

## The Influence of *Meloidogyne incognita* Density on Susceptible Tomato

M. Kamran,<sup>1</sup> S. A. Anwar,<sup>2</sup> N. Javed,<sup>1</sup> S. A. Khan,<sup>1</sup> H. Abbas,<sup>1</sup> M. A. Iqbal<sup>3</sup> and A. Zohaib<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, University of Agriculture Faisalabad, Punjab 38040, Pakistan.

<sup>2</sup>Institute of Agricultural Sciences, University of the Punjab, Quid-e-Azam Campus, Lahore, 54590, Pakistan.

<sup>3</sup>Pulses Research Institute, AARI, Faisalabad, Punjab 38000, Pakistan.

**Abstract.-** The purpose of this study was to assess the effects of increasing initial population density (Pi) of *Meloidogyne incognita* on nematode reproduction and growth of susceptible tomato (*Lycopersicon esculentum* cv. Round-41) at 25°C± 2 in growth chamber. 21-days-old tomato seedlings transplanted in 13-cm dia. earthen pot was inoculated with Pi including 250, 500, 1000, 1500 eggs of *M. incognita*. Nematode reproduction was assessed by determining the number of galls, eggs masses, eggs per root system, and reproduction rate values per root system at 60 days after inoculations. Egg-masses on Phloxine B- stained roots were quantified and 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 Pi. Reproduction rate (Pf/Pi, where Pf = final number of eggs / initial egg density) for Pi's of 250, 500, 1000 and 1500 eggs/plant was lowest when Pi = 250 (2.23) but similar when Pi = 500, 1000 or 1500. Shoot and root growth were inversely related to Pi. Strong liner relationship existed between root length, root galling index and foliage growth but very poor between root weight and foliage growth. High Pf = final eggs per root system than at all Pi suggests that Round-41 is a good host for *M. incognita*

**Key words:** *Meloidogyne incognita*, population dynamics, tomato.

### INTRODUCTION

Recent studies have shown that *Meloidogyne incognita* (Kofoid and White) Chitwood can invade, develop, and reproduce on tomato (*Lycopersicon esculentum* Mill) under field conditions in the Punjab and cause 40% yield damage (Anwar and McKenry, 2012). Our glasshouse host range tests indicate that *M. incognita* can reproduce on many commercial cultivars of tomato although the reproduction rates were not assessed (Kamran *et al.*, 2011). Damage to plants is influenced by root penetration, development, reproduction potential and inoculum density of *M. incognita* in adjacent soil (Shahab and Sharma, 2011). High densities of the nematode at planting induce loss of foliage and root growth, and severe root galling (Barker, 1998). Nematode feeding can also alter uptake of water and nutrients, interfere with translocation of photosynthates (Anwar, 1995; Williamson and Hussey, 1996) and increase incidence and severity of *Fusarium*

*oxysporum* f. sp. *lycopersici* diseases (Stirling *et al.*, 2004).

The mere presence of plant-parasitic nematodes in soil does not guarantee crop damage or yield loss, since a nematode population may remain below the damage threshold level for a specific field (Schomaker and Been; Khan, 2008). Factors such as environmental conditions, soil type, cropping history, the specific nematode species and race present, race distribution, prevailing nematode distribution pattern, nematode multiplication rate, and plant cultivar that is grown will all have a bearing on whether crop damage and yield reduction will be inflicted (Schomaker and Been; Khan, 2008).

The relationship between yield loss and initial *M. incognita* density has been illustrated in susceptible cultivars of soybean (Fourie *et al.*, 2010), cotton (Davis and May, 2005) and tomato (Jaiteh, 2012). Kinloch (1982) reported that *M. incognita* suppressed yields of susceptible soybean cultivars by more 90% in the USA. Bridge and Starr (2007) found that initial population densities of 150 J2 of this parasite per 500 cm<sup>3</sup> soil can result in yield suppression of 10%. Yield losses of between 73 - 100% have been reported on susceptible tomato cultivars due to root-knot nematode (Hemeng,

\* Corresponding author: [mkamran.uaf.pk@gmail.com](mailto:mkamran.uaf.pk@gmail.com)

0030-9923/2013/0003-0727 \$ 8.00/0

Copyright 2013 Zoological Society of Pakistan

1981).

The greater reduction in harvested yield in susceptible over that of resistant might be due to faster and higher rate of adult female development on susceptible cultivars although both are equally invaded, independent of the number (McClure, 1974). It happens because in susceptible cultivars, nematodes depend entirely on functional syncytia to acquire nutrients to develop into reproductive adult males or females. J2 also penetrate roots of resistant cultivars and initiate syncytia. However, resistance soon manifests itself by a degeneration of the young syncytia and a failure of the nematode to develop further (Endo, 1991; 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. The objective of this study was to determine: (i) the ability of *M. incognita* to reproduce on tomato cv. Round-41 and (ii) the effect of nematode reproduction at different initial inoculum densities on tomato growth.

## MATERIALS AND METHODS

Our green host screening test has revealed that tomato cv. Round-41 is highly susceptible to *M. incognita* (Kamran *et al.*, 2011). This cultivar was used to evaluate the impact of four increasing initial population densities ( $P_i$ ) including 250, 500, 1000, 1500 eggs of *M. incognita* on nematode reproduction and plant growth. Eggs were obtained from a culture of nematode infected roots of tomato. 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, NaOCl) solution shaken vigorously by hand for 4 min (Hussey and Barker, 1973).

One tomato seedling of 21-days-old was transplanted per 13-cm dia. earthen pot containing sterilized sandy loam soil (70% sand, 22% silt, and 8% clay). One week after recovery from transplant shocks, these pots were inoculated with 250, 500, 1000, 1500 eggs per pot by pipette. The check plant for comparison was inoculated with water. Each treatment was replicated twenty times. The pots were arranged on a green house bench in a

completely randomized design. The experiment was repeated to authenticate the results.

The plants were carefully up-rooted at eight weeks after inoculation and plant roots were rinsed with running tap water to free adhering soil and debris, blotted on towel paper, and weighed. The number of egg-masses per root system was counted on stained roots 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 included foliage and root growth 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. The nematode reproduction rate ( $P_f/P_i$  = final egg density/initial inoculum density) was computed.

Data were subjected to ANOVA and differences among the means were partitioned test at  $P=0.05$  according to Least significant difference test.

## RESULTS AND DISCUSSION

*M. incognita* reproduced at all four  $P_i$  and successfully induced root galls and egg masses on roots of susceptible plants of tomato cv. Round-41. Shoot and root growth were significantly affected by *M. incognita*  $P_i$  as indicated by control plants. This suggests that eggs inoculum was viable.

Reproduction rate ( $P_f/P_i$ , where  $P_f$  = final number of eggs / initial egg density) for  $P_i$ 's of 250, 500, 1000 and 1500 eggs/plant was lowest ( $P=0.05$ ) when  $P_i = 250$  (2.23) but similar when  $P_i = 500$ , 1000 or 1500. Higher reproduction rates at 1000  $P_i$  over that of 1500  $P_i$  suggests high susceptibility of this cultivar to *M. incognita*, which agrees with results of a study by Nardacci and Barker (1979). The relationships between  $P_i$  and  $P_f$  in roots, number galls, egg masses, eggs per root system and per gram was 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 proportionate to  $P_i$  levels. The roots of

**Table I.- Production of root galling, number of egg masses and total eggs on the roots of tomato line (Round-41) inoculated at four levels of eggs of *Meloidogyne incognita*.**

Inoculum levels (eggs)	Nematode reproduction parameters						
	Root galls	Gall index <sup>1</sup>	Egg masses	Egg mass index <sup>1</sup>	Eggs per root system	Eggs per gram of root	Rate of reproduction <sup>2</sup>
250	7 d <sup>3</sup>	2 c	5 d	2 b	557 d	107 d	2.23 b
500	16 c	3 b	12 c	3 a	1340 c	209 c	2.68 ab
1000	27 b	3 b	22 b	3 a	3014 b	365 b	3.01 a
1500	31 a	4 a	27 a	3 a	4373 a	459 a	2.91 a

<sup>1</sup> 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).

<sup>2</sup> Rate of reproduction = Pf/Pi (Final Population / Initial Population)

<sup>3</sup> Means with in a column sharing the same letter are not significantly different from each other at  $P = 0.05$  according to Least significant difference test.

**Table II.- Impact of *Meloidogyne incognita* at five levels of eggs on growth of tomato line (Round-41) under greenhouse conditions.**

Infection level (eggs)	Plant growth response					
	Length (cm)		Fresh weight (g)		Weight of whole plant (g)	% Reduction in growth
	Shoot	Root	Shoot	Root		
0	38.58 a <sup>1</sup>	25.45 a	46.39 a	4.332 e	50.72 a	-
250	34.50 b	19.88 b	38.87 b	5.19 d	44.06 b	13.13
500	33.13 c	17.23 c	35.40 c	6.412 c	41.81 c	17.56
1000	26.35 d	13.25 d	31.37 d	8.245 b	39.61 d	21.90
1500	22.83 e	11.23 e	27.33 e	9.528 a	36.85 e	27.34

<sup>1</sup> Means with in a column sharing the same letter are not significantly different from each other at  $P = 0.05$  according to Least significant difference test.

plants inoculated with 1500 Pi carried significantly ( $P = 0.05$ ) greater numbers of root galls and eggs compared to that inoculated with all other three Pi (Table I). Number of galls, egg masses and eggs were lowest at 250 Pi and intermediate at 500 and 1000 Pi's. Similarly, gall index was highest on roots of plants inoculated with highest Pi and lowest at the lowest Pi, but intermediate on roots inoculated with 500 and 1000 Pi. The egg mass index was equal at 500, 1000 and 1500 Pi, all being significantly higher than that of 250 Pi.

*M. incognita* significantly ( $P = 0.05$ ) suppressed plant growth at all four Pi levels over that of check plants but root growth (g) improved with increasing Pi. Plant shoot growth and root growth (length) differences among all Pi inoculated plants varied significantly ( $P = 0.05$ ), and were inversely related to Pi. The plant growth parameter

including shoot and root length and shoot weight was tremendously reduced at 1500 Pi compared to the other three corresponding Pi levels. However the growth reduction in shoot growth and root (length) was minimum at 250 Pi, the lowest inoculum level (Table II). The increased root weight with increased Pi suggests that weight is a not good parameter to evaluate the effects of root knot nematode as reported in 1993 (Anwar and 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 (Seinhorst, 1967). High reproduction rates and predicted equilibrium density

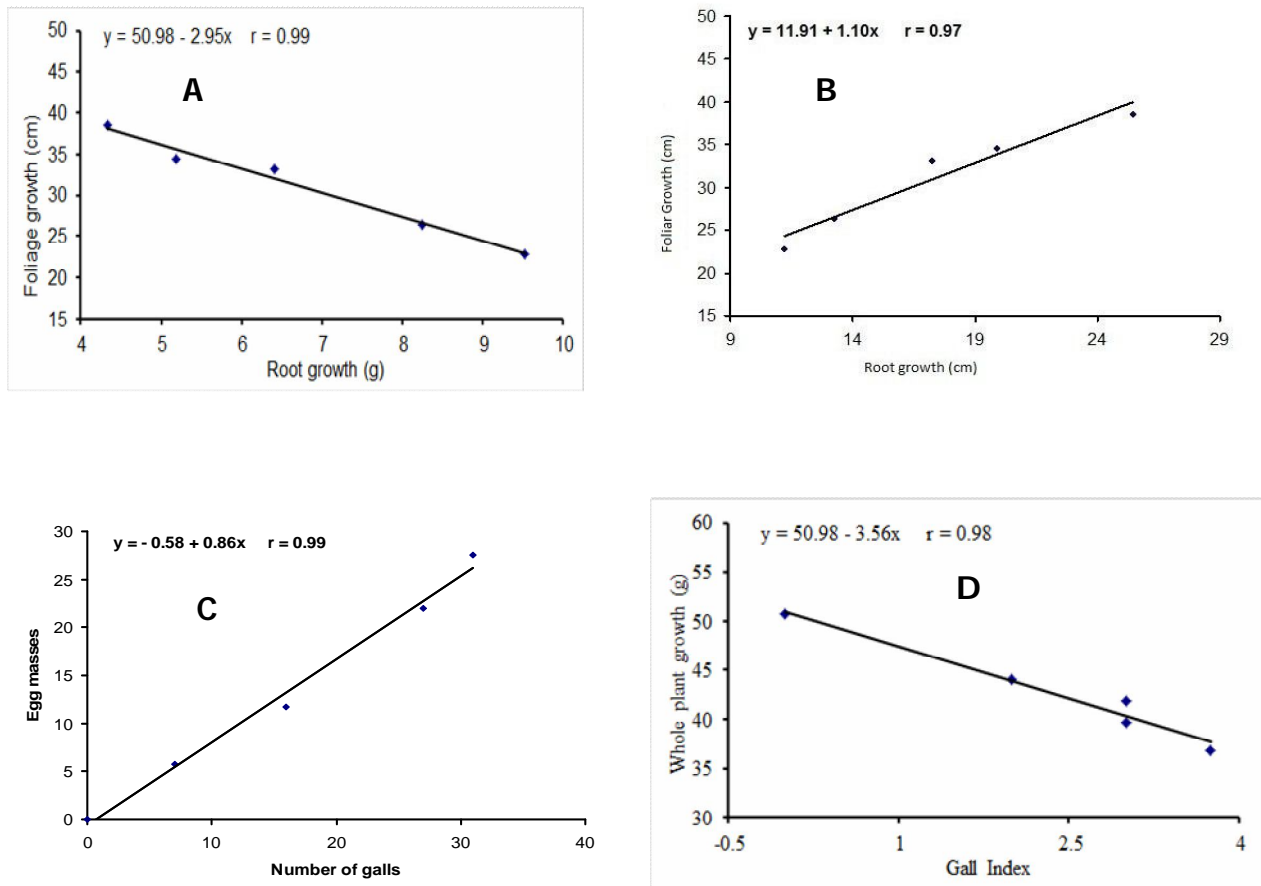


Fig. 1. Relationship between A, root growth (g) and foliage growth (cm); B, root growth (cm) and foliage growth (cm); C, number of galls and egg masses; and D, gall index and whole plant growth (g) at four inoculum densities (Pi) of *Meloidogyne incognita* on tomato cv.Round-41.

values for *M. incognita* at temperatures 25°C confirm that “Round-41” tomato is a good host for *M. incognita*, which agrees with our previous results (Kamran *et al.*, 2011). The reproduction rates on tomato 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 foliage growth of susceptible tomato cv. Round-41 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 to acquire

nutrients to develop into reproductive adult males or females (McClure *et al.*, 1974). The higher the 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 consequent plant growth.

Nematode damage limited the root system, which leads to reduced 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 interferes with translocation of food and water to foliage (Davis, 2003; Anwar and Van Gundy, 1993). Other

effects include reduced photosynthetic efficiency which caused reduction in light interception and carbohydrate synthesis and hence the capacity of the plant to generate more roots to overcome the limitations imposed by nematode damage (Anwar, 1995; Trudgill, 1992). 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.

A significant direct relationship was found between the inoculum level and root growth (g) (Table II). As inoculum density increased, the root weight also increased which might be due to the larger amount of growth substances such as tryptophan and other amino acids when compared 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 foliage length (Fig. 1A). Regression analysis revealed a linear relationship in which increasing root weight was associated with increasing *M. incognita* Pi ( $r = 0.99$ ). These findings did not agree with the hypothesis of Wareing (1970) that root and shoot are mutually dependent upon each other for exchanging nutrients, carbohydrates, 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 the regression analysis between root length and foliage length showed direct relationship ( $r = 0.97$ ), which are mutually dependent on each other as suggest by (Anwar and Van Gundy, 1993) and also justify the hypothesis that foliage growth and root growth in terms of length are better parameters to evaluate the impact of stress (Wareing, 1970) (Fig. 1B).

Regression analysis exhibited that post-harvest root gall ratings, gall and egg mass indices, and eggs per root system were positively correlated to increase in Pi (Fig. 1C). However, the gall index and whole plant growth (g) measurements displayed an inverse relationship with value of  $r = 0.98$ . (Fig. 1D), which suggests that the increase in gall index

proportionately caused reduction in plant growth.

The gradual reduction in plant growth coupled with increased nematode multiplication with increasing Pi on susceptible cv. Round-41 is comparable with that of bitter melon and round melon (Pankaj and Siyanand, 1990, cucurbits (Khan *et al.*, 2004), cowpea (Haseeb *et al.*, 2005) and on beans (Nadary *et al.*, 2006).

## CONCLUSIONS

The findings of this study contribute important information that nematode reproduction rate was maximum at harvest at Pi = 1000 eggs but beyond that reproduction potential of egg-laying-females declined because the population in the root system reached its peak and could not support further reproduction. This might be due to greater competition for infection courts and food reserves at high initial densities of nematodes (El-Sherif *et al.*, 2007). It also revealed that root length has a direct impact on foliage growth, but root weight is a very poor parameter to represent root growth in relation to foliage growth.

## REFERENCES

- ANWAR, S.A., 1995. Influence of *Meloidogyne incognita*, *Paratrichodorus minor* and *Pratylenicus scribneri* on root-shoot growth and carbohydrate partitioning in tomato. *Pakistan J. Zool.*, **27**: 105-113.
- ANWAR, S.A. AND DIN, G.M., 1986. Nematodes: Biotic constrains to plant health. *Parasitology*, **3**: 48-53.
- ANWAR, S.A. AND MCKENRY M.V., 2012. Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses. *Pakistan J. Zool.*, **44**: 327-333.
- ANWAR S.A. AND VAN GUNDY, S.D., 1993. Effect of *Meloidogyne incognita* on root and shoot growth parameters of susceptible and resistant varieties of tomato. *Afro-Asian J. Nematol.*, **3**: 152-160.
- BARKER, K.R., PEDERSON, G.A. AND WINDHAM, G.L., 1998. *Plant and nematode interactions*. ASA, CSSA, SSA Publishers, Madison, WI.
- BRIDGE, J. AND STARR, J.L., 2007. *Plant nematodes of agricultural importance*, Academic Press, Boston.
- DAVIS, R.F., EARL, H.J. AND TIMPER, P., 2003. *Interaction of root-knot nematode stress and water stress in cotton*. University of Georgia cotton research and extension report, pp. 312-315.
- DAVIS, R.F. AND MAY, O.L., 2005. Relationship between

- yield potential and percentage yield suppression caused by the southern root-knot nematode in cotton. *Crop Sci.*, **45**: 2312-2317.
- EL-SHERIF, A.G., REFAEI, A.R., EL-NAGAR, M.E. AND SALEM, H.M.M., 2007. The role of eggs inoculum level of *Meloidogyne incognita* on their reproduction and host reaction. *Afr. J. agric Res.*, **2**: 159-163.
- ENDO, B.Y., 1991. Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. *Rev. Nematol.*, **14**: 73-94.
- FOURIE, H., MCDONALD, A.H. AND WAELE, D.D., 2010. Relationships between initial population densities of *Meloidogyne incognita* race 2 and nematode population development in terms of variable soybean resistance. *J. Nematol.*, **42**: 55-61.
- HASEEB, A., SHARMA, A. AND SHUKLA, P.K., 2005. Effect of initial inoculum level of *Meloidogyne incognita* on root knot development and growth of *Vigna radiata* cv. ML-1108. *Indian J. Nematol.*, **35**: 93-94.
- HEMENG, O.B., 1981. Efficacy of selected nematicides for the control of root-knot nematode, *Meloidogyne* spp. on tomato in Ghana. *Ghana J. agric. Sci.*, **13**: 37-40.
- HOLBROOK, C.C., KNAUFT, D.A. AND DICKSON, D.W., 1983. A technique for screening peanut for resistance to *Meloidogyne incognita*. *Pl. Dis.*, **57**: 957-958.
- HUSSEY, R.S. AND BARKER, K.R., 1973. Comparison of methods for collecting inocula of *Meloidogyne* spp., including a new technique. *Pl. Dis. Rep.*, **57**: 1025-1028.
- JAITEH, F., KWOSEH, C. AND AKROMAH R., 2012. Evaluation of tomato genotypes for resistance to root-knot nematodes. *Afr. Crop Sci. J.*, **20**: 41-49.
- KAMRAN, M., ANWAR, S.A. AND KHAN, S.A., 2011. Evaluation of tomato genotype against *Meloidogyne incognita* infection. *Pak. J. Phytopathol.*, **23**: 31-34.
- KHAN, M.R., 2008. *Plant nematodes, methodology, morphology, systematics, biology and ecology*. Science Publishers, New Jersey.
- KHAN, T.A., NASIR, S. AND ASHRAF, M.S., 2004. Effect of population levels of *Meloidogyne javanica* on plant growth and nematode multiplication on cucurbits. *Pak. J. Nematol.*, **22**: 83-87.
- KINLOCH, R.A., 1982. The relationship between soil populations of *Meloidogyne incognita* and yield reduction of soybean in the coastal plain. *J. Nematol.*, **14**: 162-167.
- MCCLURE, M.A., ELLIS, K.C. AND NIGH, E.L., 1974. Resistance of cotton to the root-knot nematode, *Meloidogyne incognita*. *J. Nematol.*, **6**: 17-20.
- NADARY, S.N., AL-HAZMI, A.S., DAWABAH, A.A.M. AND AL-YAHYA, F.A., 2006. Relationship between the initial inoculum density of *Meloidogyne incognita* infection and reproduction on green beans. 9<sup>th</sup> Arab Congress of Plant Protection, Damascus, Syria.
- NARDACCI, J.F. AND BARKER, K.R., 1979. The influence of temperature on *Meloidogyne incognita* on soybean. *J. Nematol.*, **11**: 62-70.
- PANKAJ, S., 1990. Effect of initial inoculum levels of *Meloidogyne incognita* on bitter gourd and round melon. *Ind. J. Nematol.*, **20**: 64-66.
- QUESENBERRY, K.H., BALTENSPERGER, D.D., DUNN, R.A., WILCOX, C.J. AND HARDY, S.R., 1989. Selection for tolerance to root-knot nematodes in red clover. *Crop Sci.*, **29**: 62-65.
- SCHOMAKER, C.H. AND BEEN, T.H., 2006. Plant growth and population dynamics. In: *Plant nematology* (eds. R. Perry and M. Moens), Wallingford: CAB Inter., U.K. pp. 275-295.
- 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 WHEELER, A.W., 1968. Growth substances in roots of tomato (*Lycopersicon esculentum* Mill.) infected with root-knot nematodes (*Meloidogyne* spp.). *Annl. appl. Biol.*, **61**: 495-501.
- SHAHAB, S.S. AND SHARMA, S., 2011. Pathogenicity of root-knot nematode, *Meloidogyne incognita* and root rot fungus, *Rhizoctonia solani* on okra (*Abelmoschus esculentus* L.). *J. Sci. Technol.*, **3**: 97-102.
- SOBCZAK, M. AND GOLINOWSKI, W., 2009. Structure of cyst nematode feeding sites. In: *Cell biology of plant nematode parasitism* (eds. R.H. Berg and C.G. Taylor), Plant Cell Monographs, Springer, Berlin, pp. 153-187.
- STIRLING, G.R., GRIFFIN, D., OPHEL-KELLER, K., MCKAY, A., HARTLEY, D. AND CURRAR, J., 2004. Combining an initial risk assessment process with DNA assays to improve prediction of soilborne diseases caused by root-knot nematode (*Meloidogyne* spp.) and *Fusarium oxysporum* f. sp. *lycopersici* in the Queensland tomato industry. *Aust. Pl. Pathol.*, **33**: 285-293.
- TRUDGILL, D.L., 1992. Resistance to and tolerance of plant-parasitic nematodes in plants. *Annu. Rev. Phytopathol.*, **29**: 167-192.
- WAREING, P.F., 1970. Growth and its co-ordination in trees. In: *Symposium on Physiology of tree crops* (eds. L.C. Luckwill, and C.V. Cutting), Long Ashton Research Station, University of Bristol, Academic Press, N.Y., pp. 1-21.
- WILLIAMSON, V.M. AND HUSSEY, R.S., 1996. Nematode pathogenesis and resistance in plants. *Pl. Cell*, **8**: 1735-1745.

(Received 23 April 2013, revised 6 May 2013)