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Effect of Plant Growth Promoting Microbial Consortium in Reclaiming the Wasteland and Induction of Crop Growth

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***Abstract* - Background and Objective: In recent decades, wasteland reclamation becoming a major concern all over the world. There are many approaches to reclaim the degraded land. In that Plant growth promoting microbes (PGPM) contributing the growth and establishment of plant under alkaline conditions. The aim of the present study was to see the effect of plant growth promoting microbial consortium in reclaiming the wasteland and induction of crop growth.**

Methods: Different strains of plant-growth-promoting microbes (PGPM) were isolated from waste land soil samples. They were screened in separate trials under alkaline conditions. The three most effective strains of PGPM (Algae - Spirulina platensis [A1], Bacteria - Alcaligenes sps [B1] and Fungi - Arbuscular mycorrhizal fungi [F1]) were checked for plant growth promotion separately. Microbial consortium were prepared and evaluated for their growth-promoting activity.

Findings: The results showed that salinity stress significantly reduced plant growth but inoculation with PGPM separately and synergistically enhanced plant growth. The combination of A1+B1+F1 was the most efficient for improving seedling growth and nodulation. The effect of auxin concentrations on plant growth was also evaluated by High performance, Liquid Chromotography. The intensity of the triple replicate assay with the root and shoot lengths of inoculated mung bean seedlings increases.

Novelty: Thus, coinoculation with PGPM containing different enzymes could be a useful approach for inducing salt tolerance and thus improving growth and nodulation in mung bean under salt-affected conditions.

***Index Terms* - Plant growth promoting microbes, nodules, consortium, alkaline and waste land.**

INTRODUCTION

The idea of eliminating the use of fertilizers which are sometimes environmentally unsafe is slowly becoming a reality because of the emergence of microorganisms that can serve the same purpose or even do better. Depletion of soil nutrients through leaching into the waterways and causing contamination are some of the negative effects of these chemical fertilizers that prompted the need for suitable alternatives. [4,5,7] This brings us to the idea of using microbes that can be developed for use as biological fertilizers (biofertilizers). They are environmentally friendly as they are natural living organisms. They increase crop yield and production and, in addition, in developing countries, they are less expensive compared to chemical fertilizers. These biofertilizers are typically called plant growth-promoting bacteria (PGPB). [6-12] In addition to PGPB, some fungi have also been demonstrated to promote plant growth (PGPM). Apart from improving crop yields, some biofertilizers also control various plant pathogens. The objective of worldwide waste land management is much more likely to be achieved through the widespread use of biofertilizers for reclamation. However, to realize this objective it is essential that the many mechanisms employed by PGPB first be thoroughly understood thereby allowing workers to fully harness the potentials of these microbes.[13-17] The present study is to employ PGPM for inducing salt tolerance, and thus improving growth and nodulation in mung bean under salt-affected conditions.

METHODS

Study area

Soil sample was collected from waste disposal site of Chennai Municipality.

Collection of samples

A total of 3 soil samples were collected from waste disposal site of Chennai Municipality. Sample (soil mixed with waste) was collected in sterile zip-lock plastic maintaining aseptic conditions, stored at 4 °C and marked accordingly to their source and location. The collected samples were brought to the laboratory for soil testing.

All the 3 samples were named as KL 01, KL 02, KL 03

Isolation and Identification of Halotolerant soil microbes:

Bacterial and fungal isolation

For isolation, 1gm soil sample was serially diluted upto 10^{-4}. From the last two dilution 0.1ml was inoculated on nutrient agar and Sabouraud dextrose agar (SDA) plates. These media were obtained from Hi Media Laboratories Pvt. Ltd. In both medium, additionally added sodium chloride (NaCl) 1M. Plates inoculated with suspensions were incubated at 28^0 to 30^0C for 48h for growth of halotolerant fungi and bacteria. Resulted colonies from the plates were selected with the help of sterile wire loop and using streaking plate technique streaked the colony on the nutrient agar plate and Sabouraud dextrose agar (SDA). Same preparation was done repeatedly to obtain the pure line of isolates under 1M NaCl (5.8%) stress conditions. At last the pure lines of colonies obtained needed to be characterize and named that isolates as A1, B2 andd F3.

Algal isolation

Soil was wetted sufficiently with pipette and covered with lamella and left under light for 24 hours for algae to do phototaxi motion. After that, algae that were taken from lamella by the help of inoculation loop; were put to the solid culture medium (BG –11)

The isolated bacteria and fungi were identified on the basis of microscopic examination and biochemical analysis according to Bergey's manual of Determinative Bacteriology. For the identification of algae, the necessary resources – Prescott., 1975. [25]

Preparation of Microbial consortium

To prepare the microbial consortium, loopfuls of each isolated species stored on selective agar slant were inoculated together in 50 mL MSM [Mineral Salt Medium]

Estimation of Plant growth:

Root and shoot length were measured using ruler in cm.

Chlorophyll estimation:

Arnon's method 100 gm of fresh leaves was ground in mortar and pestle using 80% acetone. The extract was centrifuged and the supernatant was taken and made up to 10ml. The optical density of the extract was taken at 652nm. Samples were prepared in duplicates. From the OD value, chlorophyll content was calculated using the formula:

Total Chlorophyll (mg/ml) = OD 652X1000 /34.5Xv/1000Xw

Where OD = Optical Density

V= Final volume of 80% acetone (10ml)

W = Wt. of the sample taken (100mg)

Quantitative evaluation of phytohormones – HPLC:

Physicochemical techniques, such as high-performance liquid chromatography (HPLC), have enabled the quantification of hormones and their metabolites with much greater precision, sensitivity, and speed.

RESULTS

Physical and chemical characteristics of municipal solid waste

Soil testing result analysis were mentioned in table: 1

1. Moisture content of the collected 3 samples from Municipality waste dump site were about 65.32%, 64.21%, 66.32% respectively.
2. The maximum bacterial density is found in regions of fairly high moisture content and the optimum level for the activities of aerobic bacteria often is a 50%-75% of the soil moisture holding capacity.
3. The ph was 8.20 in 01 and 03 samples.
4. ph of 02 was 8.45

Table: 1 Physio-chemical characteristics of soil sample

Soil tests	Results		
	S1	S2	S3
pH	8.20	8.45	8.20

NITROGEN	6	3	1
NITRATE	0.64	0.23	0.14
AMMONIUM	1.13	2.89	1.02
PHOSPHOROUS	4	2	1.98
POTASSIUM	32	43	28
SULFUR	5	4	2
CALCIUM	91	129	87
MAGNESIUM	24	43	21
SODIUM	0.2	1	0.4
IRON	3	5	6
MANGANESE	5	3.6	2
ZINC	0.24	0.18	0.33

Isolation and Identification of Selected Halo-tolerant microbes for reclammation:

Bacterial Isolation:

A bacteria were noticed to have salt tolerant capacity, which is identified by sugar fermentation tests. Hence, reported as Alcaligenes sps. [Table: 2, 3]

Table: 2 Biochemical Identification of Bacteria

Isolates	Gram Reaction	Spore former	Motility	Morphology	pH	Indole	Methyl Red	VP	Citrate	TSI	Urease
KL B1	+	-	+	Rods	8.2	-	-	-	+	-	-

Carbohydrate fermentation of bacterial isolates from soil

Isolate	Arabinose	Fructose	Succinate	Glucose	Lactose	Maltose	Xylose	Identification of Genus
KL B1	-	-	+	-	+	-	-	Alcaligenes sp

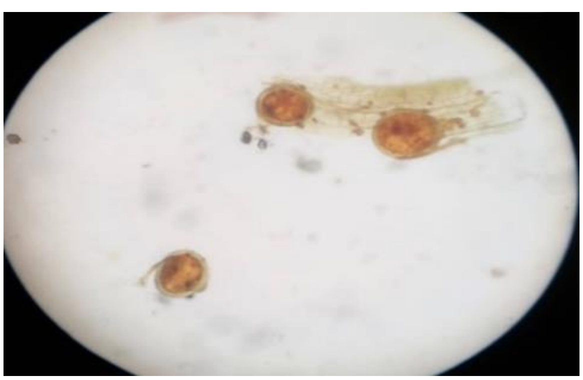


Figure: 1 Microscopic identification of fungi

Strain number	Colony Characterization	LPCB staining	pH	Identified fungi
KL F1	White creamy color elliptical shape with irregularities colonies seen	Cylindrical / slightly flared hyphae	7.5	*Rhzophagus spsi*

Carbohydrate fermentation of Fungal isolates from soil

Isolate	Arabinose	Fructose	Galactose	Glucose	Inositol	Fructose	Identification of Genus
KL F1	-	-	+	+	+	-	*Rhizophagus sps*

Algal isolation

Trichome, gelatinous substance were seen in thallus region. Psuedo vacuoles seen. pH range of the isolated algae is 8.6

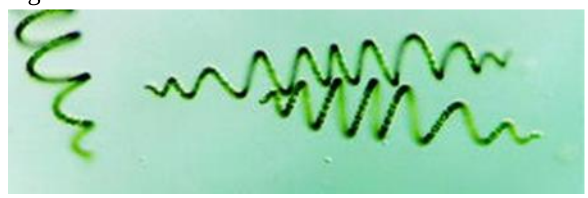


Figure: 2 Microscopic view of Spirullina sps

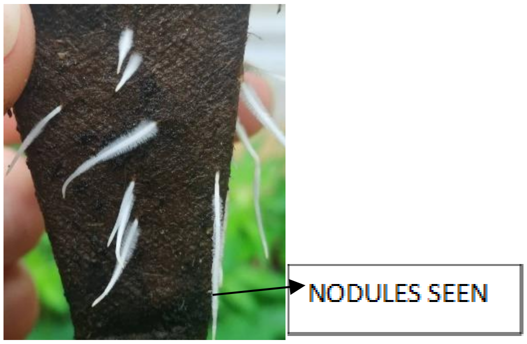
The microbial consortium were prepared together in 50 mL MSM with selected isolates. [Each selected isolates were taken 1ml]

7 days harvested plants along with consortium and its morphological parameters such as root length, shoot length and chlorophyll content of the plant were estimated and are tabulated below in table: 4 and figure: 4,

Table: 4 Physical measurements of harvested plants

Groups	Chlorophyll content	Root length	Shoot length
Consortium	95.21	4.6 cm	8.1 cm
B1	82.24	4.2cm	6.14cm
F1	86.06	4.3 cm	7.9 cm
A1	91.13	4.3 cm	8.81 cm

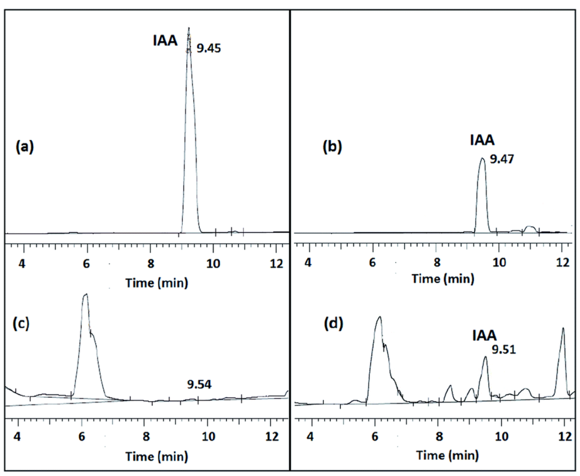
Figure: Growth seen in consortium pot



HPLC evaluation of phytohormones

Throughout 24h, Separate microbial filtrates and consortium of 3 filtrates results in showing isovitexin, phenolics, and indole acetic acid.

Indole acetic acid is a key substance in the tryptophan pathway that is Lenovo auxin biosynthesis. Biosynthesis of phenolics and flavonoids in plants initiates through the deamination of L-phenylalanine to trans-cinnamic acid and ammonia by a strategic enzyme phenylalanine PAL. In the present study, IAA increased 171% from 12 h to 24h.



DISCUSSION

The traditional triple response [Consortium] in mung bean seedlings was shown to be enhanced with increasing Auxins level in our laboratory investigation. Salt stress in the soil sample might be related to a rise in polyethylene levels. Hence, there is ethylene release when the salt stress rises in our study. Many studies have shown that Auxins has a concentration-dependent influence on seedling development, as well as an increase in ethylene production when the salinity level rises. (Shaharoona et al. 2007; Nadeem et al. 2009). [19,14]

We also discovered that inoculation and coinoculation reduced the impact of salt on mung bean seedling development. This is almost certainly owing to IAA by strains with producing capability. Mayak et al [10] findings's colloborate the findings (2004), Cheng et al.[3] (2007) and Nadeem et al. (2009) [14] both made similar observations, suggesting inoculation with PGPM diluted with ACC deaminase the negative impact of salinity on ethylene production. As a result, plant seedling growth has enhanced.

Inoculation with PGPM containing IAA increased the root length and good chlorophyll content as compared with uninoculated control. It may be due to the lowering of ethylene by these strains, thus improving the plant growth. Similarly, Elsheikh and Wood (1995) [5] observed that growth and nodulation of soybean was adversely affected by salinity and that nodulation was more sensitive than plant growth to salinity.

Many studies have found that exogenous ethylene and (or) salinity have a negative impact on plant development; however, inoculation with PGPM carrying bacteria has the opposite effect.

Plant growth is improved by IAA (Shaharoona et al. 2006; Nadeem et al. 2009; Cheng et al. 2007). [3,14,19]

Some strains of PGPM, ACC deaminase is an enzyme that cleaves ACC. ACC is converted to ammonia, which is a direct precursor to ethylene as well as a-ketobutyrate (Glick et al. 1998). It has been proven, that various bacterial species belong to distinct bacterial families - *Agrobacterium, Achromobacterium, Alcaligenes, Azospirillum, Agrobacterium, Agrobacterium, Agrobacterium, Agrobacterium, Agrobacterium, Agrobacterium, A Ralstonia, Burkholderia, Enterobacter, Pseudomonas, and Burkholderia* having a variable Auxin-producing activity should be increased the expansion of plant growth.

It was also observed that different Mycorrhizal isolates promoted growth of mung bean seedlings with different degrees of efficacy. PGPM containing IAA activity have been reported to eliminate or at least alleviate the stress-induced ethylene-mediated negative impact on plants

(Sarik et al).[18] These PGPM containing Auxins have also been studied to boost plant growth, particularly under stressed conditions, by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses (Belimov et al. 2009).[1]

Salinity had a negative impact on the presence of nodules in the root and the chlorophyll content of mung bean leaves in this study. Our findings are similar to those obtained in soybean, where it was shown that the process of nodule initiation was very sensitive to NaCl, with a concentration of 26 mmol•L–1 resulting in a 50% reduction in nodulation and total nodule mass per plant (Singleton and Bohlool 1984).[23] In Sesbania sesbane, salinity reduced the number of nodules per plant while increasing the size of nodules (Mirza and Tariq 1992).[11] Chickpea, on the other hand, has shown an increase in average nodule mass when saline levels rise, which may somewhat compensate for the decreased nitrogen content in soil.

CONCLUSION

The growth-promoting activity and nodulation of PGPM containing IAA and various halotolerant microorganisms were tested in this work in salt-affected circumstances. Bacteria, fungus, and algae of this kind might be extremely useful as inoculants to boost crop growth. Mung bean growth, nodulation, and yield under salt stress conditions. However, the extent to which these inoculants work is debatable. Benefits to plant development might vary depending on the circumstances. PGPM strains, to name a few. A PGPM strain with a variety of characteristics might be more beneficial than a strain in a variety of situations having just one characteristic

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