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Effect of biofertilizers and organic supplements on general and beneficial microbial population in the rhizosphere of black pepper cuttings (*Piper nigrum* L.)

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Abstract
The experiment was conducted at Cardamom Research Station, Pampadumpara (Kerala) during 2017 to study the influence of bio-fertilizers and organic supplements on general and beneficial microbial population in the rhizosphere of black pepper. Promising biofertilizers (*Phosphorous solubilizing bacteria*, *Azospirillum* and PGPR Mix I) and organic supplements (fish amino acid and humic acid) in sole and in combination were applied to the root zone of one month old black pepper cuttings. The results revealed that, application of bio fertilizers and organic supplements significantly increased both beneficial and non-pathogenic general microbial population (bacteria, fungus and actinomycetes) in the rhizosphere. Compared to sole application, combined application recorded maximum CFU g^{-1} soil. Among different treatments, application of *Phosphorous solubilizing bacteria* (5g) along with *Azospirillum* (5g), humic acid (0.2%) and fish amino acid (0.5%) recorded highest population of non-pathogenic general microbial population and was maximum at 45 days after treatment application. The survival of beneficial microbial population in the soil was also high for this treatment**.** The salient research findings from the study revealed the efficacy of biofertilizers and organic supplements in increasing the microbial fauna and improve soil health for better plant growth.

Keywords: Black pepper, biofertilizers, soil microbial population, growth

1. Introduction
Black pepper (*Piper nigrum* L.) is the most important spice of the world referred as 'King of Spices' cultivated for its green and dried berries. Considering its economic importance, the local and global demand is estimated to be increased from the present level. But in India, the productivity of this spice is low owing to several constraints associated with production. Nursery management techniques with more emphasis on soil health and nutrient management are essential for successful black pepper production. Biological indicators such as microbial biomass and microbial diversity are the excellent indicators of soil health (Nielsen and Winding 2002) [1]. The extent of diversity of microorganisms in soil is critical for maintenance of soil health and quality because they are involved in many important soil functions. The soil microbial biomass is the living part of the soil organic matter formed by fungi, bacteria, protozoa, and algae representing an important source of nutrients that may supply plant demands due to its rapid cycling (Sicardi *et al*., 2004) [2]. Soil microbial activity and diversity play important roles in the sustainability by keeping essential functions in soil health, involving carbon and nutrient cycling (Izquierdo *et al*., 2005) [3]. So we have to provide an environment that favors their growth by the use of organic fertilizers in which they can thrive well. Use of biofertilizers for crop production is gaining lot of importance because they are eco-friendly, low cost agriculture input, capable of improving crop yields and quality. Biofertilizers contains large population of agriculturally beneficial microorganisms in a live state and are able to mobilize the nutritionally important elements through biological processes. Bio-fertilizers like *Phosphorous solubilizer*, *Azospirillum* and PGPR Mix I is known to improve the soil fertility, crop productivity as well as establishment of seedling in several crops through atmospheric nitrogen fixation, solubilizing inorganic and organic phosphorus and other nutrients, increasing multiplication rate under the rhizosphere of the plants.

Hence the use of bio-fertilizers along with organic supplements definitely increases microbial fauna resulting in improved soil health and better growth of plants. In this context, the present investigation was undertaken to study the influence of bio-fertilizers and organic supplements on general and beneficial microbial population and their survival in the rhizosphere of rooted cuttings in the nursery.

Materials and methods

The study was conducted at Cardamom Research Station Pampadumpara during March - May 2017. The biofertilizers and organic supplements were evaluated on one month old rooted cuttings of black pepper variety *Karimunda* for their effect on growth enhancement and microbial population. Experiment had eight treatments comprised of control, single and combined application and are given below.

T_1- *Phosphorous solubilizing bacteria* (PSB) @ 10g/plant
T_2-*Azospirillum* @ 10g/plant,
T_3- PGPR Mix I @ 10g/plant,
T_4- Humic acid @ 0.2 %
T_5- Fish amino acid @ 0.5%,
T_6-*Phosphorous solubilizing bacteria* (PSB) @ 5g/plant +*Azospirillum* @5g/plant + Humic acid @ 0. 2 % + Fish amino acid @ 0.5%,
T_7-PGPR Mix I @ 5g/plant + Humic acid @2 % + Fish amino acid @ 0.5%
T_8- Control.

The experiment was laid out in completely randomized design with three replications. Commercial formulations of *Phosphorous solubilizer*, *Azospirillum*, and *PGPR* Mix I was obtained from the Department of Microbiology, College of Agriculture, Vellayani, Kerala. Humic acid containing 12 % potassium humate and fish amino acid prepared as per the procedures followed by local farmers was *in vivo* evaluated under greenhouse condition. Talc based formulation of PSB, *Azospirillum* and PGPR Mix I (5 & 10 g), humic acid @ 0.2 % and fish amino acid @ 0.5% (approximately 100 ml/polybag) were applied to the root zone of pepper cuttings grown in poly bags. The treatments were given twice at 15 days interval.

The rhizosphere soil was collected separately at 15, 30 & 45 days after treatment application for estimation of microbial load. Total microbial load *i.e*., fungal, bacterial and actinomycetes were calculated before and after the experiment. Enhancement in the microbial load of applied inoculum such as *Phosphorous solubilizing bacteria* and *Azospirillum* were recorded after the experiment. Serial dilution plate count technique (Johnson and Curl, 1972) [4] was used for estimation of microbes. One gram of soil was taken and transferred to 100 ml of sterile water to give 10^{-1} dilution. Subsequent tenfold serial dilution up to 10^{-6} was made. One ml each of 10^{-4} for fungi, 10^{-6} for bacteria, actinomycetes, *Phosphorous solubilizing bacteria* and *Azospirillum* population respectively were transferred to sterile petriplates and approximately 15 ml of pre-sterilized, cooled molten media *viz*., Martins Rose Bengal Agar, Nutrient agar, Kenknight & Munaier's Medium, Pikovoskay's medium and *Azospirillum* medium were poured for estimating fungus, soil bacteria, actinomycetes, *Phosphorous solubilizing bacteria* and *Azospirillum* respectively. After solidification of the media, plates were kept for incubation in an inverted position at 30±2ºC for a week time and emerged colonies were counted.

Results and discussion

General microbial population in the rhizosphere of black pepper rooted cuttings.

There is no significant difference in the population of bacteria, fungi and actinomycetes among different treatments before the experiment and they were on par with each other. After treatment application it was found that there is an increase in the microbial count. The total microbial population in the soil is influenced by the application of biofertilizers and organic supplements throughout the experiment period.

The population of non-pathogenic bacteria was increasing at regular intervals and was significantly high 45 days after treatment application (Table.1). The maximum population was recorded for combined application of *Phosphorous solubilizing bacteria* along with *Azospirillum*, humic acid and fish amino acid (115×10^{-6}cfu g^{-1}soil) (Plate.1) followed by combined application of PGPR Mix I along with humic acid and fish amino acid (103×10^{-6}cfu g^{-1} soil) which was higher than the sole application of both *Phosphorous solubilizing bacteria* and PGPR Mix I. The population was increased in soil even after 45 days of treatment application. Increased bacterial population in the rhizosphere of plants is because of the combined inoculation of biofertilizers and organic supplements that would have proliferated in rhizosphere of black pepper. Application of *Azospirillum* sp. to black pepper increased the population of bacteria and a final population of 106 x 10^{-5} cfu was found in the soil compared to un inoculated control (Thankamany *et al*., 2011) [5]. Humic acid and fish amino acid were absorbed directly by the crops and it stimulates the activity of microorganisms. Hence, in all the stages of plant growth, the microbial population with respect to bacteria has given significant values. The above result is in conformity with the findings of Vijendrakumar and Shankarappa (2014) [6] in garden rue (*Ruta graveolence*). They found that among all the inoculants, dual and triple inoculation of bio-fertilizers recorded maximum cfu g^{-1} soil with respect to bacterial population.

In the case of non-pathogenic fungal population(Table.1), the combined application of PGPR Mix I along with humic acid and fish amino acid showed an increase in population count after 30 and 45 days of treatment application (7×10^{-4} and 28×10^{-4} cfu g^{-1} soil respectively) (Plate.2) followed by combined application of *Phosphorous solubilizing bacteria* along with *Azospirillum*, humic acid and fish amino acid (5×10^{-4} and 25.33×10^{-4} cfu g^{-1} soil respectively). The occurrence of beneficial fungi *Trichoderma* was more in these treatments. The incidence of disease was also very less in plants treated with biofertilizers than the control plants. According to Rajasree (1999) [7] nitrogen nutrition was found to promote the soil fungal population in bitter gourd. Biedenbek *et al*. (1996) [8] observed that the soil fungal population increased with increase in the nitrogen level. Dual and triple inoculation of bio-fertilizers recorded maximum cfu g^{-1} soil with respect to fungal population in garden rue was reported by Vijendrakumar and Shankarappa (2014) [6]. Nitrogen nutrition play significant role on fungal population and here the increased population of fungus might be due to application of PGPR mix I and *Azospirillum* and above results are in conformity with the present findings.

The actinomycetes count in the soil was found increasing and is highest for combined application of *Phosphorous solubilizing bacteria* along with *Azospirillum*, humic acid & fish amino acid followed by combined application of PGPR Mix I along with humic acid & fish amino acid at 30 & 45

DAT (43×10^{-6} and 41.33×10^{-6} cfu g^{-1} soil respectively)(Table.1)(Plate.3). The lowest count was observed in untreated plants (12.66×10^{-6}cfu g^{-1} soil). Increased actinomycetes population in the rhizosphere of plants is because of the combined inoculation of biofertilizers especially *Azospirillum* and PGPR Mix I. These nitrogen fixers improves the nutrient availability in the soil ultimately leads to increased microbial activity. Application of humic acid and fish amino acid along with *Phosphorous solubilizing bacteria* also enhanced microbial activity. Nambiar (1994) [9] reported that when nitrogen was applied through organic sources, a hike in the actinomycetes population was noticed. Similar finding was recorded by Rajasree (1999) [7] in bitter gourd.

There was a significant increase in the population of PSB and *Azospirillum* in all the bio-fertilizer inoculated plants than the control (Table.2). The population was increasing and a maximum population of PSB was in soil treated with *Phosphorous solubilizing bacteria* along with *Azospirillum*, humic acid and fish amino acid on 45 days after treatment application(32×10^{-6}cfu g^{-1} soil) (Plate.4) followed by sole application of PSB @ 10g/plant (26.66×10^{-6}cfu g^{-1} soil). Minimum population was recorded in control at different growth stages of plant. In the case of *Azospirillum* sp, the maximum population was found for combined application of *Phosphorous solubilizing bacteria* along with *Azospirillum*, humic acid and fish amino acid (Plate.5) at all the three stages of observation followed by sole application of *Azospirillum* @10g/plant. The population of phosphobacteria and *Azospirillum* was highest in combined application than their sole application and they survive even after 45 days of treatment application. So the increase in rhizosphere microbial population may be attributed to the multiplication of the strains in the rhizosphere, utilizing root exudates produced by the plants (Shivakrishnaswamy, 2001) [10] and synergestic interactions between introduced microbial inoculants and also the native microorganisms in the root zone of crop. The present investigations were in conformity with the results obtained by Rajadurai *et al.* (2000) [11] in african marigold, Vijendrakumar and Shankarappa (2014) [6] in garden rue, Savitha (1996) [12] in chickpea and Yadav and Singh (1990) [13] in sugarcane.

Table 1: Effect of biofertilizers and organic supplements on general microbial population in the rhizosphere of rooted cuttings in black pepper nursery

Treatments	Total bacteria (cfu x 10^{-6})			Total fungi (cfu x 10^{-4})			Total actinomycetes (cfu x 10^{-6})		
	15DAT	30DAT	45 DAT	15DAT	30DAT	45 DAT	15DAT	30DAT	45 DAT
Phosphorous solubilizing bacteria (PSB) - 10g/plant	14.00^{d}	87.00^{b}	89.33^{c}	2.00^{b}	3.00^{c}	23.66^{ab}	25.33^{d}	33.00^{bc}	39.00^{b}
Azospirillum- 10g/plant	28.00^{c}	57.66^{d}	71.33^{d}	2.66^{b}	2.66^{c}	7.66^{c}	21.00^{e}	25.33^{d}	35.66^{c}
PGPR Mix I-10g/plant	24.66^{c}	66.00^{c}	84.00^{c}	1.00^{b}	3.00^{c}	18.66^{b}	29.00^{c}	31.33^{c}	30.33^{d}
Humic acid @ 2 %	16.66^{d}	21.66^{e}	71.66^{d}	1.66^{b}	2.00^{c}	3.33^{c}	20.00^{e}	20.33^{e}	22.00^{f}
Fish amino acid @ 0.5%	15.00^{d}	27.00^{e}	70.66^{d}	2.66^{b}	2.00^{c}	3.00^{c}	19.33^{e}	21.33^{e}	25.33^{e}
Phosphorous solubilizing bacteria (PSB) 5g/plant +*Azospirillum* 5g/plant + Humic acid @ 2 % + Fish amino acid @ 0.5%	62.00^{b}	103.00^{a}	115.00^{a}	1.00^{b}	5.00^{b}	25.33^{ab}	36.00^{a}	40.00^{a}	43.00^{a}
PGPR Mix I 5g/plant + Humic acid @2 % + Fish amino acid @ 0.5%	72.33^{a}	106.33^{a}	103.00^{b}	5.33^{a}	7.00^{a}	28.00^{a}	32.33^{b}	36.66^{ab}	41.33^{ab}
Control	12.66^{d}	11.66^{f}	54.66^{e}	1.00^{b}	1.33^{c}	2.00^{c}	8.66^{f}	10.66^{f}	12.66^{g}
CD (0.05)	5.273	6.683	7.907	2.423	1.997	7.206	3.315	3.961	2.494
CV	9.938	6.435	5.529	63.362	35.522	29.82	7.994	8.387	4.631

Table 2: Effect of biofertilizers and organic supplements on beneficial microbial population in the rhizosphere of rooted cuttings in black pepper nursery

Treatments	Phosphorous solubilizing bacteria (cfu x 10^{-6})			*Azospirillum* (cfu x 10^{-4})		
	15DAT	30DAT	45 DAT	15DAT	30DAT	45 DAT
Phosphorous solubilizing bacteria (PSB) - 10g/plant	10.66^{b}	19.33^{b}	26.66^{b}	6.66^{b}	8.33^{c}	9.66^{c}
Azospirillum- 10g/plant	8.00^{c}	8.66^{d}	10.00^{d}	**9.33^{a}**	18.66^{b}	22.33^{b}
PGPR Mix I-10g/plant	4.00^{e}	8.33^{d}	8.33^{de}	5.33^{b}	5.00^{de}	7.00^{de}
Humic acid @ 2 %	5.66^{d}	8.33^{d}	6.66^{e}	5.33^{b}	6.00^{d}	8.00^{d}
Fish amino acid @ 0.5%	6.66^{d}	11.66^{c}	13.00^{c}	5.00^{bc}	4.33^{e}	5.33^{fg}
Phosphorous solubilizing bacteria (PSB) 5g/plant +*Azospirillum* 5g/plant + Humic acid @ 2 % + Fish amino acid @ 0.5%	16.00^{a}	23.33^{a}	32.00^{a}	10.33^{a}	25.00^{a}	28.33^{a}
PGPR Mix I 5g/plant + Humic acid @2 % + Fish amino acid @ 0.5%	4.00^{e}	5.00^{e}	6.00^{e}	6.00^{b}	4.00^{e}	5.66^{ef}
Control	2.33^{f}	2.66^{e}	3.33^{f}	3.33^{c}	2.00^{f}	4.00^{g}
CD (0.05)	1.279	2.549	2.549	1.839	1.229	1.458
CV	10.264	13.486	11.101	16.527	7.718	7.455

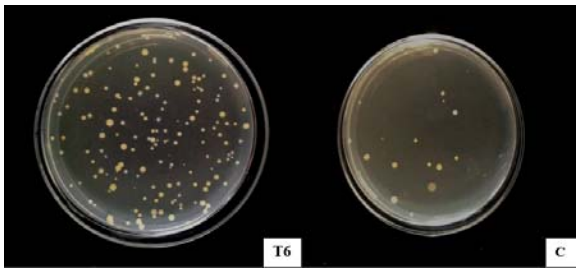

Plate 1: Effect of biofertilizers and organic supplements on general bacterial population 45 DAT

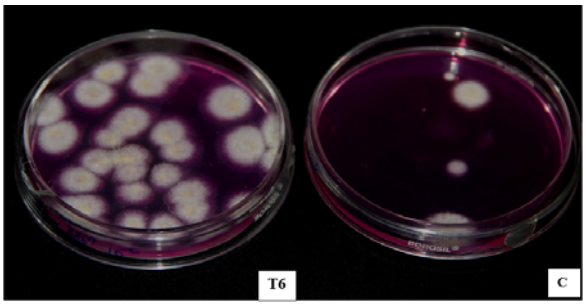

Plate 2: Effect of biofertilizers and organic supplements on general fungal population 45 DAT

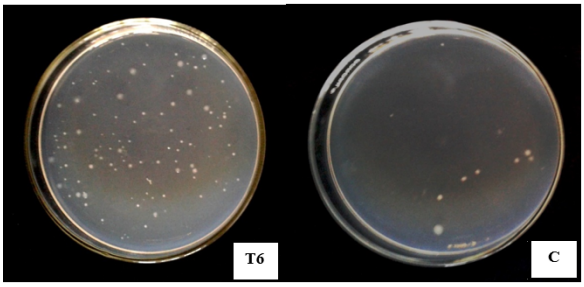

Plate 3: Effect of biofertilizers and organic supplements on Actinomycetespopulation 45 DAT

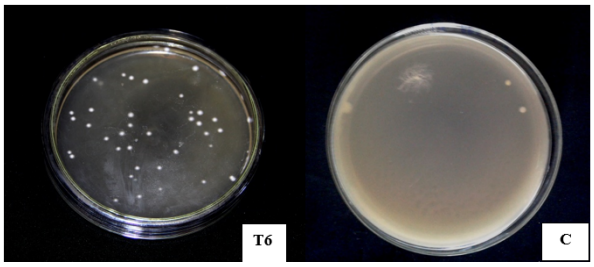

Plate 4: Effect of biofertilizers and organic supplements on Phosphorous solubilizing Bacteria 45 DAT

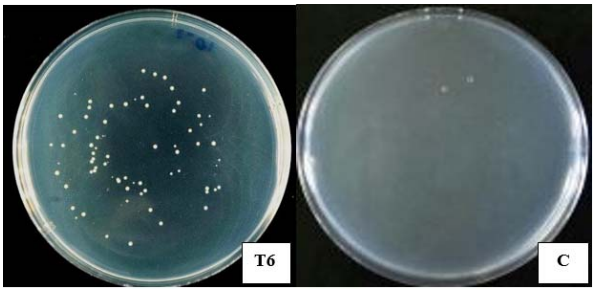

Plate 5: Effect of biofertilizers and organic supplements on *Azospirillum* populationat 45 DAT

Conclusion

The salient findings from the present study revealed the efficacy of biofertilizers and organic supplements in improving microbial fauna and soil health. The general and beneficial microbial population was more for combined application than sole application. The population was highest at 45 days after application of biofertilizers and organic supplements. Application of *Phosphorous solubilizing bacteria* (5g) along with *Azospirillum* (5g), humic acid (2 %) and fish amino acid (0.5%) resulted better growth and was more effective through increasing the general and beneficial microbial population in the rhizosphere of plants.

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