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Study of Exopolysaccharide Containing PGPRs on Growth of Okra Plant under Water Stress Conditions

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A B S T R A C T

Both biotic and abiotic stresses are major constrains to agricultural production. Under stress conditions, plant growth is affected by a number of factors such as hormonal and nutritional imbalance, ion toxicity, physiological disorders, susceptibility to diseases, etc. Plant growth under stress conditions may be enhanced by the application of microbial inoculation including plant growth promoting rhizobacteria (PGPR). These microbes can promote plant growth by regulating nutritional and hormonal balance, producing plant growth regulators, solubilizing nutrients and inducing resistance against plant pathogens. The present study had the objectives, screening the exopolysaccharide (EPS) producing bacteria from PGPR isolates and evaluating the effect of exopolysaccharide (EPS) producing bacteria on growth of Okra plants. Seven bacterial strains were collected from culture bank and tested for their drought tolerance, PGPR traits and EPS production. Out of seven cultures *Pseudomonas aeruginosa* and *Bacillus coagulans* were found to be best. Okra seeds were treated with different combination of EPS producing bacteria and shown in field. Maximum seed germination was observed in T7 (79.15%). Maximum plant height at each time interval was attained in treatment T_7 (6cm, 13.5cm, 15.8cm, 18.43cm and 22.93cm respectively). Maximum Number of leaves per plant at each time interval was attained in treatment T_7 (4.33, 6.33, 8.33, 10.33 and 10.66 respectively). Maximum leaf area graphically was observed in treatment T_7 (181.89 cm^2). Maximum root length was observed in treatment T_7 (16.56 cm). Maximum fresh weight and dry weight of root per plant was observed in treatment T_7 (7.83gm, 1.65gm respectively). Maximum fresh and dry weight of leaf per plant was observed in treatment T_7 (4.70gm, 0.86gm respectively).

Introduction

Microorganisms of soil play important role in the maintenance of quality and health of soil (Jeffries *et al.*, 2003). The direct growth promotion of plants by PGPR entails either providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect plant growth promotion occurs by PGPR due to preventing deleterious effects phytopathogenic microorganisms.

The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibbrallic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995) (ii) asymbiotic N_2 fixation (Boddey and Dobereiner, 1995), (iii) antagonism against phytopathogenic microorganisms by production of siderophore (Scher and Baker, 1982), antibiotics (Shanahan *et al.*, 1992) and cyanide (Flaishman *et al.*, 1996), (iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al.*, 1997; Gaur, 1990). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly.

A large array of bacteria including species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, *Bacillus* and Serratia have reporte to enhance plant growth (Kloepper *et al.*, 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995). Phosphorus (P), the second important plant growth-limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms (Khan *et al.*, 2009). Despite of large reservoir of P, the amount of available forms to plants is generally low. This low availability of phosphorous to plants is because the majority of soil P is found in insoluble forms, while the plants absorb it only in two soluble forms, the monobasic (H_2PO_4) and the diabasic (HPO_4^2) ions (Bhattacharyya and Jha, 2012). The insoluble P is present as an inorganic mineral such as apatite or as one of several organic forms including inositol phosphate (soil phytate), phosphomonoesters, and phosphotriesters (Glick, 2012). To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb fewer amounts of applied phosphatic fertilizers and the rest is rapidly converted into insoluble complexes in the soil (Mckenzie and Roberts, 1990). But regular application of phosphate fertilizers is not only costly but is also environmentally undesirable. This has led to search for an ecologically safe and economically reasonable option for improving crop production in low P soils. In this context, organisms coupled with phosphate solubilizing activity, often termed as phosphate solubilizing microorganisms (PSM), may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers (Khan *et al.*, 2006). *Bacterial genera like Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium and Serratia* are reported as the most significant phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012). Microbial synthesis of the phytohormone auxin (indole-3-acetic acid/indole acetic acid/IAA) has been known for a long time. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Patten and Glick, 1996). IAA produced by rhizobacteria likely,

interfere the above physiological processes of plants by changing the plant auxin pool. Moreover, bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Glick, 2012). Thus, rhizobacterial IAA is identified as an effector molecule in plant–microbe interactions, both in pathogenesis and phytostimulation (Spaepen and Vanderleyden, 2011).

Exopolysaccharides (EPS) are the active constituents of soil organic matter (Gouzou *et al.,* 1993). EPS are most important part of extracellular matrix that often represent 40–95% of bacterial weight (Flemming and Wingender 2001). Bacteria produce EPS in two forms: (1) slime EPS and (2) capsular EPS (Vanhooren and Vandamme, 1998). EPS are found in a wide variety of complex structures (Kumon *et al.,* 1994). The important roles exhibited by EPS are (1) Protective, (2) surface attachment, (3) biofilm formation, (4) microbial aggregation, (5) plant–microbe interaction, and (6) bioremediation (Manca de Nadra *et al.,* 1985). Some physical and chemical properties of EPS are useful in industries for stabilizing, thickening, coagulating, gelling, suspending, film forming, and water-retention capability in different industries like detergents, textile, paper, paints, adhesive, beverages, and food (Sutherland, 1996). Some EPS-producing bacteria like Pseudomonas have the ability to survive even under drought stress due to the production of their EPS (Sandhya *et al.,* 2009a, 2009b). The EPS protect these bacteria from desiccation under drought stress by enhancing the water retention and by regulation of organic carbon source's diffusion (Roberson and Firestone, 1992; Chenu, 1993; Chenu and Roberson, 1996).

Okra (*Abelmoschus esculentus*) is a popular vegetable which is cultivated in the tropical and sub-tropical regions of the world (Baloch, 1994). It is a semi – woody, fibrous annual crop with deep penetrating taproot and dense shallow feeder roots reaching out in all directions in the upper 45cm of the soil. The local varieties differ in growth habit such as branching, height, leaf size and arrangement, maturity period and fruit characteristics. During the vegetative phase, growth pattern of okra varieties are similar, although, the more vigorous varieties have higher leaf area and dry matter accumulation (Adelana, 1981).

It is a nutritious vegetable which plays an important role in meeting the demand of vegetables in the country when vegetables are scanty (Ahmed, 1995). Okra is grown for its immature pods which can be harvested over a relatively long period of time. Like squash, cucumber and many other vegetables, the crop must be harvested on a regular basis for best yields. If the pods are allowed to mature on the plant, flowering will be reduced and further pod production will be hindered. The green pods are rich sources of vitamins and minerals. Okra is sometimes made into soup with the addition of palm oil, fish and other condiments. It could be boiled as vegetable and served with rice and other foods. Fresh okra fruits may be consumed in the immature stage or they could be sliced, dried and stored for using during the off season. In a trial on okra using two sowing dates – 1st April and 15th April, Incalcaterra *et al.,* (2000) reported that plant height, number of pods per plant and total number of pods were higher for the 1st April sowing than the 15th April sowing (Yadav and Dhankhar, 1999).

In view of the above facts regarding the PGPRs the present study was conducted with the aim to screen exopolysaccharide (EPS) producing bacteria from rhizobacterial isolates and evaluating the effectiveness of

exopolysaccharide (EPS) producing bacteria on physiological growth of Okra plants.

Materials and Methods

Place of work

The experimental study was conducted in Post Graduate Laboratory, Department of Industrial Microbiology, SHUATS, Allahabad.

Procurement of PGPR strains

Seven strains of plant growth promoting rhizobacteria (PGPR) were collected from Microbial Collection Bank, (MCCB), Department of Microbiology and Fermentation Technology, SHUATS. Strains were preserved at 4ºC and further used for the study. The cultures were as: *Bacillus Insolitus* (MCCB 0004), *Pseudomonas aeruginosa* (MCCB 0035), *Bacillus coagulans* (MCCB0059), *Bacillus pumilus* (MCCB0011), *Bacillus licheniformis* (MCCB0012), *Pseudomonas flurescens* (MCCB0217), *Bacillus polymyxa* (MCCB0007)

In vitro assay for water stress tolerance

TSA medium (10%) with 405 g/L of sorbitol producing a lower water activity (Aw) value, corresponding to 0.919Aw at 40ºC was used for in vitro assay for water stress tolerance of seven bacterial cultures obtained from the MCCB, SHIATS, Allahabad. All the seven cultures were spot inoculated on the TSA medium and plates were incubated at 40ºC for 24 to 48h and the growth of all the cultures was observed.

Qualitative test of exopolysaccharide production

The qualitative determination of exopolysaccharide production was performed according to Paulo *et al.*, (2012). Each strain was inoculated onto 5-mm diameter paper discs disposed in a medium (2% yeast extract; 1.5% K_2HPO_4; 0.02% $MgSO_4$; 0.0015% $MnSO_4$; 0.0015% $FeSO_4$; 0.003% $CaCl_2$; 0.0015% NaCl; 1.5% agar) modified by the addition of 10% of saccharose, pH value of 7.5. The production was characterized by the size of the halo zone produced and its slime appearance. The production of EPS was confirmed by mixing a portion of the mucoid substance in 2mL of absolute ethanol, where the formation of a precipitate indicated the presence of EPS (Paulo *et al.*, 2012).

Production and extraction of EPS

EPS-producing bacteria was cultured in mineral salts medium with 12.6% K_2HPO_4, 18.2% KH_2PO_4, 10% NH_4NO_3, 1% $MgSO_4.7H_2O$, 0.6% $MnSO_4$, 1% $CaCl_2.2H_2O$, 0.06% $FeSO_4.2H_2O$, 1% sodium molybdate, 1.5% NaCl, and 0.2% of glucose in 1 litre of distilled water for 10 days (Bramchari and Dubey, 2006). After incubation for 10 days, the bacterial broth cultures were centrifuged at 10,000 rpm for 20 min at 4°C. The EPS was extracted from the supernatant by the addition of two fold ice cold ethanol (95%). The solution was chilled at 4°C for complete precipitation.

Screening of bacterial isolates for in vitro plant growth promoting traits

Phosphate solubilization index

Pikovskaya's media was poured in Petri plates under sterilized conditions. With the help of sterilized loop, a pinpoint inoculation was done on these agar plates under sterilized conditions. The plate was incubated at 28°C for 7 days. Formation of clear halo zone around the colonies was observed for phosphorus solubilization on plates. Index of phosphorus solubilization was calculated by

measuring the colony diameter and halo zone diameter with the help of following equation.

$$\text{Solubilization Index} = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

Quantitative determination of phosphate solubilization by EPS-producing PGPRs

The detection of available phosphate was performed with a colorimetric method according to Nautiyal (1999) with some modifications. Test-tubes with 10mL of NBRIP (National Botanical Research Institute's Phosphate) medium (1% glucose; 0.5% $Ca_3(PO_4)_2$; 0.5% $MgCl_2 \cdot 6H_2O$; 0.02% KCl; 0.025% $MgSO_4 \cdot 7H_2O$; 0.01% $(NH_4)_2SO_4$ was inoculated with 100µL of 10^{-8} cells/mL (OD_{550nm} = 0.1) of each isolate, in triplicate. After incubation at 180 rpm, 28ºC for 15 days, 1000µL of each sample was transferred to micro tubes of 1.5mL capacity and centrifuged at 10,000rpm for 5min. Aliquots of 145µL of supernatant of each sample was added to 570µL of distilled water and 285µL of ammonium molybdate–vanadate reagent (5%ammoniummolybdate and 0.25% ammonium vanadate, 1:1 (v/v)) (Malavolta *et al.,* 1989; Silva, 1999). Spectrophotometer was reset using negative control with 145µL of NBRIP medium without inoculum, 570µL of distilled water and 285µL of the ammonium molybdate–vanadate reagent. Optical density was taken at 420nm after 10 min of reagent addition. Standard curve was obtained using a stock solution of KH_2PO_4 (0.0875%) (0.1mg/ml).

Indole Acetic Acid production

The cultures were incubated in the peptone broth enriched with tryptophan broth to check for the production of indole acetic acid, a precursor of auxin which is an important plant hormone. The quantitative estimation of IAA was performed by using Salkowski method by using the reagent, 1 ml of $FeCl_3$ and 0.5 mM in 35% $HClO_4$. Mixtures were incubated at room temperature for 25 min and observed for pink colour production and readings were taken colorimetrically at 530 nm.

Ammonia (NH_3) production

Ammonia production was detected according to Cappuccino and Sherman (1992). Freshly grown cultures were inoculated in test-tubes with 10mL of peptone water and incubated for 48 h at 28ºC. After incubation, 1mL of each culture was transferred to microtubes of 1.5mL capacity, and 50µL of Nessler's reagent (10% $HgCI_2$; 7% KI; 50% aqueous solution of NaOH (32%) were added in each microtube. The development of faint yellow color indicates a small amount of ammonia and deep yellow to brownish color indicates maximum production of ammonia (Dey *et al.,* 2004).

Hydrogen Cyanide (HCN) production

HCN detection was performed according to Bakker and Schippers (1987), where all isolates were streaked on TSA (10%) with additionally glycine (4.4 g/L).After 24 h of growth at 28ºC, Petri dishes were inverted and on each cover, an autoclaved filter paper soaked with picric acid (0.5%) and Na_2CO_3 (2%) solution was put onto it. Petri dishes were sealed and incubated at 28ºC for 48 h. The HCN production was indicated by changes in coloration from orange to red.

Detection of siderophore production

Quantitative estimation was done by CAS-shuttle assay (Payne, 1994). Strains were inoculated in SM medium for 24 hrs at 30°C at 120 rpm. Fermented broth was then centrifuged at 10,000 rpm for 15 min. Culture supernatant (1ml) was mixed with the same

amount of CAS reagent (2ml) and absorbance was measured at 630 nm against a reference consisting of equal volume of uninoculated broth and CAS reagent. Siderphore content in the aliquots were calculated using following formula-

% Siderphore units = (Ar- As / As) × 100

Where, Ar is the absorbance of reference and As is the absorbance of the sample

Inoculation of Okra plant with EPS producing PGPRs

The cultures were allowed to grow in an orbital shaker at 120 rpm for 48 h at 30°C, thereafter centrifuged at 3000 rpm for 15–20 min. These cultures at optical density 600 nm (OD 600) equivalent to 1 were used as bioinoculant. Effect of EPS-producing bacteria on the growth of okra was studied by the inoculation of Okra seeds with EPS bacterial cells alone and in combination with their respective EPS.

Seeds of Okra collected from local market and were surface sterilized with 95% ethanol followed by shaking for 2–3 min and successively washed with sterilized water. For seeds inoculation with bacterial cells, 48-h-old cultures were prepared in NB broth media, and for inoculation of seeds with cells and their respective EPS, 10-d-old cultures were used.

Sterilized seeds were soaked in 10-d-old cultures of bacteria for 3–4 h. Seeds were sown directly in the field. After 1 week of seed germination, the seedlings were subjected to drought stress by withholding water supply for 10 d; the non-stressed plants will be kept well watered. After 10 days of drought stress, plants were harvested for further analysis.

The age of plant was 35 days at the time of harvest.

Determination of effects of EPS producing PGPR on physiological parameters of soil and plants

The bacterial isolates were tested on Okra plant growth. Okra seeds were soaked in H_2SO_4 for 3 min and washed with sterile water seven times. Seeds were then treated with bacterial isolates for 30 min. No treated seeds with any isolate were designated as control. Ten seeds were sown at 4 to 5 cm depth of soil in each plot.

Seed germination test

The germination of seeds was recorded after 7-10 days of sowing. The seed germination was calculated as follows:

$$\text{Seed germination (\%)} = \frac{\text{No. of seed germinated}}{\text{Total no. of seeds}} \times 100$$

Soil moisture test

After harvesting the plants, moisture content of rhizosphere soil of both stressed plants and non-stressed plants were measured. Fresh weight of soil sample (20 g) collected from 6-inch rhizosphere of plants was dried in oven for 72 h at 70°C. Dry weight of soil was recorded and moisture content was calculated as:

$$\text{Soil moisture (\%)} = \frac{\text{Weight of wet soil (g) - Weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

Leaf area measurement

Leaf area of randomly collected plants from each treatment was calculated graphically.

Experimental site detail

Season - Rabi

Crop - Okra (Lady's finger)
Botanical name - *Abelmoschus asculentus*
Family - Malvaceae
Design - Random Block Design
Replications – 3
Plot size - 2x2m
Total no of plots – 21
Spacing row to row – 45cm
Spacing plant to plant – 45cm
Seed rate – 20kg / hect
Total length of area – 16.4m
Total width of area – 7.2m
Main irrigation channel _ 1m
Sub-irrigation channel _ cm

Experiments was done in Triplicates

T_1: Control (without treated seeds) under normal and water stress condition

T_2-T_4: Different PGPR isolates treated seeds under normal condition

T_5-T_7: Different PGPR isolates treated seeds under water stress condition

Harvesting of the plants and analysis

Okra plants were harvested after 35 days of seed sowing by separating of plants from soil. The plants were washed through dipping into a vessel. Plant height (cm plant^{-1}) and root length (cm plant^{-1}) of each plant was recorded. Dry weights of leaves and root was recorded after drying in an oven for 1 day at 80°C.

Results and Discussion

***In vitro* assay for drought tolerance**

Seven strains of rhizobacteria were collected from Microbial Culture Collection Bank (MCCB) and in vitro assay for drought tolerance were performed on TSA medium with (10%) of sorbitol 405 g/L. All the seven cultures were spot inoculated on the TSA medium and plates were incubated at 40°C for 24h to 48h and the growth of all the cultures was observed. In the present investigation *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) showed the maximum growth on TSA medium as compared to other bacterial cultures like. *Bacillus insolitus* (MCCB0004), *Bacillus pumilus*, (MCCB0011) *Bacillus licheniformis* (MCCB0012), *Bacillus polymyxa* (MCCB0007), *Pseudomonas florescence* (MCCB0217) (Plate 1). The two strains namely *Pseudomonas aeruginosa* and *Bacillus coagulans* were further selected for exopolysaccharide production.

Plants are constantly exposed to abiotic stresses, such as salt and drought, with the latter being one of the most serious problems associated with plant growth and development affecting agricultural demands. The introduction of drought-tolerant ACC deaminase-producing microorganisms in drought-stressed soils can alleviate this stress in crop plants by lowering stress-induced ethylene production. Drought-tolerant microorganisms can survive in these habitats and bind to the seed coat or root of developing seedlings, resulting in the deamination of ACC, which is the immediate precursor of ethylene, in plant cells through the production of ACC deaminase. This in turn leads to a lowering of the plant ethylene level and thereby facilitates the growth and development of plants (Glick *et al.,* 1998). Similarly Ali *et al.,* (2013) isolated and characterized 17 bacterial isolates for their drought tolerance and out of 17 isolates 9 were showing drought tolerance.

Qualitative test of exopolysaccharide production by rhizobacterial isolates

The selected bacterial strains namely *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) which were

showing water stress tolerance were further tested for their Exopolysaccharide (EPS) production. The production of EPS by both the cultures was characterized by the size of the halo zone produced and its slime appearance on the medium on the agar medium. The production of EPS was further confirmed by observing the formation of precipitate by mixing a portion of the mucoid substance in 2mL of absolute ethanol in a test tube. In the present investigation halo zone and precipitate was observed maximum in case of *Bacillus coagulans* (MCCB0059) and minimum in *Pseudomonas aeruginosa* (MCCB0035). (Plate 2 a, b, c).

EPS material possesses unique water-retention and cementing properties and thus plays a vital role in the formation and stabilization of soil aggregates and the regulation of nutrients and water flow across plant roots through biofilm formation (Tisdall and Oades, 1982). Roberson and Firestone (1992) and Junkins and Doyle (1992) demonstrated Pa2 and *Escherichia coli* as EPS-producing organisms on the basis of mucoid colony. The production of pigmentation in all bacterial strains was also detected during the selection process.

Production and extraction of EPS from rhizobacterial strains

EPS-producing bacteria were cultured in mineral salts medium. After incubation for 10 days, the bacterial broth cultures were centrifuged at 10,000 rpm for 20 min at 4°C. The EPS was extracted from the supernatant by the addition of two fold ice cold ethanol (95%), the solution was chilled at 4°C for complete precipitation. according to the results of EPS production maximum EPS was produced by *Bacillus coagulans* (MCCB0035) followed by *pseudomonas aeruginosa* (MCCB0059) (Plate 3 a, b, c). Similar findings were observed by Hartel and Alexander (1986) between the amount of EPS produced by cowpea Bradyrhizobium strains and desiccation tolerance. Konnova *et al.*, (2001) also suggested the role for EPS material in the protection of *A. brasilense* Sp245 cells against desiccation. Chang *et al.*, (2007) suggested that a strain of *Pseudomonas putida* produce an EPS, called alginate, which influences the development of biofilm and the physical–chemical properties of EPS itself, in response to water-limiting conditions. When inoculated in plants, EPS-producing microorganisms can help plant survive under adverse conditions (Nocker *et al.*, 2012). The similar observations were also obtained by Chowdhury *et al.*, (2011) and Yuan *et al.*, (2011).

Characterization of plant growth promoting ability of rhizobacterial isolates *Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0059)

Phosphate solubilization index

Phosphate solubilization index of the bacterial cultures was studied on pikovaskiya medium. The cultures were spot inoculated on the medium and was incubated at 30ºC for 7 days. After 7 days of incubation plates were observed for halo zone formation around the colonies and the size of halo zone and diameter of colonies (Plate 4) was measured to calculate phosphate solubilization index. in the present investigation greater phosphate solubilization index was observed in case of *Bacillus coagulans* (MCCB0059) followed by *Pseudomonas aeruginosa* (MCCB0035) (Table 2; Plate 4).

Similarly there are several reports over phosphate solubilization exhibited by a variety of species: *Azotobacter* sp., *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp. (Ahmad *et al.*, 2008; Oliveira *et al.*, 2009). The highest levels of P solubilization were observed for members of the *Enterobacteriaceae* family. This was

also demonstrated by other report, in which *Enterobacter* sp. obtained from soils with low P concentration exhibited high levels of phosphate solubilization (568–642 gmL−1) (Kumar *et al.,* 2010). Several species of fluorescent *Pseudomonas* such as *P. fluorescens* NJ101 (Bano and Musarrat, 2004), *P. aeruginosa* (Jha *et al.,* 2009) were reported as good phosphate solubilizers.

Quantitative determination of phosphate solubilization by EPS-producing PGPRs

Preparation of standard curve of phosphate (K_2HPO_4)

The detection of quantity of phosphate solubilized by the bacterial cultures *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) *was* performed with a colorimetric method by preparing the standard curve of known phosphate concentration (Table 3; Figure 1 and 2; Plate 4 and 5). Amount of phosphate solubilized by the cultures was calculated by the regression equation obtained by the standard curve. According to the results obtained it was found that *Bacillus coagulans* (MCCB0059) was showing maximum phosphate solubilization (0.21 mg/ml) as compared to *Pseudomonas aeruginosa* (MCCB0035) (0.18 mg/ml).

Characterization of bacterial culture for Indole Acetic Acid (IAA) production

In the present investigation bacterial cultures namely *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) were characterized by production of Indole acetic acid, precursor of auxin hormone. In the present study all the bacterial cultures i.e. *Bacillus Insolitus* (MCCB0004), *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) showed negative Indole acetic acid production (Fig. 3; Plate 6).

Characterization of bacterial cultures on the basis of ammonia production

Ammonia production by both the isolates viz. *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) was investigated by test tube method and the development of color in the tubes was observed. In the present investigation both the cultures were observed for ammonia production. Out of these two cultures greater ammonia production was shown by *Bacillus coagulans* (MCCB0059) in comparison to *Pseudomaonas aeruginosa* (MCCB0035) (Table 5; Plate 7).

Ammonia production is another plant growth-promoting feature responsible for the indirect plant growth promotion through pathogens' control (Minaxi *et al.,* 2012). *Bacillus* species seem to be ammonia producers. Joseph *et al.,* (2007) detected ammonia production in 95% of *Bacillus* sp., followed by 94.2% of *Pseudomonas* sp., 74.2% of *Rhizobium* sp. and 45% of *Azotobacter* sp.

Characterization of rhizobacterial isolates on basis of Hydrogen Cyanide (HCN) production

The study was conducted to determine the strains that produced HCN. In present study all 3 strains were tested for HCN production and all the 3 were found to be positive i.e. producing HCN changing the colour of filter paper from yellow to orange. HCN production by both the isolates i.e. *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) was investigated by filter paper method on petri plates. The HCN production was observed by changes in coloration of filter paper from orange to red. In the present investigation both the cultures namely *Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0059) *showed production of Hydrogen*

Cyanide (HCN) Highest HCN production was observed by *Bacillus coagulans* (MCCB0059) as comparison to *Pseudomonas aeruginosa* (MCCB0035) (Table 6; Plate 8).

The production of volatile compounds is reported for several microorganisms and they can act as growth-promoting or inhibiting agents (Kai *et al.,* 2009). There are several volatiles described to date by Xu *et al.,* (2004) and HCN is one of them (Blumer and Haas, 2000). The inhibition of fungal pathogens' growth by volatiles produced by bacteria or fungi is well reported (Zou *et al.,* 2007).

Characterization of bacterial cultures (*Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0035) for siderophore production

In the present investigation both the isolates were tested for siderophore production quantitatively by CAS-shuttle assay. Both the cultures were showing positive results for siderophore production. According to the results of siderophore production maximum (153%) siderophore was produced by *Bacillus coagulans* (MCCB0035) followed by *Pseudomonas aeruginosa* (MCCB0059) (103%) and minimum (96%) in *Bacillus Insolitus* (MCCB0004) (Table 7; Fig. 4; Plate 9).

Siderophore is one of the biocontrol mechanisms belonging to PGPR groups under iron limiting condition. PGPR produces a range of siderophore which have a very high affinity for iron. Therefore the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi (Whipps *et al.,* 2001). Numerous studies of plant growth promotion through siderophore mediated Fe-uptake as a result of siderophore producing rhizobacterial inoculations have been reported by Rajkumar *et al.,* (2010). Crowley and Kraemer (2007) revealed a siderophore mediated iron transport system in oat plants. Recently (Sharma *et al.,* 2003) assessed the role of the siderophore producing *Pseudomonas* strain GRP3 on iron nutrition of *Vigna radiate*.

Effect of EPS producing bacteria on okra plant growth

In the present study the Okra plant growth was evaluated under different treatment of EPS producing bacteria namely *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059). Different growth parameters like seed germination, plant height, No. of leaves, Leaf area were evaluated in the field under water stress condition.

Effect of EPS producing bacteria (*Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0059) on seed germination of okra

The present study was conducted to evaluate the effect of EPS producing bacteria on seed germination (%) of Okra plant. In this study seven treatments of EPS producing bacterial suspension were investigated and observations were taken after 7 days of sowing of Okra seeds. T_7 treatment [Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)] showed highest mean performance on percentage seed germination followed by the treatment T_6 [(Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)] followed by treatment T_5 [(Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)] followed by treatment T_4 [(Treated seed + *B. coagulans* (MCCB0059) microbial solution)] followed by treatment T_3 [(Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution)] followed by T_2 treatment [(NPK)+ normal seed] followed by treatment T_1 [(control) + normal seed].

Table.1 Treatment schedule of Okra seeds with various inoculants

S. NO.	TREATMENTS
T1	Control +normal seed
T2	NPK + normal seed
T3	Seeds treated with *P. aeruginosa* (MCCB0035) solution
T4	Seeds treated with *B. coagulans* (MCCB0059) solution
T5	Seeds treated with *P. aeruginosa* (MCCB0035) solution + NPK (50%)
T6	Seeds treated with *B. coagulans* (MCCB0059) solution+ NPK (50%)
T7	Seeds treated with *P. aeruginosa* (MCCB0035) and *B. coagulans* (MCCB0059) solution + NPK (25%)

Table.2 Phosphate solubilization index of Different rhizobacterial isolates

S. No.	Bacterial isolates	Colony Diameter (mm)	Halo Zone Diameter (mm)	Solubilization Index
1.	*Pseudomonas aeruginosa* (MCCB0035)	6	15	35
2.	*Bacillus coagulans* (MCCB0059)	8	22	37

Table.3 Estimation of phosphate solubilisation by bacterial isolates

S. No.	Bacterial isolates	Phosphate conc. (mg/ml)
1.	*Bacillus Insolitus* (MCCB0004)	0.16
2.	*Pseudomonas aeruginosa* (MCCB0035)	0.18
3.	*Bacillus coagulans* (MCCB0059)	0.21

Table.4 Ammonia production by different rhizobacterial strains

Bacterial Strains	Observation for Ammonia production
***Bacillus Insolitus* (MCCB0004)**	++
***Pseudomonas aeruginosa* (MCCB0035)**	+++
***Bacillus coagulans* (MCCB0059)**	+++

Table.5 Hydrogen Cyanide (HCN) production by rhizobacterial strains

Strains	Results
***Bacillus insolitus* (MCCB0004)**	+++
***Pseudomonas aeruginosa* (MCCB0035)**	+++
***Bacillus coagulans* (MCCB0059)**	++

Table.6 Characterization of bacterial cultures (*Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0059) for siderophore production

S. No	Strains	Optical Density at 610nm	% Siderophore units
1.	*Bacillus Insolitus* (MCCB0004)	0.45	96
2.	*Pseudomonas aeruginosa* (MCCB0035)	0.56	103
3.	*Bacillus coagulans* (MCCB0035)	0.58	153

Table.7 Effect of different treatments of EPS producing bacteria on seed germination (%) of okra plant

Treatments	Seed Germination (%Mean value)
T_1 (Control)+normal seed	41.66
T_2 (NPK)+ normal seed	48.95
T_3 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution)	52.08
T_4 (Treated seed + *B. coagulans* (MCCB0059) microbial solution)	54.16
T_5 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)	57.29
T_6. (Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)	63.54
T_7 (Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)	79.15
F-test	S
S. Ed. (±)	6.00392
C.D. (5%)	12.8784

Table.8 Effect of EPS producing bacteria (*Pseudomonas aeruginosa and Bacillus coagulans*) on plant height (cm) of Okra plant at 7 days interval under different treatments of inoculants

Treatments	Plant height (cm) at 7 days interval				
	7days	14days	21days	28days	35days
T1. Normal seed (control)	5.0	11.33	13.03	15.43	19.4
T2. (NPK)+ Normal seed	5.0	11.4	13.06	15.83	19.66
T3. [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution]	5.16	11.6	13.36	16.16	20.13
T4. [*B. coagulans* (MCCB0059) treated seeds]	5.26	11.96	13.93	16.66	20.66
T5. [*P. aeruginosa* (MCCB0035) treated seeds+ NPK(50% of Rec. Dose]	5.3	12.5	14.13	16.86	21.16
T6. [Treated seed+ *B. coagulans* (MCCB0035) microbial solution+ NPK (50%)]	5.7	12.96	14.93	17.76	21.96
T7. [Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0035) microbial solution + NPK (25%)]	6.0	13.5	15.80	18.43	22.93
F-test	S	S	S	S	S
S. Ed. (±)	0.255	0.483	0.646	0.638	1.050
C.D. (5%)	0.548	1.036	1.386	1.368	2.253

Table.9 Effect of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on number of leaves of Okra plant at 7 days interval

Treatments	No. of leaves at different interval of days				
	7 days	14 days	21days	28days	35days
T_1.(control)+normal seed	3.66	5.66	7.33	8.66	9.33
T_2. (NPK)+ normal seed	3.66	5.66	7.66	8.33	9.33
T_3.Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution	3.66	6	7.66	9.33	9.66
T_4. [Treated seed + *B. coagulans* (MCCB0059) microbial solution]	3.66	6	8	9.33	9.66
T_5. [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)]	4	6	8	9.66	10
T_6. (Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)	4	6.33	8.33	9.66	10.33
T_7. [Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0035) microbial solution + NPK (25%)]	4.33	6.33	8.33	10.33	10.66
F-test	S	S	S	S	S
S.Ed	0.4138	0.3664	0.4249	0.6701	0.4082
C.D. (5%)	0.8877	0.7859	0.9114	1.4374	0.8756

Table.10 Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on leaf area (cm^2) of Okra plant after 35 days of sowing

Treatments	Leaf area ($cm^{2)}$
T_1 (control)+normal seed	148.41
T_2 (NPK)+ normal seed	152.30
T_3 [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution]	160.32
T_4 [Treated seed + *B. coagulans* (MCCB0059) microbial solution]	161.42
T_5 [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+NPK (50%)]	173.94
T_6 [Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)]	181.36
T_7 [Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)]	181.89

Table.11 Effect of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on root length (cm) of Okra plant after 35 days of showing

Treatments	Root length (cm)
T_1 (control)+normal seed	10.16
T_2 (NPK)+ normal seed	10.96
T_3 [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution]	12.9
T_4 [Treated seed + *B. coagulans* (MCCB0059) microbial solution]	13.6
T_5 [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)]	14.53
T_6 [Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)]	15.33
T_7 [Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)]	16.56
F-test	S
S.Ed	**1.963**
C.D. (5%)	**4.211**

Table.12 Effect of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on fresh weight of root (g) of Okra plant after 35 days of sowing

Treatments	Fresh weight of root (g)
T_1 (control)+normal seed	3.38
T_2 (NPK)+ normal seed	4.84
T_3 [Treated seed+ *P. aeruginosa*(MCCB0035) microbial solution]	5.61
T_4 [Treated seed + *B. coagulans*(MCCB0059) microbial solution]	5.08
T_5 [Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)]	6.15
T_6 [Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)]	6.75
T_7 [Treated seed+ *P. aeruginosa*(MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)]	7.83
F- test	S
S. Ed.	0.98186
CD (0.05)	2.1061

Table.13 Effect of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on dry weight of root (g) of Okra plant after 35 days of sowing

Treatments	Dry weight of root (g)
T_1 (control)+normal seed	0.76
T_2 (NPK)+ normal seed	0.94
T_3 (Treated seed+ *P. aeruginosa*(MCCB0035) microbial solution)	1.1
T_4 (Treated seed + *B. coagulans*(MCCB0059) microbial solution)	1.06
T_5 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)	1.28
T_6 (Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)	1.36
T_7 (Treated seed+ *P. aeruginosa*(MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)	1.65
F-test	S
S. Ed. (±)	0.29612
CD (5%)	0.63518

Table.14 Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on fresh weight of leaves (g) of Okra plant after 35 days of sowing

Treatments	Fresh weight of leaf (g)
T_1 (control)+normal seed	2.4
T_2 (NPK)+ normal seed	2.5
T_3 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution)	2.95
T_4 (Treated seed + *B. coagulans* (MCCB0059) microbial solution)	3.07
T_5 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)	3.66
T_6 (Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)	3.69
T_7 (Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)	4.12
F-test	S
S. Ed. (±)	0.62857
CD (5%)	1.34828

Table.15 Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on dry weight of leaves (g) of Okra plant after 35 days of sowing

Treatments	Dry weight of leaves (g)
T_1 (control)+normal seed	0.42
T_2 (NPK)+ normal seed	0.61
T_3 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution)	0.63
T_4 (Treated seed + *B. coagulans* (MCCB0059) microbial solution)	0.67
T_5 (Treated seed+ *P. aeruginosa* (MCCB0035) microbial solution+ NPK (50%)	0.69
T_6 (Treated seed+ *B. coagulans* (MCCB0059) microbial solution+ NPK (50%)	0.71
T_7 (Treated seed+ *P. aeruginosa* (MCCB0035) + *B. coagulans* (MCCB0059) microbial solution + NPK (25%)	0.77
F-test	S
S. Ed. (±)	0.09396
CD (0.05)	0.20155

Fig.1 Standard curve for phosphate

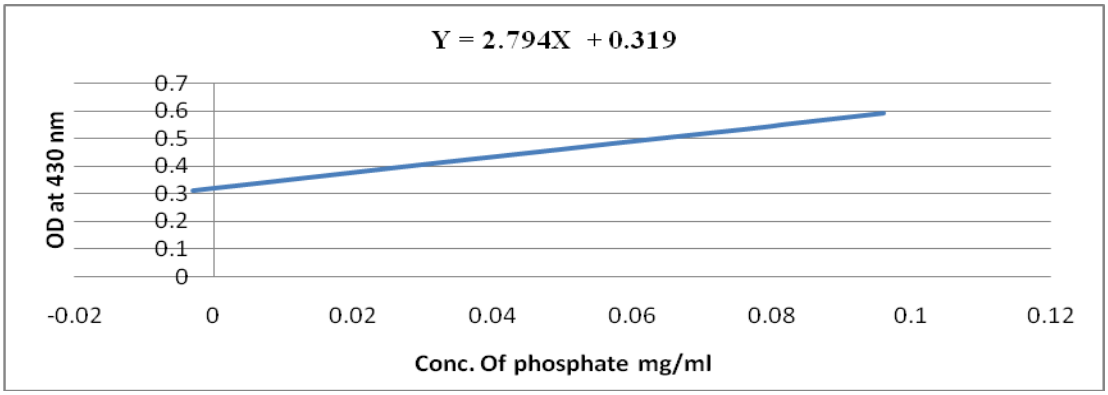

Fig.2 Quantitative determination of phosphate solubilization by EPS-producing PGPRs

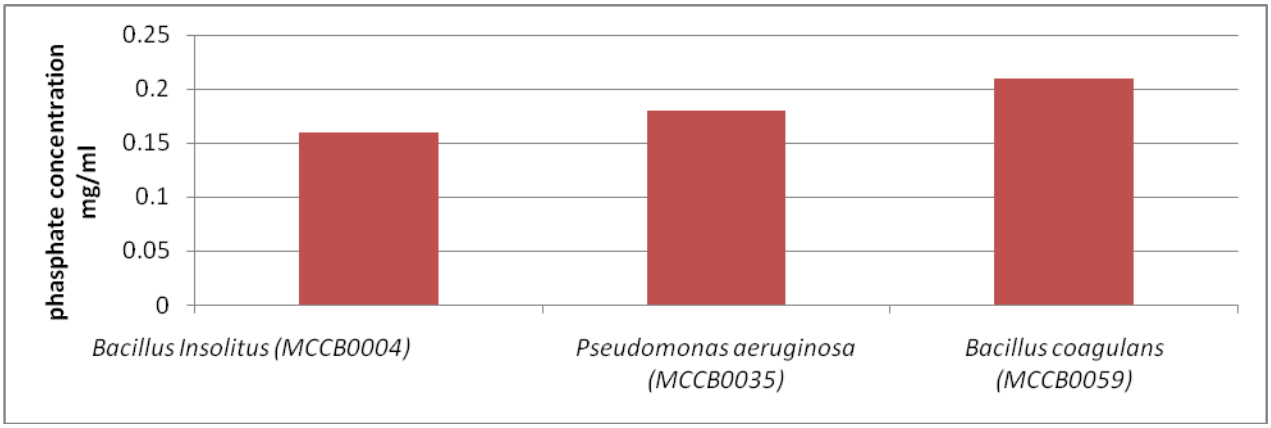

Fig.3 Standard curve of indole acetic acid

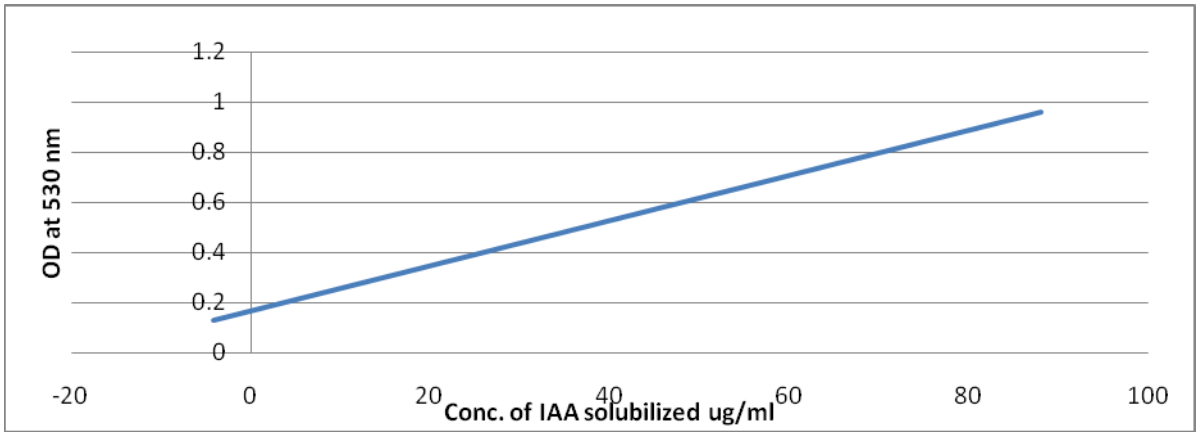

Fig.4 Characterization of bacterial cultures (*Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) for siderophore production

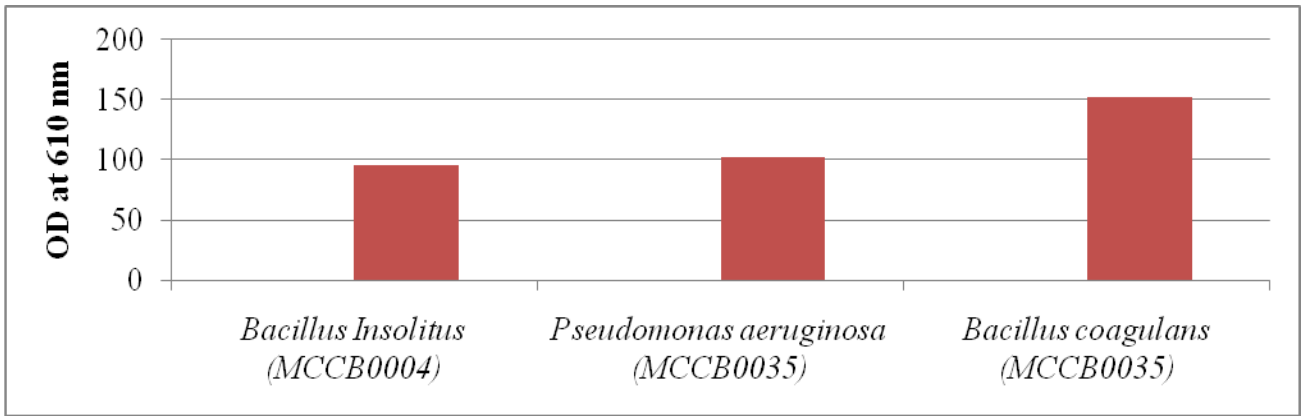

Fig.5 Variation in seed germination of Okra due to different treatments of EPS producing bacteria (*Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0059)

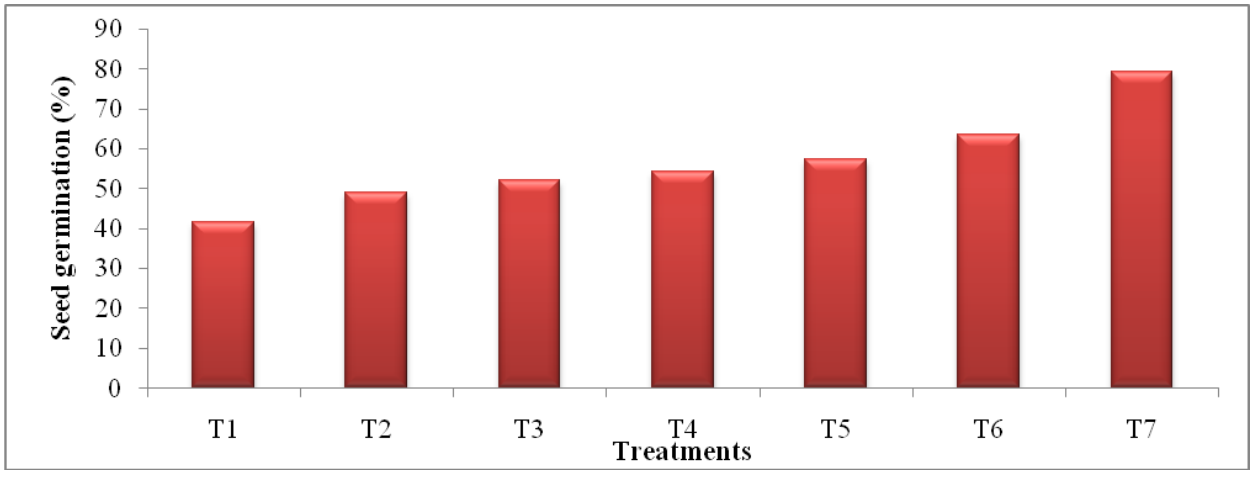

Fig.6 Variation in the plant height (cm) of okra plant at 7 days interval under different treatments of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

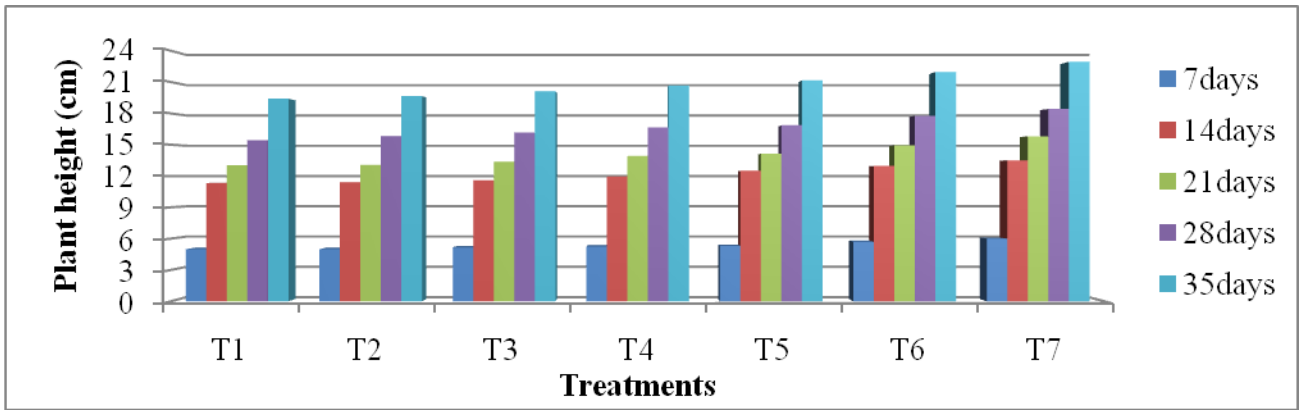

Fig.7 Variation in number of leaves of Okra plant at 7 days interval under different treatment of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

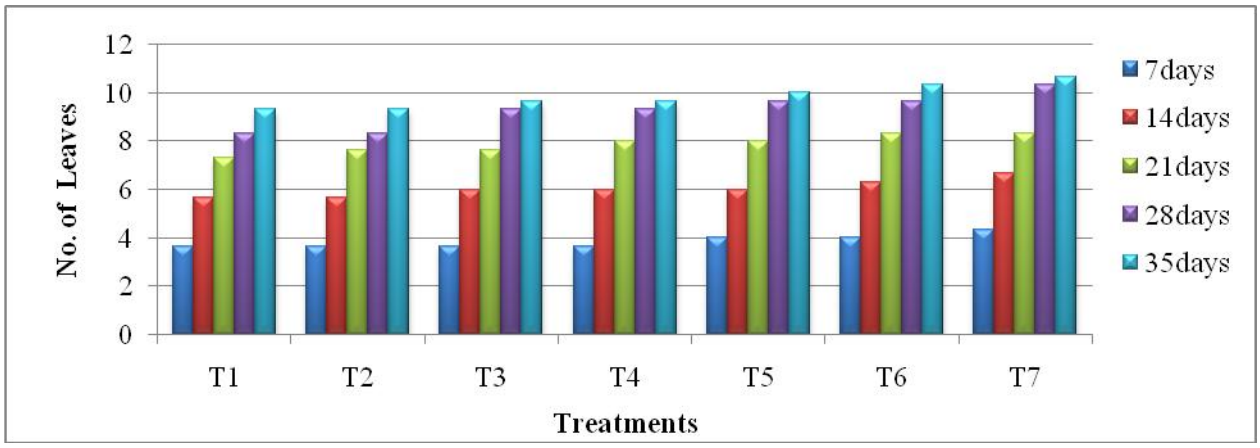

Fig.8 Variation in leaf area of Okra plant after 35 days of sowing under various treatments of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

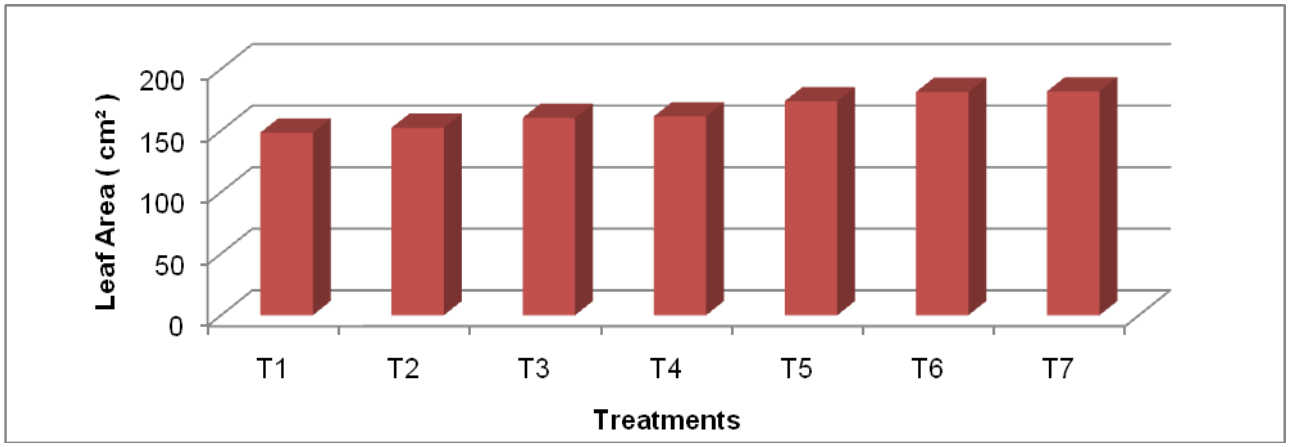

Fig.9 Variation in the root length (cm) of okra plant due to EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) after 35 days of showing

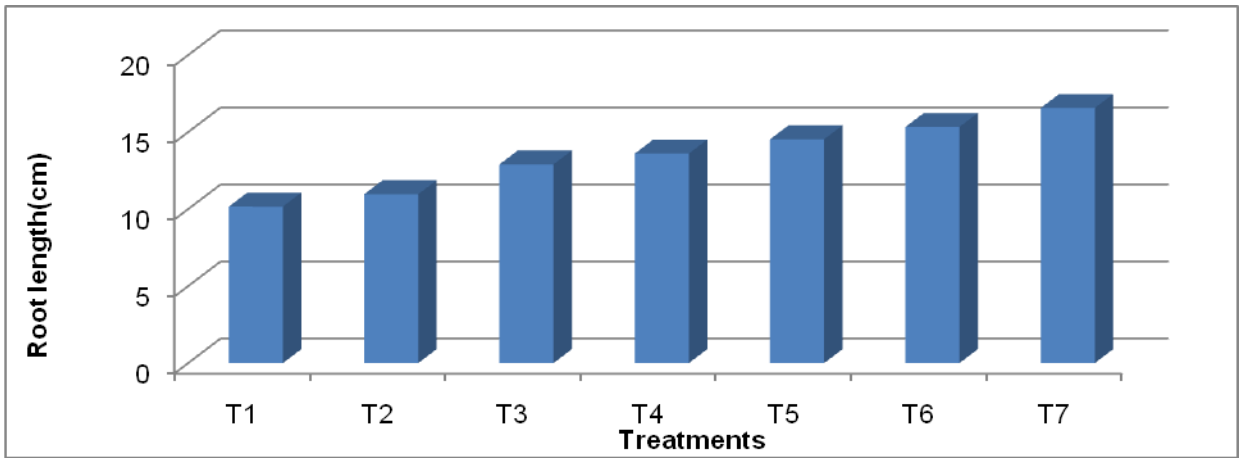

Fig.10 Variation in the fresh weight of root of okra plant due to EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

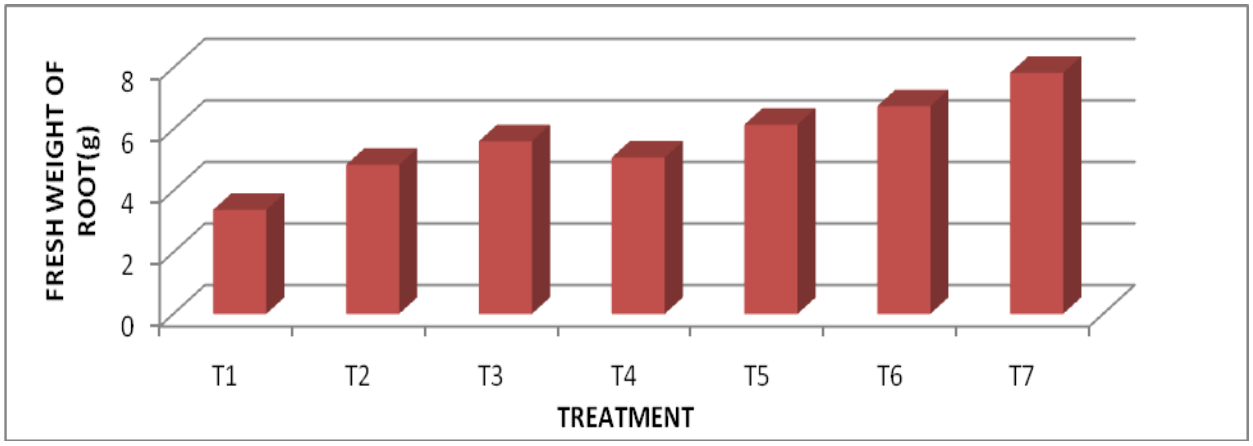

Fig.11 Variation in the dry weight of root of okra plant due to different treatment of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

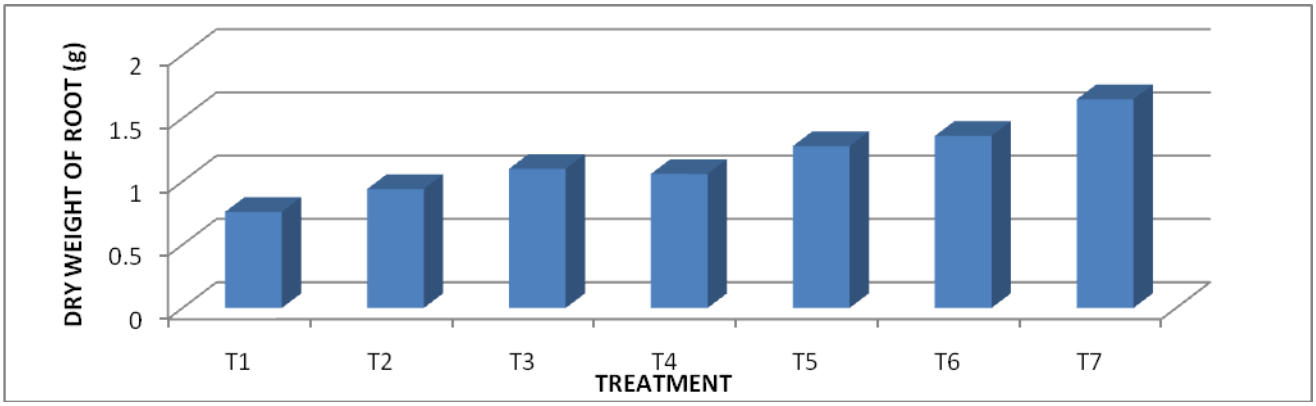

Fig.12 Variation in the fresh weight of leaves under the treatment of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

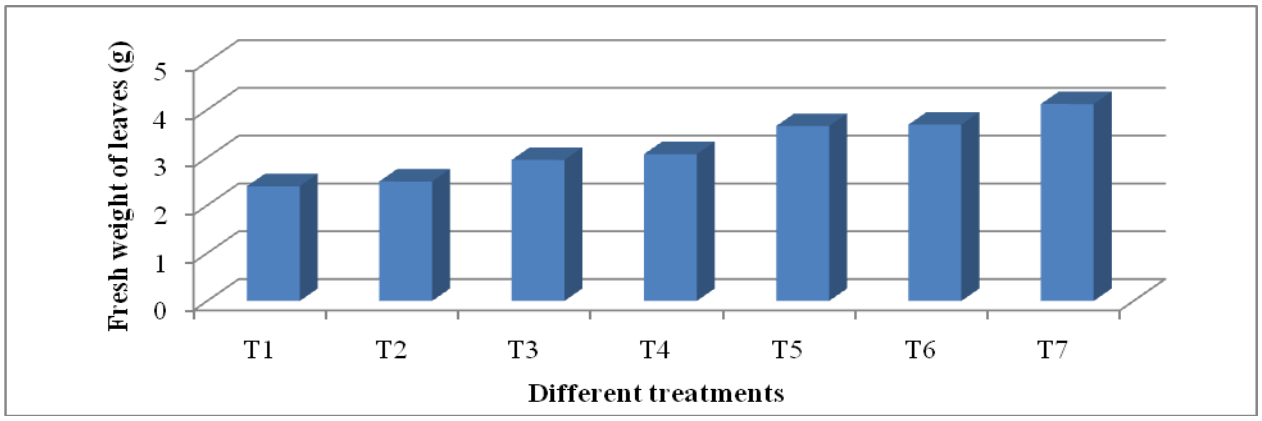

Fig.13 Variation in dry weight of leaves under the effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059)

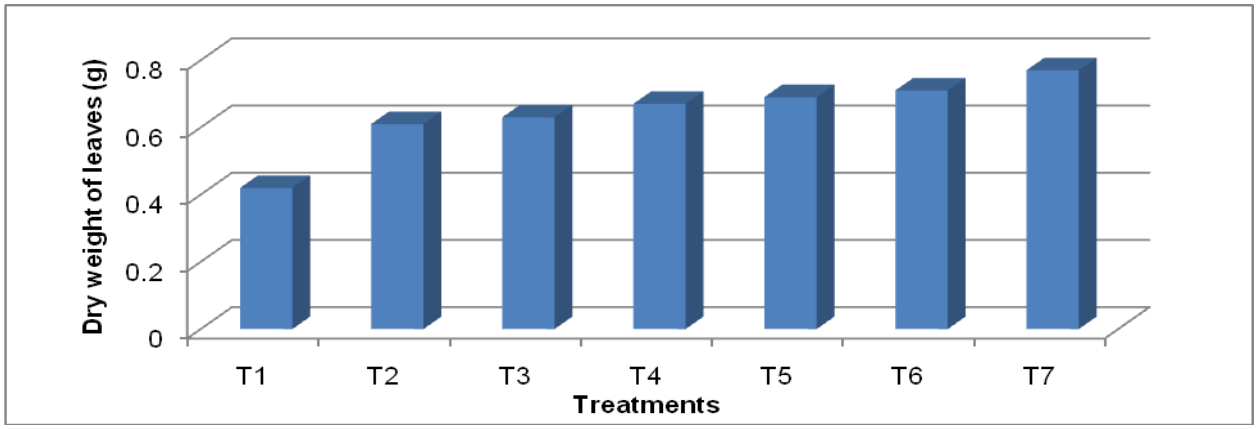

Plate.1 Screening of water stress tolerance of rhizobacterial isolates on TSA Medium

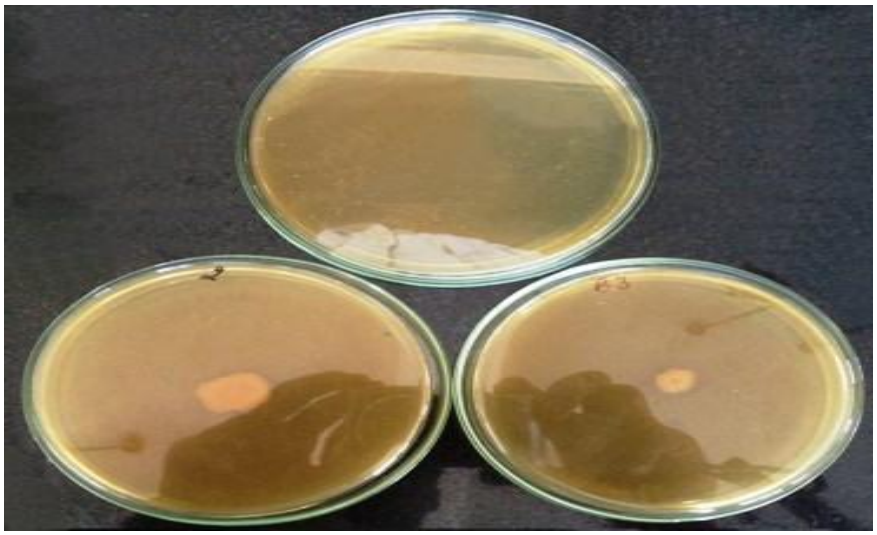

Plate.2 (a) Exopolysaccharide production on agar plate (b) Production of EPS by *P. aeruginosa* strain (MCCB0035) (c) Production of EPS by *B. coagulans* (MCCB0059)

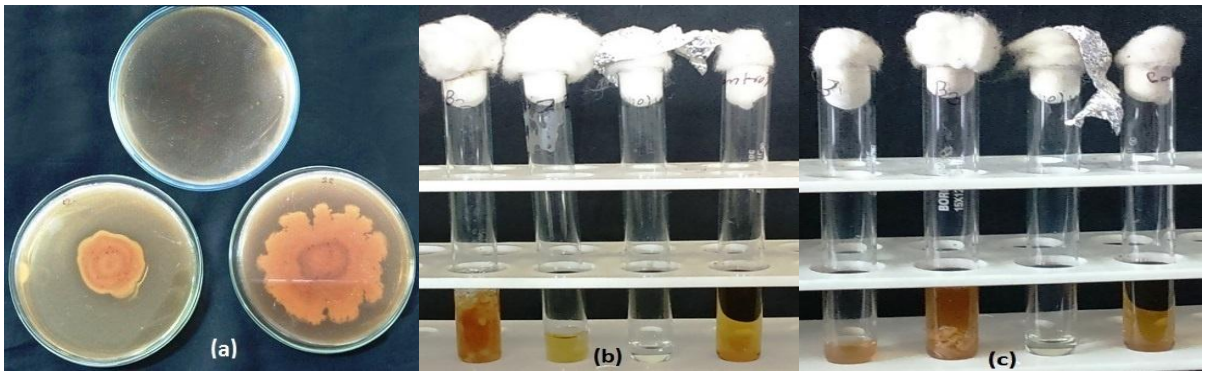

Plate.3 (a) Exopolysaccharide production and extraction (b) Production of EPS by *P. aeruginosa* strain (MCCB0035) (c) Production of EPS by *B. coagulans* (MCCB0059)

(a) (b) (c)

Plate.4 Phosphate solubilization by *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059)

Plate.5 Quantitative determination of phosphate solubilization by *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059)

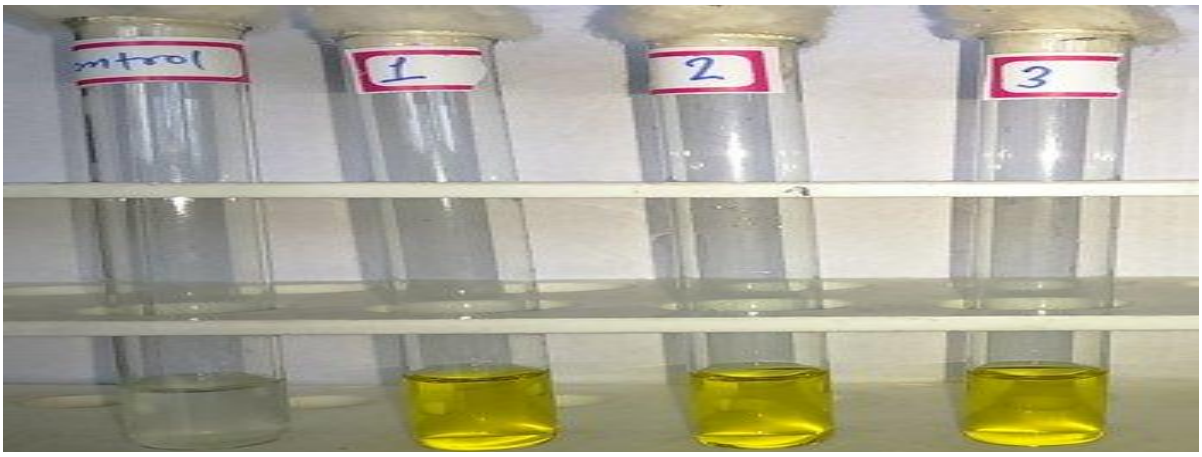

Plate.6 Test of Indole Acetic Acid (IAA)

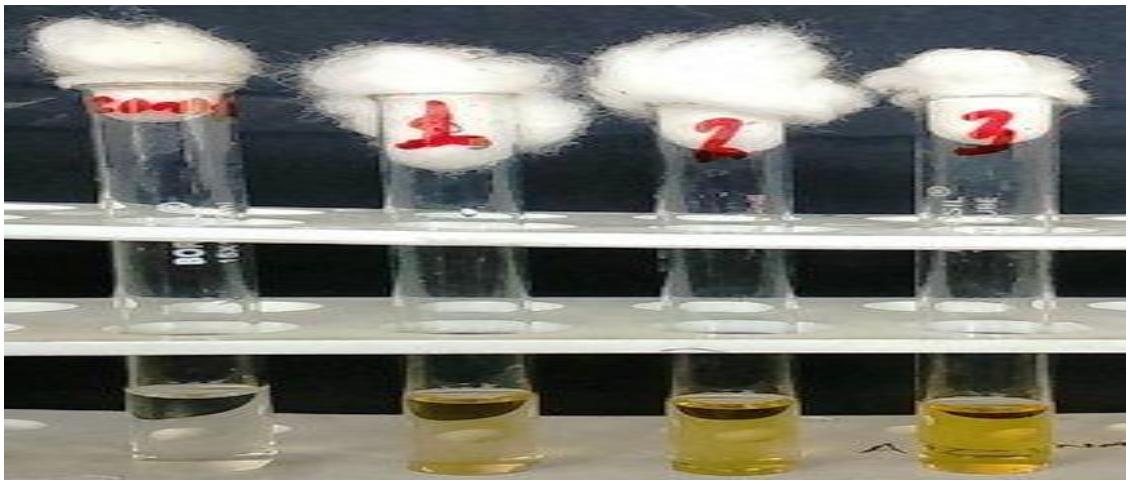

Plate.7 Production of ammonia by *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0035)

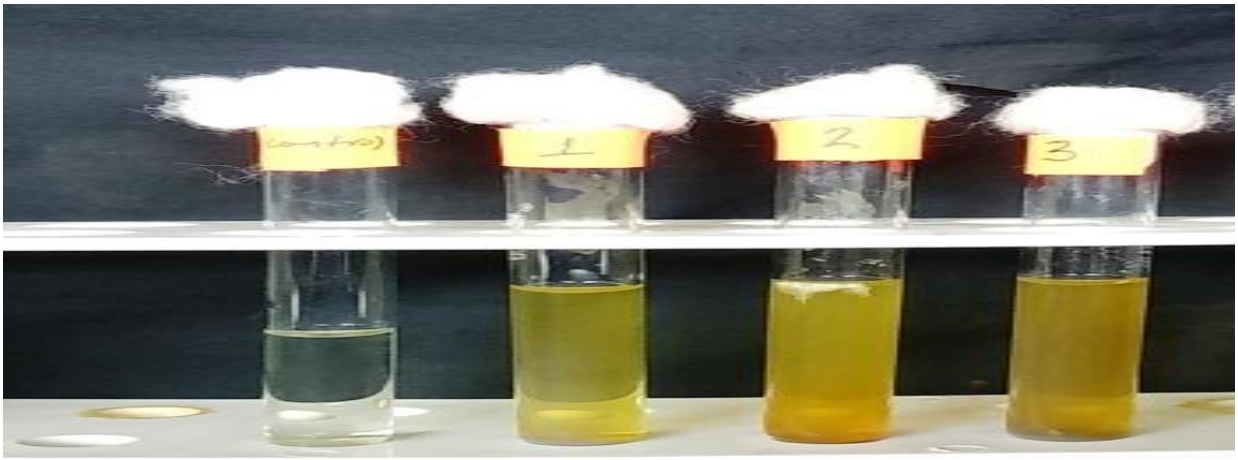

Plate.8 Bacterial culture *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) showing HCN production on TSA plates

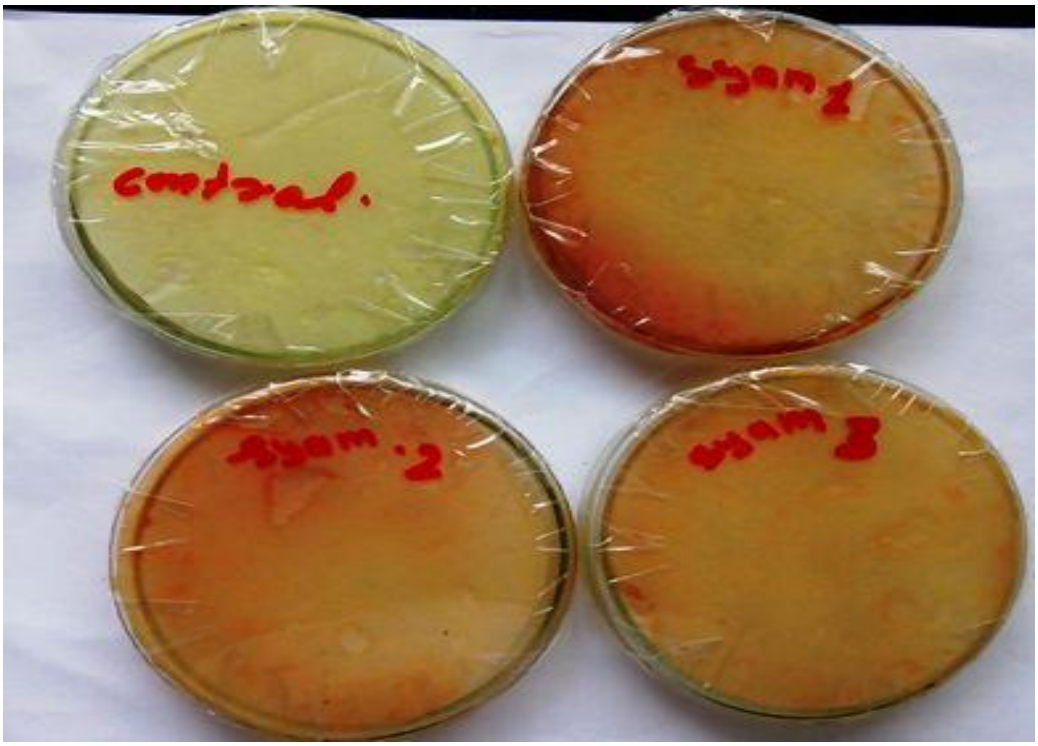

Plate.9 Siderophore Production by *Pseudomonas aeruginosa* (MCCB0035) *and Bacillus coagulans* (MCCB0059)

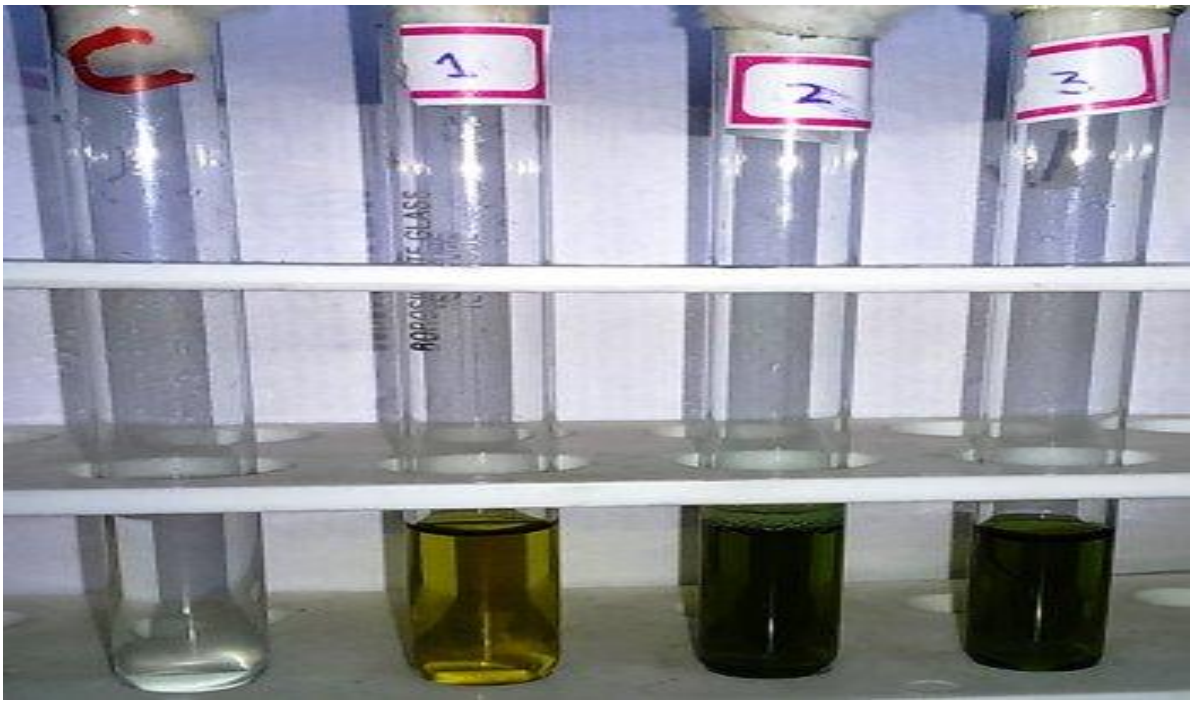

Plate.10 Growth of okra plant after 28 days

Plate.11 Okra plant biomass after 35 days of sowing

Plate.12 Different root length of okra plant under inoculants treatments after 35 days of sowing

From the observation taken the field it was found that combined bacterial solution of both the EPS producing bacteria and 25% of recommended dose of NPK gave highest seed germination. The least germination was observed in the controlplot in which only normal seed was sowed. Each EPS producing bacteria enhanced the seed germination as compared to the plants grown in the plot only treated with recommended dose of NPK. Between the two EPS producing bacterial strains, *Bacillus coagulans* treated seed given the better result as compared to *Pseudomonas aeruginosa*. Both the bacteria showed better water retention quality under water stress condition.

Combined bacterial (*Pseudomonas aeruginosa* and *Bacillus coagulans*) dose also showed better PGPR characteristics which in turn less NPK consumption with increased germination. From the data obtained the seed germination was increased approximately 95% when using the bacterial solution in combination with reduced amount of NPK. On analyzing data using ANOVA, the treatment found significant at 5% level of significance Seed germination ranged from 41.66% to 79.15%. The treatment T_7 (79.15%) had highest seed germination followed by T_6 (63.54%), T_5 (57.29%), T_4 (54.16%), T_3 (52.08%), T_2 (48.95%) and T_1 (41.66%) (Table 8 and Fig. 5).

Similarly Raza and Faisal (2013) in their study observed that inoculation of *M. luteus*-chp37 caused an increment in the seed germination of maize plant grown in pure soil. Some inhibition in seed germination was recorded in inoculated plant grown in mixed soil. Other investigators like Cezon *et al.*, (2003) also observed the enhancement in seed germination and Miche *et al.*, (2000) observed inhibition in seed germination of bacterial inoculated plants in comparison to un-inoculated ones. Inoculation with plant growth promoting bacteria had significant impact on seed germination of *Cynara scolymus* (Jahanian *et al.*, 2012; Panachali and Chanadie, 2012).

Effect of EPS producing bacteria *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) on okra plant height at 7 days interval

The experiment was conducted to test the efficacy of EPS producing microorganisms on plant height (cm) of Okra plant upto 35 days of growth. In the present investigation seven treatments were examined at different time intervals (7, 14, 21, 28 and 35 days) of growth. From the different treatments given to the plant, treatment T_7 was found to be the best combination of Bioinoculant and chemical fertilizer i.e. combination of both bacterial inoculants and reduced amount of

fertilizer i.e. 25% of recommended dose. Out of both the strains *Bacillus coagulans* inoculant was found better than the *Pseudomonas aeruginosa* when plant height was measured. In the present study the plant height was found better in case of inoculants than the untreated seeds. Both the EPS producing strain i.e. *P. aeruginosa* (MCCB0035) and *B. coagulans* (MCCB0059) showed better water retention property water stress condition. Plant height ranged from 5cm to 6cm at 7 days, 11.33 to 13.5cm at 14 days, 13.03 to 15.80cm at 21 days, 15.43 and 18.43cm at 28 days and from 19.4 and 22.93cm at 35 days under various treatments of inoculants. On analyzing the data using ANOVA two way the treatments were found significant at 5% level of significance. After 35 days Treatment T_7 (22.93cm) had highest mean performance for plant height followed by treatments T_6 followed by T_5 followed by T_4 followed by T_3 followed by T_2 and minimum in T_1 (19.4cm) (Table 9 and Fig. 6).

The findings of the present study is supported by the results of Khalid *et al.,* (1997); Biswas *et al.,* (2000a, 2000b); Hilali *et al.,* (2000, 2001), in which they reported increased plant height of various crop plants by microbial inoculation. All plants require a higher phosphate concentration at the early developmental stage for better root development (Alves *et al.,* 2001; Romer and Schilling, 1986) which in turn can influence plant height (Barber, 1977). Other studies have also reported that plant growth might be affected by the synthesis of phytohormones and vitamins, improved nutrient uptake and solubilization of inorganic phosphate (Dobbelaere *et al.,* 2003; Lucy *et al.,* 2004). Afzal *et al.,* (2014) studied the effect of *Rhizobium* and *Pseudomonas* consortium on wheat crop and stated that consortium have increased the plant height. Similarly, Cakmakci *et al.,* (2007) in barley and Javaid (2009) in blackgram [*Vigna mungo* (L.) Hepper] reported differences in terms of plant height with bacteria inoculations. Similar finding were also obtained by Ashrafuzzaman *et al.,* (2009) in his study and demonstrated that the PGPR isolates significantly affected the height of rice seedlings. The findings of the present investigation revealed that plant height increased in PGPR treated plants over uninoculated plants.

Effect of EPS producing bacteria *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) on number of leaves of Okra plant at 7 days interval

The present investigation was conducted to determine the effect of EPS producing bacteria on number of leaves of Okra plant. In this experiment seven treatments of both the inoculants namely *Pseudomonas aeruginosa* and *Bacillus coagulans* were examined at different time intervals like 7, 14, 21, 28 and 35 days. Treatment T_2 had got higher mean performance when compared to treatment T_1 and it was due to application of recommended dose of fertilizer. Treatment T_3 showed higher mean performance when compared to treatment T_2 and it was due to the application of EPS producing strain i.e. *P. aeruginosa* (MCCB0035) which have water retention qualities under water stress condition. Treatment T_4 was reported better than the treatment T_3 that might be due to the application of better EPS producing strain i.e. *B. coagulans* (MCCB0059) showing better water retention qualities in drought condition. Treatment T_5 given higher no. of leaves in comparison to the treatment T_4 and it might be due to the combined application of reduced recommended dose of fertilizer (50%) and EPS producing strain i.e. *P. aeruginosa* (MCCB0035) having water retention property under drought condition. Treatment T_6 showed greater number of leaves in comparison to treatment T_5 that might be due

to the combined application of reduced recommended dose of fertilizer (NPK 50%) and EPS producing strain i.e. *B. coagulans* (MCCB0059) showing effective water retention property under water stress condition. Treatment T_7 reported highest number of leaves in comparison to treatment T_6 and it was due to the combined application of reduced recommended dose of fertilizer (NPK 25%), and both the EPS producing strain i.e. *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) which showed better water retention qualities in drought condition. Number of leaves per plant of Okra ranged from 3.66 cm to 4.33 cm at 7 days. Treatment T_7 (4.33) had highest mean for number of leaves followed by T_6 and T_5. On the other hand T_1, T_2, T_3 and T_4 showed minimum number of leaves (3.66). Number of leaves per plant of Okra ranged from 5.66 to 6.66 at 14 days. Treatment T_7 (6.66) had highest mean performance for Number of leaves followed by T_6, T_5, T_4, and T_3. T_1 and T_2 both showed minimum number of leaves (5.66).

Number of leaves per plant of Okra ranged from 7.33 to 8.33 at 21 days. Treatment T_7 (8.33) had highest mean performance for Number of leaves followed by T_6, T5, T_4, T_3, T_2 and minimum in T_1 (7.33). Number of leaves per plant of Okra ranged from 8.33 to 10.33 at 28 days. Overall treatment T_7 (10.33) had highest mean performance for Number of leaves followed by T_6, T_5, T_4, T_3, T_2 and minimum in T_1 (8.33). The same trend was also found when the observations were taken at 35 days (Table 10 and Fig. 7).

The observations obtained in the present investigation was found similar as reported by Raza and Faisal (2013) in which they stated that the EPS producing strain *M. luteus*-chp37 showed positive enhancement for number of leaves of maize plants in both soil treatment when compared with un-inoculated respective controls.

Effect of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on leaf area of Okra plant after 35 days of sowing

The present study was examined to evaluate the effectiveness of EPS producing microorganisms on leaf area (cm^2) of Okra plant. In this investigation both the inoculants were given under seven treatments to the okra plants and leaf area (cm^2) was determined after 35 days of showing. Treatment T_7 had got highest mean performance when compared to other treatments. and it was due to the combined application of reduced recommended dose of fertilizer (NPK 25%), and both the EPS producing strains i.e. *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) showing better water retention qualities in water stress condition. Leaf area per plant of Okra ranged from 148.41 to 181.89 (cm^2) at 35 days. Treatment T_7 (181.89cm^2)) showed maximum mean for leaf area of okra plant followed by T_6 (181.36 cm^2), T_5 (173.94 cm^2), T_4 (161.42 cm^2), T_3 (160.32 cm^2), T_2 (152.30 cm^2) and minimum in T_1 (148.41 cm^2). From the two strains, *Bacillus coagulans* was found better inoculant than the *Pseudomonas aeruginosa*. On analyzing the data using ANOVA treatment found (Table 11 and Fig. 8).

In a similar study conducted by Yasmin *et al.*, (2013) also observed that the inoculation treatment with PGPR isolates 1K, 9K and KB showed increased leaf area as compared to un-inoculated control under non-stressed and water stressed conditions respectively. Naseem and Bano (2014) also reported that seeds inoculated with *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2), and *Alcaligenes faecalis* (AF3) strains in combinations with its EPS showed greater increase in leaf area as compared to control both in stressed and unstressed conditions. The inoculation of *Bacillus* spp. and *Pantoea*

sp. in *Z. mays* L. seedlings showed significant increases in leaf area under water stress condition as observed by Kavamura *et al.,* (2013).

Effect of EPS producing bacteria *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on root length of Okra plant after 35 days of sowing

The present investigation was conducted to determine the effect of EPS producing microorganisms on root length (cm) of Okra plant. In this study seven inoculants treatments were tested on the okra plant and the root length (cm) under various treatment after 35 days of sowing were observed. Treatment T_7 has got longest root length as compared to the other treatments like T_6 followed by treatment T_5 followed by treatment T_4. The root length was observed higher in Treatment T_2 as compared to treatment T_1 and it was might be due to application of recommended dose of fertilizer. Between the two bacterial isolates the *Bacillus coagulans* was found to be effective inoculants because of having better water retention property. Root length per plant of Okra ranged from 10.16 to 16.56 (cm) at 35 days. Treatment T_7 (16.56 cm)) was found to have highest mean performance for root length of Okra plant followed by T_6 (15.33 cm), T_5 (14.53 cm), T_4 (13.6 cm), T_3 (12.9 cm), T_2 (10.96 cm) and minimum in T_1 (10.16 cm). On analyzing the data by using Anova the treatment was found significant (Table 12, Fig. 9).

In a similar study conducted by Yasmin *et al.,* (2013) the inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in root length by 25-87% and 16.4-43.3% as compared to uninoculated control under non-stressed and drought stressed conditions respectively. Similarly Yadav *et al.,* (2010) also observed *Pseudomonas putida* and *Pseudomonas aeruginosa* as most efficient inoculations for enhancement of root length followed by *Bacillus subtilis*, *Paenibacillus polymyxa* and *Bacillus boronophillus* over control. Other workers have also reported the enhancement of root length by inoculation of plant growth promoting rhizobacteria for chickpea (Mishra *et al.,* 2010). Ashrafuzzaman *et al.,* (2009) in his study demonstrated that the PGPR isolates significantly increased the root length of rice seedlings ranging from 4.10 to 5.30 cm. The isolate PGB4 produced the highest root length (5.30 cm). Mehnaz and Lazarovits (2006) also showed that *P. putida* CQ179 significantly promoted root growth of two corn varieties, 39D82 and 39M27 under greenhouse conditions. When root length was considered as growth parameter, mixed inoculation of three isolates *Pseudomonas aeruginosa*, *Bacillus firmus* and *Cellulosimicrobium cellulans* gave better results showing an increase of 125%, over control (Chatterjee *et al.,* 2012).

Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on fresh weight of root (g) of Okra plant after 35 days of sowing

The study was conducted to determine the effect of EPS producing microorganisms on fresh weight of root (g) of Okra plant. In the present study 7 treatments were examined for fresh weight of root (g) after 35 days of sowing. T_2 gave higher mean performance when compared to T_1 and it was due to application of recommended dose of fertilizer. Treatment T_3 got higher fresh weight than treatment T_2 and it was due to the application of EPS producing strain i.e. *P. aeruginosa* (MCCB0035) which have water retention qualities in drought condition. Treatment T_4 showed greater fresh weight than the treatment T_3 and it was due to the application of better EPS producing strain i.e. *B.*

coagulans (MCCB0059) which showed better water retention qualities in drought condition. Treatment T_5 was found to be better than treatment T_4 it might be due to the combined application of reduced recommended dose of fertilizer (50%) and EPS producing strain i.e. *P. aeruginosa* (MCCB0035) which have water retention qualities in drought condition. Treatment T_7 had got highest mean performance when compared to treatment T_6 and it was due to the combined application of reduced recommended dose of fertilizer (NPK 25%), and both the EPS producing strain i.e. *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) showing improved water retention qualities under water stress condition. Fresh weight of root (g) per plant of Okra ranged from 3.38 to 7.83 (g) after 35 days of sowing. Treatment T_7 (7.83g) had highest fresh weight of root of Okra plant followed by T_6 (6.75g), T_5 (6.15g), T_4 (5.08g), T_3 (5.61g), T_2 (4.84g) and minimum in T_1 (3.38g) (Table 4.13 and Fig. 10).

Majeed *et al.,* (2015) observed similar results while studying the effect of different PGPR isolates on fresh weight of root. Seed inoculated with isolated PGPR strain *Cronobacter malonaticus* BR-1 increased barley fresh root weight. Similar increases in fresh root weight were observed in different crops such as potato, radish plants, sorghum and pearl millet inoculated with *Pseudomonas, Azospirillum* and *Azotobacter* strains (Bhatt and Vyas, 2015).

Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on dry weight of root (g) of Okra plant after 35 days of sowing

This experiment was conducted for the evaluation of the effect of EPS producing microorganisms on dry weight of root (g) of Okra plant. Different inoculant treatments were examined on the okra plant and dry weight of root (g) after 35 days of sowing was determined. Treatment T_7 was found to be effective when compared to all the other treatments and it was due to the combined application of reduced recommended dose of fertilizer (NPK 25%) as well as both the EPS producing strain i.e. *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) having good water retention property in drought condition. Dry weight of root (g) per plant of Okra ranged from 0.76 to 1.65 (g) at 35 days.

Treatment T_7 (1.65g) had highest mean performance for dry weight of root of Okra plant followed by T_6 (1.28g), T_5 (1.36g), T_4 (1.06g), T_3 (1.1g), T_2 (0.94g) and minimum in T_1 (0.76g). In this experiment *Bacillus coagulans* has more effect on the growth of okra plant in comparison to *Pseudomonas aeruginosa* under both conditions i.e. with reduced or without NPK. From the observation it was also found that bacterial suspension had greater effect on the growth in comparison to NPK treated or normal soil. It was also observed that around 75% chemical fertilizer can be replaced with the bacterial suspension for the growth of okra plant.

The bacterial suspensions were found to be effective even in the reduced irrigation frequency which might be due to water holding capacity of the exopolysaccharides produced by them. Upon analyzing the data using Anova the treatment found significant at 5% level (Table 14 and Fig. 11).

Yasmin *et al.,* (2013) showed that their isolates 1K, 9K and KB produced significant positive increase in dry weight of root under water stressed condition by 218-381% than under non- water stressed conditions and by 59-128.7% over uninoculated control. Similar to the present findings Chi *et al.,* (2005) reported the production of significant more root biomass of rice plants inoculated with

certain test strains of Rhizobia and accumulation of elevated levels of growth hormones (i.e. indole acetic acid and gibberellins). Moreover, Noel *et al.,* (1996) carried out a genotobiotic study where they used parent and mutant strains of *Rhizobium leguminosarum* to inoculate the seeds of canola and lettuce and observed considerable growth of seedling roots.

Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on fresh weight of leaves (g) of Okra plant after 35 days of showing

In the present study the okra plants were treated with different inoculants namely *Pseudomonas aeruginosa* (MCCB0035) as well as *Bacillus coagulans* (MCCB0059). The fresh weight of leaves was determined after 35 days of sowing. Of the two strains, Both the strains were found effective even under reduced irrigation frequency but *Bacillus coagulans* was more effective. Due to use of bacterial inoculants the chemical fertilizer could be reduced upto 70%, this was an added advantage shown by the strains.

Treatment T_7 had got highest mean performance when compared to other treatments and it was due to the combined application of reduced recommended dose of fertilizer (NPK 25%), and both the EPS producing strain i.e. *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) which showed better water retention qualities in drought condition. Fresh weight of leaves (g) per plant of Okra ranged from 2.4 to 4.12 (g) at 35 days. Treatment T_7 (4.12g) had highest mean performance for fresh weight of leaves of Okra plant followed by T_6 (3.69g), T_5 (3.66g), T_4 (3.07g), T_3 (2.95g), T_2 (2.5g) and minimum in T_1 (2.4g). Upon analyzing the data using Anova the treatment found significant at 5% level of significance (Table 15 and Fig. 12).

Effect of EPS producing bacteria (*P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) on dry weight of leaves (g) of Okra plant after 35 days of showing

In the present study the okra plants were treated with different inoculants namely *Pseudomonas aeruginosa* (MCCB0035) as well as *Bacillus coagulans* (MCCB0059). The dry weight of leaves was determined after 35 days of sowing. Of the two strains, Both the strains were found effective even under reduced irrigation frequency but *Bacillus coagulans* was more effective. Due to use of bacterial inoculants the chemical fertilizer could be reduced upto 70%, this was an added advantage shown by the strains.

Treatment T_7 showed highest performance when compared to all other treatments and this might be due to the combined application of reduced recommended dose of fertilizer (NPK 25%) as well as both the EPS producing strain i.e. *P. aeruginosa* (MCCB0035) *and B. coagulans* (MCCB0059) which showed better water retention qualities in drought condition. Dry weight of leaves (g) per plant of Okra ranged from 0.42 to 0.77 (g) at 35 days. Treatment T_7 (0.77g) had highest mean for Dry weight of leaves of Okra plant followed by T_6 (0.71g), T_5 (0.69g), T_4 (0.67g), T_3 (0.63g), T_2 (0.61g) and minimum in T_1 (0.42g). Upon analyzing the data using Anova the treatment found significant at 5% level of significance (Table 16 and Fig. 13).

Soil moisture test

After harvesting the plants, moisture content of rhizospheric soil of both stressed plants and non-stressed plants were measured. Fresh weight of soil sample (20 g) collected from 6-inch rhizosphere of plants was dried in oven for 72 h at 70°C. Dry weight of soil was recorded and moisture content was calculated as:

Soil moisture (%) = [(20 – 17.9) / 17.9] x 100 = 11.73(%)

From the above results obtained the following conclusion can be drawn:

Two bacterial isolates namely *Pseudomonas aeruginosa* (MCCB0035) and *Bacillus coagulans* (MCCB0059) were found most potent in exopolysacharide production i.e. *Pseudomonas aeruginosa* and *Bacillus coagulans* showed the maximum water stress tolerance activity on TSA medium.

These bacterial inoculants were found to be effective on the growth of the okra plant. Therefore these can be used partly with reduced chemical fertilizers doses also and by this way gradually dependency on the chemical fertilizers could also be reduced for the same crop yield.

Statistical Analysis

Field experiments were conducted in a completely randomized design. Data were subjected to ANOVA followed by a classification of means in order to compare the treatments with the control (uninoculated plants) and test of significance was calculated at 5% level of significance.

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