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Sustainable management of root-knot disease of tomato by neem cake and *Glomus fasciculatum*

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Abstract: A pot experiment was conducted during winter season of 2009–2010 in the department of Botany, AMU, Aligarh, India, to determine the nematicidal potential of organic matter, neem cake at third level of dose, and bioagent, *Glomus fasciculatum* in terms of various growth parameters of tomato, when inoculated individually as well as concomitantly with respect to root-knot development. Neem cake and *G. fasciculatum* showed potential for sustainable management while providing nutrient sources for proper plant growth. Disease intensity of root-knot nematode decreased while increasing the doses of neem cake along with the *G. fasciculatum*. Chlorophyll contents have been found to be increased in single and combined application as well. There is a progressive increase in growth parameters raised in soil amended with 10, 20, and 30 g neem cake/kg soil and inoculated with *G. fasciculatum*. Significant improvement in the plant growth was observed when *G. fasciculatum* and neem cake were inoculated simultaneously. Neem cake plus *G. fasciculatum* reduced the nematodes' multiplication and root-galling, and increased the plant growth of tomato as compared to unamended and *Meloidogyne incognita*-inoculated plants. Mycorrhyzation and agronomic parameters were increased due to application of *G. fasciculatum* alone, but enhanced further when inoculated with neem cake.

Subjects: Science; Social Sciences; Technology; Crop Science; Agriculture; Pest Management

Keywords: neem cake; tomato; *Glomus fasciculatum*; management

Rizwan Ali Ansari

ABOUT THE AUTHORS

The Aligarh Muslim University, Aligarh, has been recognized for its outstanding contributions in the several aspects related to Plant Pathology and Nematology. Rizwan Ali Ansari, has been engaged with the development and formulation of different modules by exploiting the biological organisms and organic matters against the various economically important diseases infesting several agricultural crops. He has recently received a prestigious award by the Nematological Society of India (NSI) for his outstanding contribution in organic farming. In addition, thrust area of Ansari and his groups is to promote organic farming across the world by utilizing the organic matters, mychorrhizal fungi, PGPR, biofertilizers, and some other beneficial organisms for sustainable management of plant-parasitic nematodes through biological means as well as enriching the soil with nutrients necessary for plant growth and development.

PUBLIC INTEREST STATEMENT

Tomato, *Lycopersicon esculentum* Mill. is an important vegetable crop grown worldwide for its edible fruits, responsible for correcting malnutrition in the population of third-world countries like India. Tomatoes are major source of ascorbic acid and consumed in the form of juice, paste, ketchup, puree, soup, etc. Present findings will indeed disseminate the paramount information among the non-specialist readers, especially the farmers/growers and business entrepreneurs, whose livelihood is totally depend upon the vegetable production, not only in India, but also abroad. In this way, growers and related persons may be given assistance by deploying the methodology and doses of the different treatments and results obtained from this research. This will promote the organic production of vegetables in this country and elsewhere.

1. Introduction

Tomato, *Lycopersicon esculentum* Mill. is an important vegetable crop grown worldwide for its edible fruits, responsible for correcting malnutrition in third-world countries like India. Tomatoes are consumed in the form of juice, paste, ketchup, puree, soup, etc. Fruits, besides containing the essential amino acid like tryptophan, also have citric and malic acids in appreciable amounts. Tomato contains a glucoalkaloid 'tomatine' which is used as precipitating agent for cholesterol. The estimated world production of tomato is about 125.02 million tons and the total area under its cultivation is about 45.5 lakh ha. Indian contribution to the annual world production was 10.26 million tons, with an area of 5.72 lakh ha in 2006 (Mane, Sridevi, Salimath, Deshpande, & Khot, 2010); however, a slightly increased 17.50 million tons in the recent years FAOSTAT (2012). Andhra Pradesh ranked first for tomato production. Despite great production rate globally, productivity of this vegetable is far below expectation particularly in India as compared to other countries, which could mainly be due to improper and inadequate supply of nutrients, disease incidence, and lack of adoption of new improved production technologies.

There are several constraints on the successful cultivation of tomato. Nematode alone causes about 20.6% loss in yield worldwide (Sasser, 1989). Subramaniyan, Rajendran, and Vedivelu (1990) reported yield loss of 42.05–54.42% due to *Meloidogyne incognita* from India. Jain, Dabur, and Gupta (1994) reported 47.3 and 71.9% yield loss in vegetable crops due to *M. javanica* and *M. incognita,* respectively. The crop is greatly affected with root-knot nematodes in all parts of India and elsewhere (Siddiqui & Shaukat, 2003; Sikora & Fernández, 2005) and difficult crop pests to control (Chitwood, 2002) because they have high reproduction rates (Ananhirunsalee, Barker, & Beute, 1995). Nematode-infected plants generally show foliar symptoms like nutrient deficiency, particularly nitrogen (Birat, 1963; Good, 1968), yield losses in tomato due to root-knot nematode *Meloidogyne* spp. ranges from 35 to 50% in India (Jain, 1991; Jonathan, Kumar, Devarajan, & Rajendran, 2001) and as high as 85% globally (Sasser, 1979; Taylor & Sasser, 1978).

For the successful management of plant diseases mostly, the attention was focused only on chemical pesticides which are effective but also create a lot of hazards to man and environment. In addition to the target pest, they also kill a lot of beneficial micro-organisms in the rhizosphere and also contaminate soil and water and accumulate in plant parts. So, there is a need to explore and exploit other methods of disease management. For the last one decade, biofertilizers are used extensively as an ecofriendly approach to minimize the use of chemical fertilizers, improve soil fertility status, and for enhancement of crop production by their biological activity in the rhizosphere. The best biocontrol measures have been the micro-organisms that grow in the rhizosphere. Under natural conditions, these organisms provide the frontline defense against pathogens. Among the various micro-organisms, arbuscular mycorrhizal (AM) fungi have attracted considerable attention for their usefulness in biological control. Mycorrhizae, the associations between fungi and plant roots, are among the most widespread relationships among plant communities. These are potential tools now available to improve the overall performance and productivity of plants. Arbuscular mycorrhizae are ubiquitous and occur over a broad ecological range. They constitute an integral component of terrestrial ecosystems, forming symbiotic associations with plant root systems of over 80% of all terrestrial plant species, including many agriculturally important species. They are commonly found in agricultural crops, irrespective of soil types and in turn supply the plant with nutrients (Rangaswami, 1990). It is widely accepted that AM play a recognized role in nutrient cycling in the ecosystem; Harley and Smith (1983). AM fungi also stimulate the uptake of zinc, copper, sulfur, potassium, and calcium, although not as markedly as phosphorus (Cooper & Tinker, 1978). AM fungi help the roots in better absorption of water by exploring water in wider zones in soil (Safir, Boyer, & Gerdemann, 1971, 1972). AM fungi also act as biofertilizers to supplement phosphorus and nitrogen fertilizers, and considerable improvement in the growth of several crops was observed (Marwaha, 1995; Selveraj, Sivakumar, & Bhaskar, 1996).

Among a wide variety of organic matters that have been tested as organic amendments to soil for managing plant-parasitic nematodes are oil seed cakes. Oil seed cakes are byproduct of plant seed oil-processing industries and may suppress plant-parasitic nematodes in economically important

crops (Akhtar & Malik, 2000; D'Addabbo, 1995; Hafez & Sundaraj, 1999; Jothi, Sundara Babu, & Ramakrishnan, 2004; Muller & Gooch, 1982; Radwan, El-Maadawy, Kassem, & Abu-Elamayem, 2009; Rodriguez-Kabana, 1986; Sasanelli, Greco, D'Addabbo, Coiro, & Lamberti, 2003; Tiyagi & Ajaz, 2004). Neem cake is an organic byproduct obtained in the process of cold processing of neem tree fruits and kernels, and the solvent extraction process for neem oil cake. Neem cake has an adequate quantity of NPK in organic form for plant growth. Being a totally botanical product, it contains 100% natural NPK content and other essential micronutrients as N (2–5%), P (0.5–1%), K (1–2%), Ca (0.5–3%), Mg (0.3–1%), S (0.2–3%), Zn (15–60 ppm), Cu (4–20 ppm), Fe (500–1,200 ppm), and Mn (20–60 ppm) (Puri, 1999; Schmutterrer, 2002; Seenappa, 2009; Tewari, 1992; Vietmeyer, 1992). It reduces alkalinity in soil, improves organic matter content of soil, helping improvement in soil texture, water holding capacity, and soil aeration for better root development. It contains salannin, nimbin, azadirachtin, and azadiradione as major components.

Biological control implies total or partial destruction of a pathogen by any other organism except man. The term was introduced for the first time by G.F. Von Tabuef in 1914 (Baker, 1987). Interest in biological control first arose in 1920s, when some plant pathogens were suppressed by introducing some antibiotics-producing microbes. Biocontrol of nematodes mainly alludes with the application of biological agent for the reduction of plant-parasitic nematode population, either by introduction or by stimulation of natural enemies of the nematode in the soil. The objective of the present study was to determine the efficacy of neem cake and arbuscular mycorrhizae, *Glomus fasciculatum* against root-knot nematode, *M. incognita* infecting tomato plants, and also to determine suitable application rate of neem cake.

2. Materials and methods

2.1. Preparation and sterilization of soil mixture

Soil was collected from a field of the Botany Department, Aligarh Muslim University, Aligarh, having the texture of sandy-loam. The soil was mixed with river sand and organic manure in the ratio of 3:1:1 (v/v), and 6-inch diameter pots were filled with 1 kg of soil. Water was poured into each pot to wet the soil before transferring to an autoclave for sterilization at 138 kpa for 20 min.

2.2. Raising and maintenance of the test plants

The tomato (*L. esculentum* var. Sona-21) seeds were surface sterilized in 0.01% mercuric chloride ($HgCl_2$) for 2 min and then rinsed three times with sterile water. Seeds were sown in the sterilized soil in 25 cm clay pots. One week after germination, seedlings were transplanted into each pot containing 1 kg of sterilized soil.

2.3. Preparation of nematode inoculum

Samples of nematode-infected brinjal or tomato roots were collected from the village Shankarpur, Hathras, India. Females of root-knot nematode were collected and identified as *Meloidogyne incogntia*. Pure culture of *M. incognita* was maintained in the greenhouse of the department on *Solanum melongena*. Large numbers of egg masses of root-knot nematode were hand-picked using a sterilized forceps from heavily infected eggplant root. These egg masses were washed in distilled water and then placed in 10-cm diameter 15 mesh sieves containing crossed layers of tissue paper and placed in petri dishes containing water just deep enough to contact the egg masses. The hatched juveniles were collected every 24 h and fresh water was added to the petri dishes. The concentration of second-stage juveniles (J_2) of *M. incogntia* in the water suspension was adjusted, so that each milliliter contained 200 ± 5 nematodes. Ten ml of this suspension containing 2,000 freshly hatched juveniles (J_2) was added to each pot.

2.4. AM fungus inoculum

G. fasciculatum inoculum spores were isolated and identified and the culture was maintained on *Zea mays* grown in sandy-loam soil mixed with washed river and farmyard manure in the ratio of 3:2:1 (v/v/v), respectively, in the greenhouse of the department. Later, spores of *G. fasciculatum* were

isolated by sieving from pure culture soil maintained on *Z. mays*, suspension obtained from sieving was poured on filter paper and spores of AM fungus were picked up with a fine camel hair brush or needle under a binocular. Spores of each fungus were placed in 10 ml of distilled water and poured into the pots at the rate of 600 spores per plant. Any spores remaining in the beaker that contained the spores were picked up with the brush and placed near the roots.

2.5. Soil amendment with neem cake

Oil cakes of neem were thoroughly mixed with sandy clay loam soil (51% sand, 15% silt and 34% clay; pH 7.8 and organic matter of 0.73%) at 10, 20, and 30 g/kg soil in 15-cm clay pots. Pots without amendments were kept as control. All pots were arranged in a completely randomized block design on a bench in a greenhouse that averaged 27°C minimum and 32°C maximum temperature. Each treatment was replicated five times. The pots were watered daily to ensure proper decomposition of organic matter. After 2 weeks, 7 days old tomato seedling was transplanted into each pot. The plants were then inoculated with 2,000 J_2 of *M. incognita* and 600 spores of *G. fasciculatum* after 48 h.

2.6. Inoculation technique

For inoculation, soil around the roots of the plant was carefully moved aside without damaging the roots. The inoculum suspension of both nematode and AM fungus alone and concomitantly was poured/placed around the roots, and the soil was replaced. The control treatments received sterile distilled water of a volume equal to that of the inoculum suspension.

2.7. Observations

Plants were uprooted 60 days after inoculation and the root systems were rinsed free of soil. The plants were cut with a knife just above the root emergence zone. Length of plants were measured from the top of first leaf to the end of root and recorded in cm. Fresh weight of plant was recorded in gram. For dry weight determination, shoots were put in envelopes and kept in a hot air oven at 60°C for 2–3 days before weighing. The number of galls per root system was also counted.

A 250 g subsample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting method followed by Baermann funnel extraction. Nematode suspension was collected after 24 h and the number of nematodes was counted by taking 1 ml of the suspension from each sample in a counting dish. The means of the five counts were used to calculate the population of nematodes per kg soil. To estimate the number of juveniles, eggs, and females inside the roots, 1 g subsamples of roots were macerated for 30–40 s in a waring blender and counts were made from the suspensions so obtained. The total number of nematodes present in the roots was calculated by multiplying the number of nematodes present in 1 g of root by the total weight of root.

3. Mycorrhization procedure

3.1. Clearing and staining (Phillips & Hayman, 1970)

Roots were washed with tap water and cut into 1-cm long segments and then boiled in 10% KOH solution at 90°C for 45 min. KOH solution was then poured off and roots were rinsed well in a beaker until no brown color appeared in the rinsed water. Alkaline H_2O_2, which was used to bleach the roots, was made by adding 3 ml of NH_4OH to 30 ml of 10% H_2O_2 and 567 ml of tap water. The roots were rinsed thoroughly at least three times using tap water to remove the H_2O_2. Roots were then treated with 0.05% trypan blue (in lactophenol) and kept overnight for destaining in a solution prepared with acetic acid (laboratory grade)—875 ml, glycerine—63 ml, and distilled water—63 ml. The cellular contents were removed by this method and the AM fungal structures get stained dark blue. These stained root segments were used for determining the root colonization by AM fungi.

Percentage of root colonization and percent arbuscules were determined by slide method (Giovannetti & Moose, 1980). The root segments were selected at random from the stained sample and mounted on microscopic slides in groups of ten. One hundred to 150 root segments from each

samples were used for the assessment. The presence/absence of colonization in each root segment was recorded and percent colonization was calculated as follows:

$$\text{Percent AM association} = \frac{\text{No. of mycorrhizal segments}}{\text{Total no. of segments screened}} \times 100 \quad (1)$$

Isolated spores were identified with the help of keys provided by different workers (Bakshi, 1974; Hall & Fish, 1979; Rani & Mukerji, 1988; Srinivas, Ramraj, & Sharma, 1988; Trappe, 1982).

3.2. Estimation of chlorophyll

The green leaves were taken for chlorophyll estimation and it was done according to the method of Hiscox and Israelstam (1979). One hundred milligram of tomato leaf pieces were kept in a vial containing 7 ml of a chemical named dimethyl sulfoxide (DMSO) and the chlorophyll content was extracted into the fluid by incubating for 60 min. Then this extract was transferred to a graduated tube and assayed immediately. A sample of 3 ml chlorophyll extract was transferred in cuvette and the optical density value at 645 and 663 nm was read in spectronic-1001 spectrophotometer against DMSO blank. Tomato fruit samples of 10 g in each replicate were taken for the estimation of ascorbic acid. The ascorbic acid content was estimated titrimetrically 2, 6-dichlorophenol indophenols dye (Rangana, 1976).

3.3. Estimation of N, P and K in plant

Nitrogen content in plants was determined according to the IITA (1975) procedure. Phosphate and potash contents from plants were estimated by the method of Lindner (1944).

3.4. Statistical analysis

The entire data collected during this study was statistically analyzed in simple randomized design by the method of Panse and Sukhatme (1985). Least significant differences was calculated at $p = 0.05$ and $p = 0.01$.

4. Results

4.1. Growth parameters

The growth of tomato plants in terms of length, and fresh and dry weights was increased when soil was amended with 10 g neem cake as compared to uninoculated control and this increase was further enhanced when more and more oil cakes (20 and 30 g) were added. There is a progressive increase in length, and fresh and dry weights when tomato plants were raised in soil amended with 10, 20, 30 g neem cake/kg soil and inoculated with *G. fasciculatum* (36.56, 53.41, 62.40, 50.25, 66.61, 69.74, 56.70, 72.22, and 77.37%). A significant increase in the plant growth was observed when both biocontrol agents – *G. fasciculatum* and neem cake – were inoculated. Growth of tomato plants inoculated with *G. fasciculatum* + *M. incognita* did not differ significantly from the control irrespective of the dosages of neem cake. Inoculation of *M. incognita* caused a significant reduction in the growth parameters (28.25, 31.24 and 32.38%) over uninoculated control. Neem cake and *G. fasciculatum* together reduced the multiplication and root-galling caused by *M. incognita,* which ultimately increased the plant growth of tomato as compared to unamended and *M. incognita*-inoculated plants (38.12, 48.95, 50.00, 44.92, 54.34, 56.05, 48.26, 59.51 and 64.04%) (Tables 1–3).

4.2. Chlorophyll content

Chlorophyll content was significantly increased in plants grown in amended soil with the neem cake at 10, 20, and 30 g along with the AM fungi. However, the increase was parallel to the increase in dose of neem cake along with *G. fasciculatum*. Maximum improvement in chlorophyll was recorded in those treatments receiving at 30 g + *G. fasciculatum* followed by at 20 g + *G. fasciculatum* and at 10 g + *G. fasciculatum*. Significant reduction in nematode population was recorded in those plants received at 30 g + *G. fasciculatum*. However, all treatments showed improvement in chlorophyll contents but combined application of NC + GF with at 30 g + *G. fasciculatum* exhibited significant value over control.

Table 1. Interactive effect of neem cake at 10 g/kg soil and *G. fasciculatum* on growth parameters, nematode population, and mycorrhization in tomato plants inoculated with *M. incognita*

Treatments	Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Egg masses/ root system	Number of eggs/ egg mass	Number of galls/ plant	Nematode population in soil	Mycorrhization	Chlorophyll content	N	P	K
Control	42.50	32.87	10.59	–	–	–	–	–	2.156	51.62	41.64	33.27
NC	50.63	45.10	15.43	–	–	–	–	–	2.841	83.94	70.76	57.80
MI	30.49	22.60	7.16	67.2	83.4	172.5	10,498	-	1.209	36.43	30.54	24.28
NC + MI	45.17	35.39	13.60	25.5	37.0	55.6	6,260	–	2.531	61.80	55.37	44.51
GF	48.60	43.00	13.90	–	–	–	–	47.5	2.667	68.50	60.24	48.40
GF + NC	67.00	70.55	28.17	–	–	–	–	60.7	2.961	93.78	79.07	61.33
GF + MI	43.80	33.19	11.44	30.6	46.2	64.1	7,003	42.6	2.461	54.27	48.30	37.90
NC + GF + MI	49.27	44.27	14.32	13.7	20.7	28.3	2,359	54.9	2.787	76.62	62.72	53.17
CD (p = 0.05)	**4.19**	**3.73**	**1.27**	**4.73**	**5.29**	**7.65**	**30.92**	**3.49**	**0.11**	**5.77**	**4.53**	**3.51**
CD (p = 0.01)	**5.65**	**5.03**	**1.71**	**6.63**	**7.42**	**10.73**	**43.35**	**4.89**	**0.66**	**7.78**	**6.11**	**4.74**

NC is the neem cake; MI is the *Meloidogyne incognita*; GF is the *Glomus fasciculatum*.

MI = 2,000 J_2; GF = 600 spores.

Each value is a mean of five replicates.

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Table 2. Interactive effect of Neem cake at 20 g/kg soil and *G. fasciculatum* on growth parameters, nematode population, and mycorrhization in tomato plants inoculated with *M. incognita*

Treatments	Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Egg masses/ root system	Number of eggs/ egg mass	Number of galls/ plant	Nematode population in soil	Mycorrhization	Chlorophyll content	N	P	K
Control	42.50	32.87	10.59	–	–	–	–	–	2.156	51.62	41.64	33.27
NC	58.27	56.33	19.66	–	–	–	–	–	2.927	86.56	73.25	60.17
MI	30.49	22.60	7.16	67.2	83.4	172.5	10,498	–	1.209	36.43	30.54	24.28
NC + MI	48.57	39.49	14.44	14.5	22.3	27.9	4,029	–	2.616	64.80	57.72	46.78
GF	48.60	43.00	13.90	–	–	–	–	47.5	2.724	71.26	63.15	51.04
GF + NC	85.44	98.43	35.00	–	–	–	–	74.3	3.039	96.50	82.31	63.84
GF + MI	43.80	33.19	11.44	21.2	27.1	36.3	5,062	45.2	2.536	57.36	50.49	40.37
NC + GF + MI	55.36	49.50	16.29	7.3	11.6	15.7	0520	63.5	2.858	79.40	67.98	56.19
CD (p = 0.05)	**4.72**	**3.97**	**1.50**	**5.15**	**5.39**	**8.48**	**33.17**	**4.31**	**0.13**	**5.93**	**4.74**	**3.92**
CD (p = 0.01)	**6.37**	**5.36**	**2.02**	**7.22**	**7.56**	**11.89**	**46.50**	**6.04**	**0.78**	**8.00**	**6.93**	**5.29**

NC is the neem cake; MI is the *Meloidogyne incognita*; GF is the *Glomus fasciculatum*.

MI = 2,000 J_2; GF = 600 spores.

Each value is a mean of five replicates.

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Table 3. Interactive effect of neem cake at 30 g/kg soil and *G. fasciculatum* on growth parameters, nematode population, and mycorrhization in tomato plants inoculated with *M. incognita*

Treatments	Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Egg masses/ root system	Number of eggs/ egg mass	Number of galls/plant	Nematode population in soil	Mycorrhization	Chlorophyll content	N	P	K
Control	42.50	32.87	10.59	–	–	–	–	–	2.156	51.62	41.64	33.27
NC	65.26	63.40	22.35	–	–	–	–	–	2.984	89.55	76.56	63.36
MI	30.49	22.60	7.16	67.2	83.4	172.5	10,498	–	1.209	36.43	30.54	24.28
NC + MI	53.73	42.27	15.43	8.5	14.5	20.4	2,667	–	2.679	67.32	60.00	49.33
GF	48.60	43.00	13.90	–	–	–	–	47.5	2.781	73.80	66.20	53.62
GF + NC	98.16	118.32	46.80	–	–	–	–	85.2	3.108	99.86	85.19	66.58
GF + MI	43.80	33.19	11.44	13.5	21.0	29.3	3,059	46.7	2.603	61.65	53.23	43.13
NC + GF + MI	58.93	55.82	19.91	3.4	6.7	10.2	0316	70.0	2.906	82.50	70.14	59.24
CD (p = 0.05)	**5.20**	**4.63**	**2.75**	**5.30**	**5.44**	**9.66**	**36.92**	**5.33**	**0.16**	**6.22**	**5.18**	**4.39**
CD (p = 0.01)	**7.02**	**6.25**	**3.71**	**7.43**	**7.63**	**13.54**	**51.76**	**7.47**	**0.96**	**8.39**	**6.99**	**5.924**

NC is the neem cake; MI is the *Meloidogyne incognita*; GF is the *Glomus fasciculatum*.

MI = 2,000 J_2; GF = 600 spores.

Each value is a mean of five replicates.

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4.3. Nutrient contents in plant

The results observed that individual as well as combined inoculations of neem cake and *G. fasciculatum* significantly improved the N, P, and K contents as compared to uninoculated control. The individual inoculation of organics as well as *G. fasciculatum* improved less N content in plants as compared to combined inoculations. The highest N content was observed in the combined treatment of at 30 g + *G. fasciculatum*-inoculated plants, where it was recorded as 99.86 mg/kg, while the minimum was determined in untreated control (51.62 mg/kg), but decreased significantly with the nematode-inoculated plants and recorded as 36.43 mg/kg. The different doses of organics were used to observe the difference about the performance to increase the maximum amount of nitrogen. The same order of improvement/reduction in P and K was recorded (Table 1).

4.4. Nematode population

Data given in Tables 1–3 revealed that neem cakes at the rates applied in this study from 10 to 30 g/kg soil significantly reduced the population density of nematodes in terms of number of eggmasses/root system, number of eggs/eggmass, number of galls/plant, and number of nematodes in soil. The AM fungi, *G. fasciculatum,* was found to be mitigating the losses incurred by root-knot nematode, *M. incognita*. The number of nematodes in neem cake amended soil was lesser than in the plants treated with *G. fasciculatum,* but simultaneous treatment with neem cake and *G. fasciculatum* proved to be more effective than when treated individually. Their effectiveness increases with the increase in the dosages of neem cake.

4.5. Mycorrhization

In general, the external colonization was highest when plants were inoculated with *G. fasciculatum*. The increase in mycorrhization was progressively enhanced significantly with the increase in the dosage of neem cake from 10 to 30 g/kg soil. There is a significant decline in mycorrhization when mycorrhizal plants were inoculated with *M. incognita,* but the decline in mycorrhization was further improved when mycorrhizal plants were raised in neem cake-amended soil and inoculated with *M. incognita*. The higher the dose of neem cake, the higher the mycorrhization level (Tables 1–3).

5. Discussion

The present study showed that *G. fasciculatum* is beneficial to the tomato plants, as it helps plant growth by promoting shoot and root growth of the plants. The improvement of plant growth characterized in mycorrhizal plants compared to control indicates the existence of complex physiological and biochemical beneficial relationship between the *G. fasciculatum* and tomato. AM fungi have been reported to alter the physiology of the root and reduce root-exudation (Graham, Leonard, & Menge, 1981), causing changes in phytohormone levels (Allen, Moore, & Christensen, 1980) and photosynthetic rate (Allen, Smith, Moore, & Christensen, 1981). Improved nutrient status in the mycorrhizal plants resulted in increased biomass production and growth potential. This supports the earlier findings that mycorrhizal infection can cause a beneficial physiological effect on host plants by increasing uptake of soil phosphorus (Gerdemann, 1968, 1975; Koopmans et al., 2004; Smith & Read, 1997).

There was a significant difference among the various doses of neem cake on the growth of tomato plant and also on the reduction of nematode population, both in the soil and roots. By increasing the concentration of neem cake, the total number of nematodes within roots decreased with the significant growth in tomato plant. The highest being observed when tomato plants were raised in soil amended with 30 g of neem cake. Soil treatments with neem cake significantly reduced the root-galling and number of eggs/eggmass in the root that resulted in the decrease in the soil population of root-knot nematode. The highest was observed at 30 g neem cake/kg soil. It has been reported that certain micro-organisms that contributed to the decomposition of oil cakes produce certain products like ammonia, fatty acids, formaldehyde, and phenols (Alam, Ahmad, & Khan, 1980; Alam, Siddiqui, & Khan, 1977; Khan, Alam, & Saxena, 1974; Oka et al., 2000; Sitaramaiah & Singh, 1978; Tiyagi, Khan, & Alam, 2001). The effect of these combined factors leads to reduced nematode development.

The ability of fungal biocontrol agent, *G. fasciculatum,* in the management of root-knot nematode, *M. incognita,* increased in the presence of neem cake. It has been observed by various workers that biocontrol agents of soil-borne plant pathogens are applied to soil in combination with organic materials (Goswami, Pandey, Rathour, Bhattacharya, & Singh, 2006; Harrier & Watson, (2004); Haseeb, Sharma, & Shukla, 2005; Mittal, Saxena, & Mukerji, 1995; Rodriguez-Kabana, Morgan-Jones, & Chet, 1987). It is assumed that organic materials contribute to enhanced biological activities against the target pathogen by providing the needed nutrients for the initial growth of the biocontrol agents in soil and may be used as carriers to facilitate distribution. The decomposition of organic matter released nematicidal principle(s) and the residual organic matter increased fungal activity and persistence (Ashraf & Khan, 2010; Harrier & Watson, 2004; Kerry, 1984; Mani & Anandam, 1989; Rao & Reddy, 1994).

From the results obtained, a conclusion may be drawn that the inoculation of tomato seedlings with *G. fasciculatum* and soil amended with neem cake can be used as a biological tool for managing the root-knot nematode, *M. incognita*.

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