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Bio-efficacy of Native Bioagents and Biofertilizers 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

An experiment was conducted to study the bio-efficacy of native bioagent and biofertilizer for the management of root-knot nematode *Meloidogyne incognita* infecting black gram *Vigna mungo*. For this the bioagents, *Pseudomonas fluorescens*, *Bacillus megaterium*, *Pochonia chlamydosporia* and *Purpureocillium lilacinum* and biofertilizers like *Azotobacter* sp. and *Rhizobium* sp. were screened against *M. incognita* by using seed treatment under pot condition. Further, these bioagents and bioferilizers were compared with an uninoculated check; an inoculated check and a chemical check (Carbosulfan 25 EC @ 0.2%) were used as control treatments. The results of the pot experiment revealed that all the tested bioagents and biofertilizers were improved plant growth parameters of blackgram and reduced the nematode multiplication in the soil. The maximum plant growth parameter of blackgram was recorded in the treatment with untreated and uninoculated control (T_9) followed by the treatment T_7 *i.e* seed soaking with carbosulfan 25 EC @ 0.2%. However, the minimum nematode multiplication was recorded in the treatment T_7 *i.e.*, seed soaking with carbosulfan 25 EC @ 0.2%. It observed that bacterial bioagents showed more bioefficacy than fungal bioagents. Among the bioagents the treatment T_6 *i.e.*, seed treatment with *Pseudomonas fluorescens* @ 1% (v/w) and between biofertilizers, the treatment T_4 *i.e.*, seed treatment with *Azotobacter* spp. @ 1% (w/w) were found to be the best in respect of giving the maximum shoot and root length, fresh shoot and root weight, dry shoot and root weight of blackgram and reducing the minimum number of galls per root system, egg masses per rot system and final J_2s population of *M. incognita* in the soil.

Introduction

The plant-parasitic nematodes are dominant species in the nematode world and it comprises of 4100 species of plant-parasitic nematode (PPN) (J3wqones *et al.*, 2013). Among them, the root-knot nematode *Meloidogyne incognita* attack not only more than two thousands of plant species but they also caused five per cent of global crop loss (Hussey and Janssen, 2002). These microscopic species are the hidden enemy of farmers and may not cause considerable crop loss or symptom development as other pests

and pathogens do. This nematode exhibit obligate parasitic relationship with the host plant and they produce giant cell as feeding cell and act as a metabolic sink which diverts all the nutrient towards them (Davis *et al.*, 2004). They produced galls on the roots and very easy to recognize with naked eyes. In Assam, yield losses in black gram due to *M. incognita* were recorded to the tune of 13.19-23.50 percent (Anon, 2011). But during the last few decades, the production and yield of the black gram declined and expected target could not be achieved. The root-knot nematode, *M. incognita*, is one of the major constraints in the production of black gram. The application of chemical can control the nematodes but the continuous application of chemicals can cause a harmful effect on the non-targeting species and increased their residual toxicity in the soil. However, chemical control has been adopted to diminish the pest populations, but these have not always provided a long-term suppression effect with economically feasible costs (Gomes *et al.,* 2010). Alternatives of chemical control the use of biological control agents (Siddiqui and Mahmood, 1999). Biological control is one of the possible safe alternatives to pesticides for the disease management and is likely to be free from the toxic residual effects. Application of bacteria and fungi in the rhizosphere of many plant species are known to protect the plant from an attack of diseases / pests and enhance the plant growth. Fungi like *P. chlamydosporia*, *P. lilacinum* etc., are commonly isolated from the soils and not only they found saprophytic in nature but also act as an egg parasites of plant-parasitic nematodes (Tigano-Milani *et al.,* 1993 and Arevalo *et al.,* 2009). However, such fungi are easy to mass produce and successfully colonized on the root surface. Moreover, these fungi are also interfering with space/nutrition with other microorganisms and act as a bionematicides against nematodes (De Leij and Kerry, 1991). Recently, the farmers are showing their more interests in the use of bioagents and biofertilizers than inorganic fertilizers because they are easy to apply, very cheap as compared to the inorganic fertilizers. The efficacy of biocontrol agents is varied from species to species (Irving and Kerry, 1986) and one of the means to increase the potentiality of biocontrol agents is to use the native biocontrol agents (Singh *et al.,* 2013). Such agents act as biological control agents for the exotic plant species when used in an inundative, augmentative, or conservative management strategy (Cofrancesco, 2000). In the management strategy, the delivery of biocontrol agent is an important aspect so that they can reach directly to the target pathogen. Indeed, seed treatment is the best method than other because the biocontrol agent direly landed on the seed coat and not only they protect the seedling from pathogen attack but also improve the nutrient uptake of the treated plant (Cook, 1984). Keeping this in view the potential benefits and fit fall must be examined so that effective native biocontrol agent (s) and biofertilizer (s) can be utilized. Hence, a study was undertaken on the bio-efficacy of native bioagent and biofertilizer for the management of root-knot nematode *Meloidogyne incognita* infecting black gram *Vigna mungo.*

Materials and Methods

Location of Experiment

The experiment was conducted in the net house of the Department of Nematology, AAU Jorhat during 2015-2016.

Source and maintenance of *Meloidogyne incognita,* bioagents and biofertilizers

Meloidogyne incognita egg masses were obtained from infected brinjal plants, Department of Nematology, AAU, Jorhat-13 and pure culture were maintained on tomato in pots in the Net house, Department of

Nematology, AAU, Jorhat-13. Liquid formulation of bioagents like *Pseudomonas fluorescens, Bacillus megaterium, Pochonia chlamydosporia* and *Purpureocillium lilacinum* were obtained from Department of Plant Pathology, AAU, Jorhat-13 and solid formulations of biofertilizers like *Azotobacter* sp. and *Rhizobium* sp. were obtained from Department of Soil science, AAU, Jorhat.

Collection and sterilization of soil

Required soil was collected from upland near the nematode culture house, Department of Nematology, Assam Agricultural University, Jorhat. The soil was mixed thoroughly after removing unwanted materials like stones and roots. Then the soil was mixed homogenously with finely dried cow dung and sand in the ratio of 2:1:1 respectively. The soil mixture was put in a gunny bag and sterilized in an autoclave at 121°C for half an hour.

Filling up of pots

Earthen pots with 3kg capacity were selected, cleaned and sterilized in sunshine. Few broken pieces of bricks were placed at the bottom of the pots before filling up with sterilized soil mixture. Proper labeling of each pot was done.

Source and sterilization of seed

Black gram seeds of the variety PU- 31 (susceptible to *Meloidogyne 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.

Details of the treatments

T_1- Seed treatment with *Purpureocillium lilacinum* @ 1% (v/w), T_2- Seed treatment with *Pochonia chlamydosporia* @ 1% (v/w), T_3- Seed treatment with *Rhizobium* sp. @ 1% (w/w), T_4- Seed treatment with *Azotobacter* sp. @ 1% (w/w), T_5- Seed treatment with *Bacillus megaterium* @ 1% (v/w), T_6- Seed treatment with *Pseudomonas fluorescens* @ 1% (v/w), T_7- Seed soaking with carbosulfan 25 EC @ 0.2%., T_8- Inoculated check (Nematode alone), T_9- Uninoculated check (without, nematode, bioagent and biofertilizer). Each treatment is replicate five times in the completely randomized design.

Seed treatment with fungal and bacterial bioagents

Carboxy methyl cellulose (CMC) was used as an adhesive for treating black gram seeds with fungal spore suspension and bacterial cell suspension ($1x10^8$cfu/ml.). For preparing 1% (v/w) adhesive solution, 100mg of adhesive was added to 10 ml of fungal and bacterial suspension. Now required amount of seeds was taken in a petriplate and the fungal as well as bacterial suspension with the adhesive was added drop by drop on the seeds stirring continuously. Addition of suspension was stopped when all the seeds got smeared with the suspension. After treating, the seeds were dried in shade for 6 hours and used for sowing.

Seed treatment with biofertilizers

The required amount of biofertilizers (*Azotobacter* sp. and *Rhizobium* sp.) were added to measured quantities of seeds in containers and 1% CMC was added drop by drop on the seeds stirring continuously until a uniform coating over the seeds was obtained.

Seed treatment with chemicals

The required amount of seeds was soaked in Carbosulfan 25EC @ 0.2% for 12 hours. Treated seeds were dried in shade and were sown in pots.

Inoculation of second stage juveniles of root-knot nematode, *M. incognita*

Freshly hatched second stage juveniles (J_2) of *M. incognita* were inoculated @ 3000 J_2/pot.

Observations

Shoot length (cm)

The main shoot was measured in centimeter from the ground level up to tip of the longest leaf after 60 days of sowing.

Root length (cm)

The main root length was measured in centimeter from the ground level up to tip of the longest root after 60 days of sowing.

Fresh shoot and root weight (gm)

The fresh shoot and root weight per plant was measured in gram after 60 days of sowing. These plants were weighed on the weigh balance at Nematology laboratory.

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 after 60 days of sowing.

Number of galls and egg masses per root system

The number of galls and egg masses per root system was measured after 60 days of sowing.

Final nematode population

For recording the final nematode population in soil, 200 cc of soil was collected from each pot separately and processed by modified Cobb's sieving and decanting technique (Christie and Perry, 1951).

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 bioagents and biofertilizers on plant growth parameters

The results of the present investigation (Table 1, 2; Fig. 1 and 2) showed that all the tested bioagents and biofertilizers were found to be effective in an increasing the plant growth parameters like shoot length, root length, shoot weight (fresh and dry), root weight (fresh and dry) of black gram infected by *M. incognita* as compared to the inoculated check (*M. incognita* alone) under pot conditions. However, among all the treatments, the maximum plant growth parameters were recorded in the treatment with untreated and uninoculated control. Among the rest of the treatments, the maximum shoot height, fresh and dry shoot weight, root length, and weight was recorded in the treatment, T_7 *i.e.*, seed treatment with carbosulfan 25 EC @ 0.2%. Among the fungal bioagents, seed treatment with *P. lilacinum* @ 1% (v/w) recorded maximum plant growth parameters than seed treatment with *P. chlamydosporia* @ 1% (v/w). Similar type of observations also recorded by Annapurna *et al.*, 2018 who reported that among the fungal bioagents, *P. lilacinum* found to be better than *P.*

chlamydosporia in the improving of plant growth parameters like shoot length, root length, shoot weight (fresh and dry), root weight (fresh and dry) of tomato infected by *M. incognita* under pot condition. However, among the bacterial bioagents, seed treatment with *P. fluorescens* @ 1% (v/w) recorded the maximum plant growth parameters than the seed treatment with *B. megaterium* @ 1% (v/w). Likewise, observations also reported by Ashoub and Amara (2010) who reported that *P. fluorescens*, *B. thuringiensis* and *R. leguminosarum* improved the shoot weight (fresh and dry) of the eggplant than the un-infected plants and healthy plants.

Further, they conclude that maximum shoot weight (fresh and dry) was recorded by *P. fluorescens* followed by *B. thuringiensis* and minimum shoot weight (fresh and dry) was recorded by *R. leguminosarum*. However, Khan *et al.,* 2016 reported that among the four *Pseudomonas* spp. (*P. aeruginosa, P. fluorescens, P. stutzeri* and P. *striata*), *P. fluorescens* was found to be the most effective in the improving of plant growth parameters of mung bean and concluded that seed treatment with *P. fluorescens* offers a better substitute of the nematicide in mung bean cultivation. Khan *et al.,* 2012 reported that strain of *P. fluorescens* improve the plant growth parameters of green gram because of it increased the phosphorus content of the soil and or produced more indol acetic acid (IAA) as compared to the tested bacteria like *B. subtilis* and *Paenibacillus polymyxa* and thus confirm the result of present investigation where among the bioagents *P. fluorescens* improved the plant growth parameters of black gram infected by *M. incognita* under pot condition. Whereas, among the biofertilizer, seed treatment with *Azotobacter* sp. @ 1% (w/w) was recorded the maximum plant growth parameters than the seed treatment with *Rhizobium* sp. @ 1% (w/w). Similarly, *A. chroococcum* also improved the plant growth parameters of brinjal infected by *M. incognita* (Chahal and Chahal, 1988) and wheat infected by *Heterodera avenae* (Bansal *et al.,* 1999). Whereas, *Azotobacter* spp. also improve the plant growth parameters by release of growth hormones like auxins, gibberellins, cytokinin and ethylene (Oostendorp and Sikora, 1990 and Kell *et al.,* 1989) in soil and further increased their uptake along with soil nutrients (Van Loon *et al.,* 1998 and Selvakumar *et al.,* 2009). The variable effect *Azotobacter* spp. on black gram observed in the present investigation can be attributed to possess such type of mechanism that boosts the plant growth of black gram and found to be the best biofertilizer than *Rhizobium* sp.

Efficacy of bioagents and biofertilizers on nodules per root system

In case of number of nodules per root system (Table 2, Fig. 3, and Fig. 5), maximum number of nodules (43.00) per root system was recorded in the treatment with untreated and uninoculated control (T_9) and it was significantly different from rest of the treatments. The minimum number of nodules (13.80) per root system was recorded in the treatment with *M. incognita* alone (T_8) which was found to be significantly different from the rest of the treatments. Among the rest of the treatments, maximum number of nodules per root system was recorded in the treatment, T_3 *i.e.,* seed treatment with *Rhizobium* sp. @ 1% (w/w) followed by T_6 *i.e.,* seed treatment with *P. fluorescens* @ 1% (v/w) and then T_5 *i.e.,* seed treatment with *B. megaterium* @ 1% (v/w) which were significantly different from each other and these treatments were significantly different from rest of the treatments. Furthermore, treatments with bacterial bioagents showed maximum number of nodules per root system than the treatments with fungal biaogents.

Table.1 Efficacy of bioagents and biofertilizers on plant growth parameters of black gram infected by *M. incognita* under pot condition

Treatment	Shoot length (cm)	Fresh shoot weight (gm)	Dry shoot weight (gm)
T_1	42.44^{ef}	25.62^{de}	8.90^{d}
T_2	41.50^{f}	24.00^{e}	8.08^{de}
T_3	37.80^{g}	20.70^{f}	6.82 ^{e}
T_4	43.16^{ef}	26.40^{de}	9.26^{d}
T_5	47.54^{d}	27.60^{cd}	11.64^{c}
T_6	50.10^{c}	29.44^{bc}	12.32^{c}
T_7	52.80^{b}	31.52^{b}	14.10^{b}
T_8	34.22^{h}	19.76^{g}	5.86^{f}
T_9	55.68^{a}	34.74^{a}	16.14^{a}
S. Ed (±)	1.00	1.35	0.67
CD at 0.05	2.03	2.74	1.37

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- Seed treatment with *Purpureocillium lilacinum* @ 1% (v/w), **T_2-** Seed treatment with *Pochonia chlamydosporia* @ 1% (v/w), **T_3-** Seed treatment with *Rhizobium* sp. @ 1% (w/w), **T_4-** Seed treatment with A*zotobacter* sp. @ 1% (w/w), **T_5-** Seed treatment with *Bacillus megaterium* @ 1% (v/w), **T_6-** Seed treatment with *Pseudomonas fluorescens* @ 1% (v/w), **T_7-** Seed soaking with carbosulfan 25 EC @ 0.2%, **T_8-** Nematode alone and **T_9-** Control (without nematode, bioagent and biofertilizers)

Table.2 Efficacy of bioagents and biofertilizers on plant growth parameters and number of nodules per root system of black gram infected by *M. incognita* under pot condition

Treatment	Root length (cm)	Fresh root weight (gm)	Dry root weight (gm)	Number of Nodules /root system
T_1	16.60^{e}	9.16^{d}	4.18^{d}	22.20^{ef}
T_2	16.26^{e}	9.08^{d}	4.04^{d}	20.00^{fg}
T_3	13.82^{f}	7.34^{e}	3.22^{e}	34.40^{b}
T_4	17.10^{de}	9.38^{d}	4.36^{d}	23.20^{e}
T_5	18.60^{d}	9.20^{d}	4.42^{d}	26.60^{d}
T_6	21.70^{c}	10.86^{c}	5.48^{c}	30.20^{c}
T_7	23.70^{b}	12.38^{b}	6.86^{b}	18.80^{g}
T_8	10.42^{g}	5.04^{f}	2.24^{f}	13.80^{h}
T_9	25.30^{a}	14.70^{a}	7.84^{a}	43.00^{a}
S. Ed (±)	0.77	0.57	0.39	1.33
CD at 0.05	1.58	1.15	0.80	2.69

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- Seed treatment with *Purpureocillium lilacinum* @ 1% (v/w), **T_2-** Seed treatment with *Pochonia chlamydosporia* @ 1% (v/w), **T_3-** Seed treatment with *Rhizobium* sp. @ 1% (w/w), **T_4-** Seed treatment with A*zotobacter* sp. @ 1% (w/w), **T_5-** Seed treatment with *Bacillus megaterium* @ 1% (v/w), **T_6-** Seed treatment with *Pseudomonas fluorescens* @ 1% (v/w), **T_7-** Seed soaking with carbosulfan 25 EC @ 0.2%, **T_8-** Nematode alone and **T_9-** Control (without nematode, bioagent and biofertilizers)

Table.3 Efficacy of bioagents and biofertilizers on multiplication of *M. incognita* in black gram under pot condition

Treatment	Number of galls per root system	Number of egg masses per root system	Final nematode population (200cc soil)
T_1	54.80 [c]	33.60 [c]	268.60 [c]
T_2	55.00 [c]	34.80 [c]	270.60 [c]
T_3	60.00 [b]	41.20 [b]	284.80 [b]
T_4	52.00 [c]	32.00 [c]	260.80 [d]
T_5	49.60 [d]	28.60 [d]	254.20 [e]
T_6	43.20 [e]	21.20 [e]	241.80 [f]
T_7	36.60 [f]	17.20 [f]	225.60 [g]
T_8	79.80 [a]	55.80 [a]	428.00 [a]
T_9	0.00 [g]	0.00 [g]	0.00 [h]
S. Ed (±)	0.12	0.64	2.10
CD at 0.05	0.25	1.29	4.24

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- Seed treatment with *Purpureocillium lilacinum* @ 1% (v/w), **T_2-** Seed treatment with *Pochonia chlamydosporia* @ 1% (v/w), **T_3-** Seed treatment with *Rhizobium* sp. @ 1% (w/w), **T_4-** Seed treatment with A*zotobacter* sp. @ 1% (w/w), **T_5-** Seed treatment with *Bacillus megaterium* @ 1% (v/w), **T_6-** Seed treatment with *Pseudomonas fluorescens* @ 1% (v/w), **T_7-** Seed soaking with carbosulfan 25 EC @ 0.2%, **T_8-** Nematode alone and **T_9-** Control (without nematode, bioagent and biofertilizers)

Fig.1 Efficacy of bioagents and biofertilizers on shoot and root length (cm) of black gram infected by *M. incognita* under pot condition

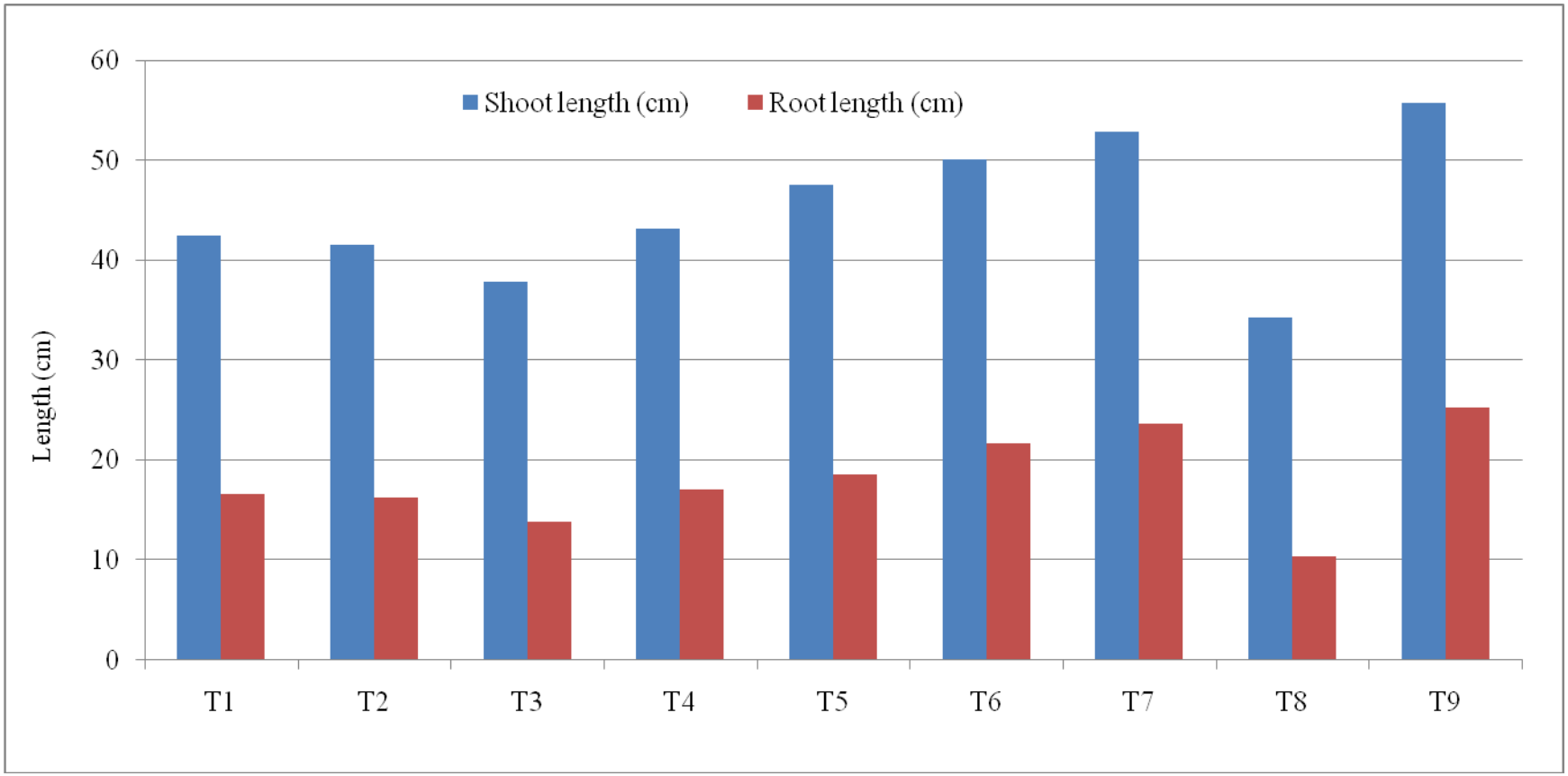


Fig.2 Efficacy of bioagents and biofertilizers on shoot weight (gm) and root weight (gm) of black gram infected by *M. incognita* under pot condition

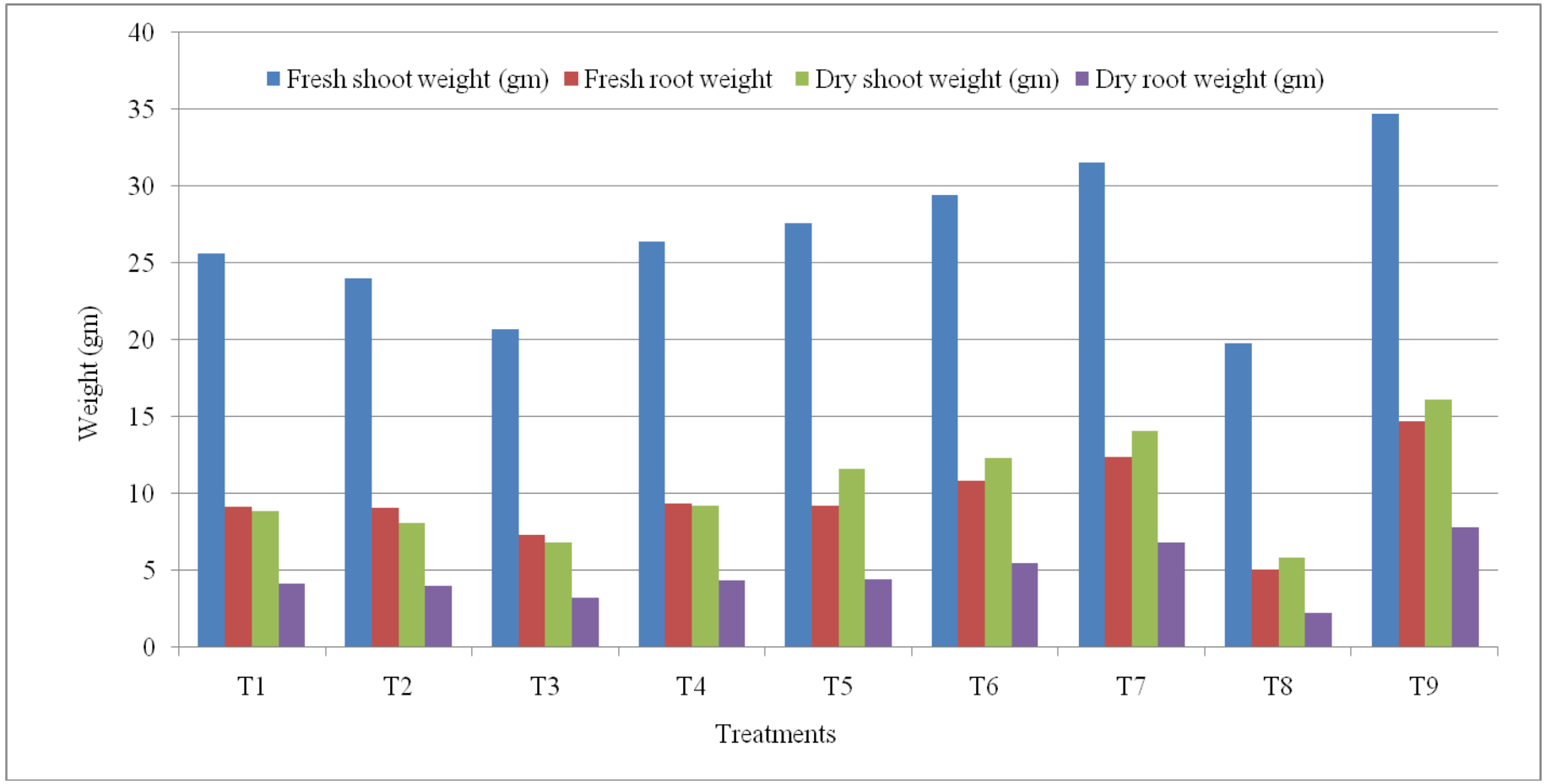


Fig.3 Efficacy of bioagents and biofertilizers on number of nodules, galls and egg mass per root system of black gram infected by *M. incognita* under pot condition

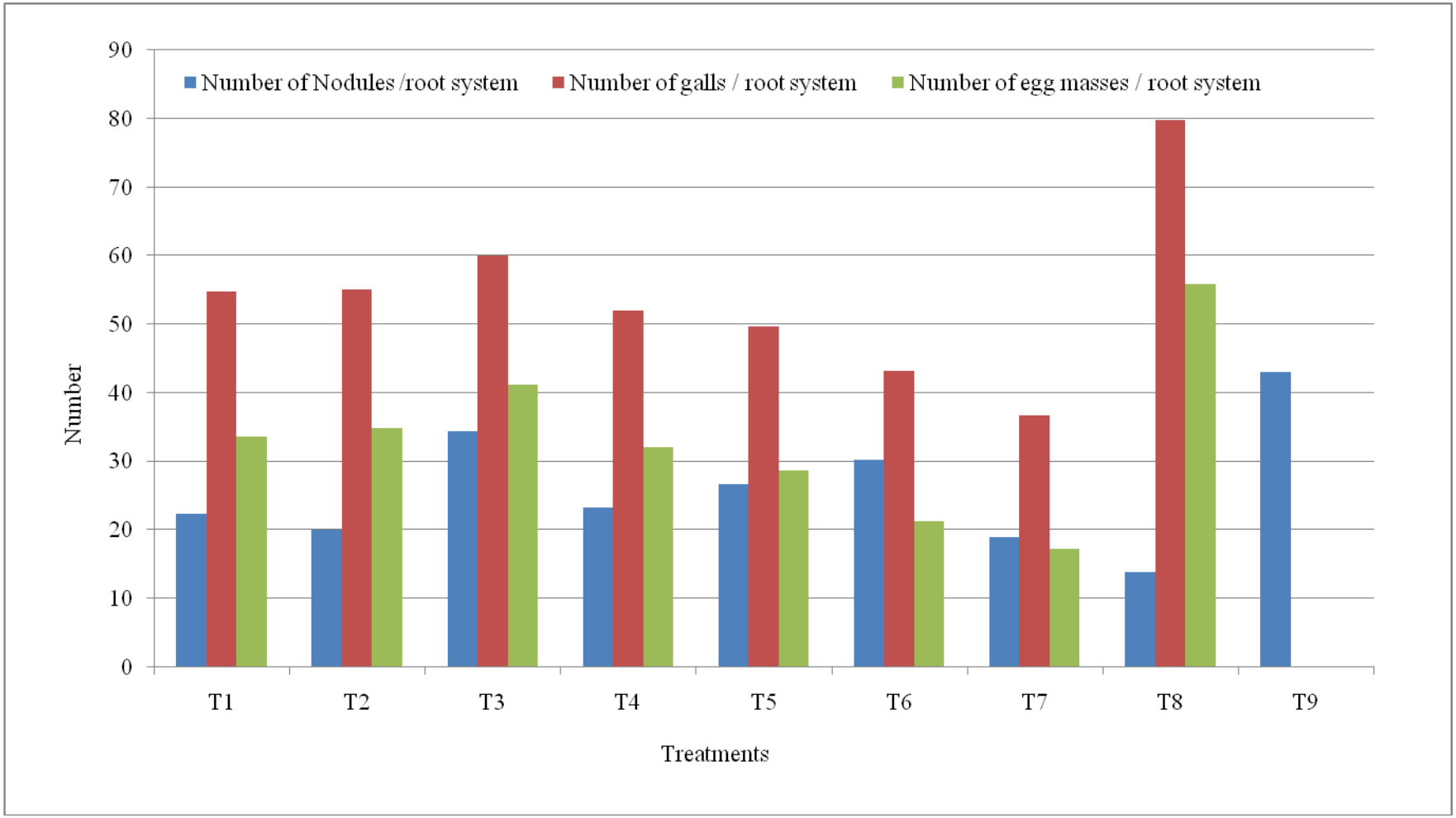


Fig.4 Efficacy of bioagents and biofertilizers on final nematode population of *M. incognita* in 200cc of soil

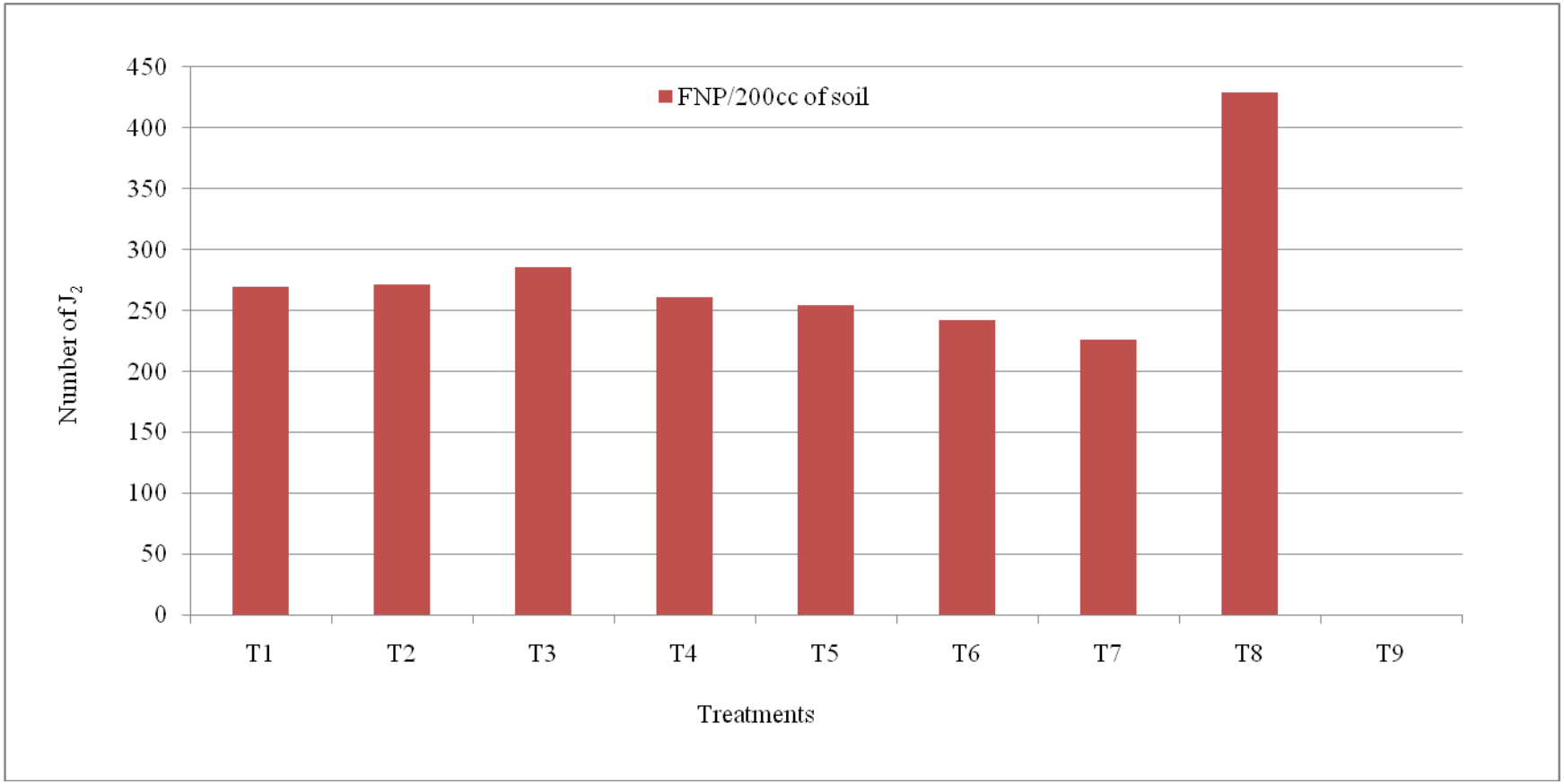
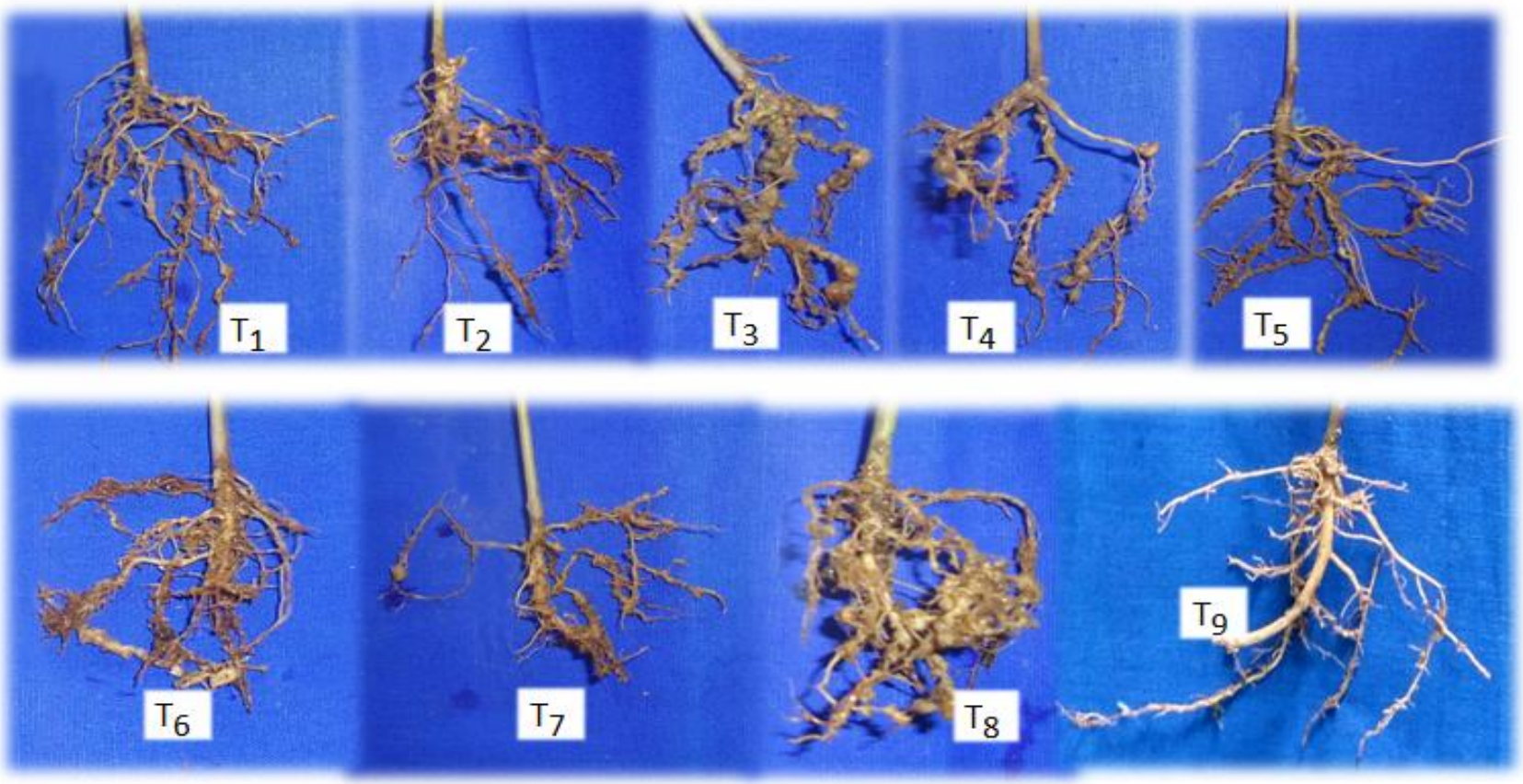


Fig.5 Efficacy of different treatments on root growth of black gram infected by *M. incognita* under pot condition



The adverse effect of root-knot nematodes on nodule formation has been recorded on peanuts (Miller, 1951), chickpea (Khan *et al.*, 1996), cowpea (Khan and Khan, 1996), soybean (Kabi, 1983) and pigeon pea (Taha, 1993). Khan *et al.*, 2002 showed a positive effect between nodulation and rhizobacteria/ fungal bioagents on nodulation of green gram infected by *M. incognita* and they concluded that root knot infected plants treated with bacterial bioagents showed more nodulation than the fungal bioagents and further they also showed that nematode infection decreased the total number of nodules/root system as compared to the uninoculated control (without root-knot nematode), thus confirming the results of the present investigation where rhizobacteria showed more nodules per root system than the fungal bioagents. The cause of reduction in the nodulation on 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). However, the application of *P. fluorescens* (Khan *et al.*, 2012) and *Azotobacter* spp. (Martinez-Toledo *et al.*, 1988) increased IAA in plant and plays a major role in the development of rhizobial nodules on the legume plant. These mechanisms might be operative in the present investigation in recording more number of nodules per root system due to application of *P. fluorescens* and *Azotobacter* spp. on black gram infected by *M. incognita*.

Efficacy of bioagents and biofertilizers on multiplication of *Meloidogyne incognita*

It is evident from the results (Table 3, Fig. 3–5), that all the treatments with bioagents and biofertilizers significantly reduced the number of galls, egg masses per root system and final nematode population in the soil as compared to control (nematode alone). The minimum number of galls and egg masses per root system and final nematode population in soil was recorded in the in the treatment T_7 *i.e.*, seed treatment with Carbosulfan 25EC @ 0.2% and the maximum was recorded in the treatment with *M. incognita* alone (T_8). The results showed that among the bioagents, the minimum number of galls and egg masses per root system and final nematode population in the soil was recorded in the treatment T_6 *i.e.*, seed treatment with *P. fluorescens* @ 1% (v/w). In line with the results of the present investigation Khan *et al.*, 2016 also recorded remarkable decrease in the galls and egg masses per root system and nematode population in the soil when *P. fluorescens* was applied as a seed treatment on green gram infected with *M. incognita*. However, among the biofertilizers, the minimum number of galls and egg masses per root system and final nematode population in soil was recorded in the treatment T_4 *i.e.*, seed treatment with *Azotobacter* sp. @ 1% (w/w). Similarly, Khan *et al.*, 2002 also observed that significant reduction in the number of galls and egg masses per roots system and nematode population in the soil when *A. chroococcum* applied as a seed treatment in the green gram. However, the rhizobacteria reduce the nematode multiplication through a different mode of action like i. control behavior of nematode (Sikora and Hoffmann-Hergarten, 1993) ii. interfering with host-nematode recognition (Oostendorp and Sikora, 1990) iii. competing for essential nutrients (Oostendorp and Sikora, 1990) iv. enhance plant growth (El-Nagdi and Youssef, 2004) v. inducing systemic resistance (Hasky-Gunther *et al.*, 1998) vi. showed direct nematicidal activity by means of the production of toxins, enzymes and other metabolic products (Siddiqui and Mahmood, 1999) like ammonia, nitrites, hydrogen sulphide and hydrogen cyanide that directly affect egg-hatch or the mobility of juveniles which cause the mortality of the nematodes

(Rodriguez-Kabana *et al.*, 1986). Hence the present study concluded that among the bioagents *P. fluorescens* and among the biofertilizers *Azotobacter* sp. were found to be more effective in the improving plant growth parameters like shoot length, root length, shoot weight (fresh and dry), root weight (fresh and dry) of black gram and reduced the number of galls, egg masses per root system and final nematode population in the soil.

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