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# Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby. by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*

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A glass house experiment was conducted for the effectiveness of vermicompositing and rhizotrophic micro- organisms (arbuscular mycorrhizal fungus (AMF) *Glomus aggregatum* and mycorrhiza helper bacterium (MHB) *Bacillus coagulans*) for the management of *Meloidogyne incognita* on tomato cv Pusa Ruby. Among the different treatments evaluated, vermicompost and *G. aggregatum* alone and in combination with *B. coagulans* recorded the maximum growth, biomass and nutrients of tomato cv Pusa Ruby with decreased root- knot nematode population and root- knot index. But amending the soil with application of vermicomposting + *B. coagulans* + *G. aggregatum* in tomato was significantly increased the plant growth, biomass and nutrients of tomato cv Pusa Ruby. Similarly reduction in root- knot nematode population, root- knot index (RKI), nematode reproduction rate (NRR) number of galls and egg masses per plant were recorded in the above treatment. Highest mycorrhizal colonization of 92.5% and minimum nematode population of 145.0/ 250cc soil was observed in the same treatment. It can be concluded that application of vermicompost + *G. aggregatum*+ *B. coagulans* increased plant growth characters and reduced RKI, NRR, number of galls and egg masses on tomato cv Pusa Ruby.

**Key words**: Biological control, *Meloidogyne incognita*, vermicompositing, AM fungus, *Glomus aggregatum*, mycorrhizal helper bacterium, *Bacillus coagulans*

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## Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important commercial and widely grown vegetable crops in both tropics and sub- tropics, which is often severely attacked by root-knot nematode, *Meloidogyne incognita*, a predominant and widely prevalent species inflicting serious loss in tomato (Sasser, 1990). A yield loss of 35 - 39.7 percent has been reported due to root-knot nematode infestation (Reddy, 1985; Jonathan *et al.,* 2001). Chemicals that are being used for controlling plant parasitic nematodes are costly and hazardous in nature. Researchers all over the world are engaged in standardizing the nematode management strategies by following non-chemical and ecofriendly approaches such as cropping systems soil amendments (botanicals) (Sukul *et al*., 2001; Rajendran and Saritha, 2005), organic soil amendment (Singh *et al*., 1990; Vedhera *et al*., 1998; Nagesh and Reddy, 1997). biological control agents (Babu *et al.,* 2000; Krishnappa, 2002; Kantharaju *et al*., 2005; Sumathi *et al*., 2006) and judicious use of nematicides (Taylor and Sasser, 1978) to stabilize crop production. Among the various biocontrol agents, arbuscular mycorrhizal fungi (AMF) are being widely used in nursery seedling as it enhances nutrient availability (Jeffries, 1987). The role of AMF in reducing harmful effect of root infection by many parasitic nematodes in crop plants is well recognized (Hussey and Roncadori, 1982; Mahaveer *et al.,* 1994; Jothi and Sundarababu, 2002; Shreenivasa *et al.,* 2007). The use of vermicompost, as a source of organic manure is supplementing chemical fertilizer is becoming popular among the farmers of the country. Increase in crop yield, soil nutrient status and nutrient uptake was reported due to application of vermicompost (Vasanthi and Kumaraswamy, 1999; Ansari and Ismail, 2001). Investigations carried out, so far, had been mostly on the management of root- knot nematode by utilizing AM fungi which have introduced from other centers and made to utilize indigenous isolates (Mishra and Shukla, 1997; Kantharaju *et al*., 2005). Limited efforts have been to utilize plant growth promoting rhizo- microorganisms and organic manures against virulent populations of root-knot nematode (Rao *et al.,* 1993). Hence, the present investigation was conducted to evaluate the effectiveness of vermicompost, AM fungus, *Glomus aggregatum* and Mycorrhiza Helper Bacterium (MHB), *Bacillus coagulans* against population of *M. incognita* on tomato cv Pusa Ruby under glass house conditions.

## Materials and methods

The experiment was conducted in PVC pots (20 cm x 16 cm) containing 5 kg autoclaved sandy loam soil (pH 6.8, P (NaHCo extractable) 9.2mg/kg, total N 0.4 g/kg, silt 110 g/kg) and mixed with rock phosphate @ 21.40 mg P/kg. The soil was

made nematode sick by thoroughly mixing freshly hatched second stage juveniles (J2) of Meloidogyne incognita @ 4000 J2/ plant. The AM fungus, *Glomus aggregatum* was mass multiplied on Rhodes grass (*Chloris gayana* Kunth.) and an inoculum rate of 1000 chlamydospores/ plant was used. The vermicompost used had 11.5 percent organic carbon, 1.3 percent total N, 1.3 percent P and 2.6 percent K. Vermicompost @ of 650 g/pot were thoroughly mixed with the soil before filling the pots (Shivaputra *et al.*, 2004). *Eudrilus eugeniae* was the earthworm species used for making the compost. *Bacillus coagulans* was grown in Pikovskaya medium (Pickovskaya, 1948) for 3 days at 30±2 °C to a cell density of 2.3 x 10 cells/ml. Inoculation by various combinations of *B. coagulans* under study was done by soaking the surface sterilized seeds of tomato cv Pusa Ruby in the liquid culture of an organism was mixed in equal proportion and then the seeds were soaked in it. Tomato seedlings were raised in PVC pots and later one seedling was transplanted per pot. Inoculation of *G. aggregatum* and application of vermicompost were done at the root zone of each 21- day- old tomato seedlings. The treatments included nematode infested soil check, vermicompost (Vc), *Glomus aggregatum* (Ga), *Bacillus coagulans* (Bc), Vc+Ga, Vc+Bc, Ga+Bc and Vc+Ga+Bc. Autoclaved soil without infestation served as non- infested soil check. The glass house temperature ranged between 27 and 32°C. They were watered daily with 50 ml tap water per pot. The plants were completely randomized block design with five replicates on glass house bench. After 60 days of transplantation, five plants from mycorrhiza treated pots were depotted; roots cleared of soil and washed in water. Rest of the plants harvested after 90 days of transplantation for plant height, dry weight of root and shoot, N, P, K content of plants, number and size of galls, number of egg masses per plant and nematode reproduction rate. AM fungal colonization of roots was estimated by trypan blue staining technique (Phillips and Hayman, 1970) along with root- knot index was scored by using 0- 5 scale (Taylor and Sasser, 1978).

For estimating the N, P, K content, tomato plants inoculated with different treatments were harvested after 90 days and dried in oven at 60 °C for three days. Nitrogen, phosphorus and potassium content of tomato plants were determined by micro- Kjeldahl, vanadomolybdate- phosphoric acid yellow colour and flame photometry methods (Jackson, 1973) respectively. The soil was also analyzed for mycorrhizal chlamydospores through wet- sieving and decantation technique (Gerdemann and Nicolson, 1963). The data were statically analyzed for variance (Little and Hills, 1978).

## Results and discussion

Application of vermicompost and *Glomus aggregatum* alone significantly increased plant growth, biomass and nutrients of tomato cv Pusa Ruby

compared to that of nematode infested soil (Table 1). However, the magnitude of increase in each character under report varied with the combinations of *G. aggregatum* and vermicomposting with *B. coagulans*. Plant height under *B. coagulans* alone treatment was same as that of vermicompost alone indicating that these treatments individually give increased or stimulation to the plant in terms of plant height, whereas, *G. aggregatum* individually as well as in combination with vermicompost and *B. coagulans* increased plant height, biomass and nutrients significantly compared to infested and non- infested soil. Similar results also have been reported by Singh *et al*. (1990) in tomato, Reddy *et al.* (1996) in tomato, and Vedhera *et al*. (1998) in ginger. Further, *B. coagulans* did not individually enhanced shoot and dry weights significantly over the non- infested soil, although it increased biomass over that of infested soil (Table 1). These results are in conformity with the findings of previous reports of Sumana *et al.* (2003), who also reported that inoculation of *B. coagulans* individually did not influence the shoot and root biomass significantly in neem plants. Maximum plant height, biomass and nutrient uptake were recorded on plants treated with *G. aggregatum* + vermicompost + *B. coagulans* followed by *G. aggregatum* + vermicompost. Results further indicated that integration of vermicompost + *G. aggregatum* + *B. coagulans* promoted better growth than their individual applications. Similar observations on enhanced plant response to AM fungi in combination with organic manures were reported by Singh *et al*. (1990) in tomato, Reddy *et al*. (1995) in acid lime, and Nagesh and Reddy (1997) in *Crossandra undulaefolia.*

At harvest, the root- knot nematode populations in soil were minimum in plants treated with *G. aggregatum* + vermicompost + *B. coagulans* followed by plants treated with *G. aggregatum* + vermicompost. Combination of *G. aggregatum* + vermicompost + *B. coagulans* had minimum number of egg masses, root galls and root- knot index (Table 2). Application of vermicompost and *G. aggregatum* individually also resulted in lower RKI and number of egg masses as compared to RKI and number of egg masses under *B. coagulans*. However, RKI and number of egg masses under individual application of vermicompost and *G. aggregatum* were significantly lower as compared to RKI and number of egg masses of infested soil (Table 2). Further nematode reproduction rate was also significantly lower in *G. aggregatum* + vermicompost + *B. coagulans* treated plants followed by *G. aggregatum* + vermicompost treated plants (Table 2). These results are in conformity with the findings of previous reports of Vedhera *et al*. (1998) who reported that application of different organic manures inhibited reproduction in females and penetration of second stage juveniles into ginger roots besides increasing the

yield as per findings of Goswani and Vijayalakshmi, (1981); Rajendran and Saritha, (2005) and Kantharaju *et al*. (2005) in tomatoes.

Root colonization by *G. aggregatum* and number of chlamydospores/ 50cc soil was significantly increased when integrated with vermicompost + *G. agrregatuma* + *B. coagulans* than its individual applications (Table 2). Among different treatments, *B. coagulans* enhanced root colonization by *G. aggregatum* to the maximum and the number of chlamydospores. The observed difference in the root colonization and spore number of *G. aggregatum* among its different treatment combinations could be possibly due to the presence of nitrogen, potassium and phosphorus content in vermicompost and *B. coagulans*. This suggest a synergistic activity were in *B. coagulans* enhances the activity of *G. aggregatum* by producing organic acids which serve as a carbon source to the fungus or by hydrolytic enzymes thus enabling the AM fungus to penetrate and ramify in the root system of the host (Duponnois and Garbaye, 1991). It can be concluded that application of vermicompost + *G. aggregatum* + *B. coagulans* increased pant growth characters and reduced RKI, nematode reproduction rate, number of galls and egg masses on tomato cv Pusa Ruby in sandy loam acidic soils.

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**Table 1.** Influence of vermicompost, *Glomus aggregatum* and *Bacillus coagulans* on growth of tomato cv Pusa Ruby infested with *Meloidogyne incognita*.

| Treatments* | Plant height, cm per plant | Plant dry weight, g per plant | | | Nitrogen uptake, %/plant | Phosphorus uptake, %/plant | Potassium uptake, %/plant |
|---|---|---|---|---|---|---|---|
| | | Root | Shoot | Total | | | |
| Infested soil check | 22.45 | 7.20 | 34.50 | 41.70 | 0.9 | 0.4 | 2.8 |
| Non- infested soil check | 30.26 | 9.85 | 41.50 | 51.35 | 1.0 | 0.5 | 3.2 |
| Vermicompost (VC) | 32.06 | 17.75 | 45.00 | 62.75 | 1.2 | 0.6 | 4.4 |
| *Glomus aggregatum* (Ga) | 34.65 | 18.04 | 46.50 | 64.54 | 1.3 | 0.6 | 4.2 |
| *Bacillus coagulans* (BC) | 31.65 | 15.16 | 43.25 | 58.41 | 1.0 | 0.6 | 4.0 |
| Vc+ Ga | 36.25 | 28.50 | 52.00 | 80.50 | 1.2 | 0.6 | 4.6 |
| VC+ BC | 33.45 | 26.65 | 51.55 | 78.20 | 1.2 | 0.6 | 4.3 |
| Ga+ BC | 34.75 | 26.05 | 50.50 | 76.55 | 1.2 | 0.6 | 4.6 |
| VC+ Ga+ BC | 35.15 | 28.68 | 58.50 | 87.18 | 1.4 | 0.6 | 4.8 |
| SEM± | 0.49 | 0.34 | 2.4 | 1.42 | 0.02 | 0.02 | 0.02 |
| CD (P= 0.05) | 1.40 | 1.02 | 4.8 | 3.72 | 0.08 | 0.08 | 0.18 |

**Table 2.** Effect of vermicompost, *Glomus aggregatum* and *Bacillus coagulans* on multiplication of *Meloidogyne incognita*, % mycorrhizal root colonization and spore number in Rhizosphere soil.

| Treatments* | Number/ plant | | Root- knot index (RKI) | Final nem. Popla./ 250 cc soil | Nematode reproduction rate | AMfungal root colonization, % | AMfungal spores/ 100 cc soil |
|---|---|---|---|---|---|---|---|
| | Galls | Egg masses | | | | | |
| Infested soil check | 132.5 | 68.22 | 4.00 | 676.4 | 3.46 | - | - |
| Non- infested soil check | - | - | - | - | - | - | - |
| Vermicompost (VC) | 59.25 | 42.65 | 3.48 | 486.4 | 2.51 | - | - |
| *Glomus aggregatum* (Ga) | 48.50 | 22.50 | 3.12 | 424.2 | 2.36 | 69.35 | 704 |
| *Bacillus coagulans* (BC) | 66.24 | 46.24 | 3.82 | 462.3 | 2.42 | - | - |
| VC+ Ga | 38.50 | 20.25 | 2.72 | 382.2 | 2.12 | 74.65 | 865 |
| VC+ BC | 39.65 | 32.08 | 3.24 | 395.6 | 2.45 | - | - |
| Ga+ BC | 39.25 | 28.65 | 2.68 | 383.4 | 1.98 | 78.25 | 922 |
| VC+ Ga+ BC | 18.25 | 19.62 | 2.65 | 265.4 | 1.42 | 92.05 | 992 |
| SEM± | 1.42 | 0.72 | - | 4.08 | 0.06 | 1.20 | 5.00 |
| CD (P= 0.05) | 4.11 | 2.08 | - | 16.06 | 0.84 | 3.50 | 17.00 |