The Journal of Animal & Plant Sciences, 22(4): 2012, Page: 1014-1018
ISSN: 1018-7081

PATHOGENICITY OF CITRUS NEMATODE (*TYLENCHULUS SEMIPENETRANS*) ON *CITRUS JAMBHIRI*

U. Irshad, T. Mukhtar, M. Ashfaq, M. Z. Kayani[*], S. B. Kayani[**], M. Hanif[***] and S. Aslam

Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[*]Green Belt Project, Department of Agriculture, Rawalpindi, Pakistan
[**]Department of Wildlife Management, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[***] Department of Mathematics and Statistics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
Corresponding author's E-mail: drtmukhtar@uaar.edu.pk

ABSTRACT

Citrus nematode (*Tylenchulus semipenetrans*) causes severe damage to citrus. In the present study the relationship between different initial densities of *T. semipenetrans* and reduction in growth parameters of *Citrus jambhiri* and nematode infestations was examined in pot experiment under greenhouse conditions using completely randomized design. Seedlings of *C. jambhiri* were inoculated with *T. semipenetrans* at the rate of 0, 1,000, 2,000, 4,000 and 8,000 second stage juveniles/pot in the greenhouse. Data were collected on fresh and dry weights of the shoot and root, shoot length, nematode populations in the roots and soil and reproduction factor. All the data were subjected to analysis of variance and means were compared by Duncan's Multiple Range Test at 5%. The relationships between number of galls and growth variables and nematode infestations were determined using regression analysis. It was observed that all the inoculum densities caused reductions in growth parameters and were found directly proportional to the inoculum density. Similarly, a direct relationship was observed between root and soil populations of the nematode and inoculum levels. On the other hand, the nematode build up was the maximum at lower inoculum level and an inverse relationship was observed between reproduction factor and inoculum densities of the nematode.

Key words: *Tylenchulus semipenetrans*; citrus nematode; inoculum densities; rough lemon.

INTRODUCTION

Among the biological factors effecting the quality and quantity of citrus, nematodes are the most important (Baines *et al.,* 1962). Numerous nematode species are associated with the citrus rhizosphere (Cohn, 1972); however, relatively few have shown to be of economic importance, with the notable exception of *Tylenchulus semipenetrans*. *T. semipenetrans*, commonly known as 'citrus nematode' causes 'slow decline' disease in citrus. The pest is cosmopolitan in distribution and has been reported to cause injury to 50 species or hybrids of *Citrus* in the family Rutaceae. Garcia Teran *et al*. (1987) reported that *T. semipenetrans* caused losses up to 43.3% on *C. aurantium*. Citrus roots infested with the nematode are more readily infested with *Fusarium oxysporum* and *F. solani* (O'Bannon *et al*., 1967) and other organisms which are important in the final destruction of the feeder roots (Begum *et al*., 2012). The loss of feeder roots results in increased drought stress and decreased uptake of soil nutrients, leading to retarded growth of shoots and chlorosis and loss of leaves. Affected trees do not die, but have an unthrifty appearance and yield fewer, smaller fruits than uninfested trees. In Pakistan, its occurrence has been reported from all the major citrus growing areas with varying degrees of infestations (Iqbal *et al*., 2006; Mukhtar *et al*., 2007; Khanzada *et al.,* 2008). The invasion and reproduction is influenced by soil types (Iqbal *et al*., 2007; Kayani *et al*., 2012a) and rootstocks (Iqbal *et al*., 2005). Although citrus fruit yield is negatively correlated with numbers of *T. semipenetrans* in the roots, the exact relationship is not known. In Israel, citrus yield decreased when there were more than 4000 females per gram of feeder roots (Cohn *et al*., 1965). An economic threshold level around 1000 juveniles/100cm^3 of soil has also been reported (Bridge and Starr, 2007). In the country, the mostly used rootstock is rough lemon (*Citrus jambhiri*) which is highly susceptible to citrus nematode and root rot. Keeping in view the above mentioned facts, the present study was planned to see the effect of different inoculum levels on the growth of *Citrus jambhiri* and the reproduction of the nematode.

MATERIALS AND METHODS

Nematode inoculum: Citrus nematode**,** *T. semipenetrans*, used in the study was multiplied on six month old seedlings of rough lemon (*C. jambhiri*) in clay pots with standard soil mixture 1:1:1 (sand, silt, and clay). The juveniles were extracted using extraction trays (Whitehead and Hemming, 1965). The juveniles were standardized and concentrated in water and used in the studies.

Pathogenicity of Citrus Nematode: Pathogenicity test was carried out on six month old seedlings of rough lemon planted singly in plastic pots (13 × 15 cm). The seedlings were then inoculated with freshly hatched second stage juveniles (J2s) of *T. semipenetrans* at the rate of 1000, 2000, 4000 and 8000. Pots without nematode inoculum served as control. Each treatment was replicated five times. The pots were arranged in a Completely Randomized Design on the bench of the green house at 25 ± 2^{o}C for two months. The pots were watered when required

Data Collection: For recording data, the plants were gently removed from the pots after stipulated period. The shoots were excised from the roots. The lengths of shoots were measured with the help of a scale. The shoots and roots of individual plants were weighed in an electric balance. For estimation of total nematode population, juveniles were extracted from the roots of individual plants and from the soil of each plant from their respective pots following the method described by Whitehead and Hemming, (1965). The total number of juveniles in soil and roots constituted the total population. The reproduction factor (Rf) was calculated by dividing the final population (Pf) by the initial one (Pi). The percent reduction/increase in growth parameters were calculated as described by Hussain *et al*. (2011a) and Kayani *et al*. (2012b).

Statistical Analysis: The experiment was repeated twice. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The means were compared by Duncan's Multiple Range Test (DMRT) at 5%. The relationships between number of galls and growth variables were determined using regression analysis.

RESULTS AND DISCUSSION

All the inoculum levels of *T. semipenetrans* caused significant reductions in growth parameters of *C. jambhiri* over control. Highly significant effects of inoculum levels were observed in case of plant height, fresh and dry shoot and root weights. Maximum reductions in plant height and fresh shoot and root weights were recorded at an inoculum level of 8000 J2s. The reductions in these parameters were recorded 29.77, 48.55 and 30.30 per cent respectively. The minimum reductions were recorded at a level of 1000 J2s. Similar trends were found in dry shoot and root weights. The reductions in these parameters increased with an increase in inoculum level. Regression studies showed positive and significant relationship between inoculum levels and plant height (r = 0.999), inoculum levels and reduction in fresh shoot weight (r = 0.983), inoculum levels and reduction in dry shoot weight (r = 0.977), inoculum levels and fresh root weight (r = 0.846), and inoculum levels and dry root weight (r = 986). These relationships are shown by trend lines and equations given in figures 1, 2, 3, 4 and 5.

Significant differences were observed in root and soil populations and nematode build up. The number of juveniles in root and soil were the maximum in the treatment where 8000 juveniles were applied and were the minimum at a level of 1000 juveniles. The total populations in root and soil increased with an increase in inoculum level and were found directly proportional to inoculum levels. Regression studies showed positive and significant relationship between inoculum levels and total root population (r = 0.812) and inoculum levels and total soil population (r = 0.817) and are shown by trend lines and equations in figures 6 and 7 respectively.

On the other hand, the rate of multiplication of nematodes (Rf=Pf/Pi) was highly significant. Comparison of treatment means revealed that the rate of multiplication was the maximum (15.28) in pots inoculated with 1000 juveniles and minimum in pots where 8000 juveniles were inoculated. The rate of multiplication of nematodes decreased with an increase in the inoculum level and was found negatively correlated with inoculum levels (-0.856) and is shown by the trend line and equation given in figure 8.

The citrus nematode, *T. semipenetrans* significantly caused reductions in growth parameters of *C. jambhiri* plants at all inoculum densities. The pathogenicity of *T. semipenetrans* has also been studied by different workers on different citrus hosts. The findings of these workers confirm that the increase in nematode population and subsequent reduction in yield of crops or other manifestations of pathogenic effects, physiological responses (total leaf chlorophyll content, CO_2 exchange rate) and concentration of sodium, potassium, iron, manganese, copper and zinc are directly influenced by initial density of nematodes in soil (Wallace, 1973; Haseeb *et al*., 1990).

Al-Azzeh and Abu-Gharbieh (2004) showed that inoculation of 500 juveniles/kg soil did not affect the growth of sour orange seedlings. However, initial population densities (Pi) of 1,000 to 20,000 juveniles/pot progressively reduced vegetative growth by 9.1 to 30.3% and root weight by 9.7 to 30.9%. Also, Pi exceeding 5,000 juveniles per pot resulted in severe decline symptoms. Plant growth was not adversely affected as long as the resulting nematode infestation stayed below certain critical levels. Deka *et al*. (2003) observed a significant reduction in plant height, fresh and dry weight of shoot and root, and root length at 1000-10,000 J2s/kg of soil. Yellowing of leaves, reduction in plant height and weight are the conspicuous manifestations of the infections of nematodes and have been documented by many researchers (Vovlas *et al*., 2008; Azam *et al*., 2011). Reduction in root and shoot lengths was likely a result of nematode feeding causing root growth to stop. This shortening of the roots affects the growth and development of the plants by limiting its ability to uptake nutrients, minerals and water etc. from the soil, eventually resulting in stunting and smaller plants. Stunted plants have reduced leaf weight or top weight. Lamberti *et al*. (1974) tested the pathogenicity of *T. semipenetrans* populations from three regions in Italy on a number of *Citrus* species and related genera, and on olive and grapevine. No differences in host preferences were found between the populations. All the populations reduced root weights of most of the plants. Al-Azzeh and Abu-Gharbieh (2004) also studied the pathogenicity of three populations of *T. semipenetrans*, collected from Northern, Southern, and Central Jordan Valley, on 'Valencia' sweet orange, 'Troyer citrange', 'Pomeroy' and 'Rubidoux' *Poncirus trifoliata*, 'Thompson seedless' grape, and 'Manzanillo' olive. The three populations were not found pathogenic to olive or *P. trifoliata*. *T. semipenetrans* Infected plants grow poorly, their leaves turn yellowish and drop early, their twigs die back, and fruit production is gradually reduced to unprofitable levels. The female second-stage juveniles usually attack young feeder roots and feed on their surface cells. There, they undergo three additional molts and produce females. The young females then penetrate deeper into the cortex and may reach as deep as the pericycle. The head of the nematode creates a tiny cavity around it and feeds on enlarged parenchyma cells known as nurse cells. Later on, cells around the feeding site become disorganized and break down resulting into decaying of young roots (Agrios, 2005). The damage to roots is associated with feeding or invasion, withdrawal of nutrients, and/or to more subtle physiological effects. The damage reduces the rate of root extension, uptake of nutrients and water causing reduction in top growth of plants. Due to reduction in top growth, the rate of carbohydrates synthesis is also reduced and the capacity of the plants to generate more roots to overcome the limitations imposed by nematode damage is greatly affected. Kallel *et al*. (2004) observed significant reduction in leaf area of the nematode infested plants along with defoliation of the spring shoots. The physiology of young trees was also affected by the nematode as it reduced stomatal conductance and leaf transpiration (Kallel and B'Chir, 2006). The lower rate of nematode build up at higher inoculum levels may be due to overcrowding of the larvae which compete for food. A similar observation was made by Di Vito *et al*. (1983) and Hussain *et al*. (2011b) who also attributed low final population densities to the severely damaged root systems being unable to support large nematode populations. It is concluded that *T. semipenetrans* is pathogenic to *C. jambhiri* at all population levels and is responsible for reductions in growth variables. Therefore, necessary control measures (Ahmad *et al*., 2004; Mukhtar *et al*., 2013a; 2013b; Vagelas and Gowen, 2012) should be adopted to minimize the damage by this nematode.

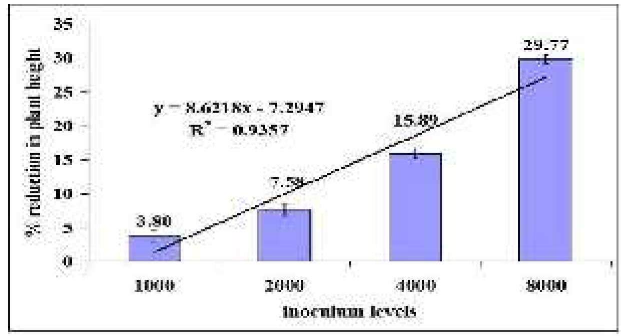

Figure 1 Effect of inoculum levels of *T. semipenetrans* on reduction in plant height.

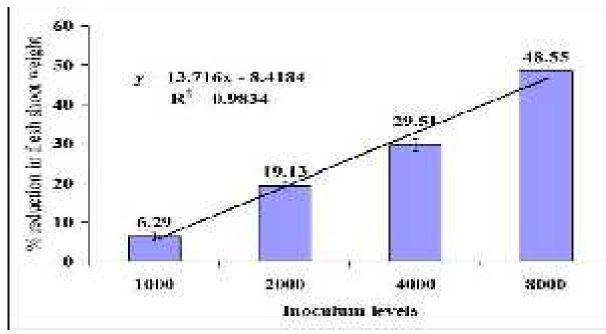

Figure 2 Effect of inoculum levels of *T. semipenetrans* on reduction in fresh shoot weight.

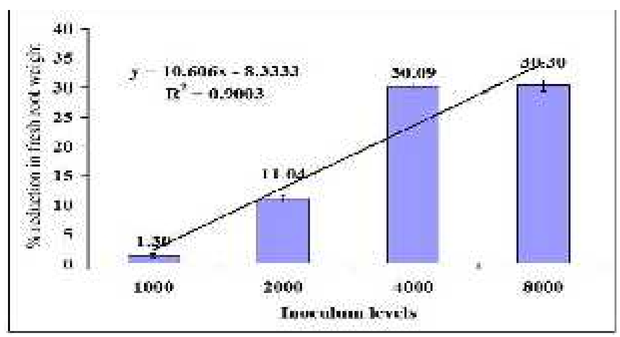

Figure 3 Effect of inoculum levels of *T. semipenetrans* on reduction in fresh root weight.

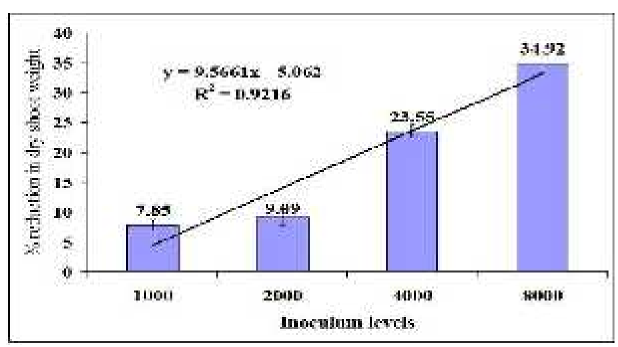

Figure 4 Effect of inoculum levels of *T. semipenetrans* on reduction in dry shoot weight.

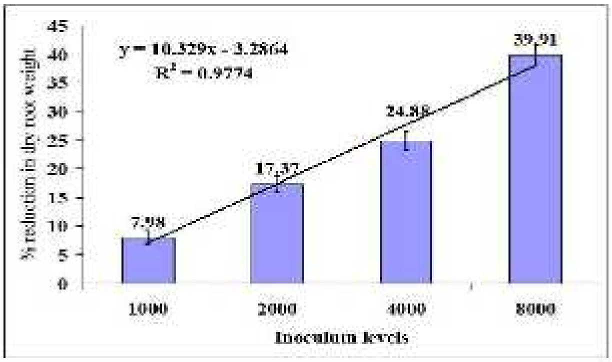

Figure 5 Effect of inoculum levels of *T. semipenetrans* on reduction in dry root weight.

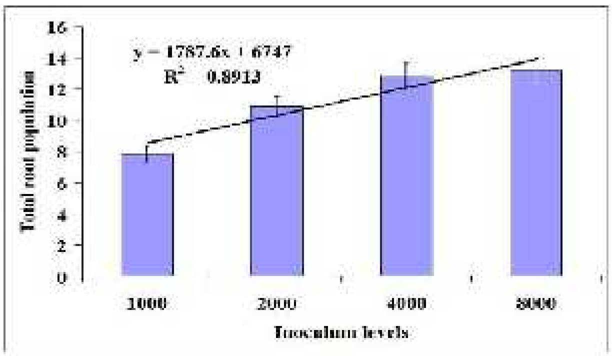

Figure 6 Effect of inoculum levels of *T. semipenetrans* on total root population.

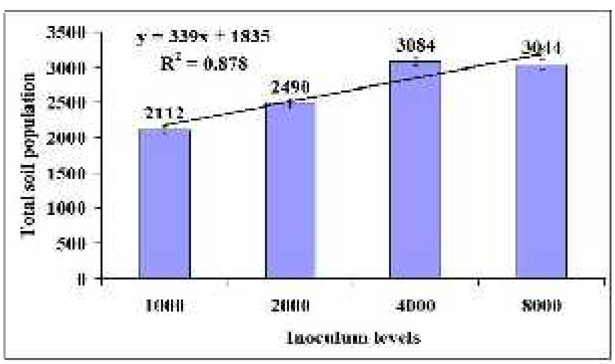

Figure 7 Effect of inoculum levels of *T. semipenetrans* on total soil population.

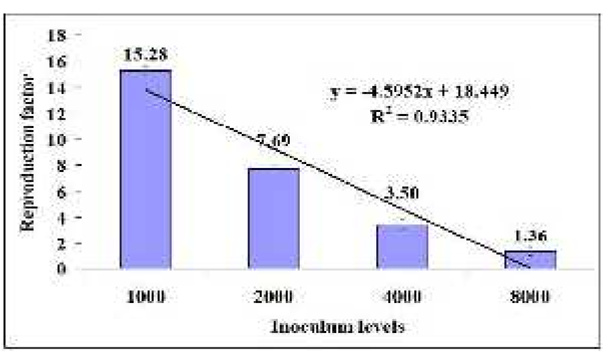

Figure 8 Effect of inoculum levels of *T. semipenetrans* on reproduction factor.

REFERENCES

Agrios, G.N. (2005). Plant Pathology. 5th Ed. Academic Press; London (UK). 848-849 pp.

Ahmad, M.S., T. Mukhtar, and R. Ahmad (2004). Some studies on the control of Citrus nematode (*Tylenchulus semipenetrans*) by extracts of three plants and their effects on plant growth variables. Asian J. Plant Sci. 3(5): 544-548.

Al-Azzeh, T.K. and W.I. Abu-Gharbieh (2004). Race identity, pathogenicity and damage threshold of *Tylenchulus semipenetrans* on sour orange in Jordan. Nematol. Medit. 32(1): 25-29.

Azam, T., Hisamuddin, S. Singh, and M.I. Robab 2011. Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Lycopersicon esculentum*, and internal structure of infected root. Arch. Phytopathology Plant Protect. 44(18): 1829-1839.

Begum, N., M.I. Haque, T. Mukhtar, S.M. Naqvi, and J.F. Wang (2012). Status of bacterial wilt caused by *Ralstonia solanacearum* in Pakistan. Pakistan J. Phytopathol. 24(1): 11-20.

Bains, R.C., J.P. Martin, T.A. De Wolfe, S.B. Boswell, and M.J. Garber (1962). Effect of high doses of D-D on soil organisms and the growth and yield of lemon trees. Phytopathology 52: 723.

Bridge, J. and J.L. Starr (2007). Plant nematodes of agricultural importance, a colour handbook. Manson Publishing Ltd, London (UK). 109 p.

Cohn, E. (1972). Nematode diseases of citrus. In: Economic Nematology (J.M. Webster, Ed.). Academic, New York, 215-244 pp.

Cohn, E., G. Minz, and S.P. Monselise (1965). The distribution, ecology and pathogenicity of the citrus nematode in Israel. Israel J. Agric. Res. 15: 187-200.

Deka, R., A.K. Sinha, and P.P. Neog (2003). Pathogenicity of the citrus nematode, *Tylenchulus semipenetrans* on *Citrus jambhiri*. Indian J. Nematol. 33(1): 63-64.

Di Vito, M., N. Greco, and A. Carella (1983). The effect of population densities of *Meloidogyne incognita* on the yield of cantaloupe and tobacco. Nematol. Medit. 11: 169-174.

Garcia Teran, O., E. Duarte, and Y.E. Jimenez (1987). Relation between the population density of *Tylenchulus semipenetrans* and the yields of 'Valencia' oranges (*Citrus sinensis*). Ciencia y Tecnica en la Agricultura, Proteccion de Plantas 10(1): 63-71.

Haseeb, A., N.K. Srivastava, and R. Pandey (1990). The influence of *Meloidogyne incognita* on growth, physiology, nutrient concentration and alkaloid yield of *Hyocyamus niger*. Nematol. Medit. 18: 127-129.

Hussain, M.A., T. Mukhtar, and M.Z. Kayani (2011a). Efficacy evaluation of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* against root-knot nematodes *Meloidogyne incognita*. Pakistan J. Bot. (Special Issue) 43: 197-204.

Hussain, M.A., T. Mukhtar, and M.Z. Kayani (2011b). Assessment of the damage caused by *Meloidogyne incognita* on okra (*Abelmoschus esculentus*). J. Anim. Plant Sci. 21(4): 857-861.

Iqbal, M.A., T. Mukhtar, and R. Ahmad (2007). Effect of soil types on the movement and infection of citrus nematode, *Tylenchulus semipenetrans*. Pakistan J. Nematol. 25 (1): 69-72.

Iqbal, M.A., T. Mukhtar, R. Ahmad, and H.U. Khan (2005). Relative susceptibility/ resistance of citrus rootstocks to citrus nematode (*Tylenchulus semipenetrans*). Pakistan J. Nematol. 23(2): 311-315.

Iqbal, M.A., T. Mukhtar, R. Ahmad, and H.U. Khan (2006). Ecological prevalence of *Tylenchulus semipenetrans* in four districts of the Punjab province, Pakistan. Pakistan J. Nematol. 24(1): 19-26.

Kallel, S. and M.M. B'Chir (2006). Effect of citrus nematode, *Tylenchulus semipenetrans*, on morphogenesis and physiology of 'Maltaise douce' orange grafted on sour orange. Nematol. Medit. 34(2): 115-128.

Kallel, S., A. Abdelwahed, M. Ammar, and M.M. B'Chir (2004). Relationship between citrus decline in orchard, population density of *Tylenchulus semipenetrans* and some soil abiotic factors. Int. J. Nematol. 14(2): 236-245.

Kayani, M.Z., T. Mukhtar, and M.A. Hussain (2012a). Association of root-knot nematodes (*Meloidogyne* spp.) with cucumber in the Pothowar region of the Punjab province of Pakistan. Int. J. Biol. Biotech. 9 (1-2): 23-29.

Kayani, M.Z., T. Mukhtar, and M.A. Hussain (2012b). Evaluation of nematicidal effects of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against root-knot nematodes, *Meloidogyne incognita*. Crop Prot. 39: 52-56.

Khanzada, S.A., A. Iqbal, A. Munir, K. Burney, S. Hameed, and H.U. Rehman (2008). Incidence and distribution of citrus nematode *Tylenchulus semipenetrans* in citrus orchards of Punjab. Pakistan J. Nematol. 26 (1): 51-58.

Lamberti, F., N. Vovlas, and A. Tirro (1974). Infectivity and pathogenicity of three Italian populations of *Tylenchulus semipenetrans* Cobb on different hosts. *Simposio Internacional (XII) de Nematologia, Sociedad Europea de Nematologos, 1-7 Septiembre, 1974, Granada, Spain:* 59.

Mukhtar, T., M.Z. Kayani, and M.A. Hussain (2013a). Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Ind. Crop Prod. 42: 447-453.

Mukhtar, T., M.Z. Kayani, and M.A. Hussain (2013b). Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Prot. 44: 13-17.

Mukhtar, T., R. Ahmad, M.Z. Kayani, and G. Irshad (2007). Occurrence of citrus nematode (*Tylenchulus semipenetrans*) in Sargodha district. Pakistan J. Phytopathol. 19(1): 40-43.

O'Bannon, J.H., C.R. Leather, and H.W. Reynolds (1967). Interaction of *Tylenchulus semipenetrans* and *Fusarium* species on rough lemon (*Citrus limon*). Phytopathology 57: 414-417.

Vagelas, I. and S.R. Gowen (2012). Control of *Fusarium oxysporum* and root-knot nematodes (*Meloidogyne* spp.) with *Pseudomonas oryzihabitans*. Pakistan J. Phytopathol. 24(1): 32-38.

Vovlas, N., A. Troccoli, A. Minuto, C. Bruzzone, N. Sasanelli, and P. Castillo. (2008). Pathogenicity and host-parasite relationships of *Meloidogyne arenaria* in sweet basil. Plant Dis. 92 (9): 1329-1335.

Wallace, H.R., 1973. Nematode ecology and plant disease. Edward Arnold (Publishers) Ltd. London. 228 pp.

Whitehead, A.G. and J.R. Hemming (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55: 25-38.