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Effect of *Azotobacter* and phosphorus solubilizing bacteria on growth and yield of okra

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ABSTRACT

A greenhouse experiment was conducted in Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur during 2017-18 with the objective to envisage the growth and yield of okra as affected by the application of bioinoculants, vermicompost as against recommended chemical fertilizers. The experiment was laid out in complete randomized design with six treatments and four replications. Treatments consisting of T_1 100% NPK, T_2 100% NPK+Vermicompost,T_3-75%NPK+Vermicompost+PSB, T_4-75%NPK+Vermicompost+*Azotob acter*, T_5-75%NPK+Vermicompost+*Azotobacter*+PSB and T_6-50% NPK+Vermicompost+*Azotobacter*+PSB. The results showed that the application of *Azotobacter* + PSB along with vermicompost and 75 % NPK produced significantly maximum growth attributes *viz.* plant height at 30, 60 DAS and at harvest, number of leaves per plant, chlorophyll content and yield and yield attributes like (Number of fruits per plant, fruit length, fruit weight, fruit yield plant^{-1}) as compared to other treatments but remained at par with *Azotobacter* + PSB along with vermicompost and 50% NPK. Both the treatments showed superior effect on okra over rest of the treatments. Similarly, results also showed that application of vermicompost (T_2) significantly increased the growth and yield parameters as compared to only inorganic treatment (T_1). The significantly highest total yield (537.34 g and 518.58g) was recorded in treatment T_5 and T_6 which proved the best treatment combination in comparison to other treatments. From the present investigation and with the cost–effective point of view it can be inferred that an application of 50% NPK + vermicompost + *Azotobacter* + PSB observed to be beneficial in enhancing vegetative growth and increasing yield of okra.

Key words: *Abelmoschus esculentus*, vermicompost, biofertilizers.

INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench.] commonly known as lady's finger or bhindi, belonging to the Family Malvaceae is one of the most important vegetable crops grown for its tender green fruits. India ranks first in the world with a production of 5972 thousand metric tones from an area of 501 thousand ha. (NHB, 8). In Chhattisgarh it occupies an area of about 26.47 thousand hectares with a total production of 269.18 thousand metric tones and productivity of 10.17 MT/ha. (NHB, 8).

Okra requires heavy nutrition and manuring to exploit its production potential. In order to increase crop production for fulfillment of requirements of growing population, the system is relying exclusively on the use of chemical fertilizers. Whereas, chemical fertilizers are quite expensive and their use over a long period deplete the soil fertility and soil health. Besides, chemical fertilization involves high cost, hence, there is a need to reduce the use of chemical fertilizers and encourage the application of biofertilizers to the maximum possible level. Biofertilizers are inputs containing micro-organisms capable of mobilizing native elements from nonusable form to usable form through biological processes (Anuradha *et.al.*, 3). Also these are cheaper and renewable sources. Application of biofertilizers results in mineral and water uptake, root development, vegetative growth and nitrogen fixation (Bhushan *et al.*, 4). The requirements of fertilizers in okra are important for the early growth and total production of fruit yield. Integrated use of organic and inorganic fertilizers can improve crop productivity (Mal *et al.*, 6). Therefore, to maintain the soil fertility and to supply plant nutrients in balanced proportion for optimum growth, yield and quality of crop, the combined judicious use of biofertilizers, organic sources and inorganic fertilizers is required (Gayathri and Reddy, 5). Among the manures, vermicompost is being a stable fine granular organic matter, when added to soil, it loosens the soil and improves the passage to the entry of air. The organic carbon in vermicompost releases the nutrients slowly and steadily into the system and enables the plant to absorb the nutrients (Abdula & Kumar, 1).

Among the biofertilizers, *Azotobacter* as nitrogen fixer and *Pseudomonas* as phosphate solublizer have gained much importance and there has been encouraging response to inoculation with *Azotobacter*

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and *Pseudomonas* as PSB. *Azotobacter* not only provides nitrogen, but also produces a variety of phytohormones i.e. indolacetic acid, and gibberellins which stimulates vegetative growth by cell division and incenses the development of large stems and leaves and tends to produce succulence in Okra fruits. The soil enriched with biofertilizers (*Azotobacter* + PSB) increased the seed germination and provide additional substances that are not found in chemical fertilizers (Sajindranath *et al*.,11). Adding of biofertilizers along with fertilizers increased crop growth and yield of okra (Anisa *et al*.,2). Naidu *et al*., 7) concluded that significant increase of microbes in soil was found with application of manures, vermicompost and biofertilizers. Inoculation with diazotrophs (*Azospirillum*, *Azotobacter* and PSB) in okra helped fixing atmospheric N, increased phosphate availability, produced growth promoting and antifungal substances and finally increased the total yield.

The present study was therefore, undertaken to assess the inoculation effect of *Azotobacter* and Phosphorus solubilizing bacteria on yield and yield attributing characters of okra and also to investigate the effectiveness of combination of biofertilizers and vermicompost when applied along with reduced dose of chemical fertilizers against the recommended inorganic fertilization. The objective of the study was to assess the effect of inoculation along with vermicompost on okra in terms of growth and yield.

MATERIALS AND METHODS

The experiment was conducted on Okra cv. Suraksha during 2017-18 in the greenhouse of The Department of Agricultural Microbiology, Indira Gandhi Agricultural University, Raipur (Chhattisgarh). Seeds of Okra, cv. Suraksha were obtained from IGKV Raipur. The location is characterized by subhumid climate with average rainfall of 1200- 1400 mm mostly concentrated during the rainy season (June to September) which is situated in plains of Chhattisgarh at $21^0$16' N latitude and $81^0$36' E longitude with an altitude of 289.60 meter above mean sea level (MSL).

The growing media used for the experiment proportionally composed of soil, sand and compost in 3:1:1 ratio, which was filled in each pot prior to sowing. Soil under study (analyzed following Page *et al*., 9) was Vertisol, free from salinity hazards (EC: 0.21 dS m^{-1}), neutral to slightly alkaline in reaction (pH: 7.2), having medium organic carbon status 6.1 g/kg soil. The whole experiment was arranged over six treatments consisting of T_1 (100% NPK), T_2 (100% NPK + Vermicompost), T_3 (75% NPK + Vermicompost + PSB), T_4 (75% NPK + Vermicompost + *Azotobacter*), T_5 (75% NPK + Vermicompost + *Azotobacter* + PSB), T_6 (50% NPK + Vermicompost + *Azotobacte*r + PSB) replicated four times and laid out in completely randomized design.

The fertilizers were given as per recommended package of practices (N: P_2O_5: K_2O - 100:50:40 kg ha^{-1}) through urea, single superphosphate and muriate of potash, respectively. The recommended dose of nitrogen was applied in two equal splits i.e. half dose of nitrogen was applied as basal dose 20 days prior to sowing of okra seeds. Also the recommended full dose of P_2O_5 and K_2O was applied as basal dose by soil incorporation. Remaining half dose of nitrogen was applied to the soil in pots as per treatment one month after sowing in liquid form with distilled water. The calculated quantities of fertilizers were applied in the respective pots as per treatment description. Vermicompost were incorporated @ 2.5 t ha^{-1} (10 g/ pot) as per treatment to respective pots prior to sowing and was thoroughly mixed into the soil. The bioinoculants, promising *Azotobacter* isolate and PSB (*Pseudomonas*) isolate were procured from Department of Agricultural Microbiology, College of Agriculture, Raipur. The *Azotobacter* and PSB cultures were revived by inoculating *Azotobacter* in Jensen's medium and PSB in Pikovskaya's medium of pH (7.0) and temperature (28^0C) each. For activation, the isolates inoculated on respective media plates and incubated at 29^0C and then biofertilizers were prepared by mixing required broth culture with sterilized charcoal as carrier material. All the biofertilizers were given as soil application @ 10 g/pot and also in seeds as per treatment description. After imposing treatments, the seeds were shade dried for 10 minutes and were sown in pots containing media 3:1:1 ratio (sand, soil, compost) at 0.5 cm depth in 2 cm apart and were kept in the green house. Uniform irrigation to all pots was given as and when required. The data on various parameters *viz.,* germination %, plant height, leaf number and chlorophyll content at flowering stage, fruit length, fruit diameter, average fruit weight, number of fruits per plant, and yield were recorded from the four labeled seedlings in each treatment. Germination percentage was calculated as below.

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total no. of seeds sown}} \times 100$$

The chlorophyll content of leaves at flowering time was determined by solubulising 0.2 g of fresh plant leaf material with 25 ml of 80% acetone and measuring the optical density of the extract at 663 and 645 nm wavelengths using spectrophotometer because at these wavelengths, maximum absorption of chlorophyll "a" and "b" takes place, respectively. The total chlorophyll was calculated using the formula.

$$\text{mg total chlorophyll/g tissue} = 20.2(A645) + 8.02(A663) \times V1000 \times W$$

where:
A= absorbance at specific wavelengths
V= final volume of chlorophyll extract
W= fresh weigh of tissue extracted

Microbial population in rhizosphere soil of okra after harvest was enumerated by serial dilution and plating technique (Subba Rao, 14). Rhizosphere soil sample (25g) from different treatments was kept as such from pot in sealed polythene bag to prevent the moisture loses and properly stored in refrigerator for quantitative analysis of microbes. Ten fold serial dilutions were prepared for each soil sample and 1 ml aliquots from the appropriate dilutions (10^{-4} for *Azotobacter* and 10^{-3} for PSB) were transferred to sterile media Petri dishes. The plates were incubated at 28^{0}C in the incubator. Colony counting was done by colony counter. After counting of colonies, the populations were expressed as cfu g^1 of dry soil using following formula (Schmidt and Caldwell, 12).

$$\text{Number of Azotobacter/ PSB per gram of oven dry soil: } \frac{\text{No. of colony forming units (cfu)} \times \text{dilution}}{\text{Dry wt. of 1 g moist soil} \times \text{aliquot taken}}$$

Data were averaged and completely randomized design (CRD) was employed to find out the significance among different treatments with the help of 'F' test as per Panse and Sukhatme ,10)

RESULTS AND DISCUSSION

Pre-sowing treatments with vermicompost and bioinoculants influenced germination characters of seeds, resulting in their improved germination (Table 1). The results of germination clearly showed that significantly highest germination was recorded in treatment T_5 and T_6 followed by T_4 which was remained at par with T_2 and T_3. Significantly lowest germination (85.73 %) was recorded in T_1 (100% NPK). Sajindranath *et al.* (11) also found the similar result while experimenting on effect of biofertilizer on okra.

Data pertaining to growth parameters like plant height, number of leaves and chlorophyll content presented inTable1 and it is clearly evident from the results that effect of dual inoculation of bioinoculants was significant for these parameters. In T_5, plants treated with (75% NPK+Vermicompost+ *Azotobacter*+PSB) recorded significantly maximum plant height 112.91cm at 90 DAS. However, progressive gain in plant height at 60 and 90 DAS was significantly highest in the plants receiving both the inoculations (Table 1). Similar results were obtained by Bhushan *et al.* (4).

The data on total chlorophyll content is depicted in Fig. 1. The maximum number of leaves per plant and total chlorophyll content (10.25 and 1.93 mg/g of fresh leaves) were recorded highest under T_5 which was at par with T_6 while it was minimum (6.87 and 1.03 mg/g) under T_1 (100% NPK) (Table 1). The organic and inorganic manures and bio-fertilizers significantly affected the total chlorophyll content because of fertilized and bioinoculated plants were photosynthetically more active. This contributes to an improved availability of moisture, nutrients and uniform distribution of nutrients in the crop root zone throughout the growth stages leading to better uptake of nutrients. Our findings are in line with those of Anisa *et al.* (2).

The perusal of Table 2 revealed that inoculation of both the biofertilizers along with vermicompost and reduced dose of NPK (T_5 and T_6) recorded the highest fruit weight and fruit girth compared to control. It was followed by T_4 where vermicompost, inorganic (¾ NPK) fertilizers and *Azotobacter* were applied. The data indicated that the treatment T6 (50%NPK+Vermi+*Azoto*+PSB) gave maximum value regarding fruit girth (5.15cm), weight of fruits (14.77g), which were at par with T_5, however treatment T_1 (100% NPK) recorded significantly minimum. Similar results

Table 1 : Effect of bioinoculants and vermicompost on plant height, number of leaves and Chlorophyll content of Okra leaf.

	Treatments	Germination Percentage (%)	Plant height (cm)			No. of leaves/ plant
			30 (DAS)	60 (DAS)	90 (DAS)	At flowering stage
T_1	100% NPK	85.73	12.40	44.37	85.43	6.87
T_2	100%NPK+Vermi	90.62	14.24	53.55	92.43	7.83
T_3	75%NPK+Vermi+PSB	91.74	14.31	57.12	94.31	9.52
T_4	75%NPK+Vermi+*Azoto*	92.34	14.61	57.23	104.81	9.23
T_5	75%NPK+Vermi+*Azoto*+PSB	96.87	16.22	61.34	112.91	10.25
T_6	50%NPK+Vermi+*Azoto*+PSB	96.75	15.56	59.78	110.00	10.00
	SEm±	1.06	0.36	0.77	1.53	0.34
	CD (0.05 %)	3.16	1.05	2.32	4.56	1.02

Table 2. Effect of bioinoculants and vermicompost on yield and yield attributing characters in okra.

Treatments	Fruit length (cm)	Fruit girth (cm)	Weight/fruit (g)	No. of fruit/ plant	Fruit yield/ plant (g)	Total yield (g)
T_1-100% NPK	10.46	4.16	10.34	8.50	95.43	251.02
T_2-100%NPK+Vermi	12.05	4.36	11.76	10.40	136.78	305.33
T_3-75%NPK+Vermi+PSB	13.26	4.76	12.87	11.75	172.03	329.78
T_4-75%NPK+Vermi+*Azoto*	13.56	4.66	13.23	12.50	184.56	384.44
T_5-75%NPK+Vermi+*Azoto*+PSB	14.56	5.12	14.17	14.50	210.87	537.34
T_6-50%NPK+Vermi+*Azoto*+PSB	14.20	5.15	14.77	14.75	198.83	518.58
SEm±	0.50	0.14	0.22	0.62	6.20	14.58
CD (0.05 %)	1.52	0.41	0.67	1.88	18.56	43.63

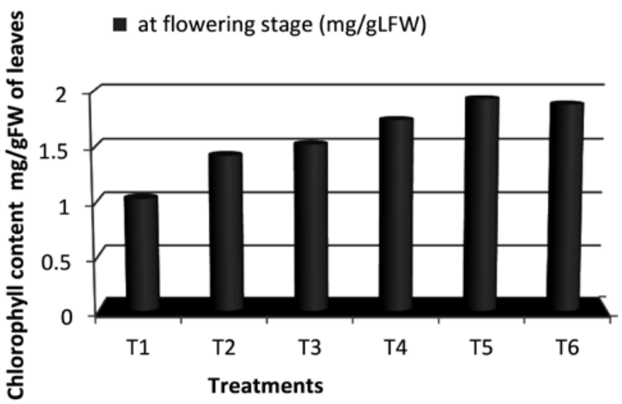

Fig. 1. Effect of bioinoculants and vermicompost on Chlorophyll content in okra leaf at flowering stage.

have been obtained by Swain *et al.* (15). Number of fruits per plant showed significant differences among the treatments and it ranged from 8.50 to 14.75. The highest number of fruits per plant recorded in T_6, which was 1.7 times higher than control and at par with treatment T_5 followed by T_4 (12.50). Bhushan *et al.* (4) also found the similar result while experimenting on effect of biofertilizer on okra. The maximum yield of okra was recorded with the treatment of T_5 (75%NPK+Vermi+*Azoto*+PSB) (537.34g) while minimum was recorded with the treatment T_1 (100% NPK) (251.02g). This is very close to the findings of Sajindranath *et al.* (11) and Sharma *et al*. (13).

Combined application of both the biofertilizers along with vermicompost and reduced NPK (T_5 and T_6) recorded the highest fruit yield per plant and total fruit yield (Table 2). Application of vermicompost might have created better soil condition due to higher rate of multiplication of inoculated microbes leading to enrichment and mobilization of bound nutrients and improvement in soil aggregation, soil physical, chemical and biological properties, which in turn helped better absorption of nutrients and expression of better biometrical parameters. These provide macro and micronutrients, increase the water holding capacity and aeration for better root formation (Anisa *et al*., 2). The higher yield observed in T_5 and T_6 might be due to the supply of additional nutrients as well as improvement in physical and biological properties of soil by organics and inorganics. Active and rapid multiplication of microorganisms in rhizosphere creating a favourable condition for nutrient availability and uptake, secretion of hormones like IAA, cytokinin, vitamin B12 and GA (Anuradha *et al*., 3) and supply of antibacterial and antifungal compounds and micronutrients might have favoured growth and yield. This suggests that recommended dose of NPK could be replaced with vermicompost along with biofertilizers. It also suggests that application of biofertilizers along with judicious application of inorganic fertilizers and compost could save 25 to 50 per cent of inorganic fertilizers.

Effect of inoculation on population dynamics of *Azotobacter* and PSB in rhizosphere soil of pot grown okra was given in Fig 2. *Azotobacter* population and PSB population density at 90 DAS increased significantly over control under different treatments. However, highest due to inoculation treatments and significantly maximum was found in T_5. These results have close conformity with the findings of Bhushan *et al*. (4). Our results are in confirmative with the findings of Anisa *et al.* (2) who reported that remarkable increase in the PSB population was observed in PSB-inoculated rhizosphere soil on comparision with uninoculated soil. Conjoint application of vermicompost, inorganic fertilizers and biofertilizers resulted in adequate and steady supply of nutrients resulting in better parameters of okra plant in T_5 and T_6.

Bioinoculants help in better translocation of soluble ions from beyond the depletion zone around root, Sajindranath *et al.* (11), thus, improved the availability and uptake of nutrients and also produced growth promoting substances. Benefits of bioinoculants

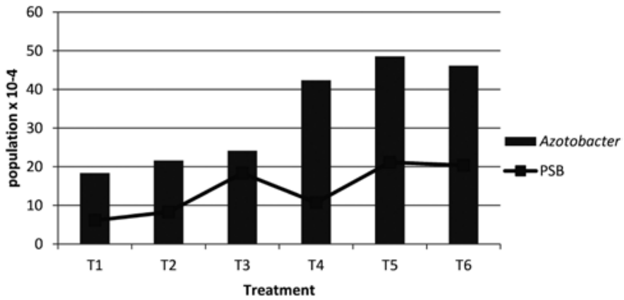

Fig. 2. Effect of bioinoculants and vermicompost on Azotobacter and PSB population after experiment completion.

and vermicompost in improving growth and yield of okra plant have been reported by Sharma *et al.* (13) , Swain *et al.* (15), Gayatri and Reddy (5).

The findings of the present study indicated that combined application of two biofertilizers (*Azotobacter* and PSB) and vermicompost along with reduced dose of NPK was the best with respect to yield signifying that at least a reduction of 25 per cent chemical fertilizers is possible by using biofertilizers. Taking the cost of input into consideration, it may be concluded that inoculation of *Azotobacter*, PSB along with vermicompost and 50% NPK improved the growth traits and enhanced yield of okra. Testing of such effective integrated fertilizers combinations in field scale studies is recommended for sustainable okra production.

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