Pakistan Journal of Nematology, 2015, Vol. 33(1): 105-112

Comparative efficacy of *Trichoderma harzianum*, neem extract and furadan on *Meloidogyne incognita* infecting tomato plant growth

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Abstract

Root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood is the virulent pathogen of tomato plants. The artificial inoculation of *M. incognita* developed a large number of galls and suppressed the tomato plant growth significantly. Three different concentrations of neem extract, furadan and *Trichoderma harzianum* were evaluated to control the *M. incognita*. All concentrations proved effective in reducing the disease development in nematode inoculated tomato plants. However, high dose of furadan became phytotoxic and plant growth was checked together with a number of galls. While, higher doses of neem extract and *T. harzianum* improved the plant growth. The combined applications of neem extract, furadan and *T. harzianum* were more effective than alone. In combined application also, the higher dose of furadan caused negative effects on plant growth. Present studies showed that incorporation of biocontrol agent like *T. harzianum* and botanical products such as neem extract against root-knot nematode provided good results and can provide an alternate of chemical pesticides which will be ecofriendly as well.

Keywords: *Solanum lycopersicum*, *Meloidogyne incognita*, *Trichoderma harzianum*, neem extract and furadan.

Tomato (*Solanum lycopersicum* Mill.) is a herbaceous plant belongs to the family Solanaceae, grown worldwide for its edible fruit. It was thought that it is first originated from South America and now cultivated throughout the world (Smith, 1994) with the production of 161 million tonnes in 2012. By production, China on the top whereas, Pakistan on 34th position with production of 560 thousand tonnes (FAOSTAT, 2012). In Sindh province, the yield of tomato per hectare 7.5 tonnes, which was the lowest from all other three provinces as well as from the national average (10.2 tonnes/hectare) (Anonymous, 2012). This lowest average yield was contributed by many factors, among them the damage caused by plant pathogens is one of the most important elements.

The tomato plant is attacked by as many as 55 diseases caused by fungi, bacteria, viruses and several species of nematodes; especially root-knot nematodes (Jones *et al*., 1993). Among them root-knot disease caused by *Meloidogyne* species are of vital importance. They are most economically damaging species of plant-parasitic nematodes on fruits, vegetables, ornamentals and field crops. The genus *Meloidogyne* includes more than 60 species, however, *Meloidogyne javanica*, *M. arenaria*, *M. incognita* and *M. hapla* are more prevalent and considered as most important (Eisenback & Triantaphyllou, 1991). Its larvae infect plant roots, causing small swellings on the roots known as knots or galls. The female deposits eggs in or on the roots, the newly hatched juveniles move toward root tips and feed on root tissues. All stages of plant growth are subject to attack of root-knot nematodes. Above ground symptoms often remain unnoticed until the disease becomes well established. The severely infected plants showed stunting growth, yellowing and overall poor growth. The host range of root-knot nematodes

includes 2000 plants and causing approximately 5% crop losses worldwide (Stirling *et al*., 1992; Sasser & Carter, 1985). In Pakistan, fairly a large number of plant species were found infected with either one or other species of root-knot nematodes (Shahina *et al*., 2009). Charchar *et al*., (2003) reported that among vegetable hosts, the tomato was highly susceptible to *Meloidogyne* spp., and causing up to 30-40% yield losses.

Various strategies have been used to control this threatening disease of crop plants. Throughout the world nematicides are found highly effective to control the *Meloidogyne* spp., (Rehman *et al*., 2006; Oduor-Owino & Waudo, 1994; Colyer *et al*., 1997). The chemical control of root-knot is costly and hazardous to agro-ecosystem and environment. Therefore, keeping in view the potential hazardous possess by the chemical control to agro-ecosystem, animals and human beings attention has been now focused to find out some alternate and effective control strategies. In this regard, use of different botanical products and biocontrol agents gain popularity (Goswami *et al*., 2008; Javed *et al*., 2007; Dababat *et al*., 2006; Haseeb *et al*., 2005). The present studies were carried out to evaluate the effect of *Trichoderma harzianum*, neem product and furadan on root-knot infection and growth of tomato plants.

Materials and Methods

Isolation, identification and multiplication of pathogen: The tomato plants showing typical symptoms of root-knot nematode infection were collected from farmer fields. The infected tomato root which has characteristic galls/knots produced by the root-knot nematodes were cut into small pieces in a watch glass with the help of fine razor. The material was then observed under the stereoscope microscope. The females of root-knot nematodes, *Meloidogyne* species were picked up with the help of drawing brush identified on the basis of female perennial pattern (Taylor & Netscher, 1974).

For multiplication of purified pathogen inoculum tomato seedlings of local variety Roma were raised in earthen pots containing sterilized soil. The newly emerging seedlings were then inoculated with single egg-mass isolated from collected diseased tomato plants. After 15 days, seedlings were transplanted into separate earthen pots containing sterilized soil. The developed galls on these plants, serve as a source of pure inoculum of root-knot nematode *M. incognita*.

Extraction of root-knot nematode: Roots of inoculated tomato plants infected with *M. incognito* showing typical galls produces by root-knot nematode were cut into small pieces with the help of fine razor/scissor and placed in a wide mouthed bottle. Commercial bleach solution (1%) was added into the bottle and then mouth tightly closed with the cap. The bottle was shaken thoroughly for a few minutes and then the contents were poured onto a 100 mesh screen fitted over a 400 mesh screen, washed under running water for 1 minute and the residual collect on 400 mesh screen. The material then transferred into a 250 ml beaker. The number of eggs and juveniles was determined with the help of the counting chamber under the stereoscopic microscope.

Multiplication/preparation of *Trichoderma harzianum*: The culture of *Trichoderma harzianum* was obtained from the culture collection center, Department of Agriculture and Agribusiness Management, University of Karachi, Karachi. The culture was revived on potato dextrose medium (PDA) and multiplied on sorghum grains as described by Kumar & Palakshappa (2009). For this purpose sorghum grains (50 g) were socked in glass beaker for 30 min, then transferred in plastic bags for steam sterilization. Each bag was separately inoculated with an inoculum disk of *T. harzianum* obtained from fresh growing colony on PDA medium. The bags were closed tightly with a rubber band, incubated for 15 days at 28 ± 2 °C and shaken daily. After 15 days, the colour of sorghum grains was changed to dark green due to abundant sporulation of *T. harzianum*. The spore load/gram of this substrate was determined with the help of hemocytometer (Weller, 1988).

Preparation of neem extract: Neem (*Azadirachta indica* L.) leaves after surface sterilization with 5% sodium hypochlorite solution, dried by placing on blotting paper. The leaves were grounded with the help of the pestle and mortar in the presence of liquid nitrogen and extracted with an equal volume (1:1 w/v) of sterilized distilled water by placing them for 2 hrs on a mechanical shaker (100 rpm) at room temperature and centrifuged at 4500 rpm. The supernatant was collected and further strained through Whatman filter paper.

Evaluation of different control agents on disease development: Tomato seedling variety Roma raised in large earthen pots containing sterilized soil. Two months old seedlings were transferred in earthen pots containing 2 kg of sterilized soil. The plants were inoculated with 100 egg-masses of *M. incognita* per pot by making a depression in the soil near the roots. After one week of transplanting, when tomato plants established well, the required amount of control agent was applied near the roots of test plants. For this purpose, small holes were made near the stem of each plant and required concentration was poured. The un-treated plants served as control. After 45 days of inoculation, treated plants were carefully uprooted so that the root system remains intact. Data on plant growth i.e., shoot length and weight, root length and weight and numbers of galls per plant were recorded. Finally, the data was analyzed by ANOVA using Statistix 8.1 software. Least significant differences (LSD) were calculated using significant level at P = 0.05.

Results and Discussion

The plants infected with root-knot nematodes appeared as pale green to yellowish in colour, remain stunted and showed poor growth as compared to the healthy plants. On affected roots, the pathogen formed typical galls of varying size and shapes which can be observed easily with naked eyes (Fig. 1). Microscopic studies of these galls revealed the presence of egg-masses and larvae of *M. incognita*. *Meloidogyne incognita* was isolated from the roots of affected tomato plants. It was identified on the basis of the perineal pattern of adult mature females as described by Taylor & Netscher (1974). The root-knot nematode is an aggressive pathogen causing root-knot disease in tomato plants, 80-85% seedling and yield losses are reported due to this pathogen (Maqbool & Ghazala, 1985; Alam & Jairajpuri, 1990).

Fig. 1. Galls of varying size produced on the roots of the tomato plant inoculated with *M. incognita*.

Pathogenicity test: The inoculation of root-knot nematode produced disease symptoms. Large number of nematode galls were observed in inoculated plants (52.66), whereas no gall was recorded on un-inoculated plants (Fig. 2). The root-knot nematode greatly checked the growth of inoculated plants. Shoot length, shoot weight and root length were significantly reduced in inoculated plants. However, root weight was higher in inoculated plants due to the presence of galls (Fig. 2).

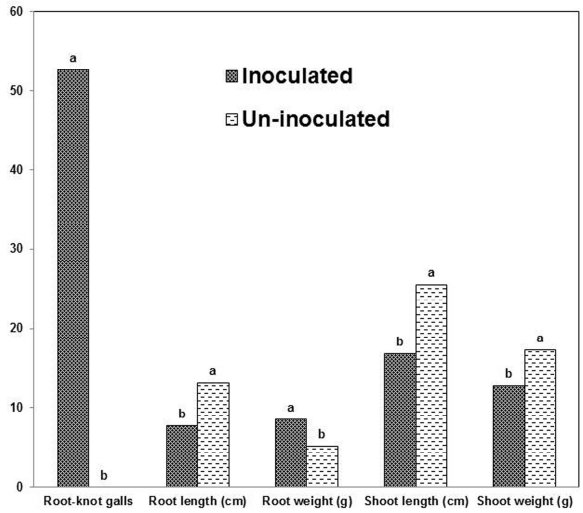

Fig. 2. Effect of pathogen inoculation on disease development and plant growth of tomato plants inoculated with *Meloidogyne incognita*. Means followed by different letters in respective bar are significantly different at P = 0.05.

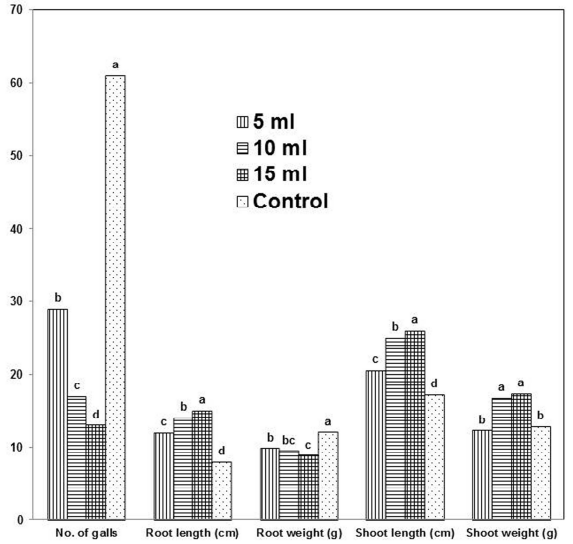

Fig. 3. Effect of different doses of neem extract on disease development and plant growth. Means followed by different letters in respective bar are significantly different at P = 0.05.

Effect of different control agents on disease development

Neem extract: Neem leaves extract applied to the tomato plants as artificially inoculated with *M. incognita*, greatly influenced the disease development and brought a significant reduction in number of nematode galls in treated plants (Fig. 3). About 50% galls reduction was recorded with the application of lower dose of neem extract. Maximum root length, shoot length and shoot weight were recorded with highest dose followed by medium and low. In contrast to root length, maximum root weight was recorded on un-treated plants (Fig. 3). Ardakani *et al*., (2009) found that water extracts of neem seed kernel were highly toxic to *M. incognita*. Neem and neem products produced better plant growth and reduced level of disease development as compared to un-treated plants (Kumar & Khanne, 2006). Similarly, Elbadri *et al*., (2009) reported that herbal powders of *Azadirachta indica* and *Acacia nilotica* effectively controlled the *M. incognita*.

Furadan: All doses of furadan significantly reduced nematode galls in treated plants as compared to un-treated plants (Fig. 4). Maximum galls reduction was observed significantly with higher dose followed by medium and lower doses. However, it appears that higher dose of furadan was highly toxic to the pathogen as well as in plants. Elsewhere, Orisajo *et al*., (2008) recorded lower population densities of *M. incognita* in soil treated with carbofuran. The maximum root length, shoot length and shoot weight was observed in plants treated with a medium dose of furadan. It was also noted that the plant growth of treated plants gradually increased with its increasing dose, but at high dose the plant growth was checked. Phytotoxic effect on the roots of tomato reported earlier when applied as 2 kg/ha (Crozzoli & Diego, 1991). Prophylactic effect of furadan has been reported by Tanimola & Gowon-Egein (2009); Enopka *et al*., (1996); Hussain *et al*., (1993) and Khalil *et al*., (1993).

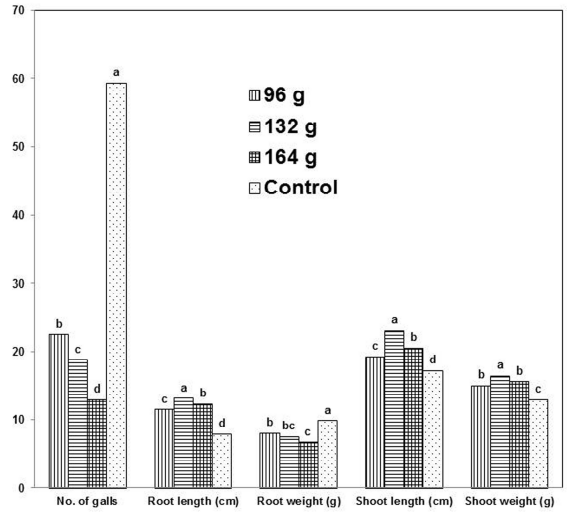

Fig. 4. Effect of different doses of furadan on disease development and plant growth. Means followed by different letters in respective bar are significantly different at P = 0.05.

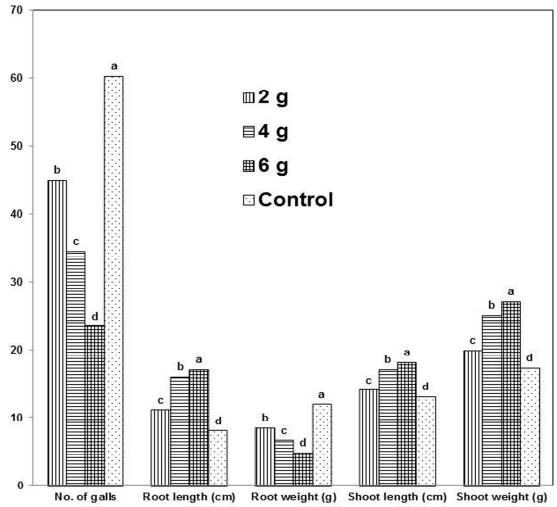

Fig. 5. Effect of different doses of *T. harzianum* on disease development and plant growth. Means followed by different letters in respective bar are significantly different at P = 0.05.

***Trichoderma harzianum*:** The application of *T. harzianum* remarkably checked the infection of *M. incognita* as treated plants produced significantly less number of nematode galls in contrast to untreated plants. It caused positive impact on plant growth as significantly higher root length, shoot length and shoot weight was recorded in treated plants as compared to un-treated plants (Fig. 5). Antagonistic fungi *T. harzianum* significantly reduced the *M. incognita* infection when applied on tomato plants. A gradual reduction in number of nematode galls was observed with increasing dose of *T. harzianum* and least nematode infection was recorded at its highest dose. It parasitized the eggs of root-knot nematode inside the egg-masses (Pandey *et al*., 2009) and controlled the *M. incognita* population (Mukhopadhyay *et al*., 2006; Spiegel *et al*., 2007; Sharma & Panday, 2009). Consequently, the application of *T. harzianum* caused positive effect on plant growth and shoot length and weight as well as root length was considerably enhanced in treated plants. Its higher dose was more effective in enhancing plant growth as compared to the medium and lower dose.

Combined application: The tomato plants inoculated with *M. incognita* was also treated with different combinations of neem extract, furadan and *T. harzianum*. All treatments checked the pathogen infection and significantly minimum numbers of galls were recorded in treated plants as compared to un-treated plants (Fig. 6). The maximum reduction in galls was recorded in plants, where a higher dose of the neem extract, furadan and *T. harzianum* were applied simultaneously. In terms of gall reduction the most effective combination was neem 15ml+Furadan 164g + *T. harzianum* 6g, which produced only 14.5 galls/plant (Fig. 6). Plant growth was significantly increased in all treated plants as compared to un-treated ones (Fig. 6). However, in treatments where furadan was applied at higher concentration (164 g) in any combination, the plant growth was decreased (Fig. 6). *T. harzianum* when applied with neem cake amended pots against *M. incognita* gave better plant status than *T. harzianum* applied alone (Kumar & Khanna, 2006). Elsewhere Bhaskar *et al*., (2007) revealed that *T. harzianum* in combination with either FYM or neem cake proved to be the most effective treatment in reducing root rot disease complex in berseem.

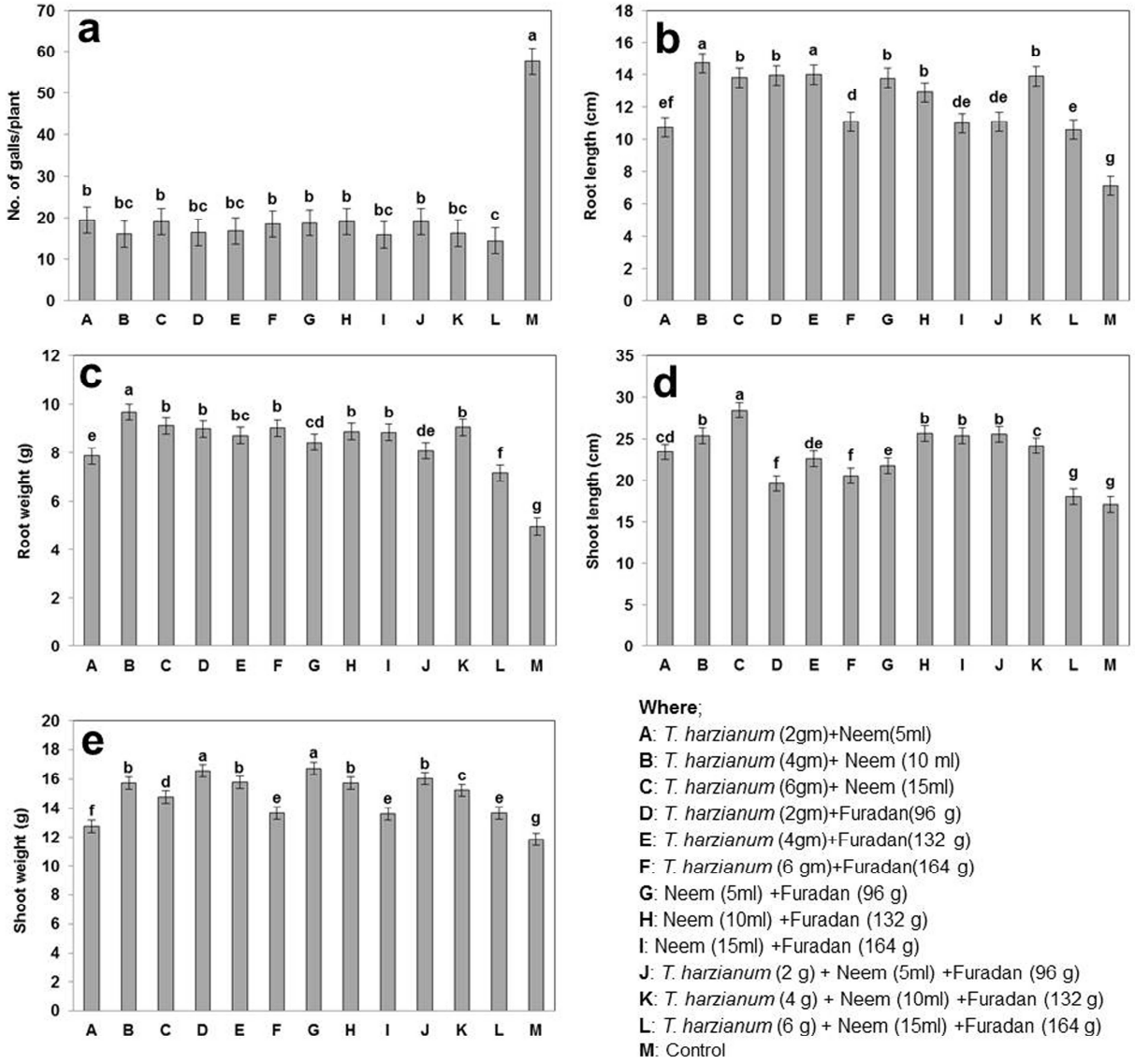

Fig. 6. Effect of combined application of neem extract, furadan and *T. harzianum* on (a) number of galls/plant, (b) root length, (c) root weight, (d) shoot length and (e) shoot weight of tomato plants. Means followed by different letters in respective bar are significantly different at P = 0.05.

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(Received: October 18, 2014)