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Effect of Microbial Consortia on Available Nutrients and Microbial Population in Soil of Soybean in Vertisol

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ABSTRACT: The research work was carried out during *Kharif* 2019 in the Experimental field, Department of Soil Science, JNKVV, Jabalpur (M.P.) under RBD with 3 replications having 16 treatments comprising different combinations as consortia of *Rhizobium* sp., PSB (*Bacillus* sp.), actinomycetes (*Streptomyces* sp.), *Rhodopseudomonas* sp., *Lactobacillus* sp., *Saccharomyces* sp. and *Aspergillus* sp. In addition, these, two types of control plots were maintained as fertilized uninoculated control (FUI) and unfertilized uninoculated control (UFUI), and the available nutrient contents (N, P & K) and populations of the microbial species were estimated at harvest. The outcome of the study revealed that Consortia of microbial inoculants T14 increased the highest content of available nutrients (N, P and K) by 50.28, 57.60 & 34.48%, respectively (FUI: 194, 14.10 & 242 kg ha^{-1}), and the population of microbial species *viz*., *Rhizobium*, Bacillus, *Streptomyces*, *Rhodopseudomonas*, *Lactobacillus*, *Saccharomyces* and *Aspergillus* by 2.43, 6.46, 1.61, 1.77, 2.25, 2.06 and 1.74 log fold, respectively over that from FUI (2.46, 2.95, 4.32, 3.12, 1.92, 2.42 and 2.77 log cfu g^{-1} soil, respectively). The ensuing performing group of treatments was T13, T11 and T12. Yield (seed and stover) of soybean at harvest were 57.79 and 65.53%, respectively over the control (1530 & 2649 kg ha^{-1}, respectively). Today, challenges are the increasing population rapidly, the environment is changing critically, and agriculture is one of the most exposed sectors to these changes and faces several some many challenges like soil pollution, pathogenic attack, soil salinity, abnormal drought, high and low temperature and these all challenges ultimately affect crop productivity. To overcome such issues, eco-friendly approaches are very forceful. Thus, it may be concluded that the application of Co-inoculation on seeds with bio-inoculants in different consortia rather than the solo-inoculation might plausibly influence the crop through direct and indirect mechanisms and enhanced soybean yield, available nutrients, and microbial population in the soil.

Keywords: *Rhizobium*, Actinomycetes, *Rhodopseudomonas*, *Saccharomyces*, Soybean, Microbial count.

INTRODUCTION

Soybean is a major staple food crop in India. On the quality aspect, it contains 40% protein (glycine, tryptophan, and lysine), 20% oil, 25-30% carbohydrate and 5% ash. Madhya Pradesh is known as a soya state that occupied 11.34 M hectares area with 13.63 MT production along with 1200 kg ha^{-1} productivity (Directors Report, IISR, 2019-20). Its growth and production can be promoted by improving soil fertility in terms of physical-chemical and biological characterization. Soybean plants require a large amount of nitrogen as the seeds contain high concentrations of protein and the total amount of nitrogen accumulating in the shoot is proportional to the seed yield (Board, 2013).

To achieve such conditions, the use of beneficial microorganisms such as *Rhizobium*, *Bacillus*, *Actinomycetes*, etc. is craze either as solo or co-inoculant (consortia). *Rhizobium* sp. is a Gram-negative soil bacterium that fixes atmospheric N_2 symbiotically in the roots of legumes in economically and agronomically effective manners (Datta *et al.*, 2015). *Rhodopseudomonas* sp. is a rod-shaped Gram-negative, purple, non-Sulphur bacterium, having the ability to switch over between four different modes of metabolism (like chemoheterotrophic, chemoautotrophic, photoheterotrophic and

photoautotrophic). The bacterium cannot use light directly as the energy source but have reduced compounds to get the energy from (Hsu *et al.*, 2021).

Bacillus sp. a Gram-positive bacterium converts insoluble forms of P (tricalcium phosphate and hydroxyl apatite) and other nutrient elements like Fe to available forms to plants and it is very useful to legume initiation in crop roots as well as suppression of pathogen as a biocontrol agent (Chen *et al.*, 2006). *Saccharomyces* sp. is saprophytic Phyto-stimulator single-celled fungi (yeast) generally larger than the bacterium which grows on the sugary solution, grapes and produces fermented wine, beer etc. It is known to contribute to the pleasant smell of bread. It is a new promising plant growth-promoting yeast with properties of good root colonizer and anti-phytopathogenicity (Venugopal, 2016), ultimately a significant increase in growth, yield quality. *Streptomyces* sp. (actinomycetes) are a versatile group of microorganisms widely distributed in arable dry soils as well as works on phytoimmunity inducer and prevent invasion of harmful microbes by the produce of IAA, siderophores, phosphate solubilizer, heavy metal tolerance, plant growth promotion (Horstmann *et al.*, 2020), decomposition of organic matter and antibiotic production. These Gram-positive mycelial bacteria have been highlighted to be the most potential candidates of biofertilizer agents (Wahyudi *et al*, 2019) and also use to produce a wide variety of industrially and medically relevant compounds (such as antibiotics, chemotherapeutics, fungicides, herbicides and immune suppressants) (Moncheva *et al.*, 2002). *Lactobacillus* sp. (lactobacterium) is a Gram-positive and saprophytic bacterium that groups in pairs and short chains, depending upon the growth conditions appears ovoid and produces lactic acid to improve the soil for controlling disease and seed germination and also reduces abiotic stress of crops (Roissart and Luquet, 1994). *Aspergillus* sp. works as an aerobic organic matter decomposer by producing citric acid and enzymes and also reduces negativity of chemical fertilizer and work as biofertilizers. It is proving its importance by enhancing plant growth and productivity by diverse plant growth-promoting traits including production of phytohormones, siderophores, and hydrolytic enzymes; making available different nutrients; and protecting plants against pathogens. It proliferates easily in two distinct forms, mycelial and pelleted (Kour *et al.*, 2019).

Inoculation of these beneficial microorganisms *viz*: diazotroph, PSB, phototroph, lactobacterium, yeast, *actinomycetes* and fungus to the soil ecosystem advances soil physico-chemical properties, soil microbial biodiversity, soil health, plant growth and crop productivity.

MATERIALS AND METHODS

The research trial was laid out during *Kharif* 2019 on the soybean crops. The global position of the site was at 23°10'N latitude and 79°57" E longitude at 411.78 meters above the mean sea level. Soils of the experimental site was medium black which belongs to the order Vertisol that was popularly known as "black cotton soil." Initial chemical properties of the soil were analyzed with 7.23 pH, 0.26 dS m^{-1} electrical conductivity and 4.9 g kg^{-1} organic carbon. The available soil status N, P and K were 218, 14.92 and 259 kg ha^{-1}, respectively. The soybean seeds (cv. JS 20-69) were sown @ 80 kg ha^{-1} with inoculation as per the prescribed treatments (Table 1) on 10.07.2019 in RBD with 16 treatment combinations and 3 replications. The crop was nourished with RDF 20:80:20 (N: P_2O_5:K_2O kg ha^{-1}) at basal dose through urea, single super phosphate and muriate of potash, respectively.

Table 1: Treatment combinations.

Treatment	Combination	Treatment	Combination
T_1	Rhizo + PSB (T1)	T_9	T9 (T2+T4)
T_2	Strepto + PSB (T2)	T_{10}	T10 (T3+T4)
T_3	Rhodo + Lacto (T3)	T_{11}	T11 (T1+T2+T3)
T_4	Saccharo + Aspergil (T4)	T_{12}	T12 (T1+T3+T4)
T_5	T5 (T1+T2)	T_{13}	T13 (T2+T3+T4)
T_6	T6 (T1+T3)	T_{14}	T14 (T1+T2+T3+T4)
T_7	T7 (T1+T4)	T_{15}	T15 (FUI)
T_8	T8 (T2+T3)	T_{16}	T16 (UFUI)

A. Seed treatment and inoculation

40 g soybean seeds were weighed separately for each plot in clean polythene bags. 1 ml of the liquid formulation of each microorganism combination transferred in bags and apply the sterilized gum acacia (2%) was used as sticker solution. Plot-wise inoculated seeds in polythene bags were first slightly moistened and then treated with carbendazim fungicide @ 2g kg^{-1} seed. Seeds were little allowed to air dry and then 1 ml of gum acacia sticker solution was poured on the seed of each polythene bag and seed treatment was done in shade then sown manually as early as possible. Necessary recommended agronomic practices were adopted.

B. Soil available nutrients

The soil samples (initial & at post-harvest) were collected from 0-15 cm depth for chemical and microbial analyses separately following by standard sampling procedure. The soil pH was determined by the Glass electrode pH meter method by the taking 1:2.5 ratio of soil and water suspension (Jackson, 1973), EC by Electrical Conductivity meter method (Jackson, 1973), available N was estimated by alkaline potassium permanganate method (Subbaiah and Asija, 1956), 5

gm soil mixed with $KMnO_4$ presence of NaOH and thus ammonia was released, and this ammonia absorbed by 2% boric acid along with mixed indicator produced ammonium borate is titrated by standard 0.02 N sulfuric acid available P extracted with 0.5 *M* $NaHCO_3$ solution (pH 8.5) and determination done by ascorbic acid used in acidic medium provides a blue color complex. The absorbance of the blue color measured at 660 nm wavelength in spectrophotometer (Olsen *et al.*, 1954) and available K by neutral 1N ammonium acetate extraction and measures with a photocell the emission intensity which is proportional and to concentration in selected wavelength (767 nm) and for a red filter is used in flame photometer (Jackson, 1973).

C. Rhizospheric population count

The soil samples were used as fresh as possible without grinding, sieving or any modifications for microbial enumeration (Table 2) purpose. The collected samples in low-density polyethylene (LDPE) (< 0.930 g cm^{-3}) bags were used as early as possible for microbial analyses and could be stored in the refrigerator at 4 °C. The fresh soil sample was taken for plating by adopting serial dilution method (David and Davidson, 2014), in this method, 10 gram of soil sample was suspended in 90 ml sterilized water aseptically in conical flasks then shake it clockwise and anticlockwise for few minutes to obtain 10^{-1} dilution.

Take sterilized water 9.0 ml in test tube alongwith the soil. The 1.0 ml of the first dilution was transferred and shaken uniformly by rolling the tube between palms of the hand to provide horizontal shaking that is called 10^{-1}dilution. The series in similar manner to get up to 10^{-9} dilution levels were prepared and marked properly for the dilutions on the test tubes. 1.0 ml of required dilution was transferred in a Petriplate; to it, 15 ml of specified melted media was poured within the aseptic environment of the laminar airflow chamber. Plates were rotated gently clockwise and anti-clockwise to mix the soil dilution within media. After solidification of media, the plates were incubated at 28°C±2°C to develop colonies of the microorganism on the media after 24 hrs., every day observed the growth development and the number of physically characteristic colonies were counted on 3-7 days.

$$\text{Viable cells (cfu g}^{-1}\text{ soil)} = \frac{\text{Number of colonies}}{\text{Oven dry weight of soil (1 g)}} \times \text{dilution factor}$$

Table 2: Initial microbial population counts of experimental soils.

Particulars	Population Counts (cfu g^{-1} soil)
Rhizobium sp.	25.26×10^{3}
*Rhodopseudomonas*sp.	9.12×10^{2}
Bacillus sp.	13.76×10^{4}
Saccharomyces sp.	63.88×10^{2}
*Actinomycetes*sp.	17.89×10^{3}
Lactobacillus sp.	5.06×10^{2}
Aspergillus sp.	4.12×10^{2}

[**Note:** Values given in parentheses are exponential values given in cfu g^{-1} soil (oven-dry basis) from plate counts]

RESULTS AND DISCUSSION

Soil is a big reservoir of nutrients but its availability for plant growth may be adversely affected due to indiscriminate use of chemical fertilizers as well as climatic and edaphic factors of soil. Availability of these nutrients slows down for plant growth and development by different soil mechanisms (Mass flow & diffusion) therefore, inclusion of microbial consortia may enhance the availability of soil nutrients which leads to plant growth, nodulation and ultimately grain production.

A. Available nutrients status (N, P and K) in soil at post-harvest of soybean

Outcomes of the study about available nutrients (N, P and K) in surface soil (up to 0-15 cm depth) at harvest of soybean are presented in Table 3. The consortium of T14 (consortia of Rhizo + PSB + Strepto + Rhodo + Lacto + Saccharo + Aspergil) performed significantly better to increase soil available **N** content of 292 kg N ha^{-1} corresponding to the response of 50.28% over FUI (194 kg ha^{-1}) followed by T13, T11, T12 and T6 for the N content of 283, 275, 269 and 266 kg N ha^{-1} which were 45.71, 41.55, 38.70 and 37.05% better response, respectively. The increased soil available N might also be attributed to the greater multiplication of soil microbes which was converted organically bound N to inorganic form soils after the crop was harvested. The mineralizable N, P and K nutrients (239, 16.6 and 302 kg/ha, respectively) were got more available by the inoculation of the microbial consortium of *Actinobacteria*, *Rhizobium* and PGPR consortium as compare to that of FUI (Amule *et al.*, 2018). The rhizobia bacterial genes involved in nitrogen turnover were affected by inoculation. The effectiveness of inoculation was related to the abundance of nif H genes in the late flowering phase to enhance the NPK content in soil at harvested stage (Babic *et al.*, 2008). Results from numerous studies have shown that both the bacterial and host genotypes impact the symbiotic interaction with soybean *Rhizobium* co-inoculation with rhizobium increases soil available N by BNF and *Pseudomonas*-induced phosphorus solubilization as well as increased production of gibberellic acid, which increases root proliferation and stimulates plant growth in soybean (Sahur *et al.*, 2018).

Table 3: Effect of microbial consortia on available nutrients (N, P and K) in soil at post-harvest of soybean.

Treatment	Available nutrient content (kg ha^{-1}) (0- 15 cm soil depth)		
	N	P	K
Rhizo+PSB (T1)	238	17.67	279
Strepto+PSB (T2)	226	17.45	275
Rhodo+Lacto (T3)	218	16.95	266
Saccharo+Aspergil (T4)	212	16.58	258
T5 (T1+T2)	242	17.72	283
T6 (T1+T3)	266	19.82	299
T7 (T1+T4)	259	19.55	295
T8 (T2+T3)	254	18.83	289
T9 (T2+T4)	244	17.83	284
T10 (T3+T4)	248	18.07	285
T11 (T1+T2+T3)	275	21.83	308
T12 (T1+T3+T4)	269	20.04	302
T13 (T2+T3+T4)	283	21.08	313
T14 (T1+T2+T3+T4)	292	22.23	325
T15 (FUI)	194	14.10	242
T16 (UFUI)	181	11.94	232
Mean	244	18.23	283
SEm±	14.558	1.098	12.842
LSD (p= 0.05)	42	3.19	37

The available phosphorous content of 22.23 kg P_2O_5 ha^{-1} was estimated and found significantly superior by the application of T14 with the response of 57.60% as compared to control FUI (14.10 kg P_2O_5 ha^{-1}) followed by T13, T11, T12, T6 and T7 for soil P content of 21.83, 21.08, 20.04, 19.82 and 19.55 kg P_2O_5 ha^{-1}, respectively along with 54.77, 49.50, 42.12, 40.54 and 38.64% response, respectively. The actinobacteria, *Bacillus*, *Rhizobium* and *Arthrobacter* influenced soil fertility through the involvement of many components and served as a nutrient enhancer. Besides producing siderophores and solubilizing phosphate, they are known to produce cocktail of enzymes which include amylase, chitinase, cellulase, invertase, keratinase, peroxidase, pectinase, phytase, and xylanase which made the complex nutrients into simple mineral forms. This nutrient cycling capacity was made an ideal natural fertilizer reported by Ghadage *et al*., (2020). The actinobacteria have the potential to mobilize insoluble inorganic phosphate for improving the growth of plants under low phosphate availability. Plant growth-promoting of Actinomycetes also well known as a solubilizer of inorganic phosphate through soil acidification process (Anwar *et al.*, 2016).

The analyzed data about of on available potassium was recorded 325 kg K ha^{-1} in soil that was observed superior performance by the application of T14 corresponding to a percent response of 34.48% as compared to FUI (242 kg ha^{-1}). This was next to the performance of T13, T11, T12 and T6 for 313, 308, 302 and 299 kg K ha^{-1} with 29.65, 27.46, 25.16 and 23.59%, response over that of FUI. The findings confirmed that bio-inoculants (*R. sphaeroides*, *L. plantarum*, and *S. cerevisiae)* significantly increased the plant height, its biomass, available nutrient contents (N, P and K) in soil, via secretion of indole acetic acid or organic acids, gibberellin, vitamin B12, antibiotics or extracellular enzymes and reducing the abscisic acid contents and significantly increased the calcium, potassium, magnesium, and phosphate contents reported by Kang *et al*. (2015).

B. Effect of microbial consortia on microbial count in rhizospheric soil at post-harvest of soybean

Diazotroph (*Rhizobium* sp.). The populations of *Rhizobium* sp. in the rhizosphere of soybean at harvest were counted and presented in Table 4. Among all the treatment combinations T14 increased maximum count by 5.98 log cfu (95.28×10^4 cfu g^{-1} soil) with the relative response 2.43 log fold increase over the control FUI (2.46 log cfu = 28.68×10^1 cfu g^{-1} soil) followed by T13, T11, T12, T6 and T7 for the population counts of 5.83 logcfu (67.75×10^4 cfu g^{-1} soil), 5.67 log cfu (46.03×10^4 cfu g^{-1} soil), 5.28 log cfu (18.94×10^4 cfu g^{-1} soil), 5.20 log cfu (15.70×10^4 cfu g^{-1} soil) and 5.07 log cfu (11.83×10^4 cfu g^{-1} soil), respectively with the responses of 2.37, 2.31, 2.15, 2.11 and 2.06 log fold increase over the FUI. The findings of Tolba *et al.*, (2016) confirmed that the consortium of *Rhizobium* and PSB secret the growth hormones and some growth-promoting substances that might induce root formation and growth of root as well as enhanced microbial population. Phosphorus has a specific role in nodules initiation, growth and function of nodules and host plant also the maximum colony counts of 9.81×10^4 cfu were observed in soil treated with *Rhizobium* + PSB. The research findings of Amule *et al*., (2018) also agreed that the inoculation of actinobacteria, *Rhizobium* and PGPR consortium inoculated plants showed a higher population of bacteria (36.5×10^5 cfu g/soil), fungi (20.6×10^3 cfu g/soil) and actinomycetes (15.4×10^5 cfu g/soil). It was again confirmed that the co-inoculation of actinobacteria, *Rhizobium* and PGPR as a consortium was found best for improving the yield of soybean and soil health too.

Table 4: Effect of microbial consortia on population of *Rhizoobium*, PSB, actinomycetes (*Streptomyces* sp.), *Rhodopseudomonas* sp., *Lactobacillus* sp., *Saccharomyces* sp. *and Aspergillus* sp. in rhizospheric soil at post-harvest of soybean.

Treatment	Microbial counts [log cfu g^{-1} soil (values in parenthesis are cfu g^{-1} soil)]						
	Rhizobium sp.	PSB (*Bacillus* sp.)	*Streptomyces* sp.	*Rhodopseudo.* sp.	*Lactobacillus* sp.	*Saccharomyces* sp.	*Aspergillus* sp.
Rhizo+PSB (T1)	4.02 ($10.38x10^3$)	4.92 ($82.49x10^3$)	5.39 ($24.54x10^4$)	3.89 ($77.62x10^2$)	2.68 ($4.74x10^2$)	3.17 ($1.49x10^3$)	3.26 ($1.80x10^3$)
Strepto+PSB (T2)	3.63 ($42.62x10^2$)	4.90 ($79.56x10^3$)	6.04 ($10.96x10^5$)	3.78 ($60.25x10^2$)	2.37 ($2.32x10^2$)	3.03 ($1.08x10^3$)	3.23 ($1.69x10^3$)
Rhodo+Lacto (T3)	3.24 ($17.47x10^2$)	4.45 ($27.92x10^3$)	5.34 ($21.87x10^4$)	4.32 ($20.89x10^3$)	3.35 ($2.25x10^3$)	2.94 ($8.77x10^2$)	3.17 ($1.49x10^3$)
Saccharo+Aspergil (T4)	3.21 ($16.08x10^2$)	4.19 ($15.36x10^3$)	5.20 ($15.84x10^4$)	3.64 ($43.65x10^3$)	2.06 ($1.13x10^2$)	3.48 ($3.04x10^3$)	3.34 ($2.17x10^3$)
T5 (T1+T2)	4.06 ($11.55x10^3$)	4.94 ($87.08x10^3$)	6.11 ($12.88x10^5$)	4.41 ($25.70x10^3$)	3.43 ($2.71x10^3$)	3.64 ($4.39x10^3$)	3.49 ($3.11x10^3$)
T6 (T1+T3)	5.20 ($15.70x10^4$)	5.86 ($72.29x10^4$)	6.45 ($28.18x10^5$)	5.12 ($13.18x10^4$)	4.11 ($1.28x10^4$)	4.12 ($1.30x10^4$)	3.94 ($8.64x10^3$)
T7 (T1+T4)	5.07 ($11.83x10^4$)	5.65 ($44.73x10^4$)	6.28 ($19.05x10^5$)	4.67 ($46.77x10^3$)	3.71 ($5.09x10^3$)	4.34 ($2.17x10^4$)	4.46 ($2.90x10^4$)
T8 (T2+T3)	4.42 ($26.54x10^3$)	5.41 ($25.89x10^4$)	6.68 ($47.86x10^5$)	4.98 ($95.49x10^3$)	4.08 ($1.21x10^4$)	3.93 ($8.51x10^3$)	3.79 ($6.21x10^3$)
T9 (T2+T4)	4.10 ($12.48x10^3$)	5.16 ($14.52x10^4$)	6.49 ($30.90x10^5$)	4.59 ($38.90x10^3$)	3.54 ($3.44x10^3$)	4.28 ($1.90x10^4$)	4.45 ($2.84x10^4$)
T10 (T3+T4)	4.18 ($15.21x10^3$)	5.08 ($12.11x10^4$)	6.33 ($21.87x10^5$)	5.04 ($10.96x10^4$)	3.96 ($9.05x10^3$)	4.21 ($1.62x10^4$)	4.28 ($1.89x10^4$)
T11 (T1+T2+T3)	5.67 ($47.03x10^4$)	5.97 ($93.86x10^4$)	6.73 ($53.70x10^5$)	5.33 ($21.37x10^4$)	4.19 ($1.56x10^4$)	4.42 ($2.61x10^4$)	4.55 ($3.57x10^4$)
T12 (T1+T3+T4)	5.28 ($18.94x10^4$)	5.89 ($77.89x10^4$)	6.80 ($63.09x10^5$)	5.18 ($15.13x10^4$)	4.14 ($1.36x10^4$)	4.51 ($3.21x10^4$)	4.70 ($5.01x10^4$)
T13 (T2+T3+T4)	5.83 ($67.75x10^4$)	6.15 ($14.17x10^5$)	6.92 ($83.17x10^5$)	5.47 ($29.51x10^4$)	4.25 ($1.76x10^4$)	4.83 ($6.81x10^4$)	4.78 ($6.07x10^4$)
T14 (T1+T2+T3+T4)	5.98 ($95.28x10^4$)	6.33 ($21.24x10^5$)	6.96 ($91.20x10^5$)	5.52 ($33.11x10^4$)	4.32 ($2.10x10^4$)	4.99 ($9.78x10^4$)	4.83 ($6.70x10^4$)
T15 (FUI)	2.46 ($28.68x10^1$)	2.95 ($90.10x10^1$)	4.32 ($21.37x10^3$)	3.12 ($13.18x10^2$)	1.92 ($8.38x10^1$)	2.42 ($2.63x10^2$)	2.77 ($5.84x10^2$)
T16 (UFUI)	2.16 ($14.59x10^1$)	2.89 ($77.69x10^1$)	4.02 ($10.47x10^3$)	2.74 ($5.49x10^2$)	1.76 ($5.74x10^1$)	2.19 ($1.54x10^2$)	2.41 ($2.59x10^2$)
Mean	4.28 ($19.13x10^3$)	5.05 ($11.13x10^4$)	6.01 ($10.23x10^5$)	4.49 ($3.09x10^4$)	3.37 ($2.32x10^3$)	3.78 ($6.04x10^3$)	3.84 ($6.91x10^3$)
$SE_m\pm$	0.535	0.632	0.367	0.287	0.290	0.294	0.229
LSD (p= 0.05)	1.55	1.84	1.06	0.84	0.84	0.85	0.67

[Note: Values given in parentheses are exponential values given in cfu g^{-1} soil (oven-dry basis) from plate counts]

Phosphorus Solubilizing Bacteria (PSB). The *Bacillus* sp. (PSB) population in rhizospheric soil is illustrated in Table 4. It was increased maximum by the treatment combination of T14 by 6.33 log cfu (21.24×10^5 cfu g^{-1} soil) with the relative response 2.14 log fold increase over that of FUI (2.95 log cfu = 90.10×10^1 cfu g^{-1} soil), followed by the response of T13, T11, T12, T6 and T7 for the population count of 6.15 log cfu (14.17×10^5 cfu g^{-1} soil), 5.97 log cfu (93.86×10^4 cfu g^{-1} soil), 5.89 log cfu (77.89×10^4 cfu g^{-1} soil), 5.86 log cfu (72.29×10^4 cfu g^{-1} soil) and 5.65 log cfu (44.73×10^4 cfu g^{-1} soil) respectively with the increment response of 2.08, 2.02, 1.99, 1.98 and 1.91 log fold, respectively over that of FUI. The co-inoculation with *B. japonicum* and *Bacillus* strains increased *B. japonicum* colonization and population on the soybean root surface and nodule number due to production of growth-promoting substances, and that co-inoculation effects were strain-dependent because soybean plants with *Bacillus* strain able to produce high levels of auxin, GA3 and salicylic acid, and natural symbiont *B. japonicum* altered plant growth parameters and significantly improved nodulation reported by Marinkovic *et al.,* (2018).

Actinomycetes (*Streptomyces* sp.). The population of Actinomycetes (*Streptomyces* sp.) in the rhizosphere of soybean at harvest was counted and given in Table 4. It was maximum by 6.96 log cfu (91.20×10^5 cfu g^{-1} soil) with the relative response 1.61 log fold increase over that of FUI (4.32 log cfu = 21.37×10^3 cfu g^{-1} soil) followed by the performance of T13, T11, T12 and T8 by 6.92 log cfu (83.17×10^5 cfu g^{-1} soil), 6.80 log cfu (63.09×10^5 cfu g^{-1} soil), 6.73 log cfu (53.70×10^5 cfu g^{-1} soil) and 6.68 log cfu (47.86×10^5 cfu g^{-1} soil), respectively with the response of 1.60, 1.57, 1.56 and 1.54 log fold. Similar research findings were also noticed by Sahur *et al.* (2018).

The researchers concluded that the association of actinomycetes, *Rhizobium* and *Trichoderma* was found to confer many advantages to the host plants such as the production of IAA that helped the promotion of roots growth or the production of siderophore that bind iron (Fe^{3+}) from the environment and subsequently help to improve nutrient uptakes, as well as the protection against plant pathogens by producing antibiotics or extracellular enzymes, increment in microbial activities as well as its population in rhizospheric soil of soybean crop. Actinobacterial strains can solubilize lignin and break down lignin-related compounds following the production of cellulose and hemicellulose-degrading enzymes, extracellular peroxidase, as well as play important role in the recycling of organic carbon and can degrade complex polymers reported by Mishra e*t al.,* (2014). Actinomycetes are considered as an important work, particularly in developing plant growth-promoting agent to increase actinomycetes activity in rhizospheric soil of soybean field (Wahyudi *et al.,* 2019).

Phototroph (*Rhodopseudomonas* sp.). The data on population of *Rhodopseudomonas* sp. in the rhizosphere of soybean at harvest are presented in Table 4 and the treatment of T14 exhibited maximum population by 5.52 log cfu (33.11×10^4 cfu g^{-1} soil) with the relative response of 1.77 log fold increase over that of FUI (3.12 logcfu=13.18×10^2 cfu g^{-1} soil) followed by T13, T11, T12, T6, T10 and T8 for the microbial population of 5.47 log cfu (29.51×10^4 cfu g^{-1} soil), 5.33 log cfu (21.37×10^5 cfu g^{-1} soil), 5.19 log cfu (15.13×10^4 cfu g^{-1} soil), 5.12 log cfu (13.18×10^4 cfu g^{-1} soil), 5.04 log cfu (10.96×10^4 cfu g^{-1} soil) and 4.98 log cfu (95.49×10^3 cfu g^{-1} soil), respectively with the respective response of 1.75, 1.71, 1.66, 1.64, 1.61 and 1.60 log fold.

The combined inoculation of *Rhodobacter*, *Lactobacillus* and *Saccharomyces* consistently enhanced growth and yield of the crop to a level equal to or greater than that achieved by single inoculation over the control. This might be due to the colonizing the hair, cortical cells and enhanced root surface area and consequently more acquisition of nutrients as well as plant hormones reported by Kang *et al.* (2015).

Lactic bacterium (*Lactobacillus* sp.). The population of *Lactobacillus* sp. in the rhizosphere of soybean at harvest was counted and given in Table 4. The treatment combination of T14 increased the maximum by 4.32 log cfu (2.10×10^4 cfu g^{-1} soil) with the relative response 2.25log fold increase over that of FUI (1.92 log cfu = 8.38×10^1 cfu g^{-1} soil). This was followed by the response of T13, T11, T12, T6 and T8 for the population of 4.25 log cfu (1.76×10^4 cfu g^{-1} soil), 4.19 log cfu (1.56×10^4 cfu g^{-1} soil), 4.14 log cfu (1.36×10^4 cfu g^{-1} soil), 4.11 log cfu (1.28×10^4 cfu g^{-1} soil) and 4.08 log cfu (1.21×10^4 cfu g^{-1} soil), respectively with the response of 2.21, 2.18, 2.15, 2.14 and 2.10 log fold increase, respectively over that of FUI. The results have shown conformity to the observation made by Kang *et al.* (2015) the inoculation of *Lactobacillus* and *Saccharomyces* on cucumber plant all three bio-inoculants significantly increased the plant height and root length as well as the microbial population in rhizospheric soil via secretion of IAA, gibberellin and reducing the abscisic acid contents and significantly increased the calcium, potassium, magnesium, and phosphate contents in soil.

Yeast (*Saccharomyces* sp.). The data on population of *Saccharomyces* sp. in the rhizosphere of soybean at harvest are presented in Table 4. The maximum increased by the treatment combination of T14 for 4.99 log cfu (9.78×10^4 cfu g^{-1} soil) with the relative response 2.06 log fold increase over that of FUI (2.42 log cfu = 2.63×10^2 cfu g^{-1} soil). This was followed by the performance of T13, T12, T11, T7 and T9 for the population of 4.83 logcfu (6.81×10^4 cfu g^{-1} soil), 4.51 log cfu (3.21×10^4 cfu g^{-1} soil), 4.42 log cfu (2.61×10^4 cfu g^{-1} soil), 4.34 log cfu (2.17×10^4 cfu g^{-1} soil), 4.28 log cfu (1.90×10^4 cfu g^{-1} soil), respectively, with the corresponding response of 2.01, 1.86, 1.83, 1.79 and 1.77 log fold increase over that of FUI. Besides, counts of yeast were also increased, it recorded 67.67 and 77.33 $\times10^4$ cfu g^{-1} dry soil after 50 and 75 days of planting, respectively by the yeasts in the rhizosphere could influence plant growth indirectly by enhancing the growth of other plant growth-promoting rhizo-microorganisms, through vitamin B12 production. The role in soil aggregate formation and maintaining the structure of soil and mineralization of organic material, growth promoters and soil conditioners to promote sustainable agriculture by the production of auxins for its role in plant cell elongation, division, and differentiation. Some soil yeast (*Saccharomyces* sp.) may also play arole in both the nitrogen and sulphur cycles and have the ability to solubilize insoluble phosphates making it more readily available top lants (Botha, 2011).

Fungi (*Aspergillus* sp.). The data on population of the fungi (*Aspergillus* sp.) in the rhizosphere of soybean at harvest is exhibited in Table 4. The treatment combination of T14 increased the maximum *Aspergillus* sp. Population by 4.83 log cfu (6.70×10^4 cfu g^{-1} soil) with the relative response 1.74 log fold increase over that of FUI (2.77 logcfu = 5.84×10^2 cfu g^{-1} soil). This was followed by the response of T13, T11, T12 and T6, for the population of 4.78 log cfu (6.70×10^4 cfu g^{-1} soil), 4.70 log cfu (5.01×10^4 cfu g^{-1} soil), 4.55 log cfu (3.57×10^4 cfu g^{-1} soil) and 4.46 log cfu (1.61×10^4 cfu g^{-1} soil), respectively with the response of 1.73, 1.70, 1.65 and 1.61 log fold increase over that of FUI. The co-inoculation of actinobacteria, *Rhizobium* and PGPR consortium inoculated plants showed the higher population of bacteria (36.5×10^5 cfu g^{-1} soil), fungi (20.6×10^3 cfu g^{-1} soil) and *Bacillus* sp. (15.4×10^5 cfu g^{-1} soil). Among the mono and co-inoculation, co-inoculation of Actinobacteria, *Rhizobium* and PGPR consortium was found best for improving the yield of soybean and also soil health reported by Amule *et al.* (2018).

C. Yields (seed and stover) of soybean

The data on seed and stover yield showed in figure 1. The seed yield (2414 kg ha^{-1}) of soybean was obtained maximum by the inoculation of consortia of T14 with 57.79% over that of FUI (1530 kg ha^{-1}). This was followed by the treatment combinations of T13, T11 and T12. The highest stover yield of 4384 kg ha^{-1} was recorded with the treatment combination of T14 by 65.53% response over the control FUI (2649 kg ha^{-1}), followed by T13, T11, T12 and T6 with 4279, 4185, 3984 and 3870 kg ha^{-1} along with the response of 61.55, 58.0, 50.42 and 46.11%, respectively. The increment in seed yield of soybean with the treatments of inoculation of rhizobia and PSB with RDF might be attributed to better nodulation, N2 fixation, crop growth and seed yield (2600 kg ha^{-1}) as compared to the uninoculated control (Kravchenko *et al*. 2013). Moreover, similar findings by Jaga and Sharma; 2015; Marinkovic *et al.* (2018). Kumawat *et al.* (2019) studied that the application of above findings of RDF+ Rhizobium + PSB to soybean crop resulted in higher seed and stover yield (2634 kg ha^{-1} and 3125 kg ha^{-1}) due to cumulative effect production of auxins, cytokinins and gibberellins on growth contributing characters finally the produce i.e., seed and stover yield. Increased root physiology, its architecture and surface area with more root hairs and nodules increased mineral uptake and plant growth of soybean.

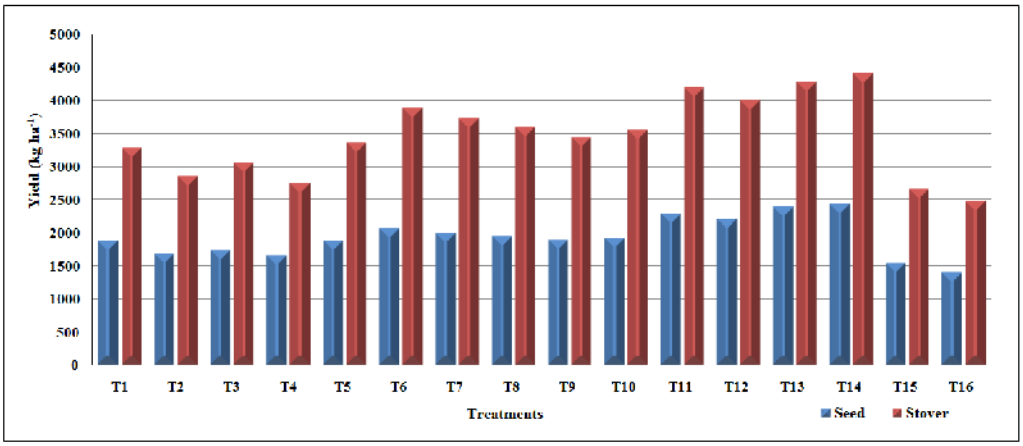

Fig. 1. Effect of microbial consortia on yields (seed and stover) of soybean.

CONCLUSIONS

The result of the investigation showed that the application of consortium T14 (consortia of Rhizo + PSB + Strepto + Rhodo + Lacto + Saccharo + Aspergil) as seed inoculation performed the best consortium for enhancing seed and stover yield of soybean. Similarly, for soil parameters, the same treatment combination performed significantly better towards soil available nutrient contents of N, P and K and rhizospheric microbial populations (*Rhizobium*, Bacillus, *Streptomyces*, *Rhodopseudomonas*, *Lactobacillus*, *Saccharomyces* and *Aspergillus*) at post-harvest. In every case, the treatment combinations of T13, T11 and T12 exhibited as the next ensuing performance group. This was attributed due to the different chemical and biochemical secretions by the microbial consortia which leads to vigorous plant growth and systemic resistance (ASR and ISR), enhancement of the protein and RNA synthesis for better production of soybean.

FUTURE SCOPE

- Actinomycetes (*Streptomyces* sp.) are able to excellent biocontrol activity against some phytopathogenic soil-borne fungi.
- The consortia of phototroph, lactobacterium, yeast, actinomycetes and fungus, popularly known as EM (Enriched Microorganism) culture, can be studied further for other leguminous crops.
- The advanced study on single-cell genomics (SCG), microfluidics, fluorescent imaging and membrane separation is also a matter of great concern.

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