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EFFECTS OF NEEM FORMULATIONS APPLIED AS SOIL DRENCHING ON THE DEVELOPMENT OF ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA* ON ROOTS OF TOMATO

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Abstract

The effects of neem formulations applied as soil drenching on the development of second stage juveniles (J2) of *Meloidogyne javanica* on roots of susceptible tomato cv. Tiny-Tim was investigated at controlled environment consisted of photoperiod of 16 h light/8 h dark at 30°C during light and 24.1°C during dark. Thirty day old seedlings were transplanted singly in 15cm diam., pots filled with autoclaved proprietary based loam. Beginning 7-days after transplant, three neem formations viz., neem cake, aza 5 mg and 10 mg were drenched @ 10 ml per pot. Water and 4% ethanol treated plants were included as check for comparison. Three days after the application, plants were inoculated with freshly hatched 250 J2 suspended in 10 ml of water surrounding root zone of each plant. Each treatment consisted of 5 replications. The experimental plants were completely randomized on a bench in a growth room. The roots of tomato plants drenched with three neem formulations and ethanol equal numbers of J2 penetrated but significantly less than that of water check plants. Three neem treatments including aza (5mg), aza (10mg) arrested the development of J2 over that of water check. The roots of plants treated with aza (10mg) allowed less number of J2 to develop into immature females than on roots of ethanol check plants. The plants treated with all three neem formulations and ethanol responded less in terms of root gall formation over that of water check. Aza at 10mg was found most effective in protecting the roots against nematode infection. These findings warrant the use of neem as biocide to manage the nematode populations.

Introduction

Plant parasitic nematodes are damaging pests of several crops of tropical and subtropical production regions of the world (Bridge & Page, 1980). The yield loss of major crops in these regions due to nematodes is estimated to be 12.3%, which are higher in developing countries due to varieties of reasons involving increased diversity of nematode genera and their higher fecundity rate due to the shorter life cycle, higher temperature, longer growing seasons, the absence of winter chilling, unawareness about nematode problems, and lack of management practices. Among the plant parasitic nematodes, root-knot nematodes, *Meloidogyne* spp., are the most important limiting factor of agricultural productivity (Sasser & Carter, 1985).

Among *Meloidogyne* spp., two economically important plant species include *M. incognita* and *M. javanica* attacking several plants of vegetables and field crops in Pakistan, because of tropical and subtropical climate. Moreover sandy and warm soils are favorable for nematode infestation, especially in irrigated areas where crops are grown continuously (Anwar *et al*., 2006).

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Tomato is an important vegetable crop of Pakistan and has been found attacked by *M. incognita* and *M. javanica*, which impose serious threat both to yield and quality (Anwar *et al*., 2006). These nematodes can cause 24-38% loss in yield if not properly managed (Luc *et al*., 1990). The losses caused by nematodes can be managed by soil fumigation with nematicides and by using resistant plants. The high cost of application of nematicides to manage the nematodes and their unfriendly effects on human and animal health and environment and unavailability of resistant tomato cultivars in Pakistan, has promoted this search for looking their alternatives.

Bioactive products from the neem tree, *Azadirachta indica* A. Juss (Family Meliaceae), have been found effective in managing the population of 16 plant parasitic nematodes species (Alam, 1993) and more than 400 species of insect pests of important food crops (Saxena, 1989; Schmutterer & Singh, 1995). Neem is widespread in Pakistan and has been found effective in bringing the nematode population below threshold level (Javed *et al*., 2008), which might provide an alternative, sustainable and inexpensive means of managing nematodes.

The bioactivity of neem materials against nematodes and insects is attributed to the presence of an array of complex compounds, triterpenes, or more specifically limonoids (Alam, 1993; Kraus, 1995). The purpose of this study was to investigate the effects of two neem formulations including aza and cake applied as soil drench on the development of J2 of *M. javanica* on roots of susceptible tomato cv. Tiny-Tim.

Materials and Methods

Preparation of neem formulations: Neem cake, a by-product left after the extraction of oil from neem seed. After proper drying the crude formulations were crushed and converted into fine powder using a ‘‘Glen Crescent’’ 240 V, grinder fitted with 2mm pore size sieve and stored in tin containers at 4^{o}C. The moisture contents of cake were 6.34%. It was allowed to decompose in 4% ethanol for three days and then passed though Whatman filter paper No 4. Aza is a refined product extracted from neem seed (Kraus, 1995). This was supplied by Cardiff Chemical Ltd. Cardiff CF3 0EF. It was also dissolved in 4% ethanol.

Nematode inoculum: Eggs of *M. javinca* were extracted from females cultured on tomato (*Lycopersicon esculentu*m) roots cv. Tiny-Tim in a glasshouse at 24-28^{o}C. The galled roots were sealed in Mason glass jars containing 800 ml 2% NaOCl (Hussey & Barker, 1973) and were agitated for 4 minutes at 200-cycle min^{-1} on a mechanical shaker (Eberbach, Ann Arbor, MI). Agitation was followed with a thorough rinse in tap water. Eggs were allowed to hatch in a mist chamber to obtain second stage juveniles (J2) for inoculation of tomato seedlings.

Tomato seedlings: Thirty day old seedlings of susceptible tomato cv. Tiny-Tim grown in transplant trays were planted singly in 15cm diam., pots filled with autoclaved proprietary based loam: a combination of loam, peat, sand, lime, fertilizers and traces of elements (John Innes No. 2. Roffey Ltd. Throop Bournemouth BHS ODF England). Beginning 7-days after transplant, neem formations were drenched in 10 ml per pot. Three days after the application, plants were inoculated with freshly hatched 250 J2 of *M. javanica* suspended in 10 ml of water by pipetting into three equidistant 3-cm-deep holes

surrounding the root zone of each plant. Inoculation holes were re-filled with steam-sterilized soil and pots were watered immediately to moisten the soil. The control plants that were inoculated with nematodes were similarly treated with sterilized water and 4% ethanol. There were four treatments (Table 1) and each treatment consisted of 5 replications. The experimental plants were completely randomized on a bench in a growth room. The growth room environment consisted of photoperiod of 16 h light/8 h dark and the temperature of 30.1°C during the light and 24.1°C during the dark. Plants were fertilized every two weeks with Hoagland's solution (Hoagland & Arnon, 1950).

Treatment effects on plant growth parameters including root and shoot weight, number of galls per root system and nematodes within the roots were assessed 21-days after inoculation. The root galling index (GI) was assessed on 0 to 5 scale, where 0 = no gall, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 galls per root system (Anwar *et al*., 2007). The roots were stained in acid fuchsin and number of nematodes were counted (Byrd *et al*., 1983). The glycerin improves optical qualities of the system, prevents drying and helps to hold the plates together. Number of J2 and immature females within the root suspension was determined under a dissecting microscope.

Data analysis: Data was subjected to analysis of variance using SAS procedures and differences among treatment means were separated by applying LSD test at $p \leq 0.05$ (SAS Institute, Cary, NC).

Results

Nematode penetration and development: The penetration of tomato roots by J2 was differentially influenced by treatments. The numbers of J2 in tomato roots of plants drenched with neem formulations as well as ethanol had statistically equal numbers of nematodes but significantly (p=0.05) less than that of in plant roots drenched with water (Table 1). The effects of neem formulations and ethanol check on advancement of J2 to J4, the immature females in tomato roots, was evident after 21 days of inculcations. Three neem treatments including Aza (5mg), Aza (10mg) and cake had similar effects on the development of J2, however all these treatments significantly (p=0.05) arrested the development of J2 over that of water check. The roots of plants treated with Aza 10mg had significantly less number of J4 but significantly more J2 compared to that of on roots of ethanol treated plants; however other two neem treatments were found similar to that of ethanol. The roots of ethanol treated plants had ca 2 times less J4 than that of water treated plants.

Table 1. Penetration and development of J2 of *Meloidogyne javanica* in roots of tomato cv. Tiny-Tim treated with aza and neem cake.

Treatments	Dose	Weight (g)		Galls per root system	Number of J2 penetrated in roots	J2 Development	
		Root	Shoot			J2	J4
Aza	5 mg	1.40	3.13			6	43
Aza	10 mg	1.55	2.96	24	49	15	34
Cake	5 % w/v	1.82	3.74	61	69	7	62
Ethanol check	4 %	1.13	2.58	66	74	4	70
Water check		1.33	2.86	97	141	9	132
LSD		0.2	1.5	21	41.31	5.1	35.1

Plant response to nematode infection: All the three neem and ethanol treatments had significantly (p=*0.05*) less number of galls per root system compared to that of water check. But Aza 10mg treatment was found most effective in protecting the roots to respond to nematode infection to form galls compared to all other treatments. There was no statistical difference among shoot growth of plants treated with neem formulations, ethanol and water. The roots of plants treated with Aza 10 mg and cake showed significant improvement over that of plant where water was used.

Discussion

Various neem products including neem cake, its oil and Nimin (containing neem triterpenes) as urea coating agents, and root-dip or seed treatment with neem extracts, have been found to be nematicidal against several species of parasitic nematodes (Alam, 1991) attacking vegetables and legumes (Haseeb *et al*., 2005) and banana (Musabyimana & Saxena, 1999). However, soil amendment with neem seems to be the most practical method for nematode control (Alam, 1993). The roots of plants raised in neem-cake amended soil appear to undergo physiological changes that render them unsuitable for nematode penetration and development, thus inducing a certain degree of resistance in plants against nematode infestation (Alam, 1993).

We tested the effects of neem cake and Aza applied as drench application on penetration and development of J2 of *M. javnica* in the roots of tomato cv. Tiny-Tim. The presence of 20% and 27% of J2 in roots treated with Aza, and cake compared to that of 56% in roots of water treated plants suggests the effectiveness of these treatments against nematode penetration. It suggests that neem metabolites were absorbed by the roots to halt the J2 penetration (Javed *et al*., 2007a). The bioactive principles in neem extracts have also been reported to inhibit the penetration of nematodes (Mojumder, 1995). These finding are in line that of neem cake decreased infestations of *M.incognita* and *Tylenchorhynchus brassicae* in vegetables and legumes (Aalam, 1991; Mishra *et al*., 1989; Mojumder, 1995) and of *Pratylenchus zeae* infestation in sugarcane (Mehta, 1997). The reduced J2 penetration in roots of ethanol treated plants indicated its toxicity against nematodes and plants as root and shoot of plant was reduced. Although it has been found effective against snails (Keshav *et al*., 1996; Ebenso, 2003.) but this is the first report that ethanol is effective against nematode

The percent of J2 advanced to J4 in roots treated with aza 5 mg, cake, ethanol and water were statistically equal in numbers (> 87%), which suggests that once the J2 had reached the vascular tissues and have established the feeding sites, then there is no barrier to stop their development. It agrees with the finding hypothesis once the J2 had passed the epidermis and cortex and had established feeding site even in resistant plants, then they can develop further (Anwar & McKenry, 2002). The low number of J4 and high number of J2 in roots treated with Aza 10 mg might be related to nematodes injury at high concentration, which could not establish the feeding sites. It confirms the claim of Khan *et al*., (1974) and Devakumar *et al*., (1985) that aza is toxic to nematodes. It also support the findings that nematode metabolites disrupt the development (Javed *et al*., 2007b).

These findings contribute important information that neem metabolites were absorbed by the tomato roots (Javed *et al*., 2007b), so J2 penetration was reduced. Our finding, also demonstrate that once J2 passed the epidermis then could be able to establish feeding courts and proceed to adult stage (Anwar & McMckenry, 2002). This study also provides an effective tool for the grower and scientists to use the neem product as soil drench for the management of root knot nematode.

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