supporting the augmentative role of Piriformospora indica in mitigation of salinity in Cucumis melo L.," *Acta Biochim. Biophys. Sin. (Shanghai)*, vol. 51, no. 3, pp. 301-312, 2019 [doi:10.1093/abbs/gmz007].
27. A. Ahmad et al., "Fenugreek a multipurpose crop: Potentialities and improvements," *Saudi J. Biol. Sci.*, vol. 23, no. 2, pp. 300-310, 2016 [doi:10.1016/j.sjbs.2015.09.015].
28. J. D. Hiscox and G. F. Israelstam, "A method for the extraction of chlorophyll from leaf tissue without maceration," *Can. J. Bot.*, vol. 57, no. 12, pp. 1332-1334, 1979 [doi:10.1139/b79-163].
29. *The Determination of Nitrogen According to Kjeldahl Using Block Digestion and Steam Distillation (AN 300, EN ISO 20483:2006)*.
30. S. Gupta and S. Pandey. ACC, "ACC Deaminase Producing Bacteria With Multifarious Plant Growth Promoting Traits Alleviates Salinity Stress in French Bean (Phaseolus vulgaris) Plants." *Front. Microbiol.*, vol. 10, p. 1506, 2019 [doi:10.3389/fmicb.2019.01506].
31. R. Hajiboland et al., "Physiological responses of halophytic C4 grass, Aeluropus littoralis to salinity and arbuscular mycorrhizal fungi colonization," *Photosynthetica*, vol. 53, no. 4, pp. 572-584, 2015 [doi:10.1007/s11099-015-0131-4].
32. M. Khalloufi et al., "The interaction between foliar GA3 application and arbuscular mycorrhizal fungi inoculation improves growth in salinized tomato (Solanum Lycopersicum L.) plants by modifying the hormonal balance," *J. Plant Physiol.*, vol. 214, pp. 134-144, 2017 [doi:10.1016/j.jplph.2017.04.012].
33. K. M. Elhindi et al., "The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.)," *Saudi J. Biol. Sci.*, vol. 24, no. 1, pp. 170-179, 2017 [doi:10.1016/j.sjbs.2016.02.010].

34. H. I. Mohamed and E. Z. Gomaa, "Effect of plant growth promoting Bacillus subtilis and Pseudomonas fluorescens on growth and pigment composition of radish plants (Raphanus sativus) under NaCl stress," *Photosynthetica*, vol. 50, no. 2, pp. 263-272, 2012 [doi:10.1007/s11099-012-0032-8].

35. A. S. Soliman et al., "Improving salinity tolerance of Acacia saligna (Labill.) plant by arbuscular mycorrhizal fungi and Rhizobium inoculation," *Afr. J. Biotechnol.*, vol. 11, pp. 1259-1266, 2014.

36. B. Giri et al., "Improved tolerance of acacia nilotica, to salt stress by arbuscular mycorrhiza, Glomus fasciculatum, may be partly related to elevated K/Na ratios in root and shoot tissues," *Microb. Ecol.*, vol. 54, no. 4, pp. 753-760, 2007. doi:10.1007/s00248-007-9239-9.

37. R. Hajiboland et al., "Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato Solanum Lycopersicum L. plants," *Plant Soil*, vol. 331, no. 1-2, pp. 313327, 2010. doi:10.1007/s11104-009-0255-z.

38. R. Hajiboland et al., "Physiological responses of halophytic C4 grass, Aeluropus littoralis to salinity and arbuscular mycorrhizal fungi colonization," *Photosynthetica*, vol. 53, no. 4, pp. 572-584, 2015. doi:10.1007/s11099-015-0131-4.

39. A. Hashem et al., "Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in Cucumis sativus L.," *Saudi J. Biol. Sci.*, vol. 25, no. 6, pp. 1102-1114, 2018. doi:10.1016/j.sjbs.2018.03.009.

40. M. Khalloufi et al., "The interaction between foliar GA3 application and arbuscular mycorrhizal fungi inoculation improves growth in salinized tomato Solanum Lycopersicum L. plants by modifying the hormonal balance," *J. Plant Physiol.*, vol. 214, pp. 134-144, 2017. doi:10.1016/j.jplph.2017.04.012.