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PLANT GROWTH PROMOTING AND ANTAGONISTIC ACTIVITY OF CONSORTIA OF HALOTOLERANT *BACILLUS* AND *RHIZOBIUM* SPP

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Abstract

Salinity prevents plants from taking up water and exposing them to draught stress, to overcome this, studies have shown that halotolerant organism can be effective in plant growth promotion in saline condition. Studies have also shown that consortium strains are more effective in plant growth promotion and bio control activity. In the present study, from various soil samples total 11 isolates were obtained. Morphological and biochemical analysis confirmed that 8 were Rhizobium and 3 were Bacillus spp.

Halotolerant analysis confirmed that 3 isolates could tolerate salt concentration up to 6%, hence these three were used for further studies. The isolates were screened for plant growth promotion assays; it includes Indole acetic acid production, Phosphate Solubilization and Zinc Solubilization. The isolates were also screened for lytic enzymes production like (Chitinase, Protease and Cellulase).

In pot assay, plant growth promotion for leguminous (Methi plant) and non- leguminous (rice plant), with single and consortium strains under halotolerant condition were done, the result showed subsequent increase in growth of shoot length and root length. Application of mixture of strains resulted with notable increase in plant growth compared to the individual strain. In pot assay, in bio-control activity consortium organism were found to inhibit plant pathogens and hence can be used as bio-control agents. Thus from the above study it can be concluded that the use of consortium organisms in field application can be used as biocontrol and bio-fe rtilizer agents.

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Key words- *Consortia, Halotolerant, Leguminous, Non-leguminous, Bio-control activity, Plant stress (Salinity), Plant promotion activity*

Introduction

For centuries, agriculture in arid and semiarid environments has faced an increase in soil salinity. Salinity is one of the major abiotic stress factor limiting plant growth and productivity. The total salt-affected land worldwide is estimated to be 900 million ha, 6% of the total global land mass [Flowers, 2004]. According to the Food and Agricultural Organization (FAO), if corrective measures are not taken, salinization of arable land will result in 30%
land loss in the next 25 years and up to 50% by the year 2050 [Munns, 2002]. Salinity prevents plants from taking up water, exposing them to drought stress. These stresses have an adverse effect on plants, hampering their growth and finally production. Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in the areas devoted to agriculture; therefore, the urgent need of biological agents (bio-preparations) is accepted worldwide.

Salinity affects plant growth. The reduction of plant growth is the result of the alteration of many physiological activities in the plant, such as photosynthetic activity, mineral uptake and nutrient deficiency. Soil salinity particularly disturbs the symbiotic interaction between legumes and Rhizobia. Thus, the development of salt-tolerant symbioses is an absolute necessity to enable cultivation of leguminous crops in salt-affected soils. Plant growth promoting rhizobacteria (PGPR) has potential to alleviate salt stress. Various studies have demonstrated PGPR under salt stress, but there are very few studies which have focused on the interaction between Rhizobium and PGPR under salt stress, mainly the *Rhizobium-Bacillus* interaction. Under laboratory condition Rhizobium-Bacillus interaction need to be studied to have potent bio-preparation so that plant can grow efficiently and yield more even under saline condition as well.

Methodology

Isolation of potential isolates: Methi plants with healthy root nodules were collected from local market. Soil samples were collected from different places like farm soil, root soil of

mangrove, rhizosphere soil of mangrove, garden soil etc. in sterile plastic bags. Healthy Root nodules were washed with tap water twice and then surface sterilized externally using 95% alcohol [1-4 minutes] and then followed by sodium hypochlorite [5 minutes] treatment. They were further washed with sterile water, crushed and streaked on CRYEMA plates. The plates were then incubated at 28°C in the dark for 48 hours [Monica Niste, *et al* (2015)].

Morphologically different colonies were picked from these plates and re-streaked on CRYEMA plates for several days for purification of the isolates. Bacterial isolates were identified on the basis of morphological & biochemical characters [Ambika R., *et al* (2014)].

Salt tolerance ability of isolates or osmo-adaptation assay: The ability of the isolates to grow at different concentrations of salt was tested by inoculating isolates in nutrient broth media containing 0.5%, 1%, 2%, 3%, 3.5%, 4%, 5%, 6% & 7% (w/v) NaCl and incubated at R.T for 24 hrs (Trivedi, R., & Arora, S.).Osmotic potential of broth medium was measured by colorimeter at 550
nm [Waraich, E. A. (2017)].

Assay of plant growth promoting factors in consortia of halotolerant *bacillus* and *rhizobium* spp.:

The bacterial isolate were grown in consortium in tryptone broth and incubated at 30◦C for 24 hr and centrifuged to collect supernatant. Two drops of O-phosphoric acid and 4 ml Salkowski's reagent were added to 1 ml of supernatant, appearance of pink colour confirmed the production of IAA [Mandal S., *et al* (2017)].

Phosphate solubilization test was performed by spot inoculation of consortium test organisms on Pikovskaya's medium. The plates were incubated at 30±1°c for 4-5 days. [Mandal S., *et al* (2017)].

Zinc solubilization ability of the isolates were detected by spotting the log phase culture of consortium bacterial strains on Tris-minimal medium plates having zinc phosphate, and zinc carbonate as source of insoluble inorganic zinc along with bromophenol blue as pH indicator or modified Pikovskaya medium which contain zinc oxide as source of zinc. The inoculated plates were then incubated at 30◦C for3days, and observed for the clearing zone around the colonies [Mandal S., *et al* (2017)].

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Exopolysaccharide production in consortia of halotolerant *bacillus* and *rhizobium* spp.: Experiment was done using 250 ml flasks each containing 100 ml of basal and malt medium inoculated with consortium test organism i.e halotolerant

Bacillus and halotolerant *Rhizobium* spp.The flaks were then incubated at 37°C for 72 hrs [Vijayabaskar, P., *et al* (2011)]. Samples from flasks were separated and concentrated to small volumes. The EPS was then precipitated from the supernatant by addition of equal volume of alcohol and left over night at 4°C before centrifugation at 7000 rpm for 20 min. After centrifugation, the precipitate was collected in Petri plates and dried at 60°C.

Enzymatic assay of consortia of halotolerant *bacillus* and *rhizobium spp.*

Organisms were spot inoculated on Chitinase detection agar (CDA), Starch agar medium plate, Carboxyl methyl cellulose (CMC) agar plates and Skim milk agar plates and incubated at 30±1 º C for 5days. After the incubation the clearance zone formation surrounding colonies indicates the production of respective enzymes [Mandal S., *et al* (2017)].

Antifungal activity of consortium

Well diffusion assay: Antifungal effects of consortium test organisms were evaluated against the pathogenic fungi (*Aspergillus niger*) by agar well diffusion method. Saline was used as negative control. The plates were incubated for 3-4 days at 28°C and the zone of inhibition was recorded [Pawar, V. A, *et al* (2014)]. After obtaining zone of inhibition the experiment was carried out using leguminous plants.

Pot Assay for antifungal activity: Approximately 15 Methi seeds were treated with consortium organism and sown in pots containing 50 gms of autoclaved soil. The pots were monitored for 15 days. To check antifungal activity *Aspergillus* culture was spread on Methi leaves and the plants were observed for 30 days [Pawar, V. A, *et al* (2014)].

Plant Growth promotion test:

The experiment was run in two sets. Methi seeds were treated with consortium organism and sown in pots containing 50 g autoclaved soil. Seeds without inoculum served as control. The pots were monitored for 15 days [Pawar, V. A, *et al* (2014)].

Set A: Pot A (soil + untreated seeds], Pot B (Soil + seeds treated with *Rhizobium* culture inoculums), Pot C (Soil + seeds treated with *Bacillus* culture inoculums), Pot D (Soil + seeds treated with *Rhizobium* culture + *Bacillus* culture inoculums).

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Set B: Pot A (soil + untreated seeds + NaCl] Pot B (Soil + seeds treated with *Rhizobium* culture inoculums + NaCl).Pot C (Soil + seeds treated with *Bacillus* culture inoculums+ NaCl), Pot D (Soil + seeds treated with *Rhizobium* culture + *Bacillus* culture inoculums+ NaCl) [Bhattacharya, C., & Pandey, B. (2015)]. The same sets (SET A and SET B) experiments were repeated for non leguminous plants also.

Results and Discussion:

The potential isolates were identified by morphological and biochemical tests. By comparing the biochemical results with standard biochemicals as mentioned in Bergeys manual of Systemic Bacteriology, isolates showed similarity with *Rhizobium spp.* and *Bacillus spp.*Out of the total isolates obtained IS 1, IS 3 & IS 8 were able to tolerate high salt Concentration .ISI was identified as *Bacillus spp* and the other two as *Rhizobium Spp*(IS3, IS8), All three isolates were found to tolerate salt upto 6%

Table No. 1: Salt tolerance for Isolates: *Bacillus* and *Rhizobium spp*

	O.D (nm) Salt Concentrations (IN %)										
	0.5%	1%	2%	3%	3.5%	4%	5%	6%	7%	8%	8.5%
IS 1	**0.70**	**0.38**	**0.34**	**0.30**	**0.37**	**0.24**	**0.28**	**0.03**	**0.00**	**0.00**	**0.00**
IS 2	0.11	-	-	0.03	-	-	-	-	-	-	-
IS 3	**0.48**	**0.42**	**0.35**	**0.33**	**0.30**	**0.35**	**0.33**	**0.03**	**0.00**	**0.00**	**0.00**
IS 4	0.68	0.68	0.43	0.38	0.38	0.29	0.29	0.00	0.00	0.00	0.00
IS 5	0.66	0.64	0.38	0.48	0.51	0.35	0.33	0.01	0.00	0.00	0.00
IS 7	0.24	0.24	0.21	0.17	0.17	0.14	0.12	0.00	0.00	0.00	0.00
IS 8	**0.37**	**0.37**	**0.34**	**0.33**	**0.32**	**0.30**	**0.29**	**0.02**	**0.00**	**0.00**	**0.00**
IS 9	0.49	0.51	0.29	0.26	0.24	0.26	0.25	0.02	0.00	0.00	0.00
IS 10	0.44	0.51	0.30	0.28	0.29	0.25	0.28	0.00	0.00	0.00	0.00

Assay of Plant growth promoting factors in consortia of Halotolerant *Bacillus* and *Rhizobium spp*.

The potential isolates in consortia were analyzed for various plant growth promotion characters, like IAA, Phosphate solubilization, Zinc solubilization. It was observed that

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consortia of *Bacillus* and *Rhizobium spp* were more effective in plant growth promotion than individual isolates. This indicates that consortia enhance plant growth promotion.

Table No.2 Indole Acetic Acid Production by Isolates

Sr. no	Test	Total volume	2 drops of o-phosp horic acid	Reagent	Incubate at room temperature for 30 minutes	O.D (530nm)
1	Bacillus	2		4		0.12
2	IS3	2		4		0.14
3	IS8	2		4		0.16
4	**B + IS3**	**2**		**4**		**0.18**
5	**B + IS8**	**2**		**4**		**0.22**

Garph:1 Indole Acetic acid Production by Isolates

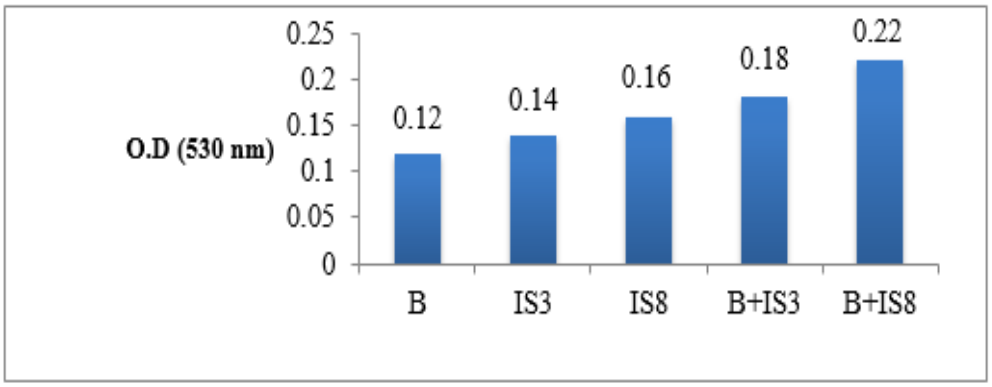

Table No: 3 Phosphate and Zinc Solubilization by single strain and consortium

Isolate code	Phosphate(solubilisation) Zone size (mm)	Zinc (Solubilization) Zone size (mm)
Bacillusspp.	25	26
IS3	32	28
IS8	28	30
B + IS3	**36**	**32**
B + IS8	**34**	**36**

Exopolysaccharide production in consortia of halotolerant *bacillus* and *rhizobium* spp.: All isolates were able to produce exopolysaccharide. *Bacillus spp.* (IS1)produced 0.005 gm/10 ml exopolysaccharide *Rhizobium spp*. (IS 3 & IS 8) was produced 0.011 &0.07 gm/10 ml exopolysaccharide respectively. Consortium (B + IS3 & B + IS8) produced 0.112 &0.12 gm/10 ml exopolysaccharide which was found to be more than the individual strain.

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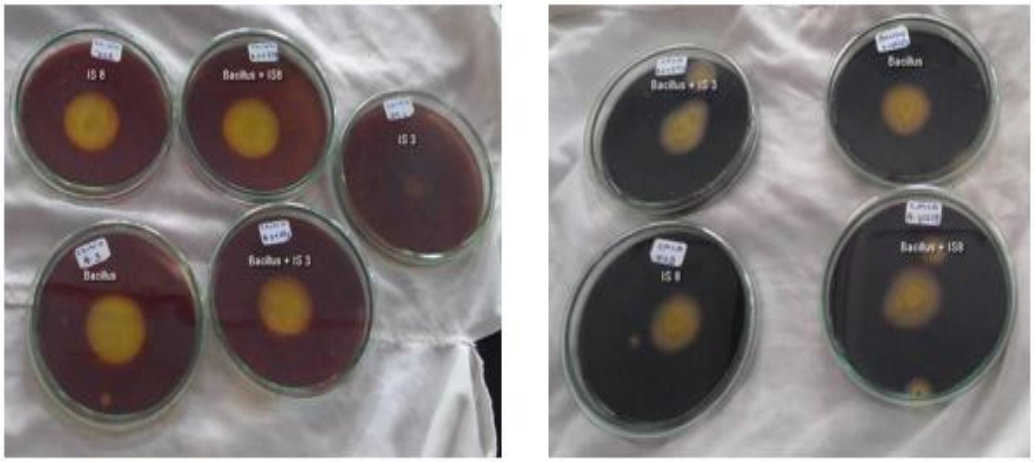
Table No. 4: Exopolysaccharide produced by single strain and consortium

Isolate code	Weight of filter paper (before) gm	Weight of filter paper (after) gm	Difference
Bacillusspp.	0.418	0.423	0.005
IS3	0.367	0.378	0.011
IS8	0.374	0.381	0.07
B + IS3	**0.367**	**0.479**	**0.112**
B + IS8	**0.367**	**0.487**	**0.12**

Enzymatic assay of consortia of halotolerant *bacillus* and *rhizobium spp.:* In Enzymatic assays it was found that consortium produced more amounts of enzymes (Chitinase, cellulose and protease than individual strains. It means consortium can be considered as better biocontrol agent than individual strains

Isolate code	Chitinase Zone size (mm)	Cellulase Zone size (mm)	Protease Zone size (mm
Bacillusspp.	34	32	32
IS3	11	30	28
IS8	34	29	29
B + IS3	**36**	**34**	**33**
B + IS8	**38**	**35**	**34**

Figure 1: Chitinase and Cellulase produced by single strain and consortium



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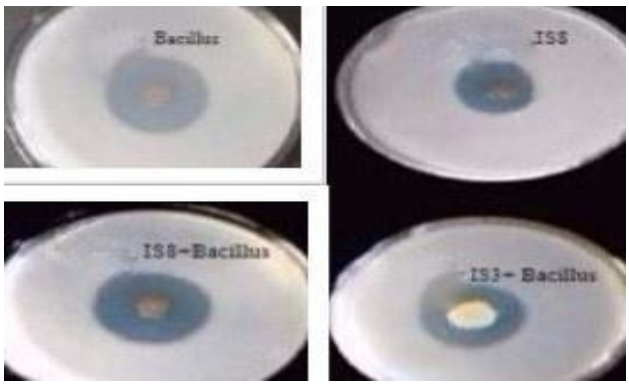

Antifungal activity of consortium on leguminous plants by pot assay: Agar well diffusion assay was conducted to analyze the antifungal activity of the consortium (Bacillus and Rhizobium). Zone diameter of 60mm around the well indicates antagonistic activity of consortia against the fungus. Further to confirm pot assay was performed. After analyzing it was found that pots without application of consortia were affected by fungus and showed symptoms like burning of young leaves and shoot rot. The pots which were applied with consortia organisms were not affected by fungus and hence can be commented that consortia shows antagonist activity against the fungus.

Table No. 6: Antifungal activity of consortium by agar well diffusion assay

Isolate code	Zone size (mm)
Bacillusspp.	43
IS3	34
IS8	38
B + IS3	**50**
B + IS8	**60**

Table No. 7:Antifungal activity of consortium on leguminous plants

Isolate code			Observation
Control	-	pot 1	Burning of young leaves , shoot rot
B + IS 8	-	**pot 2**	**No effect like control and individual strain plant**
B + IS3	-	**pot 3**	**No effect like control and individual strain plant**
IS8	-	pot4	2-3 leaves were burned
IS 3	-	pot5	2-3 leaves were burned
Bacillus spp .-		pot 6	2-3 leaves were burned

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Plant growth promoting effect of consortium on leguminous and non- leguminous plants by pot assay:

Pot assay was performed to check the efficacy of consortia on leguminous and non leguminous plants in saline conditions. Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigor and crop yield. Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (Munns 2002). The co-inoculation of legumes with *Rhizobium* and Bacillus *strains* were able to alleviate salt stress of plants grown in salt-affected soils. Individual and consortium plant growth promoting activities (PGPA) were compared where PGPA was found to be more in consortium than the individual isolate. The root and shoot length of leguminous and non-leguminous plants is more of consortia than individual strains Thus co-inoculation of *Bacillus subtilis* with *Rhizobium* can be a biopreparation as biofertilizer for agriculture-use.

Isolate code	Root height (cm)Non saline soil	Root height (cm) in saline soil	Shoot height (cm) Non saline soil	Shoot height (cm) saline soil	Complete Plant height (cm)non saline soil	Complete Plant height (cm) Saline soil
control	3	1	3	3	6 .5	5
Bacillusspp.	4	2	5	6.5	10.5	9
IS3	5	4	4.5	4	12	11
IS8	6	4.5	5	5	14	13
B + IS3	**7**	**5.5**	**9**	**5**	**17.5**	**14**
B + IS8	**8**	**6.5**	**9**	**7**	**18.5**	**16**

Fig :4 Plant Growth promotion of consortia on leguminous plants

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Table No.9: Plant growth promoting effect of consortium on Non-leguminous plant (Rice plant)

Isolate code	Root height (cm)	Shoot height (cm)	Complete Plant height (cm)
control	2	9	11
Bacillusspp.	4	13	17
IS3	6	16	22
IS8	7	17	24
B + IS3	**9**	**22**	**31**
B + IS8	**10**	**24**	**34**

Fig 5: Plant growth promotion of consortia in non leguminous plants

Discussion

High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity. Together, these effects reduce plant growth, development and survival. Plant growth promoting Rhizobacteria (PGPR) can be used to remediate contaminated soils in association plants, with increasing osmotic stresses, however

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work done on this co-inoculation is of very few extent. Salt tolerant, non -pathogenic PGPR co-inoculated with Rhizobium can serve as the good biofertilizer. *Bacillus spp* was found to tolerate salt tolerant up to 6%. IAA production by isolates and consortium was studied .Highest IAA production was seen in consortium test organism than individual organisms. Phosphate and zinc solubilization by isolates and consortium was studied. Zone of clearance around the colonies indicates solubilization of Zinc and phosphate. All the isolates and consortium were found to produce exoplosaccahride, (B+IS3) produced 0.112 gm/10 ml, and (B + IS8) produced 0.12 gm /10 ml. Consortia organisms showed secretion of various enzymes (Chitinase, protease and cellulase). All three microorganisms possess plant growth promoting activities and biocontrol activities This study demonstrates the interaction between *Rhizobium-Bacillus* under laboratory condition as well as direct interaction with plant by pot assay. This study did not involved interaction with plant in open field which need to be study further.

Conclusion

Agricultural productivity is severely affected by soil salinity because it affects plant growth in large terrestrial areas of the world. The damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. The loss of farmable land due to salinization is directly in conflict with the needs of the world population, which isprojected to increase by 1.5 billion over the next 20 years, and the challenge of maintaining the world food supplies. (Munns 2002).Soil salinity represent a serious threat to plant production. To overcome this, studies have shown that halotolerant organism can be effective on plant growth promotion in saline condition. By performing various experiment related to plant growth promotion it could be concluded that consortium is more effective in plant growth promotion than single strain. Biofertilizer most commonly refers to the use of soil microorganisms to increase the availability and uptake of mineral nutrients for plants. Plant growth promoting *Rhizobacteria* are the soil bacteria residing around/on the root surface and facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents thus can act as biofertilizer. The use of microorganisms with the aim of improving nutrients availability for plants is an important

practice and necessary for agriculture. But numerous studies have indicated that PGPR have great potential in biocontrol and plant growth promotion but most of the studies focus on plant growth promotion by using single/ individual strains. To overcome this, Studies have shown that consortium strains are more on plant growth promotion and bio control activity as compared to the single strain. By performing various experiment related to bio control activity it could be concluded that consortium is more effective in bio control activity than singlestrain.

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