PLANT NEMATODES AND THEIR MANAGEMENT ASSOCIATED WITH ALMOND (*PRUNUS AMYGDALUS* BATSCH) SEEDLINGS IN BALOCHISTAN, PAKISTAN

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Abstract

A survey of plant parasitic nematodes associated with Almond was conducted encompassing six almond nurseries of Khuzdar and Kalat districts Balochistan Province. Sixteen different nematodes were recorded. The nematodes found in all six nurseries were *Scutylenchus rugosus*, *Meloidogyne javanica* larvae and *Zygotylenchus guevarai*. Similarities between six localities on the basis of association of nematode species in the assemblages was determined.

Different treatments were used to control population of *Scutylenchus rugosus*, *Zygotylenchus guevarai* and *Meloidogyne javanica* larvae. Amendments including sugarcane bagasse, neem leaf powder, sawdust, Fertinemakil, Marigold flower powder were employed alone and in combination whereas for comparison a chemical nematicide was used.

Introduction

Almond (*Prunus amygdalus* Batsch) is a tree species native to South Asia and the Middle East. The half of the world's almond crop emanates from within the Sacramento and San Joaquin valleys of California (McKenry and Kretsch, 1989). The almond trees start bearing fruit in the 3rd year of plantation. The fruit matures in autumn almost 7 months after flowering (Griffiths and Huxley, 1992). Balochistan being an important fruit growing province of Pakistan has fertile valleys having almond tree orchards. A number of constraints are responsible for lowering the production and quality of fruit. Plant parasitic nematodes are one of the biological constraints which cause severe damage to productivity of fruits in Balochistan (Qasim *et al.*, 1988).

Management of nematode associated with almond is of great importance especially when the export of dry fruits is increasing in international markets especially Bangladesh, U.A.E. and Sri Lanka (Azim, 2015).

Sethi and Gaur (1986) suggested that if seedlings are properly treated and are made nematode free, the spread of nematodes to uninfected areas is reduced.

In the present study nematodes associated with almond seedlings in Balochistan are investigated and different amendments are employed in a pot experiment to control the nematode population.

Materials and Methods

Survey: The survey of almond seedling nurseries was conducted during March 2013. Sixty six samples were collected from six nurseries of almond (*Prunus amygdalus* Batsch) seedlings var. Kaghazi across Khuzdar and Kalat districts, Balochistan. The samples were composed of a composite of subsamples containing soil directly from the root and from the rhizosphere of the seedlings. About 200 mL of combined soil and roots were collected from each sample. Additional samples were collected within each nursery from plants exhibiting distinct symptoms of abnormal growth (chlorosis, necrosis and stunting) and combined with the systematic samples, and then a well mixed composite sample was studied for nematode extraction from each nursery. Eighty percent of the soil samples were texturally either sandy-loam or loamy sands. Average daytime temperature ranged from 15 to 22°C during the sampling period. Nematodes were extracted from the samples using Baermann funnel technique (Southey, 1970). Isolated nematodes were fixed in TAF, dehydrated in 1.2 percent glycerine and finally transferred to pure glycerine for preparing permanent slides for identification according to Siddiqi (1986). Similarities between the six nursery localities using nematode assemblage (counts) were computed using the index of Bray and Curtis (1957) and the matrix prepared.

Control: The control experiment was conducted during February 2014 at a Khuzdar nursery, Balochistan in plastic pots filled with 250 g of sandy loam soil. Six samples were taken from soil associated with uneven plant growth located approximately 3 km from the nursery and nematode population present in the soil were determined (Cobb, 1918) as 123 ± 6.0 *Scutylenchus rugosus*, 109 ± 20 *Zygotylenchus guevarai* and 38 ± 19 *Meloidogyne javanica* larvae were already present in the soil. The three nematodes comprised 75 percent of total

plant parasitic nematode populations. One week later, Almond seedlings var. Kaghazi were transplanted in pots. The amendments which included sawdust, sugarcane bagasse, neem (*Azadirachta indica*) leaf powder and marigold (*Tagetes erecta*) flower powder were used alone and in combination with Fertinemakil (a pesticide containing neem cake (97.5%) product from Pakistan Council of Scientific and Industrial Research Complex in collaboration with Crop Diseases Research Institute, PARC, University of Karachi (Pakistan). The neem leaves and marigold obtained from Winder, Balochistan, were air-dried for six weeks and powdered using a Willey mill (Thomas Scientific). Untreated pots were kept as control. For comparison the carbamate nematicide Carbofuran (a.i. 44% Agricultural product Group, Philadelphia, PA) was used. The treatments and control were replicated four times each. The dose of Fertinemakil was 2.5 g/pot and the other four amendments were applied 4.08 g/pot and Carbofuran was applied at 0.2 g/ (from this 250 mL solution poured in each pot). The pots were irrigated regularly with distilled water to avoid any contamination with fungi or nematodes.

Eight weeks after treatment soil in pots were collected for nematode populations and placed in polythene bags until the nematodes were extracted. Each sample was processed using a modified Cobb's (1918) decanting and selective sieving method. The nematode population was counted under a stereoscopic binocular microscope by shaking the nematode – counting suspension thoroughly and transferring 2 mL aliquots to a counting dish. Four aliquots were counted. Data was subjected to a factorial analysis of variance (ANOVA) followed by least significant difference (LSD) at $p < 0.05$ (Zar, 1999).

Results and Discussion

Sixteen different plant nematodes were found to be associated with almond. The three nematodes found in all the six nurseries examined were *Scutylenchus rugosus*, *Meloidogyne javanica* larvae and *Zygotylenchus guevarai*. The nematodes recorded from only one nursery were *Aphelenchus avenae*, *Boleodorus* sp., *Filenchus* sp., *Merlinius brevidens*, *Macroposthonia* sp. juveniles and *Pratylenchus zeae* (Table 1). The seedlings roots at Rod Abdullah were completely blackened where population of *M. javanica* was in high frequency.

Similarities between six locations the basis of associations of nematode species in the assemblages are given in (Table 2). Highest similarity was found in localities Kaley Abdullahjan and Daniyal nursery, Khuzdar and in localities Rod Abdullah and Kalat town nursery and Rod Abdullah and Daniyal nursery, Khuzdar. Least similarity was recorded in nurseries Kalat Town nursery and Jamiatabad. Other pairs showed intermediate levels of similarities ranging from 30 to 44.4 percent.

The factorial ANOVA showed treatments to be highly significant ($p < 0.001$) and the nematodes were also shown highly significant ($p < 0.001$). The interaction of treatments × nematodes was found to be significant ($p < 0.001$) (Table 3). With regard to nematode *Scutylenchus rugosus* the best control was obtained in pots treated with neem powder + Fertinemakil followed by neem powder and least in pots treated with sugarcane bagasse. For *Zygotylenchus guevarai* the best control was obtained in pots treated with sugarcane bagasse. For *Zygotylenchus guevarai* the best control was obtained in pots treated with sugarcane bagasse. However, for *Meloidogyne* larvae best result was obtained in pots treated with both neem powder + Fertinemakil and neem powder and least with sawdust (Figs. 1–3).

Plant parasitic nematodes are problem for almond in many parts of the world (Sindhan, 1976, Gomez-Aporisi *et al.*, 2002, Aliramgi *et al.*, 2006, Abad, 2014) including Balochistan, Pakistan (Khan *et al.*, 2008, 2009, 2010). Important nematode infecting almond are root-knot nematodes (*Meloidogyne incognita* and *M. javanica*, dagger nematodes (*Xiphinema* spp.) and lesion nematode (*Pratylenchus* spp.). Dagger nematode becomes vector for several viruses whereas Ring nematodes (*Criconemella* spp.) are associated with predisposition of young almond trees to bacterial canker (McKenry and Kretsch, 1987). The reported root-knot nematodes are common in warmer parts of the world while *Pratylenchus vulnus* was detected in one fourth of the almond production region in 17 almond producing countries of California. Howsoever, *Xiphinema americanum* was most prevalent in cooler Sacramento valley region.

Preference must be given to select those varieties seedlings for plantation which are resistant to these nematodes or free from nematodes at the time transplanting in the orchards. Sandy loam or loamy sand as often found in Khuzdar and Kalat districts have higher nematode population and may have stratified layers or hardpans.

Control of nematodes at seedling stage is manageable and relatively inexpensive. Seedlings which are uninfected when transplanted with fields may exhibit greater tolerance and adaptability to changed habitat condition and survival in greater proportion compared to the infected seedlings of untreated nurseries.

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Table 1. Nematodes associated with Almond seedlings in Khuzdar and Kalat districts, Balochistan.

	Nurseries					
	1*	2	3	4	5	6
Aphelenchus avenae	+	−	−	−	−	−
Aphelenchoides sp. juveniles	−	−	+	+	+	−
Basiria graminophila	−	+	−	−	+	−
Boleodorus sp.	−	−	−	+	−	−
Boleodorus pakistanensis	+	−	−	−	+	+
Ditylenchus sp. *juveniles*	+	+	−	−	−	−
Filenchus sp.	−	−	−	+	−	−
Helicotylenchus digonicus	−	−	+	−	−	+
Helicotylenchus indicus	+	+	−	+	+	+
Meloidogyne javanica	+	+	+	+	+	+
Merlinius brevidens	+	−	−	−	−	−
Macroposthonia sp. juveniles	−	−	−	−	+	−
Paratylenchus nainianus	−	−	−	+	+	−
Pratylenchus zeae	−	−	−	−	−	−
Scutylenchus rugosus	+	+	+	+	+	−
Zygotylenchus guevarai	+	+	+	+	+	+

*, Name of nurseries: 1 = Kalat Town nursery; 2 = Rod Abdullah; 3 = Jamiatabad; 4 = Kaley Abdullahjan; 5 = Daniyal nursery, Noorani chowk, Khuzdar; 6 = Mangochar nursery.

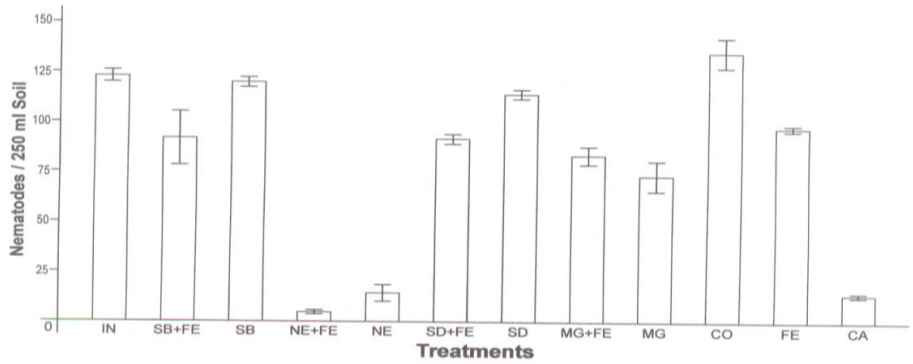

Fig. 1 Effect of different treatments on *Scutylenchus rugosus* associated with almond seedlings (IN = Initial, SB = Sugarcane bagasse, FE = Fertinemakil, NE = Neem leaf powder, SD = Sawdust, MG = Marigold flower powder, CO = Control, CA = Carbofuran).

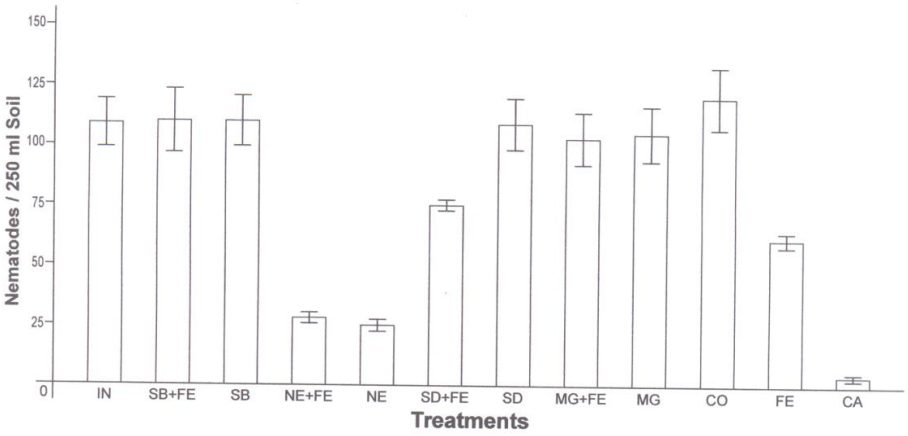

Fig. 2 Effect of different treatments on *Zygotylenchus guevarai* associated with almond seedlings (Legends as in Fig. 1)

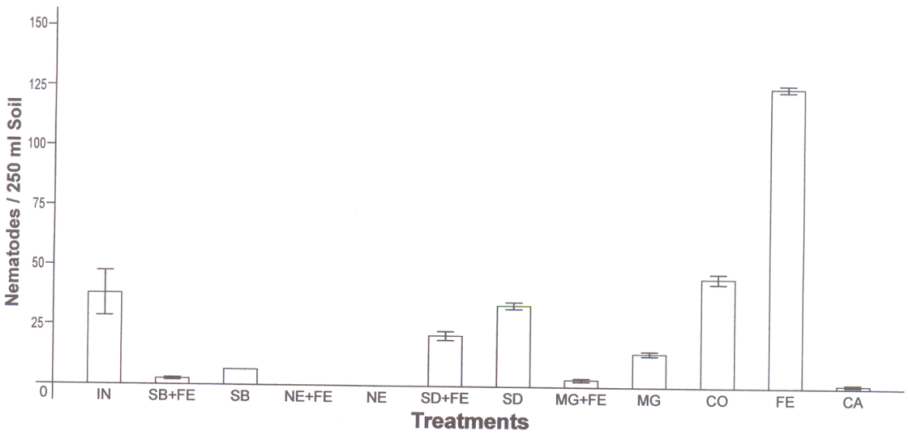

Fig. 3 Effect of different treatments on *Meloidogyne javanica* associated with almond seedlings (Legends as in Fig. 1)

Table 2. Matrix of Bray-Curtis similarity between nurseries on the basic of the nematode species recovered from six nurseries of Almond in Balochistan.

Localities	1*	2	3	4	5
2	50	-	-	-	-
3	27.27	37.5	-	-	-
4	30.76	40.0	44.4	-	-
5	38.46	50.0	40.0	54.54	-
6	40	37.5	42.85	30	40.0

*Localities as given in Table 1.

Table 3. Factorial ANOVA of various treatments and three nematode species associated with Almond seedlings.

Source	SS	Df	MS	F	P
Treatments	124610.16	10	12461.01	21.43	< 0.001
Nematodes	122274.41	2	61137.20	105.15	< 0.001
Treat. × Nematode	41893.42	20	2099.67	3.60	< 0.001
Error	57558.75	99	581.40	-	-
Total	346336.75	131	-	-	-

Treatment $LSD_{0.05}$ = 19.53, Nematode $LSD_{0.05}$ = 10.20

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