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Temporal Fluctuations in the Population of Citrus Nematode (*Tylenchulus semipenetrans*) in the Pothowar Region of Pakistan

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Authors' Contribution
MS and TM designed the study, conducted the surveys, executed experimental work, analyzed the data and prepared the manuscript. MAR provided technical assistance. TM supervised the work.

Key words
Population dynamics, Seasonal fluctuations, Citrus nematode, Pathogenicity, Pothowar region.

ABSTRACT

The study of the population dynamics of citrus nematode (*Tylenchulus semipenetrans*) in a production area is required for the assessment of damaging potential of the nematode to citrus, identification of key factors that influence population densities, and to devise effective management strategies. In the present study, seasonal fluctuations in the population densities of *T. semipenetrans* were studied in two citrus orchards naturally infested with citrus nematode during the year 2014. The effect of soil temperature was also evaluated on the populations of the nematode. The nematode populations differed significantly at both the soil depths. The populations were significantly higher at a depth of 30 cm as compared to 45 cm throughout the year at both the orchards. Similarly, females per gram of roots also followed the same pattern. The number of nematodes in the soil and females in the roots were the higher during the months of April to June and August-September showing two peaks throughout the year. The regression analysis between temperature and number of nematodes in the soil and females in the roots showed highly significant results at both the orchards. A direct relationship was observed between nematode population and temperature. Maximum nematode and female populations were observed at a temperature ranging between 26°C to 29°C at a soil depth of 30 cm. On the other hand, minimum populations were recorded at a temperature range of 9°C to 12°C. Similar trends were observed at the soil depth of 45 cm. It is concluded from the present study that the management of nematode including application of nematicides should be started in the spring season, just prior to the first root flush, to protect the new roots from nematode infection.

INTRODUCTION

Citrus fruit ranks in the second position after grapes in the world production of fruits. About 73 million tons of citrus fruits are produced annually in all parts of the tropical world. Pakistan stands in the top ten citrus producing countries of the world with negligible exports. In Pakistan, citrus is the largest group of fruits produced over an area of 176.4 thousand hectares with an annual production of 1688.7 thousand tons. Botanically, citrus is named for the genus in the family *Rutaceae* and refers to all edible, rootstock and few ornamental species. Common edible fruits include sweet orange, mandarins, grapefruit, lemons, limes, pomelos while the main rootstock species are rough lemon and sour orange. Citrus is enjoyed primarily as either fresh fruit or in the form of juice.

Citrus is affected by large number of fungi (Fateh *et al*., 2017), bacteria (Aslam *et al*., 2017a, b, 2019a), nematodes (Hussain and Mukhtar, 2019; Kayani *et al*., 2017; 2018, 2019; Kayani and Mukhtar, 2018; Khan *et al*., 2017; Mukhtar *et al*., 2017a, b, 2018; Mukhtar, 2018; Tariq-Khan *et al*., 2017; Mukhtar and Kayani, 2019), mycoplasmas, viruses and viroids (Ashfaq *et al*., 2017) and insect pests (Javed *et al*., 2017a, b; Aslam *et al*., 2019b) which are considered to be the possible causes of citrus decline. Numerous nematode species are associated with the citrus rhizoshpere. To date, however, relatively few have been shown to be of economic importance. With the notable exception of *Tylenchulus semipenetrans* Cobb, most nematode species capable of damaging mature citrus tend to be regional or local problems, due either to edaphic conditions or to the natural distribution of a particular nematode. Citrus nematode causes slow decline of citrus and an estimated 7% loss of production (Ahmad *et al*., 2007; Mukhtar *et al*., 2006, 2007; Irshad *et al*., 2012). It often passes unnoticed because the above ground symptoms are not very specific or inconspicuous. The nematodes debilitate the plants and predispose them to other problems (Chaudhry *et al*., 1992). Being a semi-endoparasite, it grows and multiplies slowly and develops high populations which become problematic. Feeder roots are damaged, rotted and destroyed. Affected trees show reduced terminal growth, chlorosis and shedding of terminal leaves, dieback of branches and considerable

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reduction in number and size of fruits (Chaudhry *et al*., 1992). Roots show brownish discoloration and unusual adherence of soil particles at the root surface. Heavily infected fresh roots of citrus may harbor over a hundred nematodes per centimeter of root, causing numerous lesions. The uptake of water and minerals from the soil is reduced and secondary invasion of soil fungi such as *Fusarium solani* and *F. oxysporum* aggravate disease infestation.

The study of the population dynamics of citrus nematode in a production area is required for the assessment of damaging potential of the nematode to citrus, identification of key factors that influence population densities, and to devise effective management strategies. In the present study, seasonal fluctuations in the population densities of *T. semipenetrans* were studied in two citrus orchards naturally infested with citrus nematode during the year 2014. The effect of soil temperature was also evaluated on the populations of the nematode.

Table I.- Population dynamics of citrus nematode (*Tylenchulus semipenetrans*) during the year 2014 at Site 1 (UAAR).

Date of sampling	Nematodes at soil depth of		Females at soil depth of	
	30 cm	45 cm	30 cm	45 cm
1st January	280	195	8	5
16th January	308	185	12	8
1st February	399	265	14	9
16th February	449	285	14	9
1st March	669	443	21	14
16th March	759	485	24	16
1st April	1289	795	39	25
16th April	1361	927	45	29
1st May	1550	996	56	36
16th May	1800	1180	56	37
1st June	1692	1045	53	34
16th June	1472	1011	50	33
1st July	1401	910	40	26
16th July	1301	846	44	29
1st August	1291	820	41	27
16th August	1191	792	43	28
1st September	1458	1099	54	35
16th September	2024	1127	58	38
1st October	1949	500	26	17
16th October	1433	396	19	12
1st November	711	358	16	10
16th November	301	300	18	12
1st December	284	172	11	7
16th December	218	152	3	2

Table II.- Population dynamics of citrus nematode (*Tylenchulus semipenetrans*) during the year 2014 at Site 2 (Khanpur).

Date of sampling	Nematodes at soil depth of		Females at soil depth of	
	30 cm	45 cm	30 cm	45 cm
1st January	336	212	11	7
16th January	380	238	13	8
1st February	516	324	17	11
16th February	532	336	18	11
1st March	770	484	26	16
16th March	858	540	30	19
1st April	1450	914	52	33
16th April	1380	868	51	32
1st May	1864	1174	64	40
16th May	1910	1204	65	41
1st June	1916	1208	68	43
16th June	1848	1164	64	40
1st July	1510	952	50	32
16th July	1492	940	49	31
1st August	1632	1028	56	35
16th August	1650	1040	57	36
1st September	1822	1148	63	40
16th September	1942	1224	65	41
1st October	880	554	31	20
16th October	810	510	29	18
1st November	568	358	21	13
16th November	618	390	22	14
1st December	436	274	16	10
16th December	284	178	11	7

MATERIALS AND METHODS

The fluctuations in the populations of citrus nematode in soil and roots were studied in two naturally infested citrus orchards in the Pothowar Region of Pakistan. The orchards were located at the University of Arid Agriculture Rawalpindi and Khanpur which were about 60 kilometers away from each other. The orchards were under cultivation for the last 15 years. Root and soil samples were taken from these two citrus orchards at 15 days interval from January 2014 to December 2014 as described by Iqbal *et al*. (2014a). Samples were collected from five randomly selected plants from each orchard, each weighing about 1 kg with the help of soil sampler at the depths of 30 and 45 cm from root zone. The samples from each tree were placed in polyethylene bags separately. Samples of feeder roots were also taken. Soil temperature was recorded at the time of sampling. Samples were taken at a distance of 120 cm from the tree trunks. The samples were immediately brought to the laboratory and processed for further evaluation (Iqbal *et al*., 2004a).

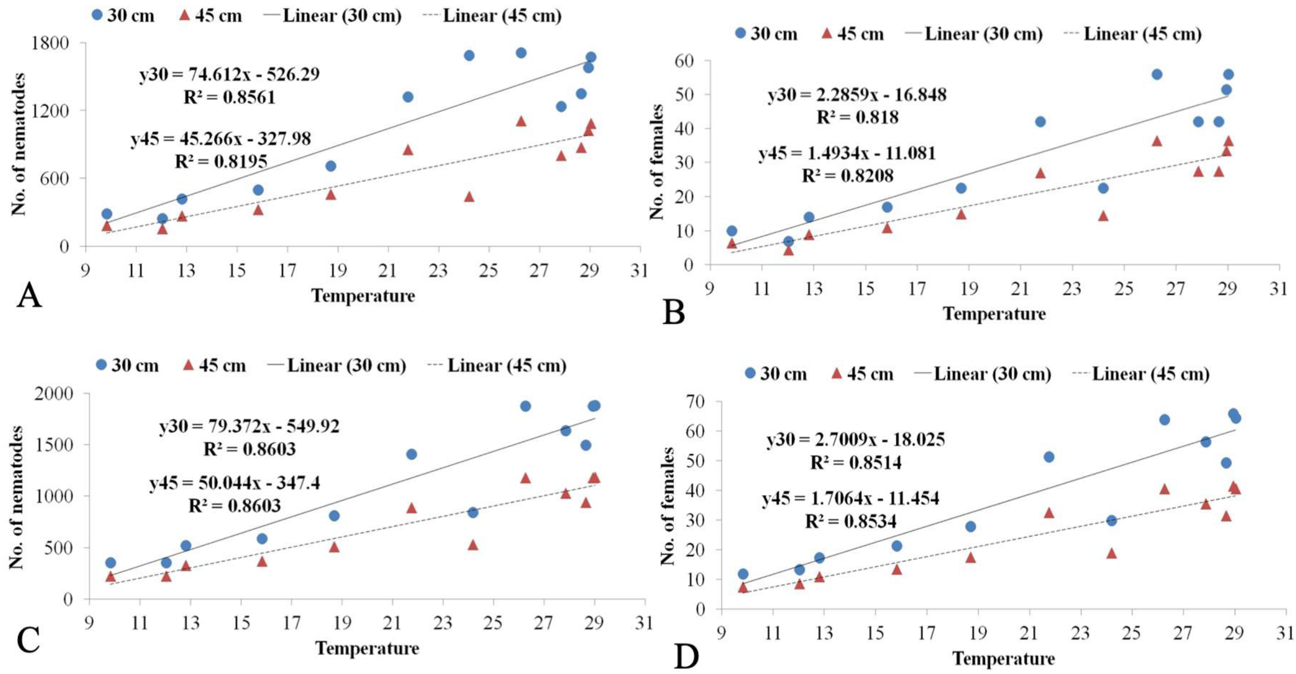

Fig. 1. Relationship between monthly mean temperature and nematode population (A) at site 1, number of females (B) at site 1, nematode population (C) at site 2, number of females (D) at site 2.

Juveniles of *T. semipenetrans* were extracted from the soil samples by modified Whitehead and Hemming tray method (Whithead and Hemming, 1965). One gram of fresh feeder roots from each sample was taken out and carefully washed under gentle stream of water to remove soil particles, stained in acid fuchsin lactophenol. The stained roots were macerated in a blender for 30 seconds. The root suspensions were sieved through 100 and 325 mesh sieves into beakers. The materials were then centrifuged to concentrate the females in a volume of 10 ml. The suspensions from each sample were placed in counting dishes and females were counted under stereomicroscope and expressed as number of females per gram of roots (Iqbal *et al*., 2014a).

The linear relationships between mean monthly number of nematodes in the soil and females per gram of roots and mean monthly temperature were calculated in Microsoft Excel 2007 by taking mean monthly temperature as independent variable (x) and nematode and female numbers as dependent variables (y).

RESULTS

The nematode populations differed significantly at both the soil depths. The populations were significantly higher at a depth of 30 cm as compared to 45 cm throughout the year at both the orchards. Similarly, females per gram of roots also followed the same pattern. The number of nematodes in the soil and females in the roots were the higher during the months of April to June and August, September showing two peaks throughout the year as shown in Tables I and II.

The regression analysis between temperature and number of nematodes in the soil and females in the roots showed highly significant results at both the orchards. A direct relationship was observed between nematode populations and temperature. Maximum nematode and female populations were observed at a temperature ranging between 26°C to 29°C at a soil depth of 30 cm. On the other hand, minimum population was recorded at a temperature range of 9°C to 12°C. Similar trends were observed at the soil depth of 45 cm as shown in Figure 1.

DISCUSSION

Citrus nematode is widespread in the world. Since its first report in roots of citrus trees in California in 1912, its occurrence has been reported from all over the major citrus growing regions of the world. The population density of *T. semipenetrans* has been reported to fluctuate throughout the year (Sweelam, 1995; Singh, 1999; Sorribas *et al*., 2000) and often exhibits two distinct periods of growth (Duncan and Noling, 1987; Al-Qasem and Abu-Gharbieh, 1995). O'Bannon *et al*. (1972) found that peak populations developed during corresponding periods of increased root growth that occurred in April-May and November-December. Infection and subsequent population cycles are restricted to primary roots because citrus nematodes feed only in the cortex of primary roots (Van Gundy and Kirkpatrick, 1964). Vilardebo (1964), Iqbal *et al*. (2004a) and Al-Azzeh and Gharbieh (2005) also recognized two high and low population periods. Several other investigators observed some seasonal variations (Yokoo, 1964; Prasad and Chawla, 1965); however, Cohn (1966) found no correlation between population fluctuations in Israel. Duncan and Cohn (1990) found that population densities of citrus nematode generally increase in autumn and spring, presumably in response to flushes in the growth of new fibrous roots on which the nematode feeds. Duncan *et al*. (1993), however, found that root availability did not adequately explain population decline in nematode levels in summer, since root mass density increased by 75% during that period. Amylytic activity in homogenates of the juveniles of *T. semipenetrans* and reduced starch levels in the nurse cells compared to surrounding cortical cells suggest that starch is a nutrient source for the nematode (Cohn, 1965). Citrus fruits represent a major carbohydrate sink with which the nematode must compete. During summer, available carbohydrates in the roots are relatively low, which can be linked to nematode population decline due to high carbohydrate demand by fruit (Duncan and Eissenstat, 1993). Dry-matter accumulation by fruit is nearly complete by midsummer, coincident with the beginning of nematode population growth (Duncan *et al*., 1993).

Citrus nematode occurs over a wide range of soil conditions throughout the world. Certain soil factors readily influence infection and reproduction. Van Gundy *et al*. (1964) found that growth and reproduction of citrus nematodes occurred on citrus seedlings in soils containing 50% clay. The rate of reproduction, however, was significantly lower in soils of 50% clay than in soils containing 5, 15 or 30% clay. Generally, citrus nematode invasion and reproduction is slower in very sandy, coarse-textured soils than in other soil types (Vilardebo, 1963; Van Gundy *et al*., 1964; O'Bannon, 1968; Baines, 1974; Ahmad *et al*., 2007; Iqbal *et al*., 2007). Soils containing organic matter up to 9% favored infection and rapid increase in nematodes that resulted in early damage. Van Gundy (1958) found that organic debris created a thin protective cover over citrus roots that enhanced nematode infectivity. Soil type apparently has little influence on citrus nematode migration. Baines (1974) and Tarjan (1971) found that nematode mobility in various soils was limited. Pandey *et al.* (2004) observed fluctuation of *T. semipenetrans* populations at different soil pH levels. Maximum population of male was recorded at pH 7.4-7.6. Female population was the maximum at pH 7.8, and the larval population increased at pH 7.6. Maximum total population of the nematode was observed at pH 7.4-7.8; optimum pH for maximum population (male, female, larva, and total) was 7.6.

CONCLUSIONS

It is concluded from the present study that population of citrus nematode showed wide variations throughout the season. The number of nematodes in the soil and females in the roots were the higher during the months of April to June and August to September showing two peaks throughout the year corresponding to the appearance of new flushes. This means that the management of nematode including application of nematicides (Ahmad *et al*., 2004; Iqbal *et al*., 2004b, 2005a, b) should be started in the spring season, just prior to the first root flush, and continue during the growing season to protect the new roots from nematode infection.

Statement of conflict of interest
The authors declare no conflict of interest.

REFERENCES

Ahmad, M.S., Mukhtar, T. and Ahmad, R., 2004. Some studies on the control of citrus nematode (*Tylenchulus semipenetrans*) by leaf extracts of three plants and their effects of plant growth variables. *Asian J. Pl. Sci*., **3**: 544-548. https://doi.org/10.3923/ajps.2004.544.548

Ahmad, R., Khan, M.A., Mukhtar, T. and Javed, N., 2007. *Infestation of citrus orchards by citrus nematode (Tylenchulus semipenetrans) and screening of some rootstocks for resistance or susceptibility.* Proceedings of the International Symposium on Prospects of Horticultural Industry in Pakistan. 28th to 30th March, 2007. Institute of Horticultural Sciences, University of Agriculture, Faisalabad. pp. 165-169.

Al-Qasem, M.S. and Abu-Gharbieh, W.I., 1995. Occurrence and distribution of citrus nematode, *Tylenchulus semipenetrans* in Jordan. *Nematol. Medit*., **23**: 335-339.

Al-Azzeh, T.K. and Gharbieh, W.A., 2005. Factors related to seasonal changes in numbers of *Tylenchulus semipenetrans* in the Jordan valley. *Pak. J. Nematol*., **23**: 241-250.

Ashfaq, M., Saleem, A., Waqas, M. and Mukhtar, T., 2017. Natural occurrence and host range studies of cucumber mosaic virus (CMV) infecting ornamental species in the Rawalpindi-Islamabad area of Pakistan. *Philipp. Agric. Scient.*, **100**: 55-61.

Aslam, M.N., Mukhtar, T., Ashfaq, M. and Hussain, M.A., 2017a. Evaluation of chili germplasm for resistance to bacterial wilt caused by *Ralstonia solanacearum*. *Australas. Pl. Pathol*., **46**: 289-292. https://doi.org/10.1007/s13313-017-0491-2

Aslam, M.N., Mukhtar, T., Hussain, M.A. and Raheel, M., 2017b. Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. *J. Pl. Dis. Prot*., **124**: 585-590. https://doi.org/10.1007/s41348-017-0100-1

Aslam, M.N., Mukhtar, T., Jamil, M. and Nafees, M., 2019a. Analysis of aubergine germplasm for resistance sources to bacterial wilt incited by *Ralstonia solanacearum. Pak. J. agric. Sci.,* **56**: 119-122.

Aslam, M.A., Javed, K., Javed, H., Mukhtar, T. and Bashir, M.S., 2019b. Infestation of *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera) on soybean cultivars in Pothwar region and relationship with physico-morphic characters. *Pak. J. agric. Sci.,* **56**: 401-405.

Baines, R.C., 1974. The effect of soil type on movement and infection rate of larvae of *Tylenchulus semipenetrans*. *J. Nematol*., **6**: 60-62.

Chaudhry, N.A., Ansari, M.A. and Mukhtar, T., 1992. Diseases infecting citrus plants and their control. *Progres. Farm.*, **12**: 27-34.

Cohn, E., 1965. The development of the citrus nematode on some of its hosts. *Nematologica*, **11**: 593-600. https://doi.org/10.1163/187529265X00744

Cohn, E., 1966. Observation on the survival of free-living stages of the citrus nematode. *Nematologica*, **12**: 321-327. https://doi.org/10.1163/187529266X00743

Duncan, L.W. and Cohn, E., 1990. Nematode parasites of citrusIn: *Plant parasitic nematodes in subtropical and tropical agriculture* (eds. M. Luc, R.A. Skora and J. Bridge). CAB International, Wallingford, UK, pp. 321-346.

Duncan, L.W. and Eissenstat, D.M., 1993. Response to *Tylenchulus semipenetrans* to citrus fruit removal: implications for carbohydrates composition. *J. Nematol*., **25**: 7-14.

Duncan, L.W. and Noling, J.W., 1987. The relationship between development of the citrus root system and infestation by *Tylenchulus semipenetrans*. *Rev. Nematol*., **10**: 61-66.

Duncan, L.W., Graham, J.H. and Timmer, L.W., 1993. Seasonal pattern associated with *Tylenchulus semipenetrans* and *Phtophthora parasitica* in the citrus rhizosphere. *J. Phytopathol*., **83**: 573-581. https://doi.org/10.1094/Phyto-83-573

Fateh, F.S., Mukhtar, T., Kazmi, M.R., Abbassi, N.A. and Arif, A.M., 2017. Prevalence of citrus decline in district Sargodha. *Pak. J. agric. Sci*., **54**: 9-13. https://doi.org/10.21162/PAKJAS/17.5643

Mukhtar, T. and Kayani, M.Z., 2019. Growth and yield responses of fifteen cucumber cultivars to root-knot nematode (*Meloidogyne incognita*). *Acta Sci. Pol. Hortorum Cultus,* **18**: 45–52.

Hussain, M.A. and Mukhtar, T., 2019. Root-knot nematodes infecting okra in major vegetable growing districts of Punjab, Pakistan. *Pakistan J. Zool.,* **51**: 1137-1143.

Iqbal, M.A., Ahmad, R., Mukhtar, T. and Khan, H.U., 2004a. Interrelationship of citrus nematode (*Tylenchulus semipenetrans* Cobb) population and seasonal fluctuations in Sargodha district. *Pak. J. Phytopathol*., **16**: 60-62.

Iqbal, M.A., Ahmad, R., Mukhtar, T. and Khan, H.U., 2004b. Evaluation of various nematicides against citrus nematode (*Tylenchulus semipenetrans* Cobb) on highly and slightly diseased trees in the citrus orchards. *Pak. J. Phytopathol*., **16**: 17-22.

Iqbal, M.A., Mukhtar, T. and Ahmad, R., 2005b. Potentiality of some plants to control Citrus nematode (*Tylenchulus semipenetrans*). *Pak. J. Phytopathol*., **17**: 36-42.

Iqbal, M.A., Mukhtar, T. and Ahmad, R., 2007. Effect of soil types on the movement and infection of citrus nematode, *Tylenchulus semipenetrans*. *Pak. J. Nematol*., **25**: 69-72.

Iqbal, M.A., Mukhtar, T., Ahmad, R. and Khan, H.U., 2005a. Relative susceptibility/ resistance of citrus rootstocks to citrus nematode (*Tylenchulus semipenetrans*). *Pak. J. Nematol*., **23**: 311-315.

Irshad, U., Mukhtar, T., Ashfaq, M., Kayani, M.Z., Kayani, S.B., Hanif, M. and Aslam, S., 2012. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on *Citrus jambhiri*. *J. Anim. Pl. Sci*., **22**: 1014-1018.

Javed, H., Hussain, S.S., Javed, K., Mukhtar, T. and Abbasi, N.A., 2017a. Comparative infestation of brinjal stem borer (*Euzophera perticella*) on six aubergine cultivars and correlation with some morphological characters. *Pak. J. agric. Sci*., **54**: 753-758.

Javed, H., Mukhtar, T., Javed, K. and Ata ul Mohsin, 2017b. Management of eggplant shoot and fruit borer (*Leucinodes orbonalis* Guenee) by integrating different non-chemical approaches. *Pak. J. agric. Sci*., **54**: 65-70. https://doi.org/10.21162/PAKJAS/17.5282

Kayani, M.Z. and Mukhtar, T., 2018. Reproductivity of *Meloidogyne incognita* on fifteen cucumber cultivars. *Pakistan J. Zool*., **50**: 1717-1722. https://doi.org/10.17582/journal.pjz/2018.50.5.1717.1722

Kayani, M.Z., Mukhtar, T. and Hussain, M.A., 2017. Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. *Crop Protec*., **92**: 207-212. https://doi.org/10.1016/j.cropro.2016.09.007

Kayani, M.Z., Mukhtar, T. and Hussain, M.A., 2018. Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of *Meloidogyne incognita*. *Pakistan J. Zool*., **50**: 897-902. https://doi.org/10.17582/journal.pjz/2018.50.3.897.902

Khan, A.R., Javed, N., Sahi, S.T., Mukhtar, T., Khan, S.A. and Ashraf, W., 2017. *Glomus mosseae* (Gerd & Trappe) and neemex reduce invasion and development of *Meloidogyne incognita*. *Pakistan J. Zool*., **49**: 841-847. https://doi.org/10.17582/journal.pjz/2017.49.3.841.847

Mukhtar, T. and Hussain, M.A., 2019. Pathogenic potential of Javanese root-knot nematode on susceptible and resistant okra cultivars. *Pakistan J. Zool*., **51**: 1891-1897.

Mukhtar, T., 2018. Management of root-knot nematode, *Meloidogyne incognita*, in tomato with two *Trichoderma* species. *Pakistan J. Zool*., **50**: 1589-1592. https://doi.org/10.17582/journal.pjz/2018.50.4.sc15

Mukhtar, T., Ahmad, R. and Khan, M.A., 2006. *Studies on the distribution, ecology and management of citrus nematode,* Tylenchulus semipenetrans. Proceedings of International Symposium on Sustainable Crop Improvement and Integrated Management, September 14-16, 2006. pp. 223-230.

Mukhtar, T., Ahmad, R., Kayani, M.Z. and Irshad, G., 2007. Occurrence of citrus nematode (*Tylenchulus semipenetrans*) in Sargodha district. *Pak. J. Phytopathol*., **19**: 40-43.

Mukhtar, T., Arooj, M., Ashfaq, M. and Gulzar, A., 2017a. Resistance evaluation and host status of selected green gram germplasm against *Meloidogyne incognita*. *Crop Protec*., **92**: 198-202. https://doi.org/10.1016/j.cropro.2016.10.004

Mukhtar, T., Hussain, M.A. and Kayani, M.Z., 2017b. Yield responses of 12 okra cultivars to southern root-knot nematode (*Meloidogyne incognita*). *Bragantia*, **76**: 108-112. https://doi.org/10.1590/1678-4499.005

Mukhtar, T., Jabbar, A., Raja, M.U. and Javed, H., 2018. Re-emergence of wheat seed gall nematode (*Anguina tritici*) in Punjab, Pakistan. *Pakistan J. Zool*., **50**: 1195-1198. https://doi.org/10.17582/journal.pjz/2018.50.3.sc4

O'Bannon, J.H., 1968. The influence of organic soil amendment on infectivity and reproduction of *Tylenchulus semipenetrans* on two citrus rootstocks. *Phytopathology*, **58**: 597-601.

O'Bannon, J.H., Radewald, J.D. and Tomerlin, A.T., 1972. Population fluctuation of three parasitic nematodes in Florida citrus. *J. Nematol*., **4**: 194-199.

Pandey, R.K., Goswami, B.K. and Singh, S., 2004. Influence of soil pH on population dynamics of *Tylenchulus semipenetrans* infesting citrus and its bio-management using VAM fungi. *Int. J. Nematol*., **14**: 174-176.

Prasad, S.K. and Chawla, M.L., 1965. Observation on the population fluctuation of citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913. *Indian J. Ent*., **27**: 450-454.

Singh, B., 1999. Occurrence and distribution of *Tylenchulus semipenetrans* in Nagpur mandarin orchards of Vidarbha, Maharashtra. *Indian J. Nematol*., **29**: 149-153.

Sorribas, F.J., Verdejo-Lucas, S., Forner, J.B., Alcaide, A., Pons, J. and Ornat, C., 2000. Seasonality of *Tylenchulus semipenetrans* Cobb and *Pasteuria* sp., in citrus orchards of spain. *J. Nematol*., **32**: 622-632.

Sweelam, M.E., 1995. Seasonal population dynamics and chemical control of *Meloidogyne javanica*, *Rotylenchulus reniformis* and *Tylenchulus semipenetrans* in vineyards. *Menofiya J. agric. Res*., **20**: 2283-2295.

Tariq-Khan, M., Munir, A., Mukhtar, T., Hallmann, J. and Heuer, H., 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. *J. Pl. Dis. Protec*., **124**: 201-212. https://doi.org/10.1007/s41348-016-0045-9

Tarjan, A.C., 1971. Migration of three pathogenic citrus nematodes through two Florida soils. *Soil Crop Sci. Soc. Flor. Proc*., **31**: 253-255.

Van Gundy, S.D., 1958. The life history of the citrus nematode, *Tylenchulus semipenetrans* Cobb. *Nematoloica*, **3**: 283-294. https://doi.org/10.1163/187529258X00049

Van Gundy, S.D. and Kirkpatrick, J.D., 1964. Nature of resistance in certain citrus rootstocks to citrus nematode. *Phytopathology*, **54**: 419-427.

Van Gundy, S.D., Martin, J.P. and Tsao, P.H., 1964. Some soil factors influencing reproduction of sweet orange seedlings. *Phytopathology*, **54**: 294-299.

Vilardebo, A., 1963. Studies on *Tylenchulus semipenetrans* Cobb in Morocco. I. *Al Awamia* (Rabat), **8**: 1-23.

Vilardebo, A., 1964. Study on *Tylenchulus semipenetrans* Cobb in Morocco. II. *Al Awamia* (Rabat) **11**: 31-49.

Whitehead, A.G. and Hemming, J.R., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annls. appl. Biol*., **55**: 25-38. https://doi.org/10.1111/j.1744-7348.1965.tb07864.x

Yokoo, T., 1964. Studies on the citrus nematode *Tylenchulus semipenetrans* Cobb, 1913) in Japan. *Agric. Bull. Saga Univ.,* **20**: 71-109.