POPULATION DYNAMICS AND CONTROL OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH POMEGRANATE (*PUNICA GRANATUM* L.) SEEDLINGS IN BALOCHISTAN, PAKISTAN

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ABSTRACT

Surveys were conducted in Rabia Road nursery, Daniyal Nursery, Noorani chowk and Gujrabad nursery, Khuzdar; Umrani nursery, Wadh, Kalat Town and Surab nursery during February and March 2013 and 2014 to study the occurrence and control of nematodes associated with pomegranate nurseries in Balochistan, Pakistan. Among them *Helicotylenchus digonicus* was the most frequently observed nematode. Different treatments were used to control the nematode associated with pomegranate seedlings and the results obtained were subjected to factorial analysis of variance (ANOVA), followed by least significant difference (LSD) at $p < 0.05$.

Keywords: Plant parasitic nematodes, pomegranate (*Punica granatum* L.), survey, control, Balochistan, Pakistan

INTRODUCTION

The pomegranate (*Punica granatum* L.) is a fruit-bearing shrub or small tree growing between 5 and 8 m. The pomegranate originated in the region of Iran and has been cultivated since ancient times throughout the Mediterranean region and Indo-Pakistan (Mortan, 1987).

In Balochistan pomegranate are often grown in conjunction with other permanent crops such as Almond. Relatively low rainfall, insufficient for commercial farming during the summer months make the tree prone to a number of pathogens including plant parasitic nematodes.

The fruit trees are subject to various factors throughout their lives, which interfere with the normal development. Plant parasitic nematodes are one of these factors and a major one which may cause considerable losses to fruit production.

Control of nematode in nursery is of great importance components of nematode management. It is highly practical and feasible to effectively treat a small area involved for producing nematode free seedlings/rootstock which grow better in orchards and also have tolerance in the farmer's field against nematode invasion after transplanting compared to infected seedlings from untreated nurseries. Sethi and Gaur (1986) suggested that if seedlings are properly treated and are made nematode free the spread of nematodes to uninfected area is reduced. Bell (2013) reported that the seedlings which are sown in sterile nurseries in pots full of compost and sand or treated soil as compared to land they would not be infected with disease.

In the present investigation nematodes associated with pomegranate seedlings in Balochistan are being reported and different amendments are being used to control the population of *Helicotylenchus digonicus*, *Meloidogyne incognita* and *Merlinius khuzdarensis* associated with pomegranate seedlings.

MATERIALS AND METHODS

Survey: The survey of pomegranate seedling nurseries was conducted during February and March 2013. Sixty six samples were collected from 6 localities of pomegranate (*Punica granatum* L.) seedlings var. Kandhari across Khuzdar and Kalat districts, Balochistan. 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 (stunting, chlorosis and necrosis) and combined with the systematic samples, then a well mixed composite sample for nematode extraction from each nursery. Soil samples (85%) were texturally either sandy-loam or loamy sands. Average daytime temperature ranged from 14 to 22°C during the sample 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 experiment was conducted at a Khuzdar nursery, Balochistan in plastic pots filled with 250 g of sandy loam soil. Six samples were taken from soil of orchard with uneven growth of pomegranate trees located 20 km from the nursery and the nematodes present in the soil were determined (Cobbs, 1918) as 47.20 ± 2.8 *Meloidogyne incognita* 73 ± 4.0 *Helicotylenchus digonicus* and 165 ± 7.7 *Merlinius khuzdarensis*. These three nematodes comprised 75 percent of the total plant parasitic nematode population. Ten days later pomegranate (*Punica granatum* L. var. Kandhari) seedlings were transplanted in pots. Amendments including sugarcane bagasse, neem (*Azadirachta indica*) leaf powder, sawdust and marigold (*Tagetes erecta*) flower powder was used alone and in combination with Fertinemakil (a nematicide containing neem cake and fungicide) produced by Pakistan Council of Scientific and Industrial Research Laboratories Complex in collaboration with CDRI, PARC, University of Karachi. Neem leaves and marigold flowers were obtained from Hub chowki and were air dried for six weeks and powdered using a Wiley mill (Thomas Scientific). Untreated pots were kept as control. For comparison a chemical nematicide Carbofuran (a.i 44% Agricultural group, Philadelphia, PA) belonging to carbamate group of pesticide was used. The treatments and control were replicated four times each. The dose for Fertinemakil was 25 g/pot, for the other four amendments 4.08 g/pot and Carbofuran was applied at 0.2 g/L (from the 250 mL solution poured in each pot). Pots were irrigated regularly with distilled water to avoid any contamination with fungi or nematode. Eight weeks after treatment the soil in pots were collected for nematode populations and placed in polythene bags until nematode extracted. The nematode population were counted under a stereoscope binocular microscope by shaking the nematode containing suspension thoroughly and transferring 2 ml aliquots to a containing dish. Four aliquots were counted. Data was subjected to factorial analysis of variance (ANOVA) followed by least significant difference (LSD) at $p < 0.05$ (Zar, 1999).

RESULTS AND DISCUSSION

Eight different nematodes were recorded from pomegranate seedlings. The nematode recovered from all the six nurseries were *Helicotylenchus digonicus* followed by *Xiphinema basiri* from five nurseries localities (Table 1).
Similarities between six nematodes in the assemblages are given in (Table 2). Highest similarity in nematode assemblages was found in localities Umrani Nursery, Wadh and Kalat town nursery, followed by Daniyal nursery, Noorani chowk and Surab nursery. The lowest similarity of 25% was recorded between Daniyal nursery and Gujrabad nursery, Khuzdar. The rest of the pairs of nurseries showed intermediate similarities ranging from 28.57 to 66.66 percent.

The factorial ANOVA resulting from the experiment showed treatments to be significant ($p < 0.001$) (Table 3). While the nematodes were also highly significant ($p < 0.001$). The interaction of treatments × nematodes was also highly significant ($p < 0.001$). For nematode *Meloidogyne incognita* larvae best control was obtained in neem powder + Fertinemakil followed by sugarcane bagasse + Fertinemakil and least in sawdust + Fertinemakil. In relation to nematode *Helicotylenchus digonicus* the order of control was found to be: neem powder + Fertinemakil > marigold + Fertinemakil and least by sugarcane bagasse alone. For *Merlinius khuzdarensis* best result was given by marigold + Fertinemakil followed by neem powder + Fertinemakil and least by sawdust (Figs. 1–3).

The root-knot nematode and the root-lesion nematode encountered in the present survey are considered to be important nematode associated with pomegranate crop in different countries (La Rue, 1977; Darekar *et al.*, 1989; Mani *et al.*, 1997 and Sudheer *et al.*, 2007).

Balochistan, Pakistan is extremely suitable for pomegranate cultivation but recently it is becoming an expensive venture for local farmers due to various pests which effect health of the plant (Khan *et al.*, 2005, 2008). Nematode management at seedlings stage can reduce losses as by avoiding introducing nematode along with seedlings that were previously not present in the orchard or buying material certified to be free of nematodes pest. Thus treating seedlings/rootstock with amendments (organic material) may provide benefits, for their survival and help them to grow better when transplanted in the fields.

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

	Nurseries					
Nematodes	1*	2	3	4	5	6
Aphelenchus sp. larvae	+	+	−	−	−	−
Helicotylenchus digonicus	+	+	+	+	+	+
Helicotylenchus indicus	−	−	−	−	+	+
Merlinius khuzdarensis	−	−	−	−	+	+
Meloidogyne incognita	−	+	−	−	−	+
Meloidogyne javanica	+	+	−	−	+	+
Pratylenchus penetrans	+	+	+	+	−	−
Xiphinema basiri	+	−	+	+	+	+

Name of nurseries: 1 = Rabia Road nursery, Khuzdar; 2 = Gujarabad nursery, Khuzdar; 3 = Umrani nursery, Wadh; 4 = Kalat Town; 5 = Daniyal nursery, Noorani chowk, Khuzdar; 6 = Surab nursery.

Table 2. Matrix of Bray-Curtis similarity of nematode in six nurseries of Pomegranate in Balochistan.

	1*	**2**	**3**	**4**	**5**
2	66.6	-	-	-	-
3	60.0	33.33	-	-	-
4	60.0	33.33	100	-	-
5	42.8	2	33.33	33.33	-
6	37.5	37.5	28.57	28.57	33.33

*Localities as in Table 9

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

Source	SS	Df	MS	F	P
Treatments	169862.07	10	16986.20	164.76	< 0.001
Nematodes	37646.01	2	18823.00	182.58	< 0.001
Treat. × Nematode	62893.15	20	3144.65	30.50	< 0.001
Error	10206	99	103.01	-	-
Total	280607.22	131	-	-	-

Treatment $LSD_{0.05}$ = 8.224, Nematode $LSD_{0.05}$ = 4.295

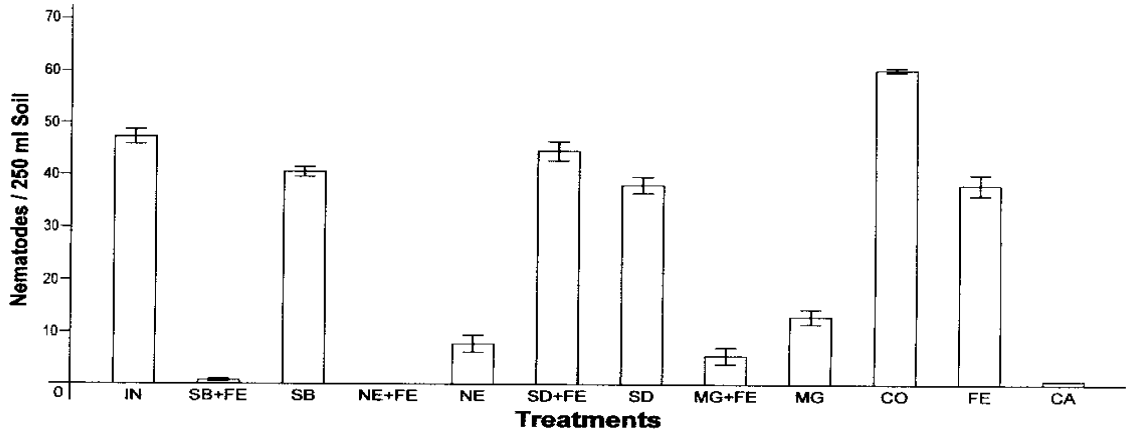

Fig. 1. Effect of different treatments on *Meloidogyne incognita* associated with pomegranate seedlings.

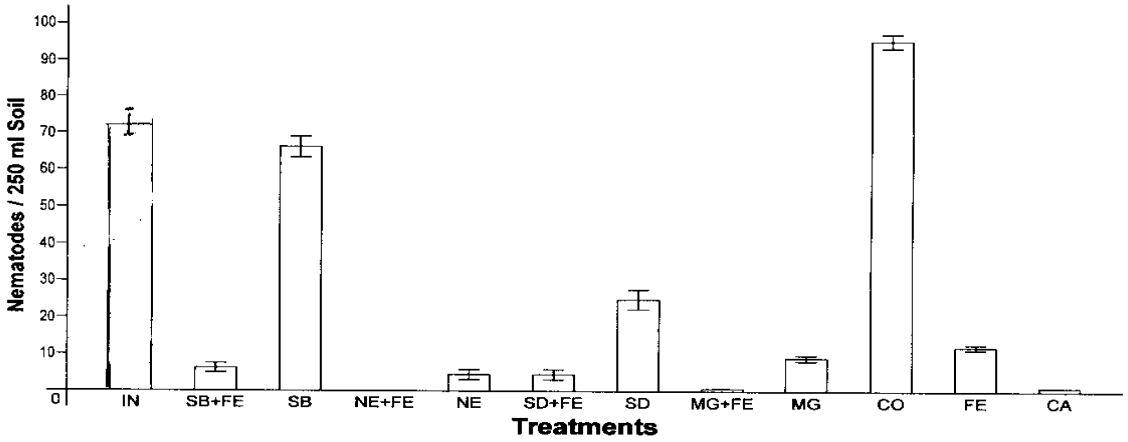

Fig. 2. Effect of different treatments on *Helicotylenchus digonicus* associated with pomegranate seedlings.

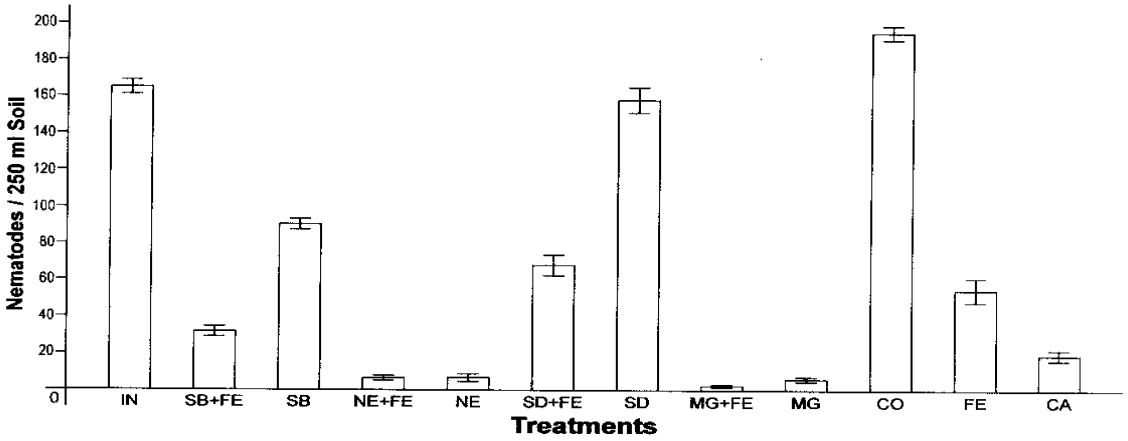

Fig. 3. Effect of different treatments on *Merlinius khuzdarensis* associated with pomegranate seedlings.

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