



SOIL & CROP SCIENCES | RESEARCH ARTICLE

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*Corresponding author: Rizwan Ali Ansari, Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh 202002, India
E-mail: rizwans.ansari@gmail.com

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Dynamic role of organic matter and bioagent for the management of *Meloidogyne incognita*–*Rhizoctonia solani* disease complex on tomato in relation to some growth attributes

Rose Rizvi¹, Rizwan Ali Ansari^{2*}, Anam Iqbal¹, Safiuddin Ansari¹, Aisha Sumbul¹, Irshad Mahmood¹ and Sartaj A. Tiyaqi¹

Abstract: A disease complex involving *Meloidogyne incognita*–*Rhizoctonia solani* was studied on tomato (*Lycopersicon esculentum* var. Pusa Ruby) under glass-house conditions to determine their concomitant effect on plant growth variables. Biofertilizers *Nerium indicum* and *Trichoderma harzianum* were tested against both pathogens individually as well as concomitantly and found its role in minimizing disease severity. Inoculation of *M. incognita* and *R. solani* resulted a significant reduction in plant growth variables over control. The plant growth variables reduction was more pronounced by *M. incognita* as compared to *R. solani*. However, *T. harzianum* exhibited their potential against the disease complex but was less effective than *N. indicum*. A manifold improvement in plant growth parameters was observed when plants were treated with biofertilizers, *N. indicum* and *T. harzianum* simultaneously. The present work has revealed that the combined application of *N. indicum* and *T. harzianum* may be a better option for the management of disease complex *M. incognita*–*R. solani* on tomato. Application of these biofertilizers after field trials may be suitable module of organic farming.



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. Corresponding author, 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 to promote organic farming across the world by utilizing the organic matters, mycorrhizal fungi, PGPR, biofertilizers, and some other beneficial organisms for sustainable management of plant-parasitic nematodes through biological means as well as enrich the soil with nutrients necessary for plant growth and development.

PUBLIC INTEREST STATEMENT

Tomato, *Lycopersicon esculentum* Mill., is an important vegetable crop being grown across the world including India. It is consumed in diverse way including as an ingredient in many dishes, sauces, salads, and drinks. The fruit is rich in lycopene, which may have beneficial health effect. Present findings will indeed disseminate the paramount informations among the non-specialist readers especially the farmers/growers and business entrepreneurs who are getting hurdles in tomato cultivation across the world, especially in India. 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 globally.

Subjects: Bioscience; Environment & Agriculture; Environmental Studies & Management; Food Science & Technology

Keywords: tomato; *Meloidogyne incognita*; *Rhizoctonia solani*

1. Introduction

Tomato is one of the most important vegetable crop which is being cultivated worldwide. India is the second largest producer of tomato in the world after China with annual production of 17,500,000 metric tons (FAO, 2012). Tomato belongs to the family solanaceae, is a rich source of lycopene, used in the cancer treatment especially in prostate cancer (Giovannucci, 1999). According to the National Cancer Institute, there is enough data to show that people who consume large amount of tomato products have significantly decreased risk of prostate, lung, and stomach cancer. A large number of phytopathogens have been encountered in relation to productivity reduction of tomato crop. Among them, root-knot nematodes are the major problem (Keshari & Gupta, 2015; Singh, Rai, Singh, & Singh, 2011).

Meloidogyne incognita causes root-knot disease in tomato plant by abnormal expansion of root cell and forming giant cells (Singh & Patel, 2013). *M. incognita* is one of the key nematode which is supposed to be widely distributed (Siddiqui & Shahid Shaukat, 2003; Sikora & Fernández, 2005) and difficult to control (Chitwood, 2002) because of its high reproduction rate (Ananhirunsalee, Barker, & Beute, 1995). A yield loss of tomato ranging from 32 to 40% due to root-knot nematode has been reported by Anwaar and Mckenry (2012).

Many soil fungi also caused innumerable diseases on tomato plants and hampered its production. Fungi are spore-forming and cause local or general necrosis as well as chlorosis of plant tissue. Among them, *Rhizoctonia solani* causes root rot and stem rot on tomato plants (El-Mohamedy, Jabnoun-Khiareddine, & Daami-Remadi, 2014).

Existence of *M. incognita* and/or *R. solani* was monitored in areas where tomato is cultivated in large scale (Aligarh, Uttar Pradesh). The interactive nature of these pathogens has already been reported (Chahal & Chhabra, 1984; Golden & Van Gundy, 1974; Goswami, Seth, Gupta, & Singh, 1975; Kumar & Haseeb, 2009; Safiuddin, Tiyagi, Rizvi, & Mahmood, 2014; Sagar, Rao, & Varaprasad, 2012). It has been advocated by several research that the pernicious effects of *R. solani* become more pronounced in the presence of *M. incognita* (Abuzar, 2013; Anwar & Khan, 2002; Bhagawati, Das, & Sinha, 2007; Mokbel, Ibrahim, Shehata, & El-Saedy, 2007).

Application of synthetic pesticides to control phytopathogens warrants immediate results. However, its application has changed the food web of natural biodiversity. Also, application of chemical pesticides causes many diseases to mankind and other vertebrates. On the other hand, natural products proved to be environmentally safe which may replace synthetic pesticides (Kim et al., 2005). Hence, there is a need to identify these eco-friendly plant products (Duke, 1990). *Nerium indicum* is a well-known alternative to chemical pesticides specially for the purpose of protecting crops against nematodes and also for the conservation of biodiversity (Ahmad, Karim, & Khan, 1990; Elbadri, Lee, Park, Yu, & Choo, 2008; Hameed, 1990; Singh & Patel, 2013). Large number of informations are available about botanicals which are being utilized for the eco-friendly and sustainable management of plant parasitic nematodes and soil-pathogenic fungi (Rizvi, Mahmood, Tiyagi, & Khan, 2012). Beneficial mycoflora are also found in the rhizosphere which protect the crops against harmful pathogens. Biomangement of root-knot nematodes (*Meloidogyne* spp.) by *Trichoderma* spp. has been found safe to soil biota (Affokpon et al., 2011; AL-Shammari, Bahkali, Elgorban, El-Kahky, & Al-Sum, 2013; Mascarin, Junior, Filho, & de, 2012; Naserinasab, Sahebani, & Etebarian, 2011; Rao, Reddy, & Nagesh, 1998; Sharon, Chet, & Spiegel, 2011; Sharon et al., 2001, 2007; Spiegel, Sharon, & Bar-Eyal, 2007). *Trichoderma harzianum* is used frequently as an antagonistic (Montealegre et al., 2010). That acts as indirectly by releasing toxic metabolites, or by competing other pathogens for food and space, directly by releasing toxic antibiotic substances (Kumar, 2013). Large number of information on this

important cash crop has generated an impetus to assess the significant role of *N. indicum* and *T. harzianum* against the disease complex involving *M. incognita* and *R. solani* in relation to some growth parameters of tomato plants.

2. Materials and method

2.1. Preparation and sterilization of soil mixture

Soil was collected from the field of the Botany Department, Aligarh Muslim University, Aligarh having the texture of sandy-loam. Before experimentation, the soil was processed to assess some important features of the soil e.g. pH 6.5, particle size (sand: 70.3, silt: 20.5, clay: 9.2), and organic carbon: 1.08%. The pH was measured with the help of pH meter (Mc Lean, 1982). The texture of soil in relation to particle size was determined by hydrometer method (Allen, Grimshaw, Parkinson, & Quarmby, 1974) and % organic carbon by Walkley (1947). The soil was mixed with river sand and organic manure in the ratio of 3:1, and 15-cm diameter pots were filled with 1 kg of soil (Rizvi et al., 2015). The chemical composition of organic manure in the form of Farm Yard Manure (FYM) used in this experiment comprises N (0.50%), P₂O₅ (0.20%) and K₂O (0.28%), bulk density (1.50), water holding capacity (43.62) and porosity (47.32%). About 250 mL of water was poured into each pot to wet the soil before transferring to an autoclave for sterilization at 138 kpa for 20 min. Soil moisture content was maintained in each pot by weighing each vial and adding water (by weight) as required (200 mL). Volumetric moisture content was calculated according to the procedure described by Campbell, Nicholaichuk, Davidson, and Cameron (1977).

2.2. Raising and maintenance of the test plant

The seeds of tomato (*Lycopersicon esculentum* var. Pusa Ruby) were surface sterilized in 0.01% mercuric chloride (HgCl₂) for two minutes and then rinsed three times with sterile water. Seeds were sown in the sterilized soil in 25-cm clay pots. One-week old seedlings were transplanted per pot.

2.3. Preparation of nematode inoculum

Root-knot infected brinjal roots were collected from the infested fields of Aligarh and its adjoining areas. Females of root-knot nematode were collected and identified as *M. incognita* on the basis of perineal pattern. Pure culture of *M. incognita* was maintained on *Solanum melongena* in the greenhouse of the department. Large number of egg masses of root-knot nematode were handpicked, 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 juveniles hatched were collected after every 24 h and fresh water was added to the Petri dishes. The amount of second-stage juveniles in the water suspension was adjusted so that each mL contained 200 nematodes. Ten milliliters of such suspension containing 2,000 freshly hatched juveniles were inoculated to each pot.

2.4. Procurement and maintenance of *R. solani*

Pure culture of *R. solani* was procured from Indian Type Culture Collection of the Plant Pathology Unit, IARI, New Delhi. The culture was then subcultured and maintained on PDA and Richard's liquid (Riker & Riker, 1936) as and when required. The Richard's liquid medium was prepared in 250-mL Erlenmeyer flasks, each flask containing about 80 mL of Richard's liquid medium. Small bits of the mycelium of the fungus were transferred to the conical flasks. Inoculated flasks were incubated at 28 ± 2°C for about 15 days to allow growth of the fungus. The pure culture of the fungus was continuously maintained on PDA contained in the test tubes by reinoculation of the fungus after every 15 days. After incubating the conical flasks for about 15 days, the liquid medium was filtered through Whatman filter paper No.1. Thereafter, mycelial mat was washed in distilled water to remove the traces of medium and gently pressed between the folds of blotting paper to remove the excess amount of water. Ten gram fungal mycelium was mixed in 90 mL of sterile water (1:10) and blended for 30 s in a waring blender to prepare the inoculum. Two gram mycelial mat of *R. solani* was inoculated to each pot.

2.5. Preparation of *T. harzianum*

Pure culture of *T. harzianum* was purchased from IARI, New Delhi and further revived on Potato Dextrose Agar (PDA) containing 200-g peeled potato, 20-g dextrose, and 20-g agar agar. The Petri dishes were then placed in an incubator at 24°C for 1–2 weeks. Ten mycelia were carefully scraped from the media and suspended in 90-mL distilled water (1:10). Two gram mycelial mat of *T. harzianum* was used for each treatment.

2.6. Preparation of *N. indicum*

Leaves of *N. indicum* were handpicked and rinsed with distilled water. After washing, the leaves were chopped with sharp sterile knife and incorporated in soil at the rate of 20 g/kg soil/pot. These pots were immediately watered with equal amount to prepare the compost of *N. indicum* leaves which were left for 20 days.

2.7. Estimation of chlorophyll content

Chlorophyll content of leaf was estimated by the method of Hiscox and Israelstam (1979). One hundred milligrams of leaf pieces were placed in a vial containing 7 mL of dimethyl sulfoxide (DMSO) and the chlorophyll was extracted into the fluid by incubating for 60 min. The extracts was transferred to a graduated tube and made up to 10 mL with DMSO and assayed immediately. A sample of 3 mL of chlorophyll extract was transferred to a cuvette and the optical density (OD) values at 645 and 663 nm were read using a spectrophotometer (Spectronic 1001) against a DMSO blank.

2.8. Percent pollen fertility

The percent pollen fertility was estimated by the method of Brown (1949) when the plants attained the subsequent stage using stainability of pollen grains in 1% acetocarmine solution.

2.9. Inoculation techniques

Just before inoculations, roots of tomato seedlings were exposed by carefully removing the top layer of soil and the required quantity of nematode suspension and fungus inoculum was poured uniformly around the exposed roots using sterilized pipette. Exposed roots were immediately covered with soil properly. There were five replicates of each treatment, uninoculated plants served as control. Plants were regularly watered in equal amount as and when required with proper thinning in appropriate time.

2.10. Experimental design

Treatments

- Control
- NI (*Nerium indicum*)
- MI (*Meloidogyne incognita*)
- RS (*Rhizoctonia solani*)
- TH (*Trichoderma harzianum*)
- NI + MI
- NI + TH
- NI + RS
- MI + RS
- MI + TH
- RS + TH
- RS + TH + MI
- RS + TH + MI + NI
- RS + MI + NI

The 14 treatments each with five replicates were arranged in a completely Randomized Block Design (RBD) and maintained in a glasshouse with air temperature. All the plants were watered up to the soil capacity. The tomato plants were uprooted 60 days after nematode inoculation for determining the plant growth and nematode-related parameters.

2.11. Parameters

After termination of the experiment, the following parameters were determined for each treatment;

- Plant length (cm)
- Plant fresh weight (g)
- Plant dry weight (g)
- Fruits per plant
- Fresh weight of fruits (g)
- Percent pollen fertility
- Chlorophyll content (mg g^{-1} fresh leaves)
- Nematode population
- Number of gall root system⁻¹
- Number of egg masses root system⁻¹
- Percent root rot

2.12. Nematode related parameters

2.12.1. Nematode population

A 250-g subsample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting method, followed by Baerman's funnel extraction to determine the final nematode population in soil (Southey, 1986). The mean of five counts was used to calculate the population of nematode per kg soil.

2.13. Galls, egg masses, and root-rot percentage

At termination of the experiment, roots of harvested plants were washed under the tap and examined for the presence of the galls. For the assessment of egg masses, plant roots were immersed for 15 min in 0.015% Phloxine B, which specifically stains the gelatinous matrix of nematode egg masses bright red and the egg masses per root system were counted. Percentage of root-rot was calculated by visual observation on root system/plant.

2.14. Statistical analysis

The entire data collected during the study were statistically analyzed in simple randomized design by the method of Panse and Sukhatme (1985). Critical difference (CD) was calculated at 5% and Duncan's Multiple Range test (DMRT) was employed to test for significant differences between treatments.

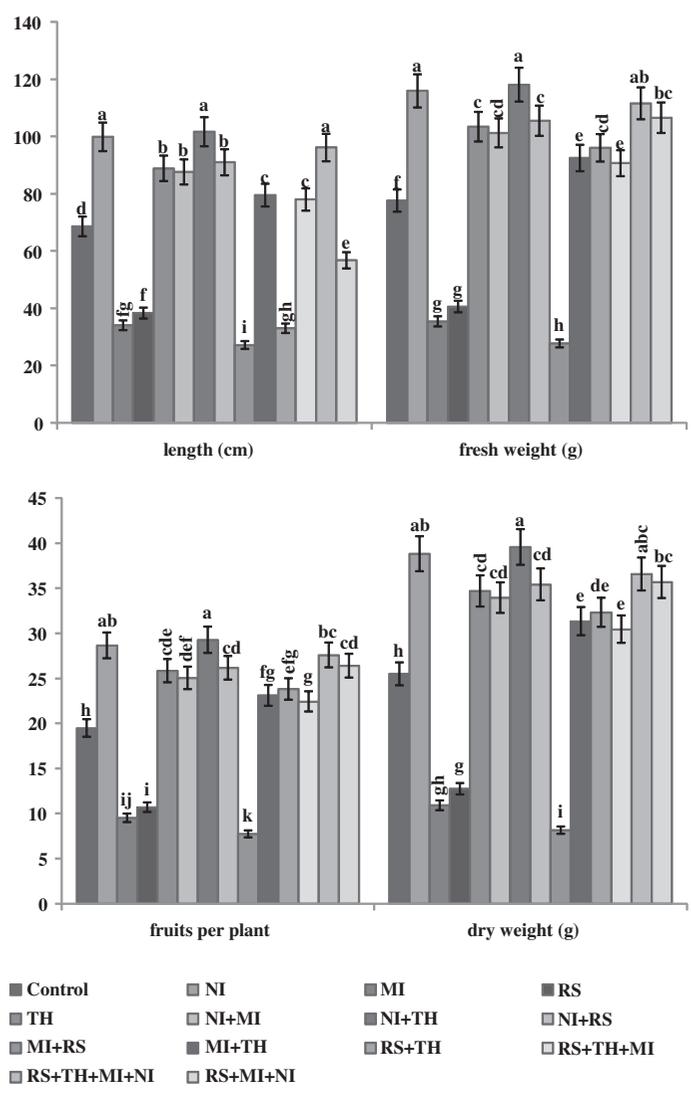
3. Results

Present study revealed that combined application of *N. indicum* and *T. harzianum* showed maximum improvement in growth variables as compared to their individual application as well as untreated control. In addition, maximum inhibition in root-rot incidence and nematode multiplication was observed in combined application of both biofertilizers. On the other hand, concomitant application of *M. incognita* and *R. solani* showed significant reduction in growth parameters; however, their individual inoculation also reduced the growth parameters but was less than their concomitant inoculation.

Amendment of organic matter, *N. indicum* significantly improved the plant growth like plant length, fresh weight, fruits per plant, and dry weight over control (Figure 1). However, maximum improvement in plant growth was observed in those plants that received the simultaneous application of *T. harzianum* and *N. indicum*. In contrast, minimum plant length was recorded in plants that

Figure 1. Soil application of *N. indicum* and *T. harzianum* on disease complex involving *M. incognita* and *R. solani* on growth parameters (Plant length, fresh weight, fruits/plant, dry weight) of tomato.

Notes: NI = *Nerium indicum*, MI = *Meloidogyne incognita*, RS = *Rhizoctonia solani*, TH = *Trichoderma harzianum*; values followed by different letters are significantly different over control.



were given concomitant application of *R. solani* and *M. incognita* followed by their individual inoculations. However, individual application of *N. indicum* was found effective and improved the plant growth significantly followed by *T. harzianum*. Manifold improvement was recorded in fresh weight of fruits, chlorophyll content, and percent pollen fertility in the experimental set those were given concurrent application of *N. indicum* and *T. harzianum* (Table 1). However, drastic decreased in plant growth was recorded in concomitantly applied (nematode + fungus) plants (Table 1; Figure 1). Moreover, maximum chlorophyll contents were recorded in plants that received combined application of *N. indicum* plus *T. harzianum*; however, in other treatments, improvement was also recorded but their effectiveness was found to be less than NI + TH. Maximum reductions in chlorophyll content were observed in plants treated with *M. incognita* + *R. solani* followed by individual inoculation of *M. incognita* and *R. solani* (Table 1). To support this hypothesis, a correlation analysis was performed between chlorophyll content and number of galls per root system (Figure 2).

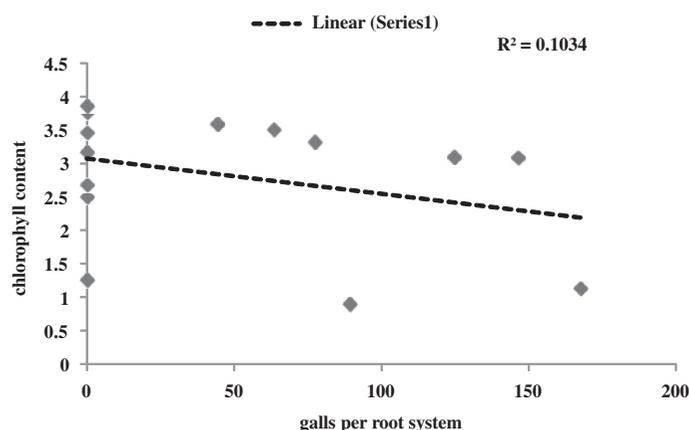
Significant reduction in root galls and egg masses/root system was recorded in all the tomato plant treated with various combinations of organic matter and bioinoculant like *N. indicum* and *T. harzianum*, respectively (Table 2). The multiplication rate of nematode population was also reduced in the tomato plants that were treated with NI + TH. Besides, other treatments were also effective against the disease complex, but their effectiveness was less than the NI + TH (Table 2).

Table 1. Soil application of *N. indicum* and *T. harzianum* on disease complex involving *M. incognita* and *R. solani* on growth parameters of tomato (five replicates)

| Treatments | Fresh weight of fruits (g) | Chlorophyll Content (mg/g) | Pollen fertility (%) |
|----------------------|----------------------------|----------------------------|-----------------------|
| Control | 156.49 ^j | 2.497 ^f | 79.17 ^{ef} |
| NI | 280.26 ^b | 3.761 ^{ab} | 93.26 ^{ab} |
| MI | 100.20 ^k | 1.1326 ^{gh} | 67.44 ^{gh} |
| RS | 120.00 ^j | 1.253 ^g | 73.66 ^{fg} |
| TH | 249.54 ^e | 2.669 ^f | 86.34 ^{bcde} |
| NI+MI | 233.93 ^f | 3.315 ^{de} | 85.80 ^{cde} |
| NI+TH | 295.84 ^a | 3.857 ^a | 96.45 ^a |
| NI+RS | 256.45 ^{de} | 3.458 ^{cd} | 88.17 ^{bcd} |
| MI+RS | 77.37 ^l | 0.893 ⁱ | 63.50 ^h |
| MI+TH | 200.19 ^g | 3.094 ^e | 84.72 ^{cde} |
| RS+TH | 223.60 ^f | 3.166 ^e | 85.90 ^{cde} |
| RS+TH+MI | 178.66 ^h | 3.077 ^e | 82.24 ^{de} |
| RS+TH+MI+NI | 275.19 ^{bc} | 3.588 ^{bc} | 91.37 ^{abc} |
| RS+MI+NI | 264.74 ^{cd} | 3.504 ^{cd} | 89.55 ^{abcd} |
| C.D. (p=0.05) | 12.47 | 0.249 | 7.33 |

Notes: NI = *Nerium indicum*, MI = *Meloidogyne incognita*, RS = *Rhizoctonia solani*, TH = *Trichoderma harzianum*; values followed by different superscript letters are significantly different over control.

Figure 2. Correlation analysis between chlorophyll content and root gall.



Application of *N. indicum* accounted for a significant reduction in the root-rot percentage (Table 2). However, maximum root-rot was recorded in the treatment that received the combine application of *M. incognita* plus *R. solani* over control (Table 2).

4. Discussion

The results presented in Table 2 emphasized that NI and TH significantly reduced the damaging potential of *M. incognita* and *R. solani* and subsequently improved the growth parameters of tomato. This might be partly due to reduction in nematode population and percent disease severity, and partly due to the additive effects of organic matter and TH which also served as manures.

Application of biofertilizer in various combinations constantly improved the plant growth in comparison to untreated control and reduced the damaging potential of *M. incognita* and *R. solani* either alone or in combinations. Our results are in conformity with those of (Harish, Saravanakumar, Radjacommar, Ebenezar, & Seetharaman, 2008; Mervat, Shawky, & Shaker, 2012). Amendments of *N. indicum* have been found to improve plant growth and yield which has been reported by several

Table 2. Soil application of *N. indicum* and *T. harzianum* on disease complex involving *M. incognita* and *R. solani* on disease incidence and nematode population affecting tomato (five replicates)

| Treatments | Number | | Nematode population | Percent rotting/ root system |
|------------------------|---------------------|---------------------|---------------------|------------------------------|
| | Galls/root system | Eggmass/root system | | |
| Control | 0 ^h | 0 ^h | 0 ^h | 0 ^g |
| NI | 0 ^h | 0 ^h | 0 ^h | 0 ^g |
| MI | 167.54 ^a | 264.26 ^a | 16,347 ^a | 0 ^g |
| RS | 0 ^h | 0 ^h | 0 ^h | 36.30 ^c |
| TH | 0 ^h | 0 ^h | 0 ^h | 0 ^g |
| NI+MI | 77.37 ^e | 161.49 ^d | 10,056 ^d | 0 ^g |
| NI+TH | 0 ^h | 0 ^h | 0 ^h | 0 ^g |
| NI+RS | 0 ^h | 0 ^h | 0 ^h | 13.20 ^f |
| MI+RS | 89.33 ^d | 117.24 ^f | 5,079 ^g | 49.27 ^a |
| MI+TH | 124.60 ^c | 200.35 ^c | 12,580 ^c | 0 ^g |
| RS+TH | 0 ^h | 0 ^h | 0 ^h | 43.56 ^b |
| RS+TH+MI | 146.29 ^b | 230.18 ^b | 14,627 ^b | 44.31 ^b |
| RS+TH+MI+NI | 44.30 ^g | 103.26 ^g | 6,190 ^f | 27.51 ^d |
| RS+MI+NI | 63.45 ^f | 134.77 ^e | 8,677 ^e | 22.20 ^e |
| C.D. (p = 0.05) | 6.98 | 9.63 | 27.65 | 2.77 |

Notes: NI = *Nerium indicum*, MI = *Meloidogyne incognita*, RS = *Rhizoctonia solani*, TH = *Trichoderma harzianum*; values followed by different superscript letters are significantly different over control.

other workers (Issac & Abu-Tahan, 2014; Moosavi, 2012; Radwan, El-Maadawy, & Abu-Elamayem, 2007). *N. indicum* has been found to be toxic to various kinds of microbes including phytonematodes (Asif et al., 2014; Singh & Singh, 1988, 1999; Tiwari & Singh, 2003). It is known fact that nematotoxic substances present in the organic matter reduce the nematode population upon decomposition. Various theories have been put forwarded by different researchers to explain possible mode of action of decomposed organic additives leading to the control of phytoparasitic nematodes. Management of phytonematodes may be due to nematicidal/nematostatic substances present in such botanicals released after decomposition (Khan, Khan, & Saxena, 1974), changes in physical and biological properties of soil (Ramesh, Panwar, Singh, & Ramana, 2009), or toxicants released or produced during microbial decomposition. Southey (1978) reported that organic manures may suppress the soil population of nematodes and subsequently enhance crop tolerance and growth variables as well. The pernicious effects of botanicals may be due to the chemicals present in the organics such as alkaloids, flavonoids, saponins, amides including benzamides and ketones that check the rate of nematode reproduction (Adegbite & Adesiyun, 2005). Alam (1976) propounded that compounds such as ammonia, H₂S, fatty acids, aldehyde, formaldehyde, amino acids, and carbohydrates are released when organic matter partially or fully decomposed. These phytochemicals were found toxic to the plant-parasitic nematodes (Alam, Khan, & Saxena, 1977). The biological activity and virulence of *N. indicum* have been proved to be lethal to *M. incognita* (Asif et al., 2014; Elbadri et al., 2008; Xiujuan, Yuxian, Furu, & Tong, 2002). Wang et al. (2009) reported that 3β-O-(β-D-diginosyl)-14,15α-di hydroxyl-5α-card-20(22)-enolide, uzarigenin and cardenolide N-1 have the lethal properties to other tylenchids such as *Bursaphelenchus xylophilus*, *Panagrellus redivivus*, and *Caenorhabditis elegans*. Our findings are in agreement with those of Srivastava and Yadav (2008). They reported that leaf extract of neem (*Azadirachta indica*) inhibited the mycelial growth of *Fusarium oxysporum*. Singh, Prajapati, Srivastava, Pandey, and Gupta (2007) stated that some botanicals in the form of marigold leaf extract inhibited the growth of *Sclerotium rolfsii*.

Application of *N. indicum* and *T. harzianum* decreased the disease intensity in the nematode–fungus infested plants. The detrimental effects of biofertilizers against the disease complex have been observed by earlier workers. (Sahebani & Hadavi, 2008; Youssef, 2013). In our study, *T. harzianum* and *N. indicum* reduced the disease severity of *M. incognita*–*R. solani* disease complex. Montealegre et al. (2010) observed the efficacious nature of some mutants of *T. harzianum* against *R. solani*. Application of *T. harzianum* and *N. indicum* helped to improve the plant growth parameters over control. A similar result against root-knot disease was also observed by Tiyagi, Mahmood, and Rizvi (2009). The plants inoculated with *N. indicum* and *T. harzianum* were found to be statistically significant as compared to other treatments which show the antagonistic effect of *T. harzianum* on nematode parasitism. Rao, Reddy, and Nagesh (2000) reported that *Trichoderma* sp. individually or in combination with castor or neem cake was able to parasitize the egg masses and ultimately reduced the galling and nematode population of *M. incognita*. Application of *Trichoderma* sp. starts penetrating the larval cuticle through hyphae and also the nematodes eggs through some enzymatic activity which dissolves the chitin layer which is supposed to be protective layer of the nematodes against many pathogenic agents inhabiting the soil. Our results are in agreement with Freitas, Pedrosa, Mariano, and Maranhão (2012), who demonstrated that *Trichoderma* spp. reduced gall index and nematode reproduction which advocates the high potential to control root-knot nematode, *M. incognita*. *T. harzianum* can alter the physiology of roots including the root exudates. Soil application of *T. harzianum* drastically decreased the rotting caused by *R. solani* on tomato (Strahnoy, Elad, Sivan, Rudich, & Chet, 1985). *Trichoderma* spp. has been reported to be efficient biological agent against various phytopathogenic soil fungi including *R. solani* and *Pythium* spp. (Harman, 1996; Howell, 2006). The efficiency of *T. harzianum* to inhibit fungal growth may be due to competition for food and space, mycoparasitism and the production of antibiotic compounds. It has been found that the hyphae of the bioagent penetrate the host mycelium through degrading cell wall by secretion of hydrolytic enzymes followed by assimilates of cell contents (Howell, 2006; Siameto, Okoth, Amugune, & Cheg, 2011). Chlorophyll content was found increased in those plants inoculated with *N. indicum* and *T. harzianum* individually and simultaneously as well. The improvement in chlorophyll content in leaves may be due to enhanced nutrient uptake by the addition of organic components which increased photosynthetic efficiency, translocation of nutrients and other metabolites.

5. Conclusion

The present study concludes that disease complex involving *M. incognita* and *R. solani* caused significant reduction in various plant growth variables. The effect of biofertilizer *N. indicum* and *T. harzianum* either alone or in combination reduced the severity of disease complex. Incorporation of the biofertilizers significantly reduced the nematode multiplication, root-galls, egg mass/ root system, and also reduced percent root-rot of *R. solani*. Hence, combined application of *N. indicum* and *T. harzianum* may be recommended against the disease complex involving *R. solani* and *M. incognita* on tomato to the farmers/growers to the developing countries especially in areas where environmental conditions and tomato culture practices may favour the development of these pathogens. Further study may be needed to understand various mechanisms of action of both biofertilizers and their possible synergism with other compounds applied in organic agriculture. In this way, this study may pave the way toward the disease management of sustainable agriculture.

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Competing interests

The authors declare no competing interest.

Author details

Rose Rizvi¹
E-mail: rose.amu@gmail.com
Rizwan Ali Ansari¹
E-mail: rizwans.ansari@gmail.com
Anam Iqbal¹

E-mail: rizsaria@gmail.com

ORCID ID: <http://orcid.org/0000-0003-1632-4627>

Safiuddin Ansari¹

E-mail: safiuddin7ansari@gmail.com

Aisha Sumbul¹

E-mail: aishasumbul92amu@gmail.com

Irshad Mahmood¹

E-mail: irshad.mahmood2@gmail.com

Sartaj A. Tiyagi¹

E-mail: satiyagi@gmail.com

¹ Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh 202002, India.

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