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Field Efficacy of *Pseudomonas fluorescens* and *Azotobacter* sp. for the Management of Root-knot Nematode *Meloidogyne incognita* Infecting Black Gram (*Vigna mungo*)

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A B S T R A C T

The field experiments were conducted at Nematology field, ICR Farm, AAU., Jorhat during 2016 and 2017 to know the efficacy of bioagent, *P. fluorescens* and biofertilizer, *Azotobacter* sp. for that *P. fluorescens* and *Azotobacter* sp. were enriched in the substrates like farmyard manure, vermicompost and enriched compost. After enrichment of *P. fluorescens* and *Azotobacter* sp. were applied singly and in combination at the rate of 1% per hectare (ha). These treatments were compared with untreated control. It was recorded that the combined application of *P. fluorescens* and *Azotobacter* sp. found to be the best than individual application of *P. fluorescens* and *Azotobacter* sp. in all the tested substrates. However, among the tested substrates, the enriched compost found to be the best in single as well as in the combined application of *P. fluorescens* and *Azotobacter* sp. The pooled data showed that combined application of *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1%/ha not only an improved the soil organic carbon, soil microbial biomass carbon content in the soil but also either maximizes the plant growth parameters like shoot height (cm), fresh and dry shoot weight (gm), root length (cm), fresh and dry root weight (gm), number of nodules per root system of black gram or minimizes the nematode infection *i.e.* number of galls and egg masses per root system of black gram and nematode population in the soil, as a result, increases the yield of black gram than rest of the treatments under field condition during 2016 and 2017.

Introduction

Pulses are the important sources of proteins, vitamins, minerals and are popularly known as “Poor man’s meat” and “rich man’s vegetable”, contribute significantly to the nutritional security of the country. In pulse crops, black gram or urdbean (*Vigna mungo* L.) is one of the most important pulse crops belonging to the family Leguminosae and subfamily papilionaceae. This crop is grown in cropping systems as a mixed crop, catch crop, sequential crop besides growing as sole crop under residual moisture conditions after the harvest of another summer crop. Its seeds are highly nutritious with protein (25-26%), carbohydrates (60%), Fat (1.5%), minerals, amino acid and vitamins. Seeds are used in the preparation of many popular dishes like dosa, idli, vada etc. In India, the production of

black gram reached 2.93 Million tones and exhibit 21.30 per cent of growth rate in the total pulse production during 2016-17 (Anonymous, 2017). India is the largest producer, 25% of the world's production, and 27% consumer of total pulses of the world. However, under the green revolution, the pulses are left for the improvement. The greatest challenge for the Indian agriculture is to provide an adequate food to the burgeoning population in order to combat the hunger and malnutrition (Anonymous, 2017). The domestic production of the pulse is often less than the estimated demand *i.e*. 23- 24 million tons (Anonymous, 2017). However, there are many challenges like pest and diseases attack the pulse crops and weigh down their production especially black gram. Among the pest and diseases, plant-parasitic nematodes like *Meloidogyne incognita* emerged as a serious pest of black gram in Assam. This nematode causes root-knot diseases in the black gram and reduced the 15-25 per cent of yield; however, in the severe cases, it can be up to 75 per cent (Sasser *et al.,* 1983). In Assam, yield losses in black gram due to *M. incognita* were recorded to the tune of 13.19-23.50 per cent (Anonymous, 2011) but during the last few decades, the production and yield of black gram declined and expected target could not be achieved. Nematicides of chemical origin are usually used for the effective control of this pest. The haphazard use of such chemical nematicides mete out the negative effect on the environment as a result killed the beneficial microorganisms in the soil, contaminate groundwater and create health hazards to the human and animals moreover its residual effects leading to the biomagnifications in the food web. To avoid such environmental effects it is better to adopt the traditional control method and it is considered as a part of nematode-management programs (Oka *et al.,* 2010). Application of organic amendments *viz*., animal and green manures, compost, nematicidal plants and proteinous wastes *etc*., are used for the nematode control but the control efficiency is not always satisfactory (Muller and Gooch, 1982). The application of antagonistic microorganism along with organic amendments generally reduced the nematode multiplication in the soil and enhances the yield of the crop (Sidhu, 2018). However, amongst the antagonistic microorganism the rhizobacteria exhibit swift rate of multiplication than others and showed greater tendency of root colonization and compete with other pathogen for the space and nutrition as well as causes either enhancement of decomposition and mineralization of applied amendments that leads to an increase in the organic carbon and microbial biomass content in the soil (Gaind *et al.,* 2006, Dutta and Kundu 2012, Das and Singh, 2014). Such bacteria in legume crops enhance the root nodulation which helps in the fixation of more atmospheric nitrogen in the soil and provide the better plant growth.

The different substrates of organic amendments contain different composition and which determines the growth of the antagonistic microorganism. Sometimes, it is observed that instead of the application of single beneficial microorganism it is better to apply two or more microorganisms in combination (Soliman *et al.,* 2011). However, among the beneficial microorganisms, the plant growth promoting rhizobacteria (PGPR) was put forward for the first time in the early twentieth century and become the noteworthy module for the modern agricultural practice in the developed as well as developing countries (Bashan, 1998). The inoculants of PGPR are an important component of the integrated plant nutrient management for sustainable agriculture and hold a great pledge for the improvement of crops. The plant growth promoting rhizobacteria like *Pseudomonas*, *Azospirillium*, *Azotobacter* and *Bacillus etc*., play a vital role in the enhancement of plant

growth (Joseph *et al.,* 2007). Furthermore, they are also posse's direct and indirect mechanisms. A direct mechanism includes augment the production of phytohormones, solubilization of phosphates (Antoun and Kloepper, 2001), an increase in the uptake of iron for the production of siderophores (Chaiharn *et al.,* 2009) and synthesis of HCN (Reetha *et al.,* 2014) along with volatile metabolites that causes break down of life cycle of pathogen while indirect mechanisms consist of inducing systemic resistance against pathogen in plant (Dowling and Gara, 1994 and Nandakumar *et al.,* 2001). However, such type of mechanism is dependent on the substrates which are used for the growth of beneficial microorganisms and it determines their efficacy in respect of crop productivity and reduction in the inoculums density of the pathogen. Keeping this in view an experiment was conducted to find out the best treatment combination for the management of *Meloidogyne incognita* on black gram under field condition.

Materials and Methods

Source and maintenance of *Pseudomonas fluorescens* and *Azotobacter* sp

The liquid formulation of *P. fluorescens* were obtained from the Department of Plant Pathology, AAU, Jorhat-13 and solid formulations of *Azotobacter* sp. were obtained from the Department of Soil science, AAU, Jorhat.

Source of organic amendments

The organic amendments like farm yard manure (FYM) and vermicompost were obtained from the Department of Soil Science, AAU, Jorhat and enriched compost was obtained from the biofertilizer production unit, DBT, AAU, Jorhat.

Source and sterilization of seed

Black gram seeds of the variety PU- 31 (susceptible to *M. incognita*) were obtained from the Krishi Vigyan Kendra, Kamrup, Guwahati. Seeds were washed with clean tap water and were surface sterilized with 0.1 per cent mercuric chloride solution for 1-2 minutes and then washed with sterile water. The wet seeds were then dried in air.

Selection of site

The field experiments were conducted at Nematology field, ICR Farm, AAU, Jorhat during 2016 and 2017 to know the efficacy of, *P. fluorescens* and, *Azotobacter* sp. For this a rectangular medium upland plot of land infested by *M. incognita* was selected. Before layout of the experiment, initial nematode population in the experimental field was determined by collecting 10 soil samples consisting of 7-10 sub samples. The average population of the samples was taken as initial population.

Enrichment of *P. fluorescens* and *Azotobacter* sp

Enrichment of *P. fluorescens* and *Azotobacter* sp. were made in the farm yard manure (FYM), vermicompost and enriched compost @ 1% and covered with gunny sheet and kept as such for their growth for 15 days.

Occasional sprinkling of water was made for moistening. Before application they were mixed thoroughly and applied as per the rates mentioned in the treatments.

Layout of experiment and treatment details

The experiment was laid in the randomized block design with three replications. The details of the treatments are as follows:

T_1: *Pseudomonas fluorescens* enriched with FYM @ 1% (1ton FYM/ha)

T_2: *Pseudomonas fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha)

T_3: *Pseudomonas fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha)

T_4: *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha)

T_5: *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha)

T_6: *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha)

T_7: *Pseudomonas fluorescens and Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha)

T_8: *Pseudomonas fluorescens and Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha)

T_9: *Pseudomonas fluorescens and Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and

T_{10}: Untreated check.

Cultural operations

The experimental field was given thorough ploughing followed by harrowing and levelling. The experiment was laid out as per design in the experiment field measuring 284 m^2 (35.5×8) m^2. Field was divided into three blocks; each block was divided into 10 plots measuring 6 m^2 (3m x 2m) each arranged in rows. Each plot was separated with a spacing of 0.5m which accommodated a channel in the middle and the inter-block spacing was also 0.5m.

Sowing of seeds

The treated seeds were sown in line @ 25 kg/ha by maintaining the spacing of 30cm x 10cm at a depth of 5 cm. After sowing, seeds were covered with thin layer of soil.

Weeding

In both the years, hand weeding was done after 15 days interval from the date of sowing.

Harvesting

Harvesting was done at maturity of the crop.

Observations

Shoot length (cm)

The main shoot was measured in centimeter from the ground level up to tip of the longest leaf at harvesting stage of the crop. For this, 10 plants/ plot were selected at random and average was calculated.

Root length (cm)

The main root length was measured in centimeter from the ground level up to tip of the longest root at harvesting stage of the crop. For this, 10 plants/ plot were selected at random and average was calculated.

Fresh shoot and root weight (gm)

The fresh shoot and root weight per plant was measured in grams for 10 plants randomly selected from each plot. These plants were weighed on the weigh balance at Nematology laboratory and average was calculated.

Dry shoot and root weight (gm)

For recording dry weights, shoots and roots were separately cut into small pieces and kept

in an oven running constantly at 60ºC at Nematology laboratory. The materials were weighed at every 24 hrs interval until a constant weight was obtained.

Number of nodules per root system

The number of nodules per root system was measured for 10 plants randomly selected from each plot and average was calculated.

Number of galls and egg masses per root system

The number of galls and egg masses per root system was measured for 10 plants randomly selected from each plot and average was calculated.

Extraction of nematode from soil

Extraction of nematode from the soil was done by the modified Cobb's sieving and decanting technique (Christie and Perry, 1951) where a series of sieves (20, 60, 150, 250 and 350 mesh) were used. Each soil sample comprised of several sub-samples of soil which was thoroughly mixed and 250ml of soil was drawn from the homogenous mixture for processing. The drawn sample was put in a plastic bucket (bucket-1) and added one litre of water and clumps were broken gently. The suspension was stirred properly and allowed to stand for about 10-15 seconds so that the heavy soil particles settles at the bottom of the bucket.

Then the suspension was poured into bucket-2 through 20 mesh sieve to remove stones and other coarse materials. Then the soil suspension was stirred and allowed to settle for about 10 sec and poured into bucket 1 through 60 mesh sieve, leaving the heavy particle in the bucket. Similar way the suspension was then pass through 150, 250 and 350 mesh sieves and the residue from each sieve was collected in separate beakers. The residues were mixed and cleaned under running tap water using 350 mesh sieves. It is then poured gently over a double layered tissue paper stretched over an aluminium wire gauge and kept on a Petridish containing filter water. Care was taken that the aluminium wire gauge should touch the water level of the Petridish. The nematode suspension was collected after 24 hours and examined under the stereoscopic binocular microscope. The nematode population was counted and recorded by taking 1 ml of aliquot in a Hawkshley nematode counted dish. The process was repeated for three times for an average count and the calculation was made for the entire volume of nematode suspension.

Final nematode population

For recording the final nematode population in soil, five sub-samples from each plot were collected randomly to make a composite sample of about 1kg, mixed thoroughly in the laboratory and 200 cc of soil was processed by modified Cobb's sieving and decanting technique (Christie and Perry, 1951) for extraction of nematodes.

Determination of organic carbon by wet digestion (%)

Organic carbon in the soil (1g) was oxidized with a mixture of 1 N $K_2Cr_2O_7$ (Potassium dichromate), Conc. H_2SO_4 (Sulphuric acid) and Conc. H_3PO_4 (Ortho phosphoric acid) for reduction of $K_2C_{r2}O_7$ by organic compounds as per the method described by Walkey (1947). The unused $K_2C_{r2}O_7$ is back titrated with ferrous ammonium sulphate (FAS) [(NH_2) SO_4 FeSO4 6 H2O] (0.5M) using diphenylamine indicator till the colour changed from violet blue to green. Blank contained no soil but all reagents treated similarly for calculation.

Oxidizeable organic carbon (TOC%) were calculated using the following formula.

$$\% \text{ Oxidizeable organic carbon (W/W)} = \frac{(Vb - Vs) \times 0.3 \times M}{Wt}$$

% TOC (W/W) = 1.334×% Oxidizeable OC,

Where M=Molarity of ferrous ammonium sulphate (0.5M), Vb= Volume of FAS for blank (ml), Vs= Volume FAS for sample (ml), Wt= Weight of soil (g), $0.3=3\times10^{-3}\times100$ where 3 is equivalent weight of C, Oxidizeable organic carbon (TOC %) were calculated using the following formula.

Determination of soil microbial biomass carbon

Soil microbial biomass carbon (µg g-^{1}soil 24-^{1}) was determined after harvesting of crop by chloroform fumigation extraction technique following the method of Vance *et al.*, (1987). Moist samples (10g soil) in 50ml glass beakers were placed in a desiccators and a vial of soda lime. A beaker containing 50 ml ethanol free $CHCl_3$ (Chloroform) and the desiccators evacuated until the $CHCl_3$ has boiled vigorously for 2 minutes. The desiccators incubated in dark at 25°C for 24 hr. After fumigation $CHCl_3$ was removed by repeated evacuation, the soil were then extracted with 25ml 0.5 Molar K_2SO4 (5:1) for 30 min by oscillating shaking at 200 rpm and then filtered through a Whatman No 42 filter paper. Controls were prepared by extracting soils without fumigation. Organic carbon content in the extracts was measured with dichromate (66.7mM) and 15ml of the digestion mixture (2:1 conc.H_2SO_4: H_3PO_4 (v/v) was added. The mixture was gently refluxed for 30 minutes, allowed to cool and diluted with 20ml distilled water. The excess $K_2Cr_2O_7$ (Potassium dichromate) was measured by back titration with FAS (40.0mM) using 1.10 phenanthroline-Ferrous sulphate complex (25mM) solution as an indicator. MBC was calculated from the differences in extractable OC between the fumigated and non fumigated soil and expressed as µg g-^{1}on dry weight basis as MBC (µg g-^{1})=Ec Where, Ec= [(OC extracted from fumigated soil)-(OC extracted from non fumigated soil)], kEC=0.38 (Vance *et al.*, 1987).

Statistical analysis

The data were analyzed by using WASP - Web Agri Stat Package 2.0 version software. Duncan's Multiple Range Test (DMRT) was conducted to determine the significance of treatments.

Results and Discussion

Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the plant growth parameters of black gram infected by *M. incognita*

The data on the plant growth parameters of black gram in 2016 and 2017 as also the pooled data of both the years have been presented in Table 1 and 2 Figure 1 and 2. The pooled data showed that maximum shoot height (cm), fresh and dry shoot weight (gm), root length (cm), fresh and dry root weight (gm) was recorded in the treatment T_9 *i.e.*, *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost and it was significantly different from rest of the treatments. This was followed by the treatments T_8 *i.e.*, *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost and T_7 *i.e.*, *P. fluorescens* and *Azotobacter* sp. enriched with FYM, respectively and both were found to be at par with each other except in the fresh and dry root weight (gm) but differed significantly from the rest of the treatments. Minimum shoot height (cm), fresh and dry shoot weight

(gm), root length (cm), fresh and dry root weight (gm) were recorded in the treatment T_{10} *i.e.*, untreated control. The pooled data showed that combined application of *P. fluorescens* and *Azotobacter* sp. found to be the best than individual application of *P. fluorescens* and *Azotobacter* sp. in all the tested substrates. However, among the substrates, enriched compost found to be the best in single as well as combined application of *P. fluorescens* and *Azotobacter* sp. Similar type of observations also recorded by Jonathan *et al.*, (2009) who recorded that application of native isolates *viz*., *P. fluorescens* (Pfbv22) significantly increases the plant growth parameters *viz.*, plant height, shoot weight, root length and root weight of tomato. Soliman *et al.*, (2011) observed the better plant growth parameters like plant height, shoot weight (fresh and dry), root length and root weight (fresh and dry) of *Acaia famensiana* infected by *M. incongita* in the *P. fluorescens* treated soil as compared to *A. chroococcum* treated soil during 2009 and 2010. Likewise, Anwar-ul-Haq *et al.*, 2011 also reported that single application as well as the combined application of *P. fluorescens* and *A. chroococcum* significantly increased the plant growth parameters of tomato as compared to the untreated control. Further, they reported that *P. fluorescens* was found to be better than *A. chroococcum* and finally concluded that combined application of *P. fluorescens* and *A. chroococcum* synergized the plant growth parameters of tomato as compared to the single application of *P. fluorescens* and *A. chroococcum*, respectively. The reason behind an increase in the plant growth parameters of black gram is might be due to the soil application of phosphate solubilizing microorganisms (PSM) that significantly increases their population when they are enriched with phosphorus enriched compost than ordinary compost. The effect of compost or vermicompost on the plant growth depends on the source of material used for the compost preparation, the role of microorganisms and nutrient content (Jack and Thies, 2006). Unlike, FYM and vermicompost, enriched compost (Biofertilizer production unit, DBT, AAU, Jorhat) contain total N (2.10%), total P (1.20%), total K (2.30%), organic carbon (21.45%), rock phosphate (10 %), humic acid (13.50%), fulvic acid (7.95%), Fe(144 ppm), Mn (259 ppm), Cu (4 ppm), Zn (500 ppm), B (116.60 ppm), Ca (0.60 ppm), Mg (0.24 ppm) and S (0.14 ppm) at PH 7.5 that facilitate the better growth of the microorganisms *i.e. P. fluorescens* and *Azotobacter* sp. However, rhizobacteria like *P. fluorescens* and *A. chroococcum* have the ability to increase the availability of N and P content in the soil and make it available to the plants **(**Kavitha and Subramanian, 2007). The application of biofertilizers to the plant and/or incorporated in the soil are known to induce resistance against nematodes that causes in an improvement of plant growth (Durrant and Dong, 2004). Further, they also increases the growth related substance like, indole acetic acid (Okan and Hadar, 1987), B vitamins, nicotinic acid, pantothenic acid, biotin as well as compounds heteroauxin and gibberellins (Tilak, 1991) that help in the increase in the plant growth parameters. Such type of mechanism might be possessed by the tested native *P. fluorescens* and *Azotobacter* sp. which causes the improvement in the plant growth parameters of black gram than the rest of the treatments under filed condition during 2016- and 2017.

Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the number of nodules per root system of black gram infected by *M. incognita*

The data on the number of nodules per root system in 2016 and 2017 as also the pooled data of both the years have been presented in Table 3 and Figure 3. The pooled data showed

that maximum number of nodules (43.17) was recorded in the treatment T_9 *i.e. P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1%/ha and it was significantly different from rest of the treatments. This was followed by the treatment T_8 *i.e P. fluorescens* and *Azotobacter* sp. enriched in vermicompost @ 1%/ha and then T_7 *i.e P. fluorescens* and *Azotobacter* sp. enriched in FYM @ 1%/ha, respectively and they were found to be at par with each other and significantly different from the rest of the treatments. Further, the pooled data showed that the lowest number of nodules (14.33) per root system was recorded in the treatment T_{10} *i.e* untreated control. However, among the tested substrates, enriched compost found to be the best in single as well as combined application followed by the vermicompost and FYM, respectively.

The combined application of *P. fluorescens* and *Azotobacter* sp. with FYM/vermicompost/ enriched compost were found to be better than when they applied singly. Pooled data revealed that the combined application of *P. fluorescens* and *Azotobacter* sp. found to be the best than individual application of *P. fluorescens* and *Azotobacter* sp. in all the tested substrates. However, among the substrates used, enriched compost found to be the best in single as well as the combined application of *P. fluorescens* and *Azotobacter* sp. (Table 4). Similar, type of observations also recorded by Soliman ***et al.,*** (2011) who evaluated the soil application of *P. fluorescens* resulted in more number of nodules per root system of *Acaia famensiana* than *A. chroococcum* during 2009 and 2010 in *M. incognita* infested soil. The cause of reduction of the nodulation in legume plant infected by root-knot nematode might be due to a competition phenomenon that may exist between nematode larvae and root-nodule bacteria (Epps and Chambers, 1962; Ichinohe, 1961 and Malek and Jenkins, 1964) and the application of rhizobacteria in soil are known to reduce the nematodes population (Durrant and Dong, 2004). Kucey *et al.,* (1989) showed that phosphorus-containing biofertilizers could help in an increase in the availability of phosphates which accumulated in the soil and able to an augment the plant growth by increasing biological nitrogen fixation. However, the application of phosphorus biofertilizers like *P. fluorescens* (Khan *et al.,* 2012) and *Azotobacter* sp. (Martinez-Toledo *et al.,* 1988) increased IAA in plant and plays a major role in the development of rhizobial nodules (Glick, 1955) on the plant. However, these types of mechanisms might be operative in the present investigation in recording more number of nodules per root system due to the application of *P. fluorescens* and *Azotobacter* sp. on black gram infected by *M. incognita*.

Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on organic carbon and microbial biomass carbon content in the soil

The data on the organic carbon and microbial biomass carbon content in the soil in 2016 and 2017 as also the pooled data of both the years have been presented in Table 5 and Figure 4 and 5. The pooled data showed that the maximum organic carbon content in the soil (1.47 g/kg of soil) was recorded in the treatment T_9 *i.e. P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1%/ha and was found to be significantly different from the rest of the treatments. However, all the treatments were found to be statistically different from each other and the minimum organic carbon content in the soil (1.02 g/kg of soil) was recorded in the treatment, untreated check (T_{10}). As far as the soil microbial biomass carbon content in the soil is concern, the maximum soil microbial biomass carbon content in the soil (497.50 $\mu g g^{-1}$soil $24h^{-1}$) was recorded in the treatment T_9 *i.e., P. fluorescens* and *Azotobacter* sp.

enriched with enriched compost @ 1%/ha which was found to be statistically different from the rest of the treatments. This might be due to the higher availability of carbon in the applied substrate *i.e* enriched compost which improved the microbial and enzymatic activities in the soil (Gogoi, 2016). However, the minimum soil microbial biomass carbon content in soil (243.83 $\mu g g^{-1}$soil $24h^{-1}$) was recorded in the treatment, untreated check (T_{10}) due to the low quantity of organic matter present in the native soil and its utilization by crops and other soil microbes which resulted in the exhaustion of organic carbon in the soil.

In the present investigation, the treatments with bioagents and biofertilizers increased the organic carbon and soil microbial biomass carbon content in the soil as compared to the untreated control. However, dual application of *P. fluorescens* and *Azotobacter* sp. showed better results in the improving of organic carbon and soil microbial biomass carbon content in the soil as compared to their single application.

Further, it was observed that when the bioagents and biofertilizers were applied in soil enriching in enriched compost showed better results in terms of increase in the organic carbon and soil microbial biomass carbon content in the soil. Similarly, Dutta and Kundu (2012), Das and Singh (2014) reported that organic manures mixed with PGPR significantly increased the organic carbon in the soil as compared to the sole application of manures**.**

Gaind *et al.,* (2006) reported that application of rock phosphate in manure significantly increased the organic carbon and microbial biomass carbon content in the soil, thus confirm the result of the present investigation where the *P. fluorescens* and *Azotobacter* sp. were enriched in enriched compost that contains rock phosphate significantly enhance the organic carbon and microbial biomass carbon content in the soil. Further, the incorporation of organic materials provides soil organisms with a new energy source that result an increase in the diversity and activities of soil microbes, as a result, increased the organic carbon and microbial biomass carbon content in the soil.

Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the multiplication of *M. incognita* in black gram

The pooled data (Table 4 and Figure 3) showed that minimum number of galls per root system, number of egg masses per root system and final nematode population in the soil were recorded in the treatment T_9 i.e., *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost and it was significantly different from rest of the treatments. This was followed by the treatments T_8 *i.e*., *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost and T_7 *i.e*., *P. fluorescens* and *Azotobacter* sp. enriched with FYM, respectively. Pooled data revealed that combined application of *P. fluorescens* and *Azotobacter* sp. found to be the best than individual application. In the present investigation it observed that an individual application of *P. fluorescens* found to be the best than application of *Azotobacter* sp.

However, among the tested substrates, enriched compost found to be the best in single as well as combined application of *P. fluorescens* and *Azotobacter* sp. in reducing the number of galls per root system, number of egg masses per root system and final nematode population in the soil. Similar, type of observations was also recorded by Jonathan *et al.,* (2009) who recorded that application of native isolates *viz*., *P. fluorescens* (Pfbv22) significantly reduced the nematode infection and multiplication on tomato.

Table.1 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the shoot growth parameters of Black gram infected by *M. incognita* under field condition

Treatment	Shoot length (cm)			Fresh shoot weight (gm)			Dry shoot weight (gm)		
	2016	2017	Pool	2016	2017	Pool	2016	2017	Pool
T_1	22.67^{cdef}	23.00^{def}	22.83^{de}	18.07^{cde}	19.50^{de}	18.78^{def}	2.93^{e}	4.20^{de}	3.56^{e}
T_2	25.07^{cd}	26.00^{cd}	25.53^{cd}	20.63^{bcd}	21.17^{cd}	20.90^{ef}	3.33^{d}	5.00^{d}	4.16^{d}
T_3	25.67^{c}	29.00^{bc}	27.33^{c}	22.10^{bc}	23.13^{bcd}	22.62^{bcd}	5.27^{c}	7.73^{b}	6.50^{b}
T_4	20.67^{ef}	21.33^{ef}	21.00^{ef}	13.80^{ef}	15.63^{ef}	14.72^{fg}	2.23^{e}	3.30^{e}	3.88^{de}
T_5	21.33^{def}	22.67^{def}	22.00^{def}	15.47^{def}	18.47^{def}	16.97^{efg}	2.47^{e}	3.90^{g}	4.42^{d}
T_6	24.33^{cde}	25.33^{cde}	24.83^{cd}	20.23^{bcd}	22.00^{cd}	21.12^{cde}	3.67^{d}	6.33^{c}	5.00^{c}
T_7	30.17^{b}	33.67^{b}	31.92^{b}	24.13^{b}	25.00^{bc}	24.57^{bc}	6.07^{b}	7.13^{b}	6.60^{b}
T_8	32.33^{b}	34.33^{b}	33.33^{b}	25.33^{b}	27.00^{b}	26.17^{b}	7.17^{b}	8.00^{b}	7.58^{b}
T_9	38.00^{a}	40.33^{a}	39.17^{a}	31.20^{a}	32.37^{a}	31.78^{a}	8.30^{a}	9.23^{a}	8.76^{a}
T_{10}	20.67^{ef}	20.33^{ef}	20.50^{ef}	15.43^{f}	14.03^{f}	13.23^{g}	2.23^{e}	2.73^{ef}	2.48^{f}
S. Ed±	2.19	2.55	1.80	2.47	2.27	2.32	0.55	0.60	0.52
CD at 0.05	4.28	5.37	3.78	5.19	4.77	4.88	1.10	1.20	1.05

Mean with different letters in the column are significantly different from each other based on Duncan's Multiple Range Test (C.D.at 0.05)

T_1- *Pseudomonas fluorescens* enriched with FYM @ 1% (1ton FYM/ha), T_2- *P. fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha), T_3- *P. fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha), T_4- *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_5- *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_6-*Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha), T_7 - *P. fluorescens* and *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_8 - *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_9 - *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and T_{10}- Untreated check

Table.2 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the root growth parameters of black gram infected by *M. incognita* under field condition

Treatments	Root length (cm)			Fresh root weight (gm)			Dry root weight (gm)		
	2016	2017	Pool	2016	2017	Pool	2016	2017	Pool
T_1	18.87^{cde}	19.37^{def}	19.12^{def}	9.00^{de}	10.00^{efg}	9.50^{def}	3.60^{cd}	4.26^{cd}	3.93^{cd}
T_2	19.63^{cde}	21.30^{cdef}	20.47^{cde}	9.67^{cde}	11.00^{def}	10.33^{d}	3.53^{cd}	4.00^{cd}	3.76^{d}
T_3	21.80^{bc}	22.53^{bcd}	22.17^{bcd}	10.33^{cd}	11.67^{de}	11.00^{de}	3.36^{de}	3.47^{de}	3.42^{de}
T_4	16.30^{def}	17.60^{fg}	16.95^{fg}	8.67^{de}	9.00^{fg}	8.83^{ef}	2.63^{d}	2.87^{e}	2.75^{e}
T_5	15.87^{ef}	18.50^{efg}	17.18^{efg}	9.67^{cde}	10.33^{defg}	10.00^{de}	3.03^{d}	3.83^{cde}	3.43^{de}
T_6	19.73^{cd}	21.90^{bcde}	20.82^{cd}	10.00^{cd}	12.33^{d}	11.17^{d}	3.43^{cd}	3.63^{cde}	3.53^{d}
T_7	23.67^{b}	24.17^{bc}	23.92^{bc}	12.33^{c}	14.67^{c}	13.50^{c}	4.30^{c}	4.70^{c}	4.50^{c}
T_8	24.33^{b}	25.23^{b}	24.78^{b}	15.50^{b}	17.83^{b}	16.67^{b}	5.40^{b}	5.83^{b}	5.62^{b}
T_9	29.00^{a}	31.00^{a}	30.00^{a}	19.17^{a}	20.50^{a}	19.83^{a}	7.03^{a}	7.33^{a}	7.18^{a}
T_{10}	14.00^{f}	15.33^{g}	14.67^{g}	7.00^{e}	8.33^{h}	7.67^{f}	1.13^{e}	1.40^{f}	1.22^{f}
S. Ed±	1.81	1.80	1.66	1.27	1.07	1.03	0.50	0.51	0.34
CD at 0.05	3.82	3.78	3.49	2.68	2.26	2.06	1.05	1.07	0.71

Mean with different letters in the column are significantly different from each other based on Duncan's Multiple Range Test (C.D.at 0.05)

T_1- *Pseudomonas fluorescens* enriched with FYM @ 1% (1ton FYM/ha), T_2- *P. fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha), T_3- *P. fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha), T_4- *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_5- *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_6-*Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha), T_7 - *P. fluorescens* and *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_8 - *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_9 - *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and T_{10}- Untreated check

Table.3 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the number of nodules per root system of black gram infected by *M. incognita* under field condition

Treatment	Number of nodules per root system		
	2016	2017	Pooled
T_1	15.00 ^{e}	20.33 ^{def}	17.67 ^{ef}
T_2	17.33 ^{de}	22.33 ^{de}	19.83 ^{de}
T_3	24.33 ^{d}	29.33 ^{c}	26.33 ^{c}
T_4	16.33 ^{f}	15.00 ^{g}	15.66 ^{h}
T_5	15.33 ^{e}	17.00 ^{efg}	15.67 ^{fg}
T_6	21.33 ^{cd}	25.33 ^{cd}	23.33 ^{cd}
T_7	27.33 ^{bc}	37.00 ^{b}	32.17 ^{b}
T_8	30.00 ^{b}	40.00 ^{b}	35.00 ^{b}
T_9	38.67 ^{a}	50.67 ^{a}	43.17 ^{a}
T_{10}	12.67 ^{e}	16.00 ^{fg}	14.33 ^{g}
S. Ed±	1.12	1.50	1.32
CD at 0.05	2.25	3.12	2.68

Mean with different letters in the column are significantly different from each other based on Duncan's Multiple Range Test (C.D.at 0.05)

T_{1-} *Pseudomonas fluorescens* enriched with FYM @ 1% (1ton FYM/ha), T_2- *P. fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha), T_3- *P. fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha), T_4- *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_5- *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_6-*Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha), T_7 - *P. fluorescens* and *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_8 - *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_9 - *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and T_{10}- Untreated check

Table.4 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the multiplication of *M. incognita* in black gram under field condition

Treatment	Number of galls per root system			Number of egg masses per root system			Final Nematode Population (200cc soil)		
	2016	2017	Pool	2016	2017	Pool	2016	2017	Pool
T_1	63.33 ^{B}	62.00 ^{b}	62.67 ^{b}	35.33 ^{d}	33.67 ^{cd}	34.50 ^{de}	280.00 ^{bc}	270.00 ^{bc}	275.00 ^{bc}
T_2	58.33 ^{bc}	56.67 ^{bc}	57.50 ^{c}	34.33 ^{de}	30.00 ^{de}	32.66 ^{e}	270.00 ^{bcd}	260.00 ^{bcd}	265.00 ^{cd}
T_3	52.00 ^{de}	50.00 ^{de}	51.00 ^{de}	37.00 ^{cd}	32.00 ^{d}	34.50 ^{de}	263.33 ^{cd}	256.67 ^{cd}	260.00 ^{de}
T_4	64.33 ^{b}	62.67 ^{b}	63.50 ^{b}	46.33 ^{b}	44.00 ^{b}	45.16 ^{b}	286.67 ^{b}	280.00 ^{b}	283.33 ^{b}
T_5	59.67 ^{bc}	57.33 ^{bc}	58.50 ^{c}	41.33 ^{bc}	38.67 ^{bc}	40.00 ^{c}	260.00 ^{d}	246.67 ^{de}	253.33 ^{def}
T_6	55.33 ^{cd}	54.00 ^{cd}	54.67 ^{cd}	39.33 ^{cd}	36.00 ^{cd}	37.66 ^{cd}	253.33 ^{de}	243.33 ^{de}	248.33 ^{efg}
T_7	50.00 ^{de}	47.00 ^{e}	48.50 ^{e}	30.00 ^{ef}	25.00 ^{ef}	27.50 ^{f}	243.33 ^{e}	240.00 ^{de}	241.67 ^{fg}
T_8	48.67 ^{e}	50.00 ^{de}	49.33 ^{e}	27.00 ^{f}	23.00 ^{f}	25.00 ^{f}	240.00 ^{e}	236.67 ^{e}	238.33 ^{g}
T_9	39.00 ^{f}	37.33 ^{f}	38.17 ^{f}	21.00 ^{g}	18.00 ^{g}	19.50 ^{g}	220.00 ^{f}	216.67 ^{f}	218.33 ^{h}
T_{10}	75.33 ^{a}	78.00 ^{g}	76.67 ^{g}	55.67 ^{a}	63.00 ^{a}	59.33 ^{a}	366.67 ^{a}	346.67 ^{a}	356.67 ^{a}
S. Ed±	0.54	0.35	0.45	0.55	1.26	1.35	1.74	1.80	1.60
CD at 0.05	1.11	0.72	0.93	1.13	2.53	2.72	3.52	3.64	3.23

Mean with different letters in the column are significantly different from each other based on Duncan's Multiple Range Test (C.D.at 0.05). T_1- *P. fluorescens* enriched with FYM @ 1% (1ton FYM/ha), T_2- *Pseudomonas fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha), T_3- *P. fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha), T_4- *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_5- *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_6-*Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha), T_7 - *P. fluorescens* and *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_8 - *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_9 - *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and T_{10}- Untreated check

Table.5 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the organic carbon and microbial biomass carbon content in the soil infected by *M. incognita* under field condition

Treatment	Organic carbon ()			Soil microbial biomass carbon (µgg^{-1}soil 24h^{-1})		
	2016	2017	Pooled	2016	2017	Pooled
T_1	1.22^{f}	1.25^{f}	1.23^{f}	301.33 ^{e}	321.00 ^{f}	311.16 ^{f}
T_2	1.26^{e}	1.30^{e}	1.28^{e}	368.33 ^{d}	371.00 ^{e}	369.67 ^{e}
T_3	1.31^{d}	1.34^{d}	1.33^{d}	396.33 ^{bc}	398.33 ^{d}	397.33 ^{d}
T_4	1.05^{i}	1.08^{i}	1.06^{i}	251.00 ^{f}	258.00 ^{i}	254.50 ^{i}
T_5	1.11^{h}	1.15^{h}	1.13^{h}	259.67 ^{f}	267.00 ^{h}	263.33 ^{h}
T_6	1.16^{g}	1.19^{g}	1.18^{g}	280.00 ^{f}	289.00 ^{g}	284.50 ^{g}
T_7	1.37^{c}	1.42^{c}	1.39^{c}	417.00 ^{c}	425.00 ^{c}	423.00 ^{c}
T_8	1.43^{b}	1.44^{b}	1.42^{b}	473.67 ^{b}	480.00 ^{b}	477.17 ^{b}
T_9	1.47^{a}	1.48^{a}	1.47^{a}	495.67 ^{a}	499.33 ^{a}	497.50 ^{a}
T_{10}	1.01^{j}	1.04^{j}	1.02^{j}	241.67 ^{f}	246.00 ^{i}	243.83 ^{j}
S. Ed±	0.01	0.01	0.01	4.50	3.53	4.04
CD at 0.05	0.02	0.03	0.02	9.03	7.13	8.14

Mean with different letters in the column are significantly different from each other based on Duncan's Multiple Range Test (C.D.at 0.05).

T_1- *Pseudomonas fluorescens* enriched with FYM @ 1% (1ton FYM/ha), T_2- *P. fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha), T_3- *P. fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha), T_4- *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_5- *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_6-*Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha), T_7 - *P. fluorescens* and *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_8 - *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_9 - *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and T_{10}- Untreated check

Table.6 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the yield performance of black gram infected by *M. incognita* under filed condition

Treatment	Yield (q/ha)		
	2016	**2017**	**Pooled**
T_1	4.38^{cd}	5.07^{c}	4.73^{cd}
T_2	4.43^{cd}	5.33^{c}	4.88^{cd}
T_3	4.97^{bc}	5.50^{c}	5.23^{c}
T_4	4.14^{d}	5.17^{c}	4.66^{d}
T_5	4.16^{d}	5.53^{c}	4.85^{cd}
T_6	4.82^{bcd}	5.47^{c}	5.14^{cd}
T_7	5.33^{b}	6.37^{b}	5.85^{b}
T_8	5.57^{b}	6.67^{b}	6.12^{b}
T_9	6.53^{a}	7.33^{a}	6.93^{a}
T_{10}	3.27^{e}	3.43^{d}	3.35^{e}
S. Ed±	0.37	0.27	0.24
CD at 0.05	0.79	0.57	0.51

Mean with different letters in the column are significantly different from each other based on Duncan's Multiple Range Test (C.D.at 0.05).
T_1- *Pseudomonas fluorescens* enriched with FYM @ 1% (1ton FYM/ha), T_2- *P. fluorescens* enriched with vermicompost @ 1% (1ton vermicompost/ha), T_3- *P. fluorescens* enriched with enriched compost @ 1% (1ton enriched compost /ha), T_4- *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_5- *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_6-*Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha), T_7 - *P. fluorescens* and *Azotobacter* sp. enriched with FYM @ 1% (1ton FYM/ha), T_8 - *P. fluorescens* and *Azotobacter* sp. enriched with vermicompost @ 1% (1ton vermicompost/ha), T_9 - *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and T_{10}- Untreated check

Fig.1 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the shoot and root length (cm) of black gram infected by *M. incognita* under field condition

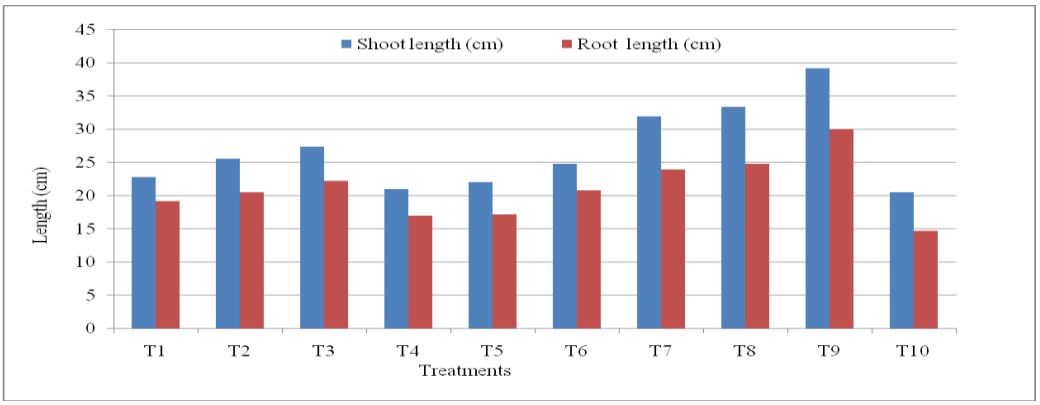


Fig.2 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the shoot and root weight (gm) (fresh and dry) of black gram infected by *M. incognita* under field condition

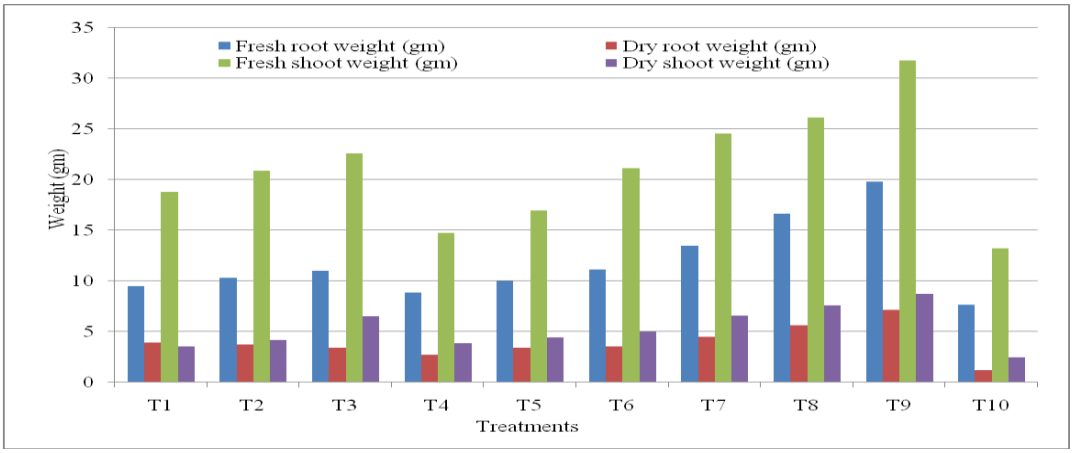


Fig.3 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the number of nodules per root system and multiplication of *M. incognita* in black gram under field condition

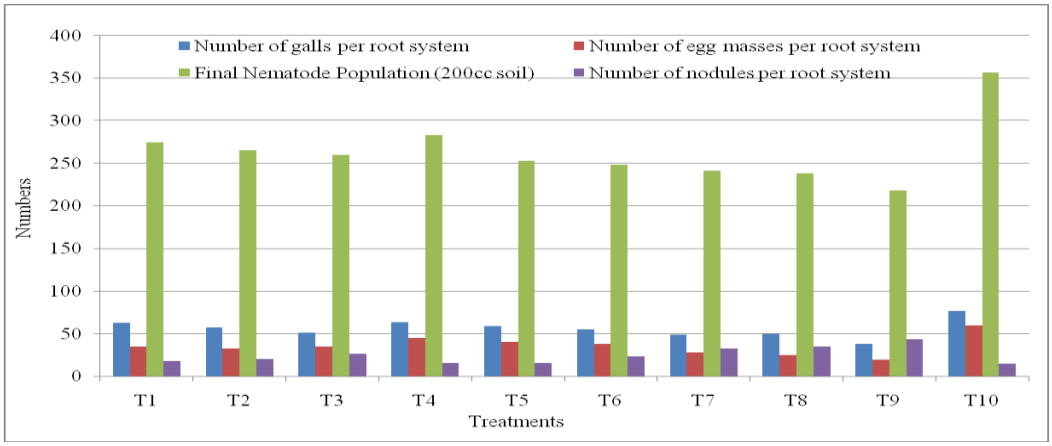


Fig.4 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on the organic carbon (g/kg of soil) content in the soil infected by *M. incognita* under field condition

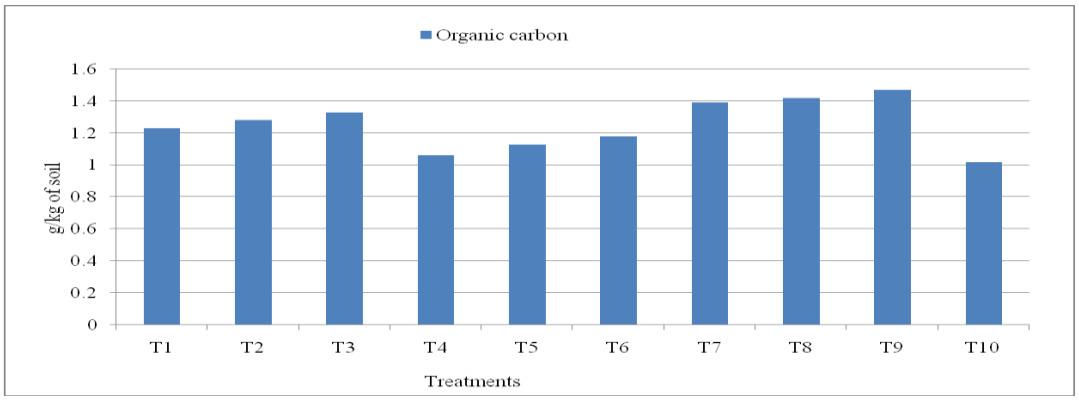


Fig.5 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on microbial biomass carbon (µgg^{-1}soil 24h^{-1}) content in the soil infected by *M. incognita* under field condition

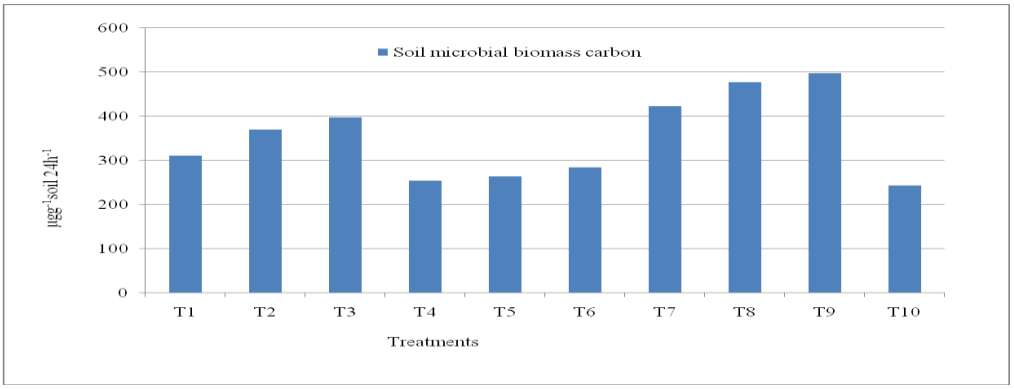
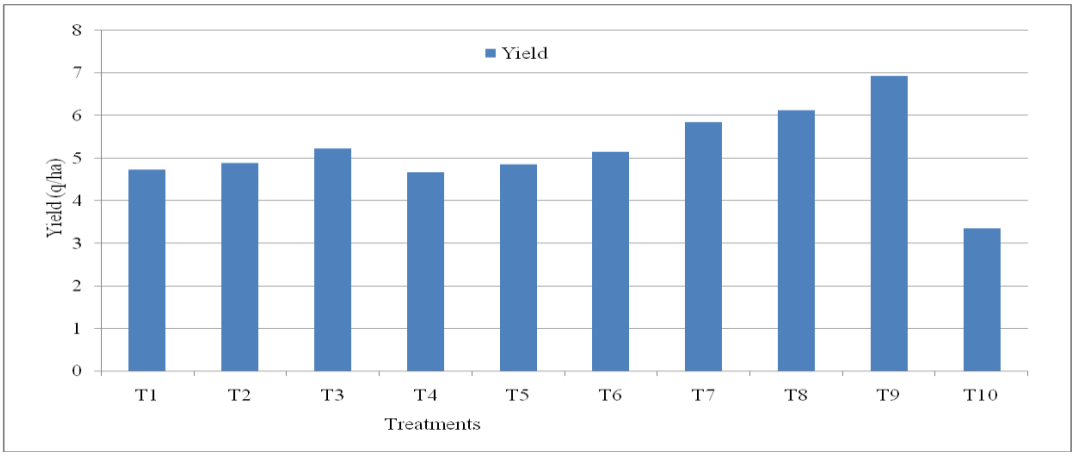


Fig.6 Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on yield (q/ha) performance of black gram infected by *M. incognita* under filed condition



Further they reported that untreated control treatments recorded the highest soil as well as root population of nematodes. Soliman *et al.,* (2011) evaluated that the soil application of *P. fluorescens*, considerably decreased the number of galls per root system, number of egg masses per root system and final nematode population in soil of *A. famensiana* infected by *M. incongita* than the soil application of *A. chroococcum* during 2009 and 2010. Likewise, Anwar-ul-Haq *et al.,* 2011 also reported that single as well as combined application of *P. fluorescens* and *A. chroococcum* significantly decreased the number of galls, number of egg masses per root system and final nematode population in the soil of tomato infested with *M. incongita* as compared to the untreated control. Further, they reported that *P. fluorescens* found to be better than *A. chroococcum* and finally concluded that combined application of *P. fluorescens* and *A. chroococcum* synergized the plant growth of tomato by reducing the nematode multiplication as compared to the single application. The reduction in the nematode population due to the soil application of *P. fluorescens* and *Azotobacter* sp. in the present investigation is might be due to (1) the release of pre-existing nematicidal compounds in soil amendments, (2) generation of nematicidal compounds, such as ammonia and fatty acids, during degradation, (3) enhancement and/or introduction of antagonistic microorganisms, (4) increase in the plant tolerance and resistance, (5) changes in soil physiology that are unsuitable for the nematode behaviour and (6) phosphate mineralization which result in an increase in the phosphate level that has an adverse effect on nematode (Pant *et al.,* 1983). The combinations of these mechanisms, rather than a single one, appear to produce nematode suppression in amended soils (Rodriguez-Kabana *et al.,* 1986). As a matter of fact, *P. fluorescens* and *Azotobacter* sp. used in the present investigation might have such type of mechanism(s) that resulted in the reduction of the final nematode population of *Meloidogyne incognita* in soil.

Efficacy of *P. fluorescens* and *Azotobacter* sp. alone and in combinations on yield of black gram infected by *M. incognita*

The data on the on yield of black gram in 2016 and 2017 as also the pooled data of both the years have been presented in Table 6 and Figure 6. The pooled data showed that the single and combined application of *P. fluorescens* and *Azotobacter* sp. enriched in FYM/vermicompost/ enriched compost gave maximum yield of black gram as compared to the untreated control during 2016 and 2017. However, the combined application of *P. fluorescens* and *Azotobacter* sp. found to be the best than individual application of *P. fluorescens* and *Azotobacter* sp. in all the tested substrates. However, among the tested substrates, enriched compost found to be the best in single as well as combined application of *P. fluorescens* and *Azotobacter* sp. Similarly, type of work also reported by several worker who showed that the single as well as combined, application of *P. fluorescens* + *A. chroococcum* increased the yield of wheat (Kumar *et al.,* 2001), tomato (Sakthivel *et al.,* 2009), chickpea **(**Rokhzadi and Toashis, 2011), strawberry (Mishra and Tripathi, 2011), lettuce (Chamangasht *et al.,* 2012) and banana (Patil and Shinde, 2013)..

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