



Augmentation of Halotolerant Cyanobacteria to Restore Soil Properties and Promote the Plant Growth in Saline Soils

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Abstract

Soil salinity caused by high concentrations of soluble salts in the soil. Soil salinity is one of the most overwhelming environmental factors that affect decrease in cultivated land area, crop productivity and quality. Also, they affect the effects on soil physicochemical properties and ecological balance of the area. Cyanobacteria are widely distributed and play a diverse role in soil as well also produce a wide range of secondary metabolites with bioactivity including plant growth promoting metabolites. The present study aims at use of halotolerant and plant growth promoting cyanobacterial inoculants to boost the growth of crops in saline soil. Factorial design (2^3) was used to check the efficacy of cyanobacterial consortium on different plantlets like brinjal, chilli and tomato. The harvested biomass of selected cyanobacterial cultures; *Oscillatoria* sp. (TCC-4), *Oscillatoria* sp. (TCC-5) and *Nostoc* sp. were homogenized and equally mixed to prepare consortia. The seeds of brinjal, chilli and tomato were brought from the local market. The seeds were sown in saline soil using nursery trays. The soil was treated as per the factorial design. These trays were exposed to natural sunlight under nursery. The trays were watered every day and kept the trays moist. After 40 days soil from trays were analyzed for their soil properties and growth of plantlets. Improved growth of plantlets has suggested those halotolerant cyanobacteria, along with organic manure and other additives, enriched soils with physical properties as well as with organic carbon, nitrogen and other nutrients. Use of cyanobacteria helps to reestablish microbial flora in salt affected soils as well as promote plant growth.

Keywords

Salinity, Cyanobacteria, Secondary metabolites, Halotolerant cyanobacterial consortium.

INTRODUCTION:

Salt affected soils are widely distributed throughout the world. The excess accumulation of salts in soil resulted in salt affected soils. Water is a primary carrier responsible for the formation of saline soil [1,2]. Commonly cations of Sodium, potassium, calcium and magnesium and anions of chlorine, sulphates, bicarbonate, carbonate, borate and nitrate are responsible for salination [3]. Salt may rise to the soil surface by transport of salt laden water and then accumulate due to evaporation [4]. According to the Central soil salinity research institute (CSSRI), Karnal, India, 6.06 Lakh hectare physiographic region of Maharashtra is under salinity [5]. Soil salinity can also become concentrated in soil due to human activity [6]. Salts are transported and ions are dissolved in water to developing saline soils. This salt containing water moves through a wetted soil zones towards dry zone. High content salts resulted in degradation of soils properties and further into the retard plant growth and decrease yield [7]. Proper temperature, water, space, light condition, good quality of soil and seed resulted into the better plant growth and yield.

Cyanobacteria is beneficial to crops by their nitrogen fixation, production of extracellular substances, production of plant growth promoting substances and production of secondary metabolites. Researchers reported previously that the cyanobacteria play a vital role in improving soil structure by increasing soil aggregation, soil aeration, water holding capacity, thus it is useful for the reclamation of soils [8,9]. Cyanobacteria produce a wide range of substances with bioactivity. Besides that, they have also been reported to benefit plants by producing auxin, gibberellins, cytokinins, vitamins, amino acid, and polypeptides which improve plant growth and productivity [10].

Anabaena, *Anabaenopsis*, *Calothrix*, *Chlorogloeopsis*, *Cylindrospermum*, *Gloeotheca*, *Nostoc*, *Plectonema*, and *Synechocystis* produce IAA which has been reported previously. It is also found that IAA increases the level of the biosynthesis of certain enzymes and stimulates nitrogen fixation in the cyanobacteria *Anabaena doliolum* and *Nostoc punctiforme* [11]. The reduction in exchangeable sodium following algalization was attributed to utilization of sodium by algae for its own metabolism and decrease in pH and exchangeable sodium in alkali soils [12, 13].

Cyanobacterial biofertilizers and its contribution to nitrogen fixation, production of plant growth promoting metabolites have significant effect on the crop yield like Barely, Oat, tomato, radish, cotton, sugarcane, maize, chili and lettuce and contributed significantly in soil fertility [14, 15]. Mazahar and Hasnain [16] studied the effect of filamentous non heterocystous *Phormidium* cyanobacterial strain from rice fields has plant growth promoting characters such as phosphate solubilization, nitrogen fixation, hydrogen cyanide and auxin production. *Phormidium* SM-14 and SM-15 strains were able to significantly enhance the growth of wheat under control conditions. In the present chapter, the selected cyanobacterial cultures were used for preparation of cyanobacterial inoculants and their effect on development of plantlet growth and soil properties.

MATERIALS AND METHODS:

Cyanobacterial culture and growth conditions:

The cyanobacterial cultures *Oscillatoria* sp. (TCC-4), *Oscillatoria* sp. (TCC-5) and *Nostoc* sp. (*Nostoc* balls was collected in rainy season from the tunnels at near the Lonavala) were selected on the basis of their plant growth promoting activity [17]. These cultures were maintained in optimized conditions BG-11 medium (pH 8.0) at 24±1°C with 16:8 hr light and dark photoperiod under 2000 lux light intensity.

Cultivation of selected cyanobacterial cultures:

The *Oscillatoria* sp. (TCC-4), *Oscillatoria* sp. (TCC-5) and *Nostoc* sp. were grown in 700 ml BG-11 medium (pH 7.4) in 1 lit Erlenmeyer flask and incubated at 24°C under 2000 lux light illumination with 16:8 light: dark photoperiod for 15 days. After 15 days the grown flaks were homogenized, and homogenized cultures was added in to the fresh 1500 ml BG-11 medium in 2 lit Erlenmeyer flasks. The flasks were again incubated for 15 days. The grown biomass was harvested and used for further experiments.

Preparation of Consortium:

The grown biomass was used to check the efficacy of cyanobacterial inoculants on growth plantlets and change in the soil properties of saline soil. The harvested biomass of selected cyanobacterial cultures was homogenized and equally mixed. The mixed consortium was used for the nursery assay.

Collection of saline soil

The saline soil field was found with the help of Krishi Vigyan Kendra, Baramati. On the basis of that information soil samples were collected from various agricultural fields. The soil samples were collected as per the standard protocols and samples were brought in polythene bag at laboratory. The collected samples were primarily characterized for their pH and electrical conductivity (EC). The (>4dS/m) electrical conductivity of soil are considered as saline soil [18].

Nursery experimental setup

The 2³ factorial design was used for cultivation of plantlets in saline soil at nursery. These 2³ factorial designs were constructed from statistician expert. The soil was brought to the nursery and filtered to remove the unwanted debris. The selected saline soil was used for this nursery experiment. The nursery trays (70 wells/tray) were brought from the M/s. Shri Balaji Agro Plast, MIDC, Satara. Brinjal, Chili and Tomato seeds have been selected as the experimental plant species and seeds are purchased from local market.

The total 8 treatments were selected as per the factorial design including control (Table 1). The one tray used for each treatment for each selected plant seeds. The mixed consortium of *Oscillatoria* sp. (TCC-4), *Oscillatoria* sp. (TCC-5) and *Nostoc* sp. was used for this experiment. Each well of tray has 50 gm capacity. One tray was used for each treatment. The trays were filled with the saline soil were treated with the organic manure, vermicompost and cyanobacterial consortia as details given in Table 2. The seed was sowed in treated soil and trays were watered. Trays will be exposed to natural sunlight under nursery. After 40 days, the tray-soil will be assessed for microbial as well as physicochemical characteristics.

Table 1-Treatment codes and description of nursery experiment

Treatments	Treatment codes	Description
T1	Control	Control (Only saline soil)
T2	CC	Cyanobacterial consortium (CC)
T3	VC	Vermicompost (VC)
T4	OM	Organic manure (OM)
T5	VC+CC	Vermicompost (VC)+ Cyanobacterial consortium (CC)
T6	OM+CC	Organic manure (OM) + Cyanobacterial consortium (CC)
T7	OM+VC	Organic manure (OM) + Vermicompost (VC)
T8	OM+VC+CC	Organic manure (OM) + Vermicompost (VC) + Cyanobacterial consortium (CC)

Table 2-Factorial (2³) design of nursery experiment for cultivation of plantlets in saline soil

Treatment	Treatment codes	Organic manure (g)	Vermicompost (g)	Cyanobacterial Consortium (g)
T-1	Control	----	----	----
T-2	CC	----	----	0.15
T-3	VC	----	10	----
T-4	OM	10	----	----
T-5	VC+CC	----	10	0.15
T-6	OM+CC	10	----	0.15
T-7	OM+VC	10	10	----
T-8	OM+VC+CC	10	10	0.15

Analysis of soil properties:

The control and treated soils were analyzed for their physicochemical properties (pH, EC and SAR) and nutrient content of soil (NPK). The determination of pH of soil is a key for soil classification on the basis of acidity or alkalinity. Soil pH was determined in 1:2.5 soil/water extract. The electrical conductivity of water extract of soil (1:2.5 soil/water) gives a measure of soluble salt content of the soil. The available nitrogen and total nitrogen of soil was estimated using Kjeldahl method. The available phosphorous present in the soil was determined by Olsen's Method [19]. Estimation of available potassium and sodium was done using flame photometer as method described by [20]. The total organic carbon content of soil was estimated by modified method of Walkley and Black [21]. The content of soluble calcium and magnesium was estimated titration method with EDTA [22] and use this calcium plus magnesium concentration for calculation of sodium adsorption ratio.

Analysis of plant growth:

The plant growth was assessed in terms of number of seeds germinated, % germination of seeds, shoot length, root length and total chlorophyll content of leaves. Chlorophyll content was determined as per protocol described by [23].

RESULTS AND DISCUSSION

Cultivation of cultures:

The selected cultures were cultivated into the one-liter medium to 5 lit medium. The growth was further inoculated in to the 1 to 15 lit tubs. The harvested biomass was used for the development of plantlets in saline soil (Fig. 1).



Fig. 1- Cultivation of selected cyanobacterial cultures

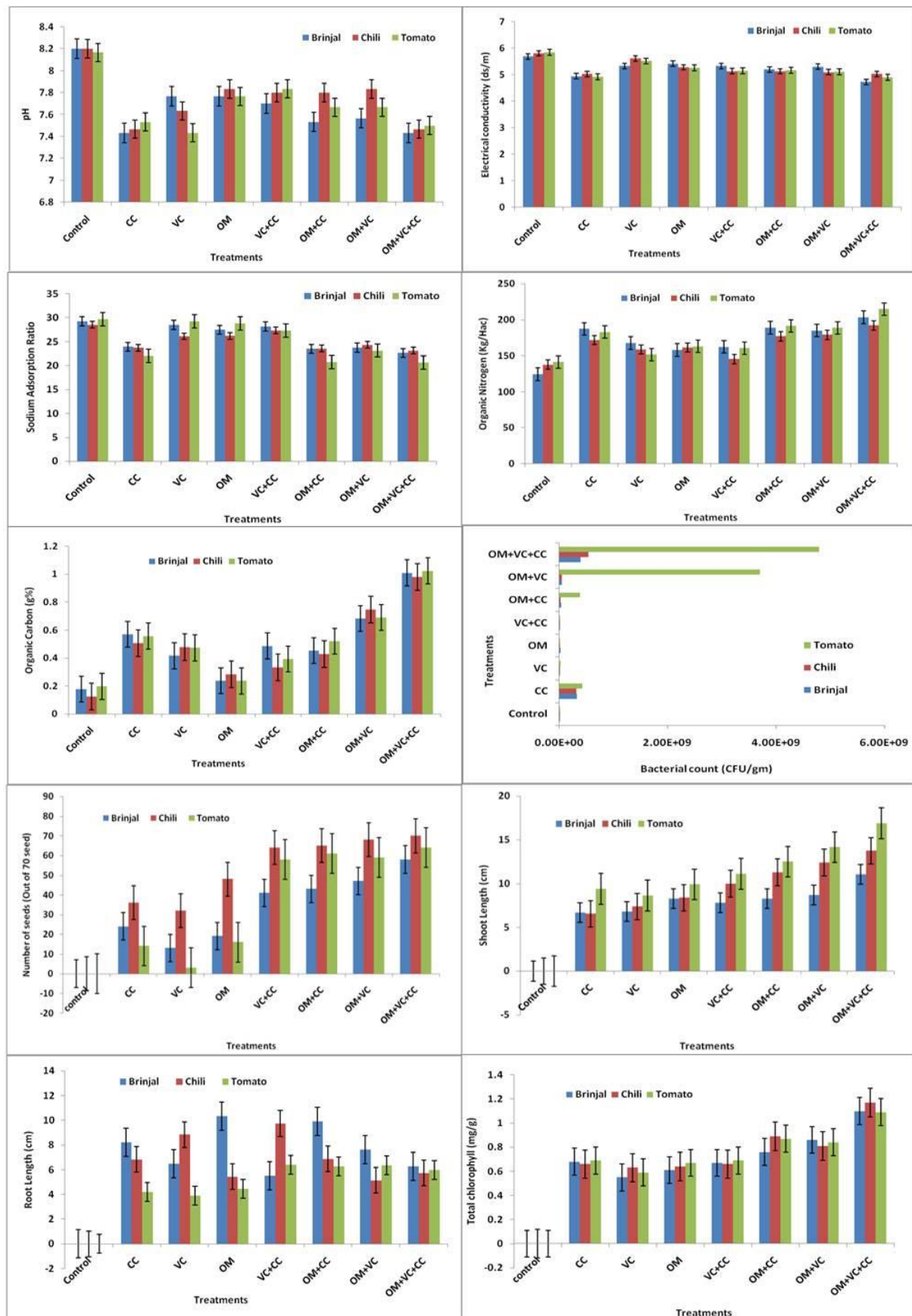


Fig. 2-Effect of various treatments on soil properties and growth of Brinjal, Chili, Tomato and wheat in saline soil

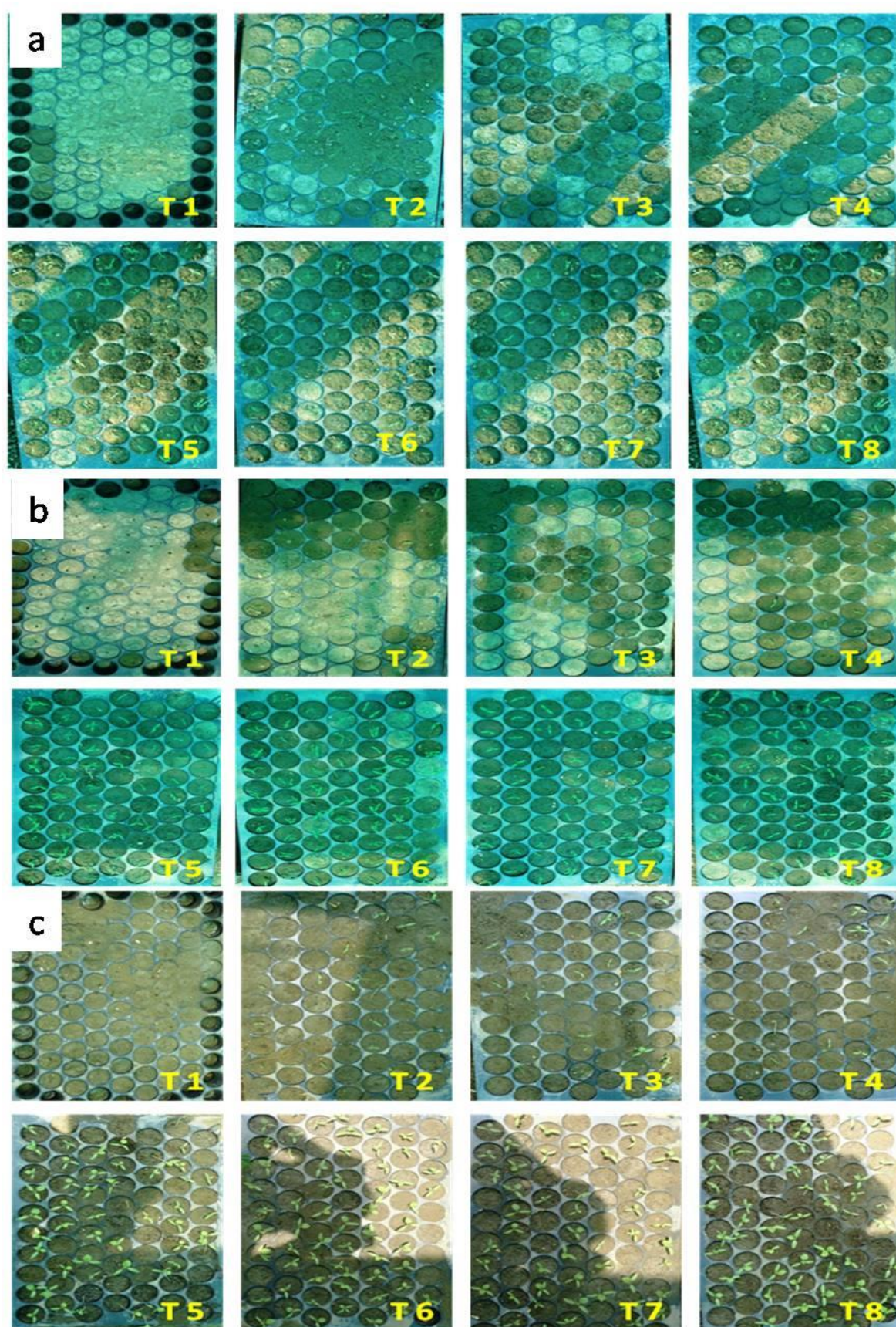


Fig. 3-Effect of various treatments on Brinjal growth after a)-1st week, b) 3rd week and c) 5th week of sowing

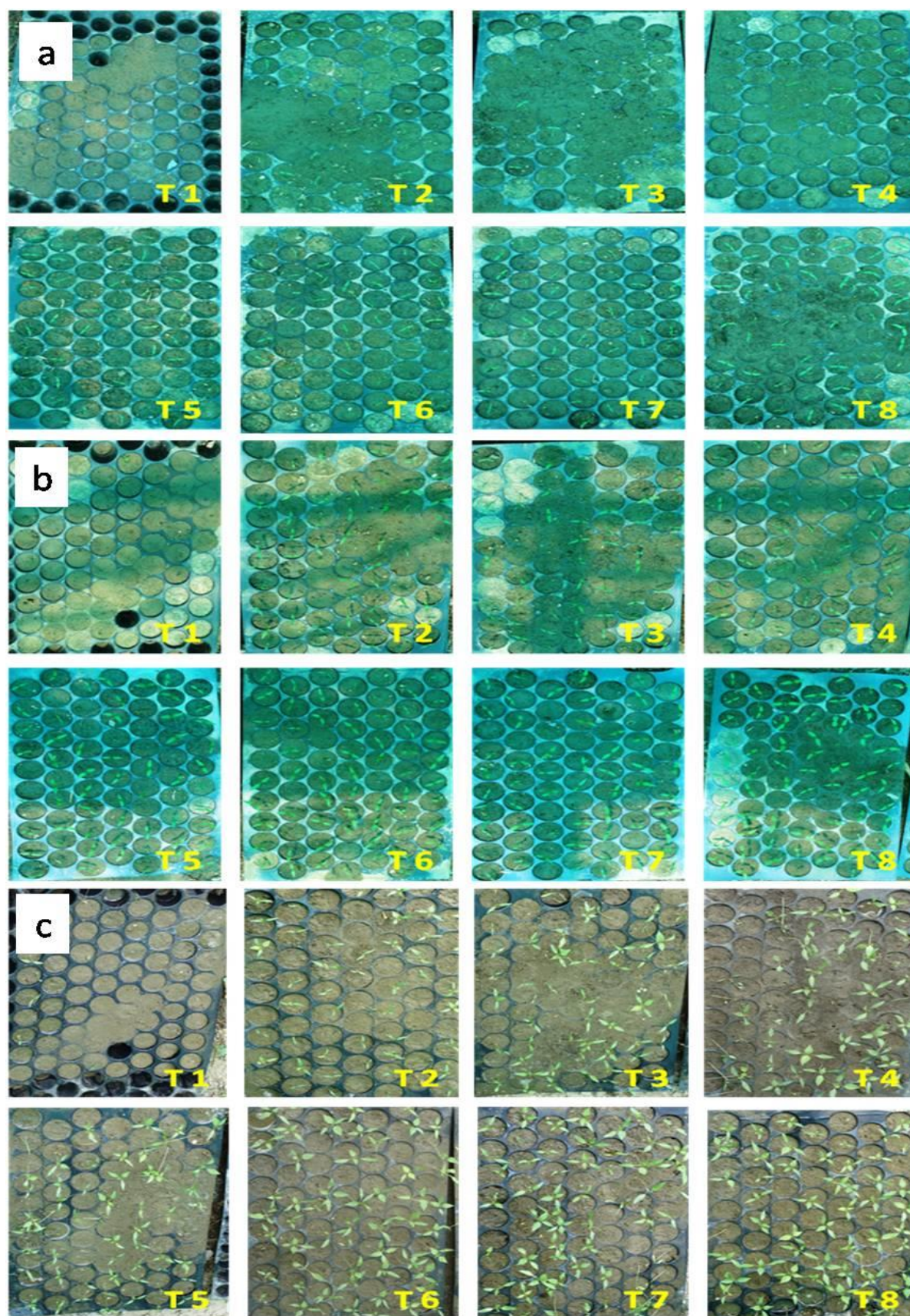


Fig. 4-Effect of various treatments on Chili growth after a)-1st week, b) 3rd week and c) 5th week of sowing

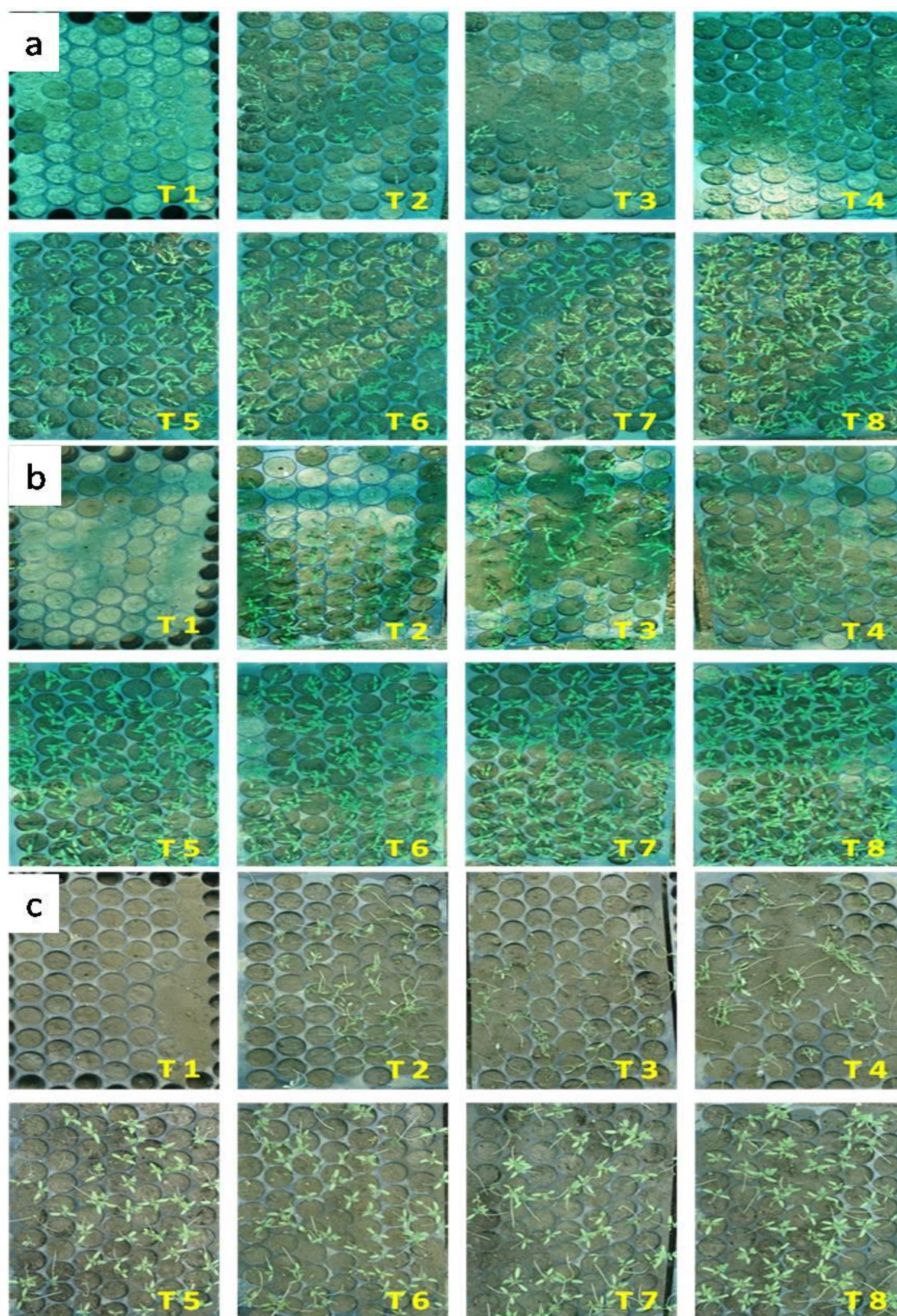


Fig. 5-Effect of various treatments on Tomato growth after a)-1st week, b) 3rd week and c) 5th week of sowing

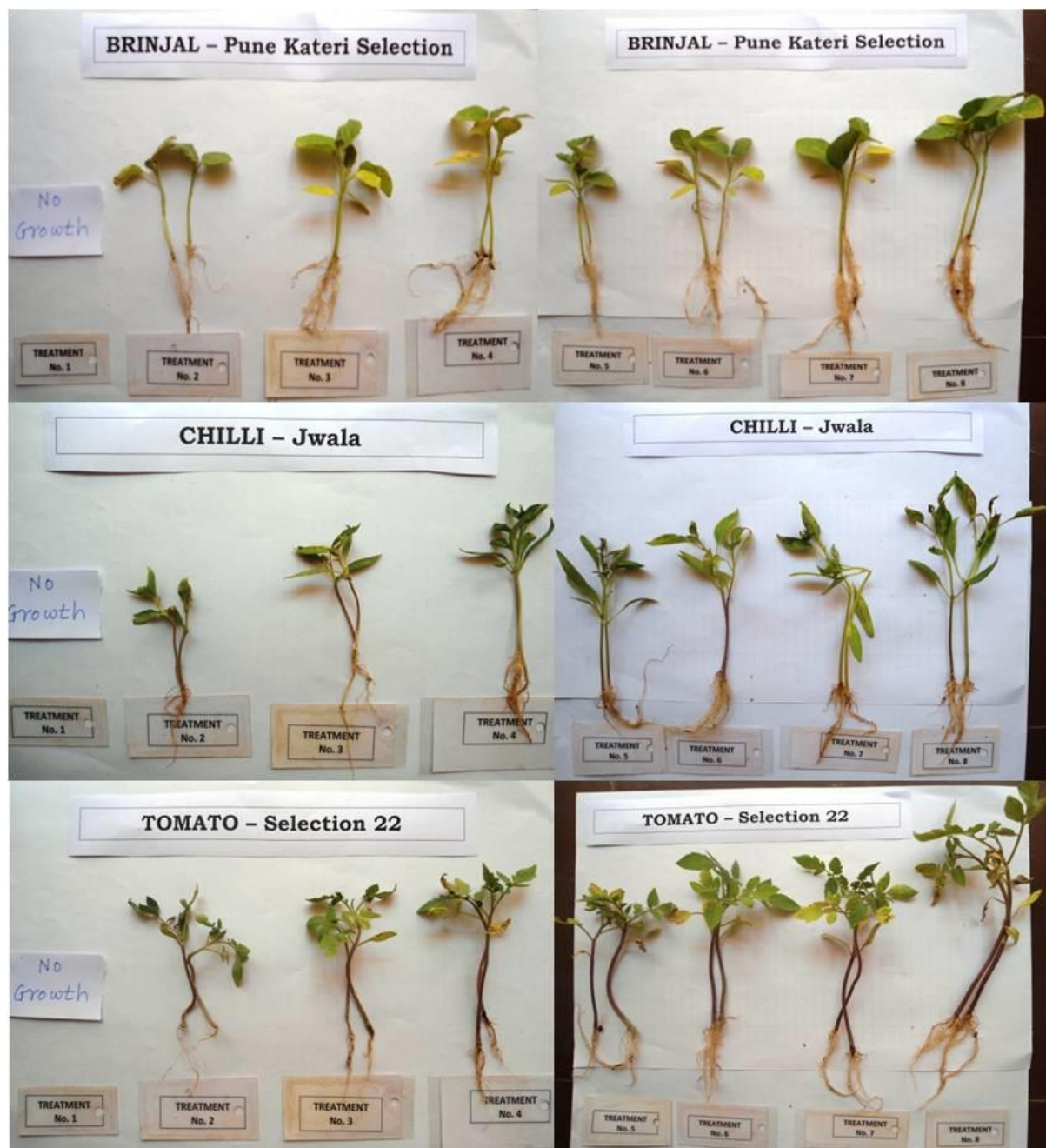


Fig. 6-Effect of various treatments on Brinjal, Chili and Tomato in saline soil

Nursery Experiment:

Effect of various treatments on pH of saline soil:

Decrease in soil pH resulted when saline soils were amended with organic manure and Vermicompost in combination with cyanobacterial consortium. The treatment of cyanobacterial consortium and all combinations was found to be more effective in Brinjal and Chili plants; whereas Vermicompost and all combinations were effective for Tomato. Combination of all amendments was found to be effective in the

experimental plants and also statistically analyzed for testing the significance of difference due to treatments.

For the effect of various treatments on pH of saline soil for Brinjal (OM, CC, OM+CC, VC+CC and OM+VC+CC), Chili (VC, CC, VC+CC and OM+VC+CC) and Tomato (VC, CC, VC+CC, OM+VC+CC) are statistically significant. Their P values are shown in Table 3. According to statistical analysis (OM, CC and VC+CC), (CC and OM+VC+CC), (VC, VC+CC and OM+VC+CC) and (OM, CC

and OM+VC+CC) are most significant for Brinjal, Chili, and Tomato, respectively. The pH values showed that all soils average were moderately alkaline, pH >7.7. Most plants prefer a soil pH between 5.5 and 7.2 and most bacteria multiply at the pH range 6.3 to 6.8; hence, the soil is too alkaline for both plants and the rhizosphere bacteria community. The soil treated with soil conditioner decreased the pH to 8.62 from initially 9.0. While soil conditioner and plant growth rhizobacteria treatment decreased pH 8.4 for growth of *Casuarina equisetifolia* [24]. Siam [25] reported the pH of faba beans grown soil slight decreases from 8.04 to 7.99 by the addition of bioinoculants combined with NPK dose, while pH 8.05 to 8.02 by the addition of only NPK fertilizer. Chikkaswamy [26] reported the cyanobacterial inoculants mixed with the farm yard manure along with the different combinations of NPK dose decreased pH of soil towards the neutrality. They recorded initial pH 8.2 of control plot and 7.3 to 8.0 pH of treated plot with different cyanobacterial inoculants.

Effect of various treatments on electrical conductivity (EC) of saline soil:

Electrical conductivity of soil was found to have decreased in all treatments for saline soils. Maximum reduction of 1.0 ds/m in electrical conductivity was seen with the treatment of combination of organic manure, Vermicompost and cyanobacterial consortium on saline soil for Brinjal and Chili. The Table 3 shows statistical values of experiment of effect of various treatments on soil electrical conductivity.

For the effect of various amendments on electrical conductivity of saline soil for Brinjal (OM, CC, OM+VC, and OM+VC+CC), Chili (OM, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC) and Tomato (CC) are statistically significant. According to statistical analysis (CC and OM+VC+CC), (OM, CC, OM+CC, VC+CC and OM+VC+CC), (CC) and (OM, CC OM+VC and OM+VC+CC) are most significant for Brinjal, Chili and Tomato, respectively. The electrical conductivity was recorded 6.75 (dS/m) and 7.67 (dS/m) due to the treatment of full dose of NPK along with the bioinoculants and without bioinoculants, respectively [25]. They also found the 0.92 (dS/m) reduction in the soil electrical conductivity due to the addition of bioinoculants like *Rhizobium* and *Bacillus*. El-Shahat [27] reported the foliar treatment of Azolla and

bacteria was reduced salinity (EC) from 16.90 to 13.30 dS/m for seasonal cultivation of barley.

Effect of various treatments on sodium adsorption ratio (SAR) of saline soil:

The Table 3 showed the statistical result of experimental data of effect of various treatments on sodium adsorption ratio in saline soil. Sodium adsorption ratio (SAR) can be taken as an indicator of soil's aggregation properties. A significant change in SAR was found when soils were amended with Vermicompost and Cyanobacterial consortium (Fig. 2). All combination was found effective for Brinjal, Chili and Tomato. Salinity alleviation due to cyanobacterial consortium may be attributed to Na⁺ removal by the production of extracellular polymers due to biosorption. Thus the osmotic as well as ionic effect of Na⁺ has an inhibitory effect on seed germination, seedling growth and chlorophyll concentration [28]. Table 3 showed the statistical data (P-value ANOVA) of effect of various treatments on sodium adsorption ratio (SAR) of saline soil for Brinjal (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC), Chili (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC) and Tomato (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC) are statistically significant. According to statistical analysis (OM, VC, CC, OM+VC and VC+CC), (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC), (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC) and (OM, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC) are most significant for Brinjal, Chili and Tomato, respectively.

Effect on organic nitrogen and organic carbon:

In the treatment of all combinations, organic carbon and organic nitrogen were increased. Cyanobacteria are a group of microorganisms that can fix the atmospheric nitrogen. Efficient nitrogen fixing cyanobacteria like *Nostoc*, *Anabaena*, *Aulosira*, *Calothrix*, *Tolypothrix*, *Scytonema* and *Oscillatoria* were identified from various agroecological regions and utilized for crop production [29]. Cyanobacteria play an important role in improvement in soil fertility and increasing crop yield. *Anabena* and *Nostoc* has potentially to fix large amount of atmospheric nitrogen (20-25 kg/ha). Adam [30] reported the application of *Nostoc muscorum* as a nitrogen fixation and plant growth promoting activities. Beside source of nitrogen fixation cyanobacteria provides other advantages such as a increasing soil microbial flora, organic matter, produces plant growth promoting substances,

tolerance of salinity and also help in reclamation of saline soil which result into the yield of crop under salinity condition [29].

Table 3 showed the statistical data (P-value ANOVA) of effect of various treatments on organic nitrogen of saline soil for Brinjal (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC), Chili (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC) and Tomato (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC) are statistically significant. According to statistical analysis (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC), (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC), (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC) and (CC and OM+VC+CC) are most significant for Brinjal, Chili and Tomato, respectively. Non heterocystous cyanobacteria can fix nitrogen aerobically [31]. Many cyanobacteria were fix nitrogen biologically and it is used as biofertilizers in agriculture, whereas cyanobacteria contribute 20-25 kg N/ha/season and also improve fertility of soil [32, 33]. Different cyanobacteria are widely used as biofertilizers in Indian rice field and it also contributes in soil fertility. In slightly alkaline pH condition is very important factor in growth of cyanobacteria in diverse rice soil ecologies of India [34]. Omar [35] was observed that the highest loss of soil properties in saline soil and increased with FYM applications. This increased parameter might be due to ensured supply of nitrogen content and favorable soil conditions.

The statistical data (P-value ANOVA) of effect of various treatments on organic carbon of saline soil showed in Table 3. The treatments for Brinjal (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC), Chili (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC) and Tomato (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC) are statistically significant. According to statistical analysis (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC), (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC), (OM, VC, CC, OM+VC, VC+CC and OM+VC+CC) and (CC) are most significant for Brinjal, Chili and Tomato, respectively. In future cyanobacteria play important role as biological agent in remediation and amelioration of soil and water environment [32]. Generally, effect of salinity on growth of crop plant related studies are available but their utilization as an agent of bioremediation is less documented [36].

Effect of various treatments on bacterial count of saline soil:

The mean data on the bacterial count is presented in Table 4. It is apparent from the Figure 2 that the saline soil was very poor in the microbial flora. Due to addition of organic manure, Vermicompost and Cyanobacterial inoculums, as soil microflora either singly or in combination, increased bacterial count by many folds. Cyanobacterial treatment was found to be the most effective in enhancing soil microflora when inoculated along with other amendments in the case Tomato. Table 4 showed the statistical data (P-value ANOVA) of effect of various treatments on bacterial count of saline soil for Brinjal (OM, CC and OM+VC), Chili (OM, CC, OM+VC, OM+CC and OM+VC+CC) and Tomato (OM, VC and OM+VC) are statistically significant. According to statistical analysis (OM+VC), (CC, OM+VC and OM+VC+CC), (OM, VC and OM+VC) and (OM+VC+CC) are most significant for Brinjal, Chili and Tomato, respectively. Patil [23] reported the initial flora of saline soil was 5.9×10^3 cfu/g of soil, while with grown *C. equisetifolia* it was increased to 4.3×10^5 cfu/g of soil and flora of saline soil was increased 5.3×10^7 to 9.8×10^9 cfu/g of soil due the treatments of different combined bioinoculants.

Effect of various treatments on the seed germination of Brinjal, Chili and Tomato in saline soil:

The data on growth of Brinjal, Chili and Tomato were monitored in terms of seed germination, root and shoot length and chlorophyll content. The effect of various soil treatments on seed germination mentioned in following figures. Table 4 showed the statistical data (P-value ANOVA) of effect of various treatments on seed germination of saline soil for Brinjal (OM+VC+CC), Chili (CC and OM+VC+CC) and Tomato (CC, VC+CC and OM+VC+CC) are statistically significant. According to statistical analysis (OM+VC+CC) is most significant for Brinjal, Chili and Tomato.

Effect on Shoot and Root length:

Among the treated groups the highest shoot were observed with the treatment all combinations for Brinjal, Chili and Tomato. Logest root were observed in Organic manure, Vermicompost + Cyanobacterial consortium, Organic manure + Vermicompost and Vermicompost + Cyanobacterial consortium for Brinjal, Chili and Tomato, respectively. The significant increase in shoot and root length of *Vigna mungo* L. had been

already reported by [37, 38, 39]. Table 4 showed the statistical data (P-value ANOVA) of effect of various treatments on shoot length of saline soil for Brinjal (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC), Chili (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC) and Tomato (OM, VC, CC, OM+CC, VC+CC and OM+VC+CC) are statistically significant. According to statistical analysis (OM, VC, CC, OM+VC, OM+CC and OM+VC+CC), (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC), (OM, VC, CC, OM+CC, VC+CC and OM+VC+CC) and (OM and CC) are most significant for Brinjal, Chili and Tomato, respectively.

Table 4 showed the statistical data (P-value ANOVA) of effect of various treatments on root length of saline soil for Brinjal (OM, VC, CC, OM+VC, OM+CC and VC+CC), Chili (OM, VC, OM+VC, OM+CC, VC+CC and OM+VC+CC) and Tomato (OM, VC, CC, OM+VC, OM+CC, VC+CC and OM+VC+CC), are statistically significant. According to statistical analysis (OM, VC, CC, OM+VC, OM+CC and VC+CC), (OM, OM+VC, OM+CC, VC+CC and OM+VC+CC), (OM) and (OM and OM+VC+CC) are most significant for Brinjal, Chili and Tomato respectively.

Table 3-Effect of various treatments on soil properties and their statistical analysis (ANOVA)

Soil Properties	pH			Electrical conductivity (dS/m)			Sodium adsorption ratio			Organic nitrogen (Kg/Hac)			Organic carbon (Kg/Hac)		
	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato
Treatment Code															
Control	8.2 (0.12)	8.2 (0.09)	8.1 (0.07)	5.68 (0.21)	5.8 (0.02)	6.8 (0.00)	29 (0.12)	28 (0.32)	29 (0.21)	124 (0.04)	137 (0.12)	141 (0.42)	0.17 (0.14)	0.12 (0.31)	0.19 (0.01)
OM	7.4 (0.00)	7.4 (0.21)	7.5 (0.18)	4.93 (0.00)	5.0 (0.00)	4.9 (0.05)	24 (0.00)	23 (0.00)	22 (0.00)	187 (0.00)	171 (0.00)	183 (0.00)	0.57 (0.00)	0.50 (0.00)	0.55 (0.00)
VC	7.7 (0.08)	7.6 (0.01)	7.4 (0.00)	5.32 (0.11)	5.6 (0.12)	5.5 (0.24)	28 (0.00)	26 (0.00)	29 (0.00)	167 (0.00)	158 (0.00)	151 (0.00)	0.41 (0.00)	0.47 (0.00)	0.47 (0.00)
CC	7.7 (0.00)	7.8 (0.00)	7.7 (0.00)	5.40 (0.00)	5.2 (0.00)	5.2 (0.04)	27 (0.00)	26 (0.00)	28 (0.00)	158 (0.00)	161 (0.00)	163 (0.00)	0.23 (0.00)	0.28 (0.00)	0.23 (0.00)
OM+VC	7.7 (0.47)	7.8 (0.58)	7.8 (0.33)	5.32 (0.01)	5.1 (0.00)	5.1 (0.53)	28 (0.00)	27 (0.00)	27 (0.00)	162 (0.00)	145 (0.00)	160 (0.00)	0.48 (0.00)	0.33 (0.00)	0.39 (0.00)
OM+CC	7.5 (0.02)	7.8 (0.36)	7.6 (0.84)	5.18 (0.85)	5.1 (0.00)	5.1 (0.08)	23 (0.14)	23 (0.62)	20 (0.00)	189 (0.04)	177 (0.01)	191 (0.27)	0.45 (0.02)	0.42 (0.00)	0.52 (0.00)
VC+CC	7.5 (0.00)	7.8 (0.00)	7.6 (0.00)	5.29 (0.09)	5.0 (0.00)	5.0 (0.19)	24 (0.00)	24 (0.00)	23 (0.00)	185 (0.00)	179 (0.00)	188 (0.00)	0.68 (0.00)	0.74 (0.00)	0.69 (0.00)
OM+VC+CC	7.4 (0.00)	7.4 (0.00)	7.5 (0.00)	4.72 (0.00)	5.0 (0.00)	4.8 (0.09)	22 (0.04)	23 (0.00)	20 (0.00)	203 (0.00)	192 (0.00)	214 (0.00)	1.01 (0.00)	0.98 (0.00)	1.02 (0.00)

The values given in table are of 'mean' of three replicates and values in parentheses are of *P*-value (ANOVA) obtained from statistical analysis

Table 4-Effect of various treatments on microbial flora and plant growth properties and their statistical analysis (ANOVA)

Plant growth Properties	Bacterial count (cfu/gm)			% Seed germination			Shoot length (cm)			Root length (cm)			Total chlorophyll (mg/g)		
Treatment Code	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato	Brinjal	Chili	Tomato
Control	7.50×10 ⁵ (0.14)	1.65×10 ⁶ (0.08)	9.93×10 ⁵ (0.22)	47 (0.08)	47 (0.05)	45 (0.13)	0 (0.01)	0 (0.05)	0 (0.08)	0 (0.09)	0 (0.14)	0 (0.04)	0.42	0.34	0.39
OM	1.94×10 ⁸ (0.00)	2.40×10 ⁸ (0.00)	1.69×10 ⁸ (0.00)	79 (0.05)	75 (0.05)	73 (0.05)	6.6 (0.00)	6.5 (0.00)	9.4 (0.00)	8.2 (0.00)	6.8 (0.00)	4.2 (0.00)	0.68	0.66	0.69
VC	4.53×10 ⁶ (0.07)	7.63×10 ⁶ (0.01)	1.64×10 ⁷ (0.00)	70 (0.8)	60 (0.8)	71 (0.8)	6.8 (0.00)	7.3 (0.00)	8.6 (0.00)	6.5 (0.00)	8.8 (0.00)	3.9 (0.00)	0.55	0.63	0.59
CC	1.83×10 ⁷ (0.00)	2.53×10 ⁶ (0.00)	2.43×10 ⁶ (0.15)	72 (0.8)	71 (0.00)	72 (0.00)	8.2 (0.00)	8.3 (0.00)	9.9 (0.00)	10.3 (0.00)	5.4 (0.00)	4.4 (0.00)	0.61	0.64	0.67
OM+VC	2.27×10 ⁶ (0.00)	1.16×10 ⁷ (0.00)	3.23E+06 (0.00)	79 (0.62)	73 (0.05)	77 (0.05)	7.8 (0.00)	10 (0.00)	11.1 (0.15)	5.5 (0.00)	9.7 (0.00)	6.4 (0.00)	0.67	0.66	0.69
OM+CC	1.18×10 ⁸ (0.42)	2.90×10 ⁷ (0.02)	2.36×10 ⁸ (0.23)	74 (0.05)	71 (0.14)	72 (0.14)	8.2 (0.00)	11.2 (0.00)	12.5 (0.00)	9.9 (0.14)	6.8 (0.62)	6.2 (0.00)	0.76	0.89	0.87
VC+CC	1.77×10 ⁸ (0.58)	4.30×10 ⁷ (0.05)	2.56×10 ⁹ (0.43)	62 (0.05)	61 (0.62)	65 (0.02)	8.7 (0.00)	12.4 (0.00)	14.1 (0.00)	7.6 (0.00)	5.1 (0.00)	6.3 (0.00)	0.86	0.81	0.84
OM+VC+CC	3.90×10 ⁸ (0.07)	5.13×10 ⁸ (0.00)	3.90×10 ⁹ (0.28)	80 (0.01)	80 (0.01)	80 (0.01)	11.3 (0.00)	13.7 (0.00)	16.8 (0.00)	6.2 (0.00)	5.7 (0.00)	5.9 (0.00)	1.1	1.17	1.09

The values given in table are of 'mean' of three replicates and values in parentheses are of *P*-value (ANOVA) obtained from statistical analysis

Effect on chlorophyll content:

The marked increase was found using soil amendment like organic manure, vermicompost and cyanobacterial consortium for Brinjal, Chili and Tomato. This result was due to the enrichment of soil fertility through application of these soil amendments that can enhance plant growth. The chlorophyll content was found *Vigna mungo* L. T6 due to adequate nitrogen supply by dual inoculants i.e. *Rhizobium* and Cyanobacteria [37]. The use of biofertilizers can be the replacement for the chemical fertilizers which offers economic and ecological benefits to the soil fertility and farmers. Biofertilizers added nutrients in the soil by natural processes of nitrogen fixation, solubilizing phosphorus and the synthesis of plant growth hormones during growth with reduction of soil salinity. The halotolerant cyanobacteria in biofertilizers restore the soil microbial flora, soil fertility and reduce the effect of salinity.

Microbial inoculants have attained special significance in modern agriculture. Responses of biofertilizers have been obtained in cereals, millets, pulses, legumes, oil seeds, sugarcane and cotton grown under difficult agro climatic conditions [40, 37]. The effect of algae on Indian paddy fields revealed that cyanobacteria accelerated seed germination and promoted seedling growth. It was also observed that both yield and quality of the grains was improved. The beneficial effect of BGA on rice crop is not only restricted to their atmospheric nitrogen fixing capacity but also lies in their capacity to produce plant growth hormones [37]. Cyanobacteria are friendly biofertilizers for rice-based cropping systems, being the major components of wetland rice ecosystems, which are easily available and serve as the cheapest sources of natural biofertilizers [41, 42].

CONCLUSION:

In this study, aim was mainly focused on the role of cyanobacteria in soil fertility and reclamation of salinity. The selected cyanobacterial cultures were cultivated successfully. Application of soil-based halotolerant cyanobacteria after growing Brinjal, Chili, Tomato and Wheat in saline soil at nursery resulted in increased physical properties of soil and crop growth. Improved growth of wheat has suggested that halotolerant cyanobacteria, along with organic manure and other additives, enriched soils with

physical properties as well as could be with carbon, nitrogen and other nutrients. Thus, the cyanobacteria can play major role in agriculture, with regard to the relationship between salinity and crop plants and also important components of arid and semi-arid ecosystems.

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REFERENCES:

- [1] Green C.J., Clerk D.R. and Gordon J.A. Laboratory exercises to demonstrate effect of salt on plant and soils. J Nat Resour Life Sci Educ, 29:41-45, (2000).
- [2] Schleiff U. Analysis of water supply of plants under saline soil condition and conclusions of research on crop salt tolerance. J Agronomy and Crop Science, 194:1-8, (2008).
- [3] Hasegawa P.M. and Bressan R.A. Zhu J.K. and Bohnert H.J. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol, 51:463-499, (2000).
- [4] Qadir M. and Oster J.D. Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. Science for the Total Environment, 323:1-19, (2004).
- [5] Sharma D.K., Gupta S.K., Singh R. and Dey P. Annual Report, 2010-2011, Central Soil Salinity Research Institute, Karnal- 132, 001, India, (2011).
- [6] Hayat S., Yadav S., Irfan M. and Ahmad A. Causes of salinity and plant manifestations to salt stress: A review. J Environ Bio, 32:667-685, (2011).
- [7] Kumar M., Joshi E. and Jat A.L. Salinization: Causes and prevention. Popular Kheti, 1:76-79, (2013).
- [8] Hedge D.M., Dwivedi B.S. and Babu S.N. Biofertilizer for cereal production in India: A review. Indian J Agricultural Sci, 69:73-83, (1999).
- [9] Malakar E. and Kalita M.C. A perspective towards development and commercialization of potential BGA biofertilizers of Assam, North East India and carrier materials for BGA mass production and inoculum development. Annals of Biological Research, 3(1):814-828, (2012).

- [10] Singh D.P., Prabha R., Yandigeri M.S. and Arora D.K. Cyanobacteria mediated phenylpropanoids and phytohormones in rice (*Oryza sativa*) enhance plant growth and stress tolerance. *Antonie Van Leeuwenhoek*, 100:557-568, (2011).
- [11] Tsavkelova E.A., Klimova S.Y., Cherdyntseva T.A. and Netrusov A.I. Microbial producers of plant growth stimulators and their practical use: a review. *Appl Biochem Micro*, 42:117-126, (2006).
- [12] Subhashini D. and Kaushik B.D. Amelioration of salt affected soils with blue green algae. I. Influence of algalization on the properties of saline-alkali soils. *Phykos*, 23:273-277, (1984).
- [13] Somani L.L. Handbook of Biofertilizers: Blue Green Algae. Agrotech Publishing Academy, Udaipur, 289-486, (2005).
- [14] Chakdar H., Jadhav S.D., Dhar D.W. and Pubbi S. Potential applications of blue green algae. *Journal of Scientific and Industrial Research*, 71:13-20, (2012).
- [15] Thajuddin N. and Subramanian G. Cyanobacterial biodiversity and potential applications in biotechnology. *Curr Sci*, 89:47-57, (2005).
- [16] Mazahar S. and Hasnain S. Screening of native plant growth promoting cyanobacteria and their impact on *Triticum aestivum* var. Uqab 2000 growth. *African Journal of Agricultural Research*, 6:3988-3993, (2011).
- [17] Bhosale A., Puranik P. and Pawar S. Screening and optimization of indole 3 acetic acid producing non-heterocystous cyanobacteria isolated from saline soil. *Scholars Academic Journal of Biosciences*, 4(9):738-744, (2016).
- [18] Pawar S.T., Bhosale A.A., Gawade T.B. and Nale T.R. Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. *J Microbiol Biotech Res*, 3:24-31, (2013).
- [19] Olsen S.R., Cole C.V., Wantanable F.S. and Dean L.A. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. United State Dept. of Agric. CIRC., Washinton, D.C., 939, (1954).
- [20] Jackson M.L. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi, (1967).
- [21] Jackson M.L. Soil chemical analysis. Verlag: Prentice Hall, Inc., Englewood Cliffs, NJ. 498 S. DM 39.40, (1958).
- [22] Mahi E.Y., Ibrahim I.S., Abdel-Majid H.M. and Eltilib A.M. A simple method for determination of calcium and magnesium carbonate in soils. *Soil Science Society of America Journal*, 51:1152-1155, (1987).
- [23] Sadasivam S. and Manickam A. Biochemical methods. 2nd Edn, New Age International (P) Ltd., New Delhi, 193-197, (1996).
- [24] Patil D.P., Kulkarni M.V., Maheshwari V.L. and Kothari R.M. A sustainable agro-biotechnology for bioremediation of saline soil. *J Sci Ind Res*, 61:517-528, (2002).
- [25] Siam H.S., Ashmaye S.H., El-Kader M.G.A. and Mahmoud A.A. Partial replacement of integrated mineral fertilizer through biofertilization to maximize economic yield of faba bean under saline soil conditions. *International Journal of Chem Tech Research*, 9(6):153-164, (2016).
- [26] Chikkaswamy B.K. Effect of cyanobacterial biofertilizer on soil nutrients and mulberry leaf quality and its impact on silkworm crops. *International Journal of Advanced Research in Engineering and Applied Sciences*, 4(1):1-15, (2015).
- [27] El-Shahat R.M., Sherif A.E.A. and Mohamed F.M. Response of barley grown in saline soil to bio-fertilizer as a partial substitutive of mineral fertilizer. *Global Journal of Scientific Researches*, 2(5):144-153, (2014).
- [28] Arora M., Kaushik A., Rani N. and Kaushik C.P. Effect of cyanobacterial exopolysaccharides on salt stress alleviation and seed germination. *Journal of Environmental Biology*, 31(5):701-704, (2010).
- [29] Sahu D., Priyadarshani I. and Rath B. Cyanobacteria - as potential biofertilizer. *CIBTech International Journal of Microbiology*, 1(2-3):20-26, (2012).
- [30] Adam M.S. The promotive effect of the cyanobacterium *Nostoc muscorum* on the growth of some crop plants. *Microbiologica polinica*, 48:163-171, (1999).
- [31] Bergman B., Gallon J.R., Rai A.N. and Stal L.J. N₂ fixation by non-heterocystous cyanobacteria. *FEMS Microbiol Rev*, 19:139-185, (1997).
- [32] Prasanna R., Jaiswal P. and Kaushik B.D. Cyanobacteria as potential options for environmental sustainability – promises and challenges. *Indian J Microbiol*, 48:89-94, (2008).
- [33] Islam M.Z., Begum S., Ara H. and Wallullah T.M. Effect of furdan on the growth and nitrogen fixation by blue green algae. *J Bio-Sci*, 15:23-35, (2007).
- [34] Nayak S. and Prasanna R. Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. *Applied Ecology and Environmental Research*, 5(2):103-113, (2007).
- [35] Omar H.K. Studies on the combined effect of organic and inorganic nitrogen sources on nutrient release, yield and quality of rice. *J Biochem Biophy Res Comm*, 65:846-856, (1995).
- [36] Prasanna R., Joshi M., Rana A. and Nain L. Modulation of IAA production in cyanobacteria by tryptophan and light. *Polish Journal of Microbiology*, 59(2):99-105, (2010).
- [37] Karthikeyan A., Nagasathya A.S. and Priya E. Hypersaline cyanobacterium: A potential biofertilizer for *Vigna mungo*. L (Black Gram). *Am.-Eur. J. Sustain. Agric*, 2(1):87-91, (2008).
- [38] Kibria M.G. Efficacy of biofertilizers and rhizobial strains in controlling foot and root rot of lentil, mungbean and chickpea. *Bangladesh Journal of Environmental Science*, 2:29-31, (2000).

- [39] Hossain I., Khan M.A. and Podder A.K. Seed treatment with *Rhizobium* in controlling *Fusarium oxysporum* and *Sclerotium rolfsii* for biomass and seed production of Lentil (*Lens culinaris* M.). Bangladesh Journal of Environmental Science, 5:61-64, (1999).
- [40] Gupta A.B. Algal flora and its importance in the economy of rice fields. Hydrobiologia, 28:222-231, (1966).
- [41] Ladha J.K. and Reddy P.M. Nitrogen fixation in rice systems: state of knowledge and future prospects. Plant Soil, 25(2):151-167, (2003).
- [42] Abdel-Raouf N., Al-Homaodan A.A. and Ibhaheem I.B.M. Agricultural importance of algae. Afr J Biotechnol, 11:11648-11658, (2012).