The Journal of Animal & Plant Sciences, 26(3): 2016, Page:739-744
ISSN: 1018-7081

RELATIONSHIP BETWEEN *MELOIDOGYNE INCOGNITA*DENSITYAND PLANT GROWTH OF OKRA

M. Hussain*[1], M. Zouhar[1], P. Ryšánek[1] and S. A. Anwar[2]

[1]Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources,Czech University of Life Sciences, Prague,Kamýcká 961/129, 165 00 Praha 6, Suchdol.[2]Institute of Agricultural Sciences, University of the Punjab, Lahore, 54590, Pakistan.
*Corresponding author: hussain@af.czu.cz; manzoor.sahi@gmail.com

ABSTRACT

Root-knot nematodes (*Meloidogyne*spp.) are the most important pests of vegetables, field crops, and ornamental plants. During our previous study, twenty eight okra cultivars were examined against resistance and Pusa Swami was ranked the most susceptible. The purpose of this study was to investigate the effects of increasing initial population density (Pi) of *M. incognita* on nematode reproduction and growth of susceptible okra cv. Pusa Swami in green house. Five Pi levels (250, 500, 750 and 1000 eggs) were used during experiment. Nematode reproduction was assessed by determining the number of galls, egg-masses,eggs per root system, and reproduction rateper root system. Egg-masses on Phloxine B stained roots were quantified by using stereomicroscopeand root systems were rated for galling and egg mass presence on a 0 to 5 scale where 0 = no gall or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >100 galls or egg masses per root system. Nematode reproduction was directly proportional to initial population density (Pi). Reproduction rate (Pf/Pi, where Pf = final number of eggs / initial egg density) increased proportionately with increased Pi. Foliage growth (cm) had inverse relation to Pi whereas root growth (g), gall and egg-mass indices were significantly increased. Our results show that root weight is poor parameter to assess the effects of root knot nematode to correlate with foliage growth.

Key words: *Meloidogyne incognita*, okra, plant growth parameters, Nematode reproduction parameters.

INTRODUCTION

Okra (*Hibiscus esculentus* L., family Malvaceae) is an important cross-pollinated vegetable crop of Indo-Pak sub-continent, cultivated extensively in tropics, sub tropics and temperate zones of the Middle East, Africa, Brazil, Turkey and the southern states of United States (Acquistucci and Francisci, 2002; IBPGR, 1990; Jideani and Adetula, 1993;Thomson and Kelley, 1937).

The global production of okra is 4.8 million ton pods, out of which India contributes 70%, Nigeria 15%, Pakistan 2%, Ghana 2%, Egypt 1.7 % and Iraq 1.7% (Gulsen *et al.,* 2007). The crop has been challenged by various yield reducing agents including fungi, bacteria, viruses, mycoplasma, nematodes, and insects. The total loss of vegetables due to these pests has been estimated up to 20-30% and unchecked growth may increase the loss up to 80-90% and it would fetch very low price in market (Hamer and Thomson, 1957)

Nematodes are the most successful and abundant animal phylum (Boucher and Lambshead, 1994) and they spread everywhere below soil line. Some nematode species are parasites of animals and plants (Blaxter and Bird, 1997) and cause serious diseases in both of them. The impact of nematodes on humans is evaluated through yield reductions in food and fiber crops, through weakness of livestock, and by direct infection; nematodes such as hookworm and Ascaris. A billion of people have been affected by this parasite. *Meloidogyne* spp. is considered a single major unmanageable biotic cause of plant stress and crop loss (Bird and Kaloshian, 2003; Hussain *et al.,* 2015). Root-knot nematodes derive nutrients symplastically from the phloem through giant cells and cause a strong nutrient sink effect on plant (Anwar and Van Gundy, 1993; Dorhout *et al.,* 1993; McClure, 1977). *Meloidogyne* species and particularly *M. incognita* are the most economically damaging plant-parasitic nematodes on vegetables as well as field crops (Baird *et al.,* 1996; Kinlock and Sprenkel, 1994) worldwide. High inoculum level of plant parasitic nematode affected loss of foliage, root growth and galling severity (Barker *et al.,* 1998). Nematode feeding below soil level can alter uptake of water and nutrients levels which reduces photosynthesis process in plants and ultimately invites many other opportunistic soil borne fungi like *Fusariumoxy sporum* (Anwar and Van Gundy, 1993; Stirling *et al.,* 2004). To establish a successful interaction between plants and nematodes not only require higher population threshold level in the soil but also environmental factors affecting crops, soil type, texture and pore size, cropping pattern and history, nematode and crop race, nematode distribution pattern and its multiplication are very important(Khan, 2008; Schomaker and Been 2006). Higher rate of nematode multiplication results in significant reduction of shoot and root growth (Dhawan and Sethi, 1978; Gaur and Prasad, 1980). The resistance level of the cultivars had significant impact on plant damage inflicted by nematodes, which might be due to genetic make-up of plants (Ammti *et al.,* 1985, Anwar and McKenry, 2002). Higher yield reduction in susceptible cultivar over that of resistant one might be due to faster development of nematode female even when both are subjected to nematodes at equal time frame (Anwar *et al.,* 2007; McClure *et al.,* 1974). In susceptible plants, nematodes could develop nursing cells faster than that in resistant plant (Sobczak and Golinowski, 2009).

Information about nematode-crop relationships is also crucial for growers to decide on economically viable management strategies within crop production systems. Therefore, in the present study we addressed the issue by determining the interaction between economically important nematode *M. incognita* and okra plant. Consequently, the ability of *M. incognita* to reproduce on okra cv. Pusa Swami showed significant interaction between both and the effects of *M. incognita* reproduction at different initial inoculum densities on okra growth.

MATERIALS AND METHODS

Okra cv. Pusa Swami is highly susceptible to *M. incognita* as shown previously (Anwar *et al.,*2007; Hussain *et al.,* 2015, 2016). Therefore, the cultivar was used to evaluate the impact of four increasing initial population densities (Pi) of *M. incognita* on nematode reproduction and plant growth. The experiment was conducted in green house (University of Agriculture, Faisalabad Pakistan)during2008-2011.

Morphological and molecular identification of nematodes was conducted at Czech University of Life Sciences, Prague. For the preparation of inoculum, eggs were obtained from a culture of nematode infected roots of susceptible tomato cv. Beril. Galled root pieces containing egg masses were cut into small pieces and placed in a container of 500 ml capacity with 200 ml of 0.5% Chlorox (Sodium hypochloride, NaOCI).Solution was shaken vigorously by hand for 4 min (Hussey and Barker, 1973). Eggs were washed several times by water and counted under stereomicroscope.

Three seeds of okra were sown in earthen pots of size of 13 x 12 cm containing sterilized soil (70% sand, 22% silt, and 8% clay). After germination of seeds, thinning was done leaving one plant per pot. The pots were placed in greenhouse at temperature of 25˚C.Plants at four leaves stage were inoculated with 1ml of water containing 250, 500,750and 1000 eggs per pot by pipette. Water inoculated plants were used as control. Each treatment containing single plant was replicated five times. The experiment was repeated. The plants were carefully up-rooted at eight weeks after inoculation and plant roots were rinsed with running tap water to remove adhering soil and debris, blotted on towel paper, and weighed. The number of egg-masses per root system was counted on roots stained with Phloxine B (Holbrook *et al.,* 1983). The root systems were rated for galling and egg mass presence on a 0 to 5 scale (Quesenberry *et al.,* 1989) where 0 = no gall or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >100 galls or egg masses per root system. Eggs from nematode inoculated root systems were extracted as above (Hussey and Barker, 1973) and counted. Plant growth parameters including shoot length (cm), root weight (g) and nematode plant response in terms of galls and egg masses per root system, gall and egg mass indices, eggs per root system and per gram of root were also recorded. The nematode reproduction rate (Pf/Pi = final egg density/initial inoculum density) was computed. Pool data from two experiments were analyzed using ANOVA and differences among the means were partitioned at $P=0.05$ according to Least significant difference by using software SPSS 10.0.

RESULTS AND DISCUSSION

Meloidogynein cognita reproduced at all four Pi’s and successfully induced root galls and egg masses on roots of susceptible plants of okra cv. Pusa Swami. Shoot and root growth was significantly affected at different *M. incognita* egg concentration as shown in (Table 1). This suggests that the egg inoculum was viable and had significant role in maintaining the interactions.

Reproduction rate (Pf/Pi, where Pf = final number of eggs / initial egg density) among Pi's of 250, 500, 750 and 1000 eggs/plant was found to be significantly ($p = 0.05$) different. Interestingly, it was found to be lowest ($p = 0.05$) when Pi = 250 (0.9), highest (3.4) at Pi = 1000 and intermediate at other two Pi’s. Similarly, increasing of inoculum level two times resulted in doubling of the reproduction rate. Higher reproduction rates at higher Pi’s suggest high susceptibility of the okra cv. Pusa Swami to *M. incognita*, which support the results by Nardacci and Barker (1979) and Kamran *et al.*(2013). This increase in rate of reproduction might be due to genetic make-up of variety which might have susceptible gene, which attracted greater number of J2 to penetrate in the roots of okra (Fenoll *et al.,* 1997; Castagnone and Sereno, 2002). The relationship between Pi and Pf in roots, number of galls, egg masses, eggs per root system and per gram observed to be highly significant ($p = 0.05$). The response of roots in terms of induction of root galls and egg production to *M. incognita* infection was directly proportional to Pi levels.

The roots of plants inoculated with 1000 eggs carried significantly greater numbers of root galls and eggs compared to that inoculated with all other three egg concentrations (Table 1). Number of galls, egg masses and eggs were found less at 250 Pi and intermediate at 500 Pi. Similarly, gall index was highest on roots of plants inoculated with highest Pi (Figure A) and lowest at the lowest Pi, but intermediate on roots inoculated with 500 and 750 eggs.

The gall and egg mass indices were similar at 750, 1000 Pi's, lowest when Pi = 250 eggs per plant and intermediate at Pi = 500 eggs per plant.

Meloidogynein cognita significantly ($p = 0.05$) suppressed plant growth at all four Pi levels as compared to the control plants however, root weight (g) observed to be increased with increasing Pilevels. Shoot length (cm) among all Pi's inoculated plants varied significantly ($p = 0.05$), and were inversely related to Pi levels, whereas root weight (g) were directly proportional to Pi levels. The plant growth parameters including foliage (cm) and root growth (cm)were tremendously reduced at 1000 Pi as compared to the other three Pi's. However reduction in plant growth was minimal at 250 Pi, due to the low inoculum level (Table 1). The increased root weight with increased Pi suggests that weight is not a good parameter to evaluate the effects of root knot nematode as reported byAnwarand Van Gundy (1993).

Seinhorst (1967) established host status by the values of the equilibrium density (E, where Pf = Pi) and the maximum rate of reproduction (the maximum Pf/Pi ratio). Plants are good hosts if both values are high, poor hosts if both are low, and the values will be influenced by external or environmental conditions for any plant and nematode combination. High reproduction rates and predicted equilibrium density values for *M. incognita* at temperatures 25°C confirmed that "Pusa Swami" is a good host for *M. incognita*, which is in line with our previous results (Anwar *et al.*,2007; Hussain *et al.,* 2016). The reproduction rates on okra are comparable to *M. incognita* reproduction on susceptible soybean (Nardacci and Barker, 1979) and the perceived reproduction rates for a range of endoparasitic and ectoparasitic nematodes reproducing on good hosts (Seinhorst, 1967).

The greater reduction in shoot length of susceptible okra cv. Pusa Swami coupled with a higher rate of nematode multiplication might be due to faster invasion of roots by greater numbers of J2 and the consequent development of adult females. It happens because in susceptible cultivars, nematodes depend entirely on functional syncytia or giant cells to acquire nutrients to develop into reproductive adult males or number of adult feeding females, the greater the stress on the plant leading to alteration in physiological functions like up-take of nutrients, photosynthesis (Anwar, 1995; Williamson and Hussey, 1996) and consequently plant growth.

Nematode damage limits, the growth of root system, which leads to reduce rate of uptake of nutrients and water (Anwar and Din, 1986). Impaired water relations appear to contribute substantially to reduced rates of top growth. This is probably because the developing giant cell systems and disruption of the developing xylem, which interfere with translocation of nutrients and water to foliage (Davis *et al.,* 2003; Anwar and Van Gundy, 1993). Other effects include reduced photosynthetic efficiency which causes reduction in light interception and saccharides synthesis and hence the capacity of the plant to generate more roots to overcome the limitations imposed by nematode damage (Anwar, 1995; Trudgill, 1992; Anwar and Mckenry, 2012). This appears to be the main mechanism of damage by *M. incognita*, and this effect was further increased by reductions in root efficiency as revealed by a decrease in root-shoot ratio. Further damage is associated with withdrawal of nutrients by the developing females.

Postharvest root galling, egg masses (Figure A), gall and egg mass indices, and eggs per root system (Table 1) were positively correlated to increase in Pi levels. However, the gall index and foliage growth (g) measurements displayed an inverse relationship (Table 1), which suggests that the increase in gall index proportionately causes reduction in plant growth. The gradual reduction in plant growth coupled with increase nematode multiplication and increasing Pi on susceptible cv. Pusa Swami is comparable with that of bitter gourd and round melon (Pankaj, 1990), cucurbits (Khan *et al.,* 2004), tomato (Kamran *et al.,* 2013), cowpea (Haseeb *et al.,* 2005) and on beans (Nadary *et al.,* 2006).

Overall, a significant direct relationship was found between the inoculum level and root weight (g) (Table 1; Figure B). As inoculum density increased four times, the root weight also increased double which might be due to the large amount of growth substances such as tryptophan and other amino acids when compared with nematode un-inoculated plants (Anwar and Van Gundy, 1993; Setty and Wheeler, 1968). These substances and diversion of photosynthate increased the root weight but had inverse impact on shoot length (Figure B).These findings did not agree with the hypothesis of Wareing (1970) that root and shoot are mutually dependent upon each other for exchanging nutrients, saccharides, growth substances and are physiologically in equilibrium and any reduction in root growth limit the shoot growth or vice versa. So these observations suggested that root weight was not a good parameter for the assessment of plant growth. However Anwar and Van Gundy (1993) reported direct relationship between root length and foliage length in *M. incognita* infected tomato, which are mutually dependent on each other, which validate the hypothesis that foliage growth and root growth in terms of length are better parameters to evaluate the impact of stress (Wareing, 1970).

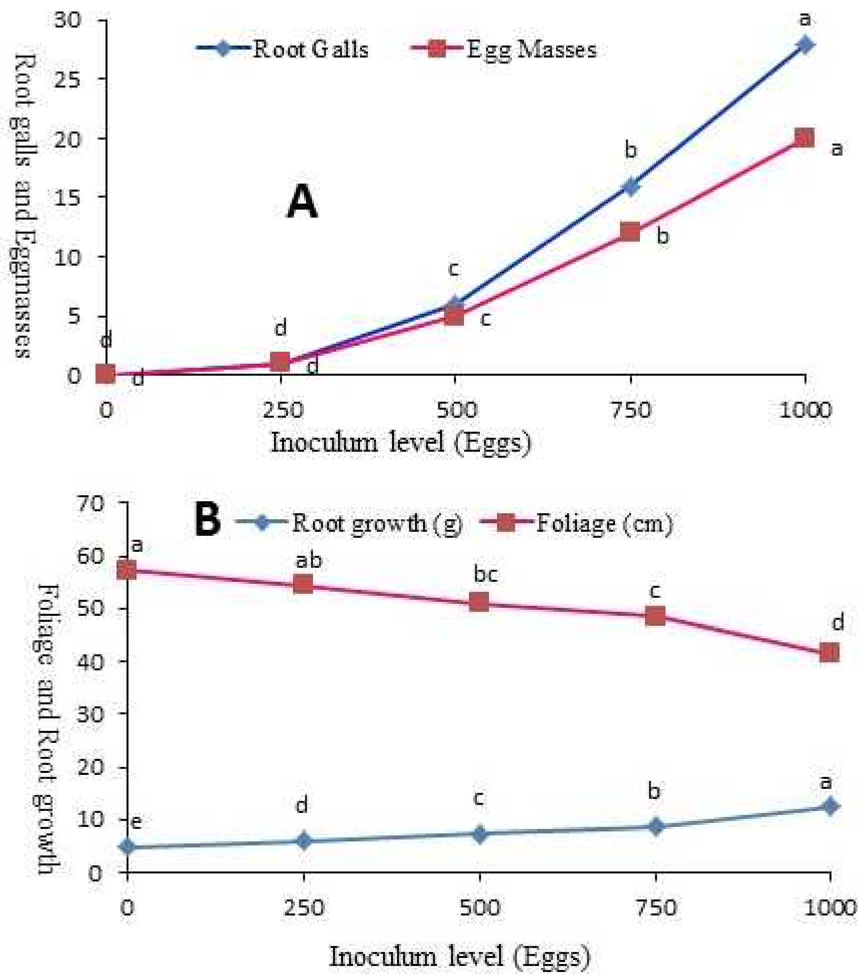

Figure 1. Relationship between A; root galls and egg masses per root system and Pi (initial nematode density), B; foliage growth and root growth at four Pi of *Meloidogyne incognita* on okra cv. Pusa Swami.

Table 1. Production of root galling, number of egg masses and total eggs on the roots of okra cv. Pusa Swami inoculated at four levels of eggs of *Meloidogyne incognita* and effects on plant growth.

Inoculum levels (eggs/plant)	**Nematode reproduction parameters**							**Plant growth parameters**	
	Root galls/root system	**Gall index[1]**	**Egg masses/root system**	**Egg mass index[1]**	**Eggs per root system**	**Eggs per g of root**	**Rate of reproduction[2]**	**Foliage [cm]**	**Root [g]**
0	0d	0	0d	0	0d	0d	0.0e	57.2a	4.8e
250	1d	1	1d	1	242d	49d	0.9d	54.4ab	6.0d
500	6c	2	5c	2	934c	156c	1.8c	51.0bc	7.4c
750	16b	3	12b	3	2180b	294b	2.9b	48.6c	8.6b
1000	28a	3	20a	3	3460a	399a	3.5a	41.4d	12.5a

[1] Gall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses, and 5 = > 100 galls or egg masses per root system (Quesenberry*et al.*, 1989).

[2] Rate of reproduction = Pf/Pi (Final Population / Initial Population)

[3] Means of ten replications with in a column sharing the same letter are not significantly different from each other at $P = 0.05$ according to Duncan Multiple Range Test.

Conclusion: Our findings contribute an important information that the magnitude of nematode reproduction rate, development of root galls, production of eggs, and gall and egg mass indices are directly related to Pi levels. Our results also reveal that root weight is a very poor parameter to represent root growth in relation to foliage growth in root-knot nematodes infected plants. We strongly suggest root length to be considered as alternate to root weight in root-knot nematode to assess the impact on plant growth as these two parameters are directly related to each other (Anwar and Van Gundy, 1993).

Acknowledgment: The authors thank Czech University of Life Sciences, Prague for financial support. The work has been supported by CIGA project number PROJ201500056.

REFERENCES

Acquistucci, R. and R. Francisci (2002). Effect of okra (*Hibiscus esculentus*L) addition on thetechnological properties of wheat flour. International J. Food Science and Nutrition.53:375-379.

Ammati, M., I.J. Thomason and P.A. Robberts (1985). Screening *Lycopersicon* spp. for new genes imparting resistance to root-knot nematodes (*Meloidogyne*spp.). Plant Disease. 69:112-115.

Anwar, S.A (1995). Influence of *Meloidogyne incognita*, *Paratrichodorusminor* and *Pratylenchusscribneri* on root-shoot growth and carbohydrate partitioning in tomato. Pakistan J. Zool. 27:105-113.

Anwar, S.A. and G.M. Din (1986). Nematodes: Biotic constrains to plant health. Parasitology. 3:48-53.

Anwar, S.A. and M.V. McKenry (2002). Developmental response of a resistance-breaking population of *Meloidogynearenaria* on *Vitis* spp. J. Nematology. 34:28-33.

Anwar, S.A. and M.V. McKenry (2012). Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses. Pakistan J. Zoology. 44:327-333**.**

Anwar, S.A. and S. D. Van Gundy (1993). Effect of *Meloidogyne incognita* on root and shoot growth parameters of susceptible and resistant varieties of tomato. Afro-Asian J. Nematology.3:152-160.

Anwar, S.A., A. Zia, M. Hussain, and M. Kamran (2007). Host suitability of selected plants to *Meloidogyne incognita* in the Punjab, Pakistan. International J. Nematology. 17:144-150.

Baird, R.E., R.F. Davis, P.J. Alt, B.G. Mullinix and G.B. Padgett (1996). Frequency and geographical distribution of plant-parasitic nematodes on cotton in Georgia. Supplements J. Nematology. 28:661-667.

Barker, K.R., G.A. Pederson and G.L Windham (1998). Plant and nematode interactions. ASA, CSSA, SSA Publishers, Madison, WI.

Bird, D.M.C.K. and I. Kaloshian (2003). Are roots special? Nematodes have their say. Physiological and Molecular Plant Pathology. 62:115-123

Blaxter, M. and D.M.C.K. Bird (1997). Parasitic nematodes. In: *C. elegans* II. Cold Spring Harbor, eds. By D.L. Riddle, T. Blumenthal, B.J. Meyer and J.R. Priess. pp. 851- 78, Cold Spring Harbor Laboratory Press.

Boucher, G. and P.J.D. Lambshead (1994). Ecological biodiversity of marine nematodes in samples from temperate, tropical, and deep sea regions. Conservation Biology. 9:1594-604.

Castagnone, S.P. and Sereno (2002). Genetic variability of nematode: a threat to durability of plant resistance gene. Euphytica. 193-199.

Davis, R.F., H.J. Earl and P. Timper (2003). Interactionof root-knot nematode stress and water stress in cotton. University of Georgia cotton research and extension report**.**

Dhawan, S.C. and C.L Sethi (1978). Observations on the pathogenicity of *Meloidogyne incognita* to eggplant and on relative susceptibility of some varieties to the nematode. Indian J. Nematology. 6: 39-46.

Dorhout, R., F. Gommers and C. Kolloffel (1993). Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. Physiological and Molecular Plant Pathology. 43:1-10

Fenoll, C.F., A. Aristizabal, S. Sanz-Alferaz and F.F. Del Campo (1997). Regulation of gene expression in feeding sites. In: Cellular and Molecular Aspects of Plant Nematode Interactions. Kluwer Academic Publishers. 133-149p.

Gaur, H.S. and S.K. Prasad (1980). Population studies on *Meloidogyne incognita* on eggplant (*Solanummelongena*) and its effect on the host. Indian J. Nematology. 10: 40-52.

Gulsen, O., S. Karagul and K. Abak (2007). Diversity and relationships among Turkish germplasm by SRAP and Phenotypic marker polymorphism. Biologia Bratislava. 62:41-45.

Hamer and C. Thomson (1957). Vegetables crops McGraw-Hill Co., Inc. N.Y. Toronto, London.

Haseeb, A., A. Sharma and P.K. Shukla (2005). Effect of initial inoculum level of *Meloidogyneincognita*on root knot development and growth of *Vignaradiate* cv. ML-1108. Indian J. Nematology. 35:93-94.

Holbrook, C. C., D.A. Knauft and D.W. Dickson (1983). A technique for screening peanut for resistance to

Meloidogyne incognita. Plant Disease. 57:957-958.

Hussain, M., S.A. Anwar, S. Sehar, A. Zia, M. Kamran, S. Mehmood and Z. Ali (2015). Incidence of plant-parasitic nematodes associated with okra in district Layyah of the Punjab, Pakistan. Pakistan. J. Zool. 47: 847-855.

Hussain, M., M. Kamran K. Singh M.Zouhar,P.Ryšánek and S.A Anwar (2016). Response of selected okra cultivars to *Meloidogyne incognita*. Crop Protection. 82:1-6.

Hussey, R.S. and K. R. Barker (1973). Comparison of methods for collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter. 57:1025-1028.

I.B.P.G.R (1990) (International Board for Plant Genetic Resources) Report on InternationalWorkshop on okra Genetic Resources held at the National Bureau for Plant Genetic Resources, New Delhi, India. 8-12 October

Jideani, V.A. and H.O. Adetula (1993). The potential of okra seed flour for weaning foods inWest Africa, Ecological Food Nutrition 29:275-283

Kamran, M., S.A. Anwar, N. Javed, S.A. Khan, H. Abbas, M. A. Iqbal and M. Zohaib (2013). The Influence of *Meloidogyne incognita* density on susceptible tomato. Pakistan J. Zoology 45:727-732

Khan, M.R. (2008). Plant nematodes, methodology, morphology, systematics, biology and ecology. SciencePublishers, New Jersey.

Khan, T.A., S. Nasir and M. S. Ashraf (2004). Effect ofpopulation levels of *Meloidogynejavanica* on plant growth and nematode multiplication on cucurbits. Pakistan J. Nematology. 22: 83-87.

Kinlock, R.A. and R.K. Sprenkel (1994). Plant-parasitic nematodes associated with cotton in Florida. Supplements J. Nematology. 26:749-752.

McClure, M.A. (1977). *Meloidogyne incognita:* A metabolic sink. J. Nematology. 9:88-90.

McClure, M.A., K.C. Ellis and E.L. Nigh (1974). Resistance of cotton to the root-knot nematode, *Meloidogyne incognita*. J. Nematology. 6: 17-20.

Nadary, S.N., A. S. Al-Hazmi, A. A. M. Dawabah and F.A. Al-Yahya (2006). Relationship between the initial inoculum density of *Meloidogyneincognita*, infection and reproduction on green beans. *In:* 9th Arab congress of plant protection Damascus, Syria NB-E. 112.

Nardacci, J.F. and K.R. Barker (1979). The influence of temperature on *Meloidogyne incognita* on soybean. J. Nematology. 11:62-70.

Pankaj, S (1990). Effect of initial inoculum levels of *Meloidogyne incognita* on bitter gourd and round melon. Indian J. Nematology. 20:64-66.

Quesenberry, K.H., D.D. Baltensperger, R.A. Dunn, C.J. Wilcox and S.R. Hardy (1989). Selection for tolerance to root-knot nematodes in red clover. Crop Science. 29: 62-65.

Schomaker, C.H. and T. H. Been (2006). Plant growth and population dynamics. In: Plant Nematology, eds. ByR. Perry and M. Moens. pp. 275-295, Wallingford: CAB Inter. U.K.

Seinhorst, J.W (1967). The relationships between population increase and population density in plant parasitic nematodes II, Sedentary nematodes. Nematologica. 13:157-171

Setty, K.G.H. and A.W. Wheeler (1968). Growth substances in roots of tomato (*Lycopersiconesculentum* Mill.) infected with root-knot nematodes (*Meloidogyne* spp.). Annals of Applied Biology. 61:495-501.

Sobczak, M. and W. Golinowski (2009). Structure of cyst nematode feeding sites. In: Cell biology of plant nematode parasitism. Plant Cell Monographs, eds. by R.H. Berg and C.G. Taylor. pp 153-187. Springer, Berlin.

Stirling, G.R., D. Griffin, K. Ophel-Keller, A. Mckay, D. Hartley and J. Currar (2004). Combining an initial risk assessment process with DNA assays to improve prediction of soil borne diseases caused by root-knot nematode (*Meloidogyne*spp.) and *Fusariumoxy sporum*f. sp. *lycopersici*in the Queensland tomato industry. Australasian Plant Pathology. 33:285-293.

Thomson, H.C. and W.C. Kelley (1937). Vegetables Crop. McGraw Hill Book Co., Inc. New York and London.

Trudgill, D.L (1992). Resistance to and tolerance of plant parasitic nematodes in plants. Annual Review of Phytopathology. 29:167-192.

Wareing, P.F (1970). Growth and its co-ordination in trees. In: Symposium on physiology of tree crops, eds. by Luckwill, L.C and C.V. Cutting. pp. 1-21. Long Ashton Research Station, University of Bristol, Academic Press, N.Y.

Williamson, V.M. and R.S. Hussey (1996). Nematode pathogenesis and resistance in plants. Plant Cell. 8: 1735-1745.