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Evaluation of different zinc solubilizing bacteria/bacterial isolates for their effects on growth and yield of Soybean (*Glycine max* L. Merrill) under greenhouse condition

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Abstract

Soybean (*Glycine max* L. Merrill) is one of the best source of proteins, carbohydrates, fat, vitamins, and other nutrients with a very high nutritional value. Zinc solubilizing microbial inoculants have a significant effect on the growth of soybean. Inoculation of zinc solubilizing microbial strain *B. subtilis* significantly increased plant height and number of leaves which was followed by *B. megaterium* which was found to be at par with *T. ferroxidans* as compared to the control pot. Significantly highest number of branches were produced with the microbial strain *B.subtilis* inoculated pot which was found to be at par with *P.fluorescens*, *T.thioxidans*, whereas the highest number of the pod was observed with the microbial strain *B.subtilis* which was followed by *B. megaterium* and found to be at par with *T. thioxidans*. Improvement in the root characteristics as compared to the un-inoculated pot and the highest increase in oven-dry weight of root and shoot was obtained with the inoculation of *B.subtilis* which was followed by *B.megaterium*. The root shoot ratio was increased significantly with different zinc solubilizing microbial strains such as *B.subtilis* and found to be at par with *T. thioxidans*. Among root density, the highest density was found in *B. subtilis*. Highest root volume was observed in *B. subtilis* which was followed by *B. megaterium* and lowest root volume observed in uninoculated control. Zinc solubilizing microbial cultures have a significant effect on grain yield and dry matter content. Inoculation of zinc solubilizing microbial strain *B. subtilis* significantly increased grain yield and dry matter which was followed by *B. megaterium*.

Keywords: *Glycine max*, zinc solubilizing bacteria, greenhouse

Introduction

Soybean (*Glycine max* (L.) Merrill) a grain legume is considered as a wonder crop due to its dual qualities viz., high protein (40-43%) and oil content (20%). It is an important oil seed crop belongs to family Leguminaceae, sub family papilionaceae and tribe phaseoleae. It helps in fixing atmospheric nitrogen in soil and improves the soil fertility and productivity. Therefore it is called as "Gold of Soil". Soybean is sensitive to Zn deficiency, which is needed for chlorophyll formation, nodulation, growth hormone stimulation, lipid and protein metabolism, carbohydrate synthesis, enzymatic activity and reproductive process (Thena *et al.*, 2014). It plays a vital role in photosynthesis, synthesizing of auxins, nitrogen fixation and production of biomass (Kobrae *et al.*, 2011) [13]. Microbes are potential alternate that could cater plant zinc requirement by solubilising the complex zinc in soil. Zinc solubilizing microbial inoculants have a significant effect on the growth of soybean. A bacterial based approach was contrived to solve the micronutrient deficiency problem. In soil it undergoes a complex dynamic equilibrium of solubilisation and precipitation that is greatly influenced by the soil pH and micro flora and that finally affects their accessibility to plant roots for absorption (Altomare *et al.*, 1999) [2].

Material and methodology

During the investigations on "Studies on zinc solubilization bacteria and their effect on growth and yield on Soybean", a series of experiments were carried out in the laboratory and greenhouse of Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

Culture Media

PVK (Pikovaskaya) medium and some other common laboratory medium were used for isolation of micro-organism from the soil samples.

Methods

Isolation and characterization of zinc solubilizing bacteria

Collection of soil samples from soybean rhizosphere

The present studies were undertaken with an objective to select a promising zinc solubilizing bacterial strains. For that soil samples were collected from university field. All the collected samples were packed in polythene bags and transported to the laboratory for preservation in refrigerator for further studies.

Isolation and of zinc solubilizers

Serial dilution method was used for isolating the zinc solubilising bacteria from the rhizospheric soil. 1.0 g soil from soybean rhizosphere sample was suspended in 10 ml of distilled water in test tubes. From the 1st dilution 1.0 ml was transferred to test tube containing 9 ml of distilled water to get 10⁻² dilution. The same method was followed for preparing up to 10⁻⁹ dilution. The PVK medium which was prepared containing glucose-10 gm, Ammonium sulphate-1gm, potassium chloride-0.2 gm, Di-potassium hydrogen phosphate-0.2 gm, Magnesium sulphate-0.1 gm, yeast extract-0.2 gm, Maganeses Sulphate-0.1 gm and Iron Sulphate-0.0001 g in distilled water. The source of insoluble zinc compounds such as zinc oxide, zinc carbonate and zinc phosphate was supplemented at 1% and 15 g agar was added into the medium and autoclaved at 121°C for 30 min. The medium was poured in sterile petri-plates under aseptic condition. After solidification, 0.1 ml from 10⁻⁷, 10⁻⁸ and 10⁻⁹ dilutions of soybean rhizosphere soil samples were taken by sterile pipette, transferred and spread on to petri-plates. The inoculated plates were incubated at 27-30°C for 48 hrs.

Purification and Characterization of the bacterial isolates.

After incubation, the grown individual colonies showing halozone formation around the colonies on PVK medium supplemented with ZnO, ZnCO₃ and zinc phosphate were picked up, transferred into nutrient agar slants for further studies. The gram staining, cell morphology of the isolates was conducted for identification. The selected bacterial isolates were examined for their morphological features.

Solubilization of insoluble zinc compounds by different microbial isolates in laboratory

Zinc solubilizing ability of different microorganisms was evaluated using zinc oxide, zinc carbonate and zinc phosphate in plate media assays. Seven microbial strains (Seven bacteria) and 0.1% of each chemical source in three replications were used. Colony and halo diameters were measured after incubating the plates for 72 h in incubator. Further, zinc solubilizing ability of microbial strains in three replications was studied with ZnO, ZnCO₃ and Zn₃(PO₄)₂ solutions on plate assay.

Screening of ZSB isolates on plate assay for Zn solubilization efficiency.

All the bacterial isolates were screened by plate assay for their efficiency to solubilize zinc on PVK medium supplemented separately with zinc oxide, zinc carbonate and zinc phosphate

at a 0.1 % Zn. After spot inoculating plates were incubated at 28°C and observed for formation of clear halozone around bacterial growth after seven days. Zinc solubilisation efficiency was calculated as described by Ramesh *et al.* (2014) [23].

$$\text{Solubilization Efficiency (SE)} = \frac{\text{Diameter of solubilisation halo zone}}{\text{Diameter of colony}} \times 100$$

The solubilization index (SI) was calculated using the following formula. (Sadiq *et al.*, 2013) [25].

$$\text{Solubilization Index (SI)} = \frac{\text{Colony Diameter} + \text{Halo zone Diameter}}{\text{Colony Diameter}}$$

Insoluble zinc sources

All the microbial isolates were screened in plate for their efficiency to solubilize zinc on medium supplemented separately with zinc oxide (ZnO), Zinc carbonate (ZnCO₃) & Zinc phosphate [Zn (PO₄)₂] at a concentration equivalent to 0.1 percent Zn (Fasim, *et al.*, 2002).

Media used to test zinc solubilisation capacity

The isolates were inoculated into modified PKV medium The ingredients g L⁻¹ are given below.

1. Glucose-10.0 g
2. Ammonium Sulphate-1.0 g
3. Potassium Chloride-0.2 g
4. Dipotassium hydrogen phosphate-0.2 g
5. Magnesium Sulphate-0.1 g
6. Yeast extract-0.2g
7. Manganese Sulphate-0.1g
8. Iron Sulphate-0.0001g
9. Agar- 15g
10. Distilled water-1000 ml

Containing 0.1% insoluble zinc compounds (ZnO, ZnCO₃ and Zn₃(PO₄)₂) (Bapiri *et al.*, 2012) [4].

Solubilization of insoluble zinc compound by different microbial isolates in pot culture

The experiment with three replications in completely randomized design (CRD) in pot was carried out to study the bio-efficacy of promising zinc solubilizing microbial isolates of *Bacillus* spp, *Pseudomonas* spp, *Thiobacillus* spp. using soybean as test crop. The sterilized soil and Hogland solution were used as a nutrient source in pots. Ten days after seedling were inoculated with 24hrs. old microbial isolates@5ml per pot as per treatment. The observations were recorded on growth parameters like plant height and number of leaves at 30, 60 and 90 days after sowing. Chlorophyll content was determined as per procedure given by DMSO method. (Hiscox and Isaeristem, 1979) [9].

Material used

Seed: The seed of soybean MUS-171 was procured from Soybean Research Station, VNMKV, Parbhani.

Soil: The soil used for pot culture experiment was zinc deficient (0.55 mg/kg) identical to that of Typic Haplusterts (Vertisol) of Parbhani series. The soil was air dried, sieved and sterilized at 121°C for 1 hr. for 3 consecutive days. The soil was filled in the pots having holding capacity of 8 kg.

The initial chemical properties of the experiment such as soil pH, EC, available Zn were given in Table.1

Inoculation of microbial inoculants

Ten days after sowing, 24 hrs. culture of microbial inoculants (*Pseudomonas striata*, *P.fleuroscens*, *Bacillus subtilis*, *B.megaterium*, *B.cereus*, *Thiobacillus thioxidans*, *T.ferrooxidans*) was inoculated @10 ml per pot as per the treatment.

Nutrient Solution

Hoagland solution without zinc source was used as the nutrient solution.

Hoagland's Solution (Plant Nutrient Solution) Components

Sr. No.	Components	Stock Solution	mL Stock Solution/1L
1	2M KNO ₃	202g/L	2.5
2	2M Ca (NO ₃) ₂ × 4H ₂ O	236g/0.5L	2.5
3	Iron (Sprint 138 iron chelate)	15 g/L	1.5
4	2M MgSO ₄ × 7H ₂ O	493g/L	1
5	1M NH ₄ NO ₃	80g/L	1
Minors			
6	H ₃ BO ₃	2.86g/L	1
7	MnCl ₂ × 4H ₂ O	1.81g/L	
8	CuSO	0.051g/L	
9	H ₃ MoO ₄ × H ₂ O or	0.09g/L	
10	Na ₂ MoO ₄ × 2H ₂ O	0.12g/L	
11	1M KH ₂ PO ₄	136g/L	
(PH to 6.0 0.5 with 3MKOH)			

Observations recorded

Biometric observations

Following biometric observations were recorded during conductance of pot experiment at various growth stages of soybean. One plant from each pot was randomly selected and tagged for the biometric observations. Observations were recorded at critical growth stages of soybean.

Height of plant (cm)

Height of plant was measured in cm from the base of the plant. i.e. ground level to the last fully opened leaf at the apex.

Number of leaves

Number of leaves per plant was measured of tagged plants.

Number of branches per plant

The total number of branches from one observational plant from each net pot counted.

Number of pod per plant

Pod was counted from one observational plant from each net pot.

Root parameters

1. Root volume

For this purpose, three plants were uprooted by digging carefully from inner border rows of plots without damaging roots. Root portion was cut off and volume was measured, with the help of measuring cylinder containing water displacement method

2. Root and shoot weight

Plants uprooted were dried in shade followed by oven drying till constant weight and oven dry weight of root and shoot were recorded on monopan electrical balance.

3. Root: Shoot ratio

Oven dry weight of shoot was divided by its respective oven dry weight of root.

4. Root density (g/cc)

Root density = Oven dry weight of root (g)/Root volume × 100

5. Yield of seed soybean per pot

The matured pod was manually picked from each net pot and the weights were recorded on electronic digital balance and converted to g/pot by multiplying with hectare factor.

Dry matter yield

The weight of dry matter accumulated in plant is an index of the plant growth. The roots of the plant uprooted for dry matter will be removed, after removing the roots the plants were air dried under sun for eight days till the final constant dry weight.

Biochemical studies in soybean

1. Chlorophyll content in leaves: The plant pigment like chlorophyll 'a', chlorophyll 'b', and total chlorophyll content in fresh soybean leaves were determined by DMSO method at maturity level as per procedure described by Hiscox and Isaeristem (1979)^[9].

The chlorophyll content was calculated using following formula

$$\text{Chlorophyll a (mg/ g tissue)} = 12.7 (A_{633}) - 2.69 (A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g tissue)} = 22.9 (A_{645}) - 4.68 (A_{633}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg /g tissue)} = \frac{(A_{652}) \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where,

A=Absorbance at specific wavelength

V=Final volume of chlorophyll extract in DMSO (ml)

W=Fresh weight of tissue extracted (g)

Result and discussion

Effect of different zinc solubilizing microbial inoculants on growth attributes of soybean. Zinc solubilizing microbial inoculation affected the early plant growth of soybean. Many of zinc solubilizing microbial strains such as *B.subtilis*, *B.megaterium* and *P.striata* had a significant effect on growth of soybean in zinc deficient poor calcareous soil, while non-treated plants by comparison, performed poorly under such conditions. The data pertaining to crop growth parameters was presented in following tables.

Plant height (cm) of soybean

Periodical plant height was measured at 30 days interval of crop growth and data presented in Table 1. After 30, 60 and at harvest of crop, plant height ranged from 17.66-33.00cm, 29.00-43.66cm and 32.66-49.66cm. Results showed that,

change in plant height was significantly highest at 30, 60 and at harvest of crop when inoculated with *B. subtilis* (33.0, 43.66 and 49.66 cm) followed by *B. megaterium* (32.66, 42.33 and 47.66cm), *P. striata* (31.00, 40.66 and 46.66cm), *P. fluorescens* (27.66, 40.33 and 41.00cm), *Bacillus cereus* (27.33, 39.66 and 42.33 cm) respectively and these treatments were found at par with each other whereas, the lowest plant height was recorded in un-inoculated control treatment (17.66, 29.00 and 32.66cm).

Table 1: Effect of different zinc solubilizing microbial inoculants on plant height (cm) of soybean

Treatments	30DAS	60DAS	At Harvest
T1 : <i>Bacillus subtilis</i>	33.00	43.66	49.66
T2 : <i>Bacillus megaterium</i>	32.66	42.33	47.66
T3 : <i>Bacillus cereus</i>	27.33	39.66	42.33
T4 : <i>Pseudomonas striata</i>	31.00	40.66	46.66
T5 : <i>Pseudomonas fluorescens</i>	27.66	40.33	41.00
T6 : <i>Thiobacillus thiooxidans</i>	23.33	35.66	38.33
T7 : <i>Thiobacillus ferrooxidans</i>	21.33	33.66	37.00
T8 : Control	17.66	29.00	32.66
SE m±	1.09	1.42	1.26
CD (P=0.01)	3.32	4.29	3.80

Number of leaves of soybean

The effect of zinc solubilizing microbial treatments on number of leaves showed significant improvement as compared to un-inoculated control (Table 2). Number of leaves ranged from 7.33-15, 9.00-18 and 7.66-14.66 at 30, 60 DAS and at harvest of crop respectively. Significantly highest number of leaves was produced with the *B. subtilis* (15.00, 18.00 and 14.66) followed by *B. megaterium* (13.66, 16.66 and 13.33), *P. striata* (12.00, 15.66 and 11.00), *B. cereus* (11.00, 14.00 and 10.66) respectively and the treatments *T. ferrooxidans* was found to be at par with rest of the treatments. The un-inoculated pot shows lowest number of leaves (7.33, 9.00 and 7.66) at different growth stages of crop.

Table 2: Effect of different zinc solubilizing microbial inoculants on number of leaves of soybean

Treatments	30DAS	60DAS	At Harvest
T1 : <i>Bacillus subtilis</i>	15.00	18.00	14.66
T2 : <i>Bacillus megaterium</i>	13.66	16.66	13.33
T3 : <i>Bacillus cereus</i>	11.00	14.00	10.66
T4 : <i>Pseudomonas striata</i>	12.00	15.66	11.00
T5 : <i>Pseudomonas fluorescens</i>	10.33	12.00	10.00
T6 : <i>Thiobacillus thiooxidans</i>	9.00	10.33	9.66
T7 : <i>Thiobacillus ferrooxidans</i>	8.66	9.33	8.33
T8 : Control	7.33	9.00	7.66
SE m±	0.55	0.72	0.35
CD (P=0.01)	1.67	2.16	1.06

The similar result were reported by many workers viz, Abbasi *et al.* (2011) [1], Pawar and Ismail (2016) [18], Islam *et al.* (2006) and Ilicic *et al.* (2017) who observed that, the improvement in soybean growth and yield trail achieved by addition of PGP strains *Bacillus* sp. and *P. chlororaphis*, *B. japonicum*. Similarly Kumar *et al.*, (2014) also reported the height of plant was significantly increased when combinely inoculated with *B. megaterium*, *A. chlorophenolicus* and

Enterobacter sp. then individually as well as in un-inoculated control under pot experiments.

Effect of different zinc solubilizing microbial inoculants on chlorophyll content in leaves of soybean.

Chlorophyll-a, chlorophyll-b and total chlorophyll content was increased in all inoculated plants than control. The data presented in Table 3. According to the results, chlorophyll-a varied from 1.23-2.26 mg/g. The highest content of chlorophyll-a was observed with inoculation of *B. subtilis*. It was significantly superior over control (1.23 mg/g) and at par with other zinc solubilizing microbial treatments while, chlorophyll-b content ranged from 1.00-1.80 mg/g. The inoculation of *B. subtilis* was found to enhance chlorophyll-b which was significantly highest (1.80mg/g) among all the treatments. While, the lowest chlorophyll-b content was observed in un-inoculated control treatments (1.00mg/g). Further, the total chlorophyll content might be increased due to the increase in rate of photosynthesis which can be ascribed to more absorption of nutrients.

Table 3: Effect of different zinc solubilizing microbial inoculants on chlorophyll content (mg/g (fw) in leaves of soybean

Treatments	Chlorophyll (a)	Chlorophyll (b)	Total chlorophyll
T1 : <i>Bacillus subtilis</i>	2.26	1.80	4.06
T2 : <i>Bacillus megaterium</i>	2.20	1.60	3.80
T3 : <i>Bacillus cereus</i>	2.10	1.40	3.50
T4 : <i>Pseudomonas striata</i>	1.90	1.33	2.96
T5 : <i>Pseudomonas fluorescens</i>	1.70	1.26	2.96
T6 : <i>Thiobacillus thiooxidans</i>	1.50	1.16	2.66
T7 : <i>Thiobacillus ferrooxidans</i>	1.30	1.13	2.43
T8 : Control	1.23	1.00	2.23
SE m±	0.06	0.04	0.15
CD (P=0.01)	0.18	0.12	0.45

fw= fresh weight

Our results corroborate with the Vidyashree *et al.* (2018) who showed that tomato seedlings treated with *Bacillus* sp. recorded highest quality parameters viz., chlorophyll content, phenol content, ascorbic acid content and lycopene content compared to all other ZSB inoculated tomato plants. Similar result were also reported by Peter *et al.* (2015) that the total chlorophyll contents level of inoculated plants with microbial treatment showed highest significance than the un-inoculated plants.

Number of branches in soybean

The effect of zinc solubilizing microbial treatments on number of branches showed significant improvement as compared to un-inoculated control (Table 4). Number of branches ranged 2.00-5.33 and 2.66-7.00 at 60 DAS and at harvest respectively. Significantly highest number of branches was observed with the *B. subtilis* (5.33, 7.00), followed by *B. megaterium* (4.66, 5.66), *P. striata* (4.33, 5.33), inoculated pot where as rest of the treatments were par with each other. The un-inoculated pot shows lowest number of branches (2.00-2.66) at different growth stages of crop.

Table 4: Effect of different zinc solubilizing microbial inoculants on number of branches of soybean

Treatments	60DAS	At Harvest
T1 : <i>Bacillus subtilis</i>	5.33	7.00
T2 : <i>Bacillus megaterium</i>	4.66	5.66
T3 : <i>Bacillus cereus</i>	3.66	5.00
T4 : <i>Pseudomonas striata</i>	4.33	5.33
T5 : <i>Pseudomonas fluorescens</i>	3.33	4.33
T6 : <i>Thiobacillus thiooxidans</i>	3.00	4.00
T7 : <i>Thiobacillus ferrooxidans</i>	2.33	3.33
T8 : Control	2.00	2.66
SE m±	0.35	0.33
CD (P=0.01)	1.06	1.00

The findings are corroborate with the Petkar *et al.* (2018) [20], Raman (2012) [22], and Islam *et al.* (2006) [11]. Where Naveen *et al.* (2012) [16] studied the effect of dual inoculation of *B.subtilis* and *Bradyrhizobium japonicum* along with graded levels of chemical fertilizers (75% nitrogen and phosphours) on plant growth parameters of soybean (*Glycine max* L.) viz, germination, height of plant at 50% flowering, number of main branches at 50% flowering, dry plant weight at 50% flowering, number of root nodules per plant at 50% flowering, dry weight of root nodules and dry matter yield at harvesting.

Number of pod in soybean: The effect of zinc solubilizing

microbial treatments on number of pods showed significant improvement on un-inoculated control (Table 5). Number of pods ranged from 2.00-4.67 and 4.66-8.67 at 60 and after harvest of crop, respectively. Significantly highest number of pod was produced with the *B. subtilis* (4.67 and 8.67), followed by *B. megaterium* (4.66- 7.67), *P. striata* (4.00-7.33) inoculated pot which was found to be at par with the *Thiobacillus thiooxidans* (3.00 and 6.33) and superior over other microbial strains. The uninoculated pot shows lowest number of pod (2.00 and 4.66) at different growth stages of crop.

Table 5: Effect of different zinc solubilizing microbial inoculants on number of pod in soybean

Treatments	60DAS	At Harvest
T1 : <i>Bacillus subtilis</i>	4.67	8.67
T2 : <i>Bacillus megaterium</i>	4.66	7.67
T3 : <i>Bacillus cereus</i>	3.66	7.33
T4 : <i>Pseudomonas striata</i>	4.00	7.33
T5 : <i>Pseudomonas fluorescens</i>	3.33	6.66
T6 : <i>Thiobacillus thiooxidans</i>	3.00	6.33
T7 : <i>Thiobacillus ferrooxidans</i>	2.67	5.66
T8 : Control	2.00	4.66
SE m±	0.33	0.33
CD (P=0.01)	1.00	1.00

Our results are in agreement with Saxsena (2010) and Navsare *et al.* (2017) [17] who reported that at harvest number of pod per plant in mungbean were highest (13.20) when inoculated with RDF + *Rhizobium* + PSB + KSB + ZSB followed by the plot which was inoculated with RDF + *Rhizobium* + PSB + KSB + ZSB (11.20). while, minimum number of pod per plant (8.8) found in the control plot.

Effect of different zinc solubilizing microbial inoculants on root characteristics of soybean: The perusal of data presented in Table 6 revealed that the inoculation of zinc solubilizing microbial strains improved the root parameters of soybean when compared with the un-inoculated root parameters.

Table 6: Effect of different zinc solubilizing microbial inoculants on root parameters of soybean

Treatments	Root Volume	Root Density (g/cc)	Root Shoot Ratio	ODW of root (g)	ODW of shoot (g)
T1 : <i>Bacillus subtilis</i>	10.00	40.28	4.63	1.51	7.00
T2 : <i>Bacillus megaterium</i>	9.33	31.36	4.00	1.50	6.00
T3 : <i>Bacillus cereus</i>	8.00	26.03	3.59	1.48	5.33
T4 : <i>Pseudomonas striata</i>	7.00	20.98	2.98	1.45	4.33
T5 : <i>Pseudomonas fluorescens</i>	6.66	21.30	2.34	1.41	3.33
T6 : <i>Thiobacillus thiooxidans</i>	5.33	18.68	2.64	1.38	3.66
T7 : <i>Thiobacillus ferrooxidans</i>	4.33	16.10	1.72	1.34	2.33
T8 : Control	3.33	15.20	1.25	1.32	1.67
SE m±	0.19	1.11	0.13	0.004	0.40
CD (P=0.01)	0.59	3.42	0.42	0.013	1.23

ODW = Oven Dry Weight

In root volume significant variation was observed which ranges in between 3.33-10.00. The highest root volume was observed in *B.subtilis* (10.00), followed by *B.megaterium*

(9.33) and the lowest root volume observed in un-inoculated control (3.33). Root density ranged between 15.29- 40.28 g/cc. Highest root density was found in *B.subtilis* (40.28g/cc) and

lowest was noticed in *T.ferrooxidans* (16.10g/cc). However, root shoot ratio which was in between 1.25-4.63 shows significant variation due to inoculation of different zinc solubilizing microbial strains.

The oven dry weight of shoot and root varied from 1.67 to 7.00 g and 1.32 to 1.51g zinc solubilizing microbial strain *B.subtilis* produced significantly highest oven dry weight of shoot and root (7.00 to 1.51g) followed by *B.megaterium* (6.00 to 1.50 g). whereas, lowest dry weight of shoot and root was noticed in un-inoculated control (1.67 to 1.32g). Significantly highest ratio was observed in *B.subtilis* (4.63) treated plant which was at par with *T.thiooxidans* (2.64), *P.striata* (2.98), *P.fluorescens* (2.34). Whereas, lowest ratio was observed in un-inoculated control (1.25). The similar result were reported by many workers viz, Jain *et al.* (2016), Shanmugaiah *et al.* (2009)^[27] and Tariq *et al.*, (2007)^[28] who observed that, plant inoculation with PGPB strain provided a significant increase in shoot and root length and biomass. A significant increase in number of lateral roots was observed over the un-inoculated control. whereas, Jayant Raman (2012)^[22] noted maximum shoot and root growth caused due to inoculation of *Azotobacter chorococcum* + *P.striata* and *Trichoderma viride*.

Effect of different zinc solubilizing microbial inoculants on yield attributes and dry matter of soybean

Grain yield

There was a significant increase in the grain yield of soybean due to inoculation of zinc solubilizing microbial culture along with recommended dose of Hoagland solution as compared to the un-inoculated control, data regarding grain yield is presented in Table 13. Grain yield was increased from 3.80-

5.40 g/plant. Significantly highest grain yield of soybean was obtained with treatment *B.subtilis* (5.40 g/ plant) followed by *B. megaterium* (5.20 g /plant) and lowest yield was found in control treatment (3.80 g/ plant).

Dry matter yield

Dry matter yield of soybean was increased during experiments with the application of zinc solubilizing microbial inoculants along with recommended dose of Hoagland solution and data is narrated in Table 7.

Dry matter yield of soybean varied from 7.33 to 13g/plant during experiment. Highest dry matter yield was obtained in *B.subtilis* (13g/plant), followed by *B.megaterium* (11.66 g/plant), *B.cereus* (10.66g/plant). However, the lowest dry matter yield was observed in un-inoculated control (7.33 g/plant).

These findings are also similar with Navsare *et al.* (2018)^[17] who investigated that highest seed yield in mungbean (3.65 g/plant) observed in the plot which was inoculated with RDF + *Rhizobium* + PSB + KSB + ZSB followed by the plot which was inoculated with RDF + *Rhizobium* + PSB + KSB + ZSB (3.23 g/plant) and RDF + *Rhizobium* + PSB + KSB + ZSB (3.05) . While, lowest seed yield (2.28 g/plant) was observed in the control plot. The similar result was reported by Sable *et al.* (2017) who revealed that kernel and haulm yield of groundnut and net returns was increased with inoculation of zinc solubilizing microorganisms along with recommended dose of fertilizers over un-inoculated control. Among all the zinc solubilizers, RDF + *Rhizobium* + *P.striata* recorded significantly highest yield and net returns.

Table 7: Effect of different zinc solubilizing microbial inoculants on grain yield and dry matter yield of soybean

Treatments	Grain yield (g/plant)	Dry matter yield (g/plant)
T1 : <i>Bacillus subtilis</i>	5.40	13
T2 : <i>Bacillus megaterium</i>	5.20	11.66
T3 : <i>Bacillus cereus</i>	5.06	10.66
T4 : <i>Pseudomonas striata</i>	5.03	9.33
T5 : <i>Pseudomonas fluorescens</i>	4.70	8.66
T6 : <i>Thiobacillus thiooxidans</i>	4.53	8.33
T7 : <i>Thiobacillus ferrooxidans</i>	4.30	7.66
T8 : Control	3.80	7.33
SE m±	0.071	0.37
CD (P=0.01)	0.21	1.12

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