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ESTIMATION OF DAMAGE TO OKRA (*ABELMOSCHUS ESCULENTUS*) BY ROOT-KNOT DISEASE INCITED BY *MELOIDOGYNE INCOGNITA*

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Abstract

The pathogenic potential of *Meloidogyne incognita* on okra (*Abelmoschus esculentus*) was determined at initial population densities of 0, 1000, 2000 and 4000 second stage juveniles per kg of soil in pots in the glasshouse inoculated after 2nd, 3rd, 4th and 5th week of emergence. Significant reductions in plant height and fresh shoot weight and increases in root weight, number of galls and egg masses were observed at all inoculum densities. With an increase in inoculum level, there was a progressive increase in height and shoot weight reductions, root weight, number of galls and egg masses. Plants inoculated after 2nd week of emergence were heavily damaged. However, with the increase in plant age at the time of inoculation, the damaging effects lowered significantly. Reductions in height and shoot weight and increase in root weight, number of galls and egg masses were found to be directly proportional to inoculum densities. On the other hand, with an increase in the initial inoculum density and plant age there was a corresponding decrease in the reproduction factor being inversely proportional to inoculum densities and plant ages.

Introduction

Okra (*Abelmoschus esculentus*) is a summer vegetable crop grown in many countries (Khan *et al*., 2013). The young tender fruits of okra are cooked in curries, stewed and used as soups. Okra is a rich source of many nutrients. A half cup of okra contains calories 25, dietary fiber 2g, protein 1.5g, carbohydrates 5.8g, vitamin A 460 IU, vitamin C 13mg, folic acid 36.5µg, calcium 50mg, iron 0.4mg, potassium 25mg, magnesium 46mg.

Okra is subjected to attack by many insects and pathogens including fungi, viruses, mycoplasmas and nematodes (Hussain *et al*., 2011a; Ahmad *et al*., 2012; Arain *et al*., 2012; Iqbal *et al*., 2012; Srivastava *et al*., 2012). The most widespread and economically important are the root-knot nematodes (*Meloidogyne* spp.). Their life cycle is completed in 25 days at 27°C, but it takes longer at lower or higher temperatures. The short life cycle enables root-knot nematode populations to survive well in the presence of a suitable host and their populations build up to a maximum as crops reach maturity.

Root-knot nematodes infect a wide range of important crop plants and are particularly damaging the vegetable crops in tropical and subtropical countries (Osman *et al*., 2012; Youssef *et al*., 2012). The infested plants are chlorotic, stunted and unthrifty (Archana & Saxena, 2012). Among over 100 described species of *Meloidogyne*, the four most commonly occurring species are *Meloidogyne incognita*, *M. arenaria, M. javanica and M. hapla*. The species of root-knot nematode attack more than 2000 species of plants including almost all cultivated plants. Root-knot nematodes are reported to cause annual losses in tropics up to 29% in tomato, 22% in okra, 24% in potato, 23% in egg plant, 25% in pepper and 28% in beans (Sasser, 1979). There are 23 species of nematodes associated with okra crop in Pakistan. Among these, *M. incognita* (Kofoid & White 1919; Chitwood, 1949) is the most destructive one and hence is of economic importance (Hussain *et al*., 2012; Kayani *et al*., 2012a, 2012b, 2013). Myriads of attempts have been made to control these insidious pathogens by employing various strategies (Rahoo *et al*., 2011; Dong *et al*., 2012; Fabiyi *et al*., 2012; Goswami *et al*., 2012; Ismail & Mohamed, 2012; Khan *et al*., 2012a, 2012b; Qureshi *et al*., 2012; Radwan *et al*., 2012; Mukhtar *et al*., 2013a; Rizvi *et al*., 2013; Shinwari *et al*., 2013) but the problem is still there. In Pakistan, the root-knot nematode problem is more damaging than in developing countries, because the country has tropical and sub-tropical regions where the climate is suitable for nematode activity throughout the year. Sandy soil in warm irrigated areas favours the infestation of root-knot nematode.

The influence of nematode numbers on plant growth and yield can often be expressed as a linear regression of growth or yield on log nematode numbers. It is possible that competition at high densities of nematodes population for invasion and feeding sites reduces the yield proportionately as the population increases (Wonang & Akueshi, 1990). The effect of different inoculum levels of *Meloidogyne* spp. on different crops have been studied by different workers (Youssef & Al-Nagdi, 2004) and such information is lacking on okra crop. Keeping in view the economic importance of *M. incognita* in reducing the quality and quantity of crop production, the present study was designed to determine the effect of different inoculum densities of *M. incognita* on okra at different ages which will help in the determination of economic threshold level.

Materials and Methods

Nematode inoculums: The root-knot nematode, *Meloidogyne incognita*, used in the experiment was collected from infected cucumber roots. The nematode was multiplied from a single egg mass on tomato cv "Money maker" and was confirmed by making perineal pattern (Taylor & Netscher, 1974). The nematode was further mass produced on tomato in pots in green house at 25°C ± 2. After 35 days roots were removed from soil, washed with tap water, cut into approximately 1-2 cm pieces. The roots were vigorously shaken in a bottle containing 0.5% NaOCI for 5 minutes. The eggs were collected on a 38 µm sieve and washed in a beaker (Hussey & Barker, 1973). The egg suspension was poured onto an extraction tray and juveniles were collected (Whitehead & Hemming, 1965). For the estimation of inoculum density, the nematode suspension was poured into a measuring cylinder. The numbers of juveniles were estimated in ten aliquots of 1 ml in a counting dish under a dissecting microscope at a magnification of 35×and their mean was calculated. Based on the total volume of the suspension, total number of juveniles was extrapolated. For concentrating the juveniles' suspension, it was left to settle down for several hours and afterwards the extra water was decanted off leaving the bottom undisturbed.

Pathogenicity test: Okra plants cv "Sabz Pari" were raised in autoclaved sterilized soil in pots at weekly intervals. The pathogenicity of *M. incognita* was evaluated at different inoculum densities. When okra plants attained ages of 2, 3, 4, and 5 weeks, these were inoculated with an initial population (Pi) of *M. incognita* @ 0, 1000, 2000, and 4000 second stage juveniles per plant. Each treatment was quadruplicated. Inoculation was done by adding the required amount of nematode suspension into four holes around the plants. The holes were covered with soil to prevent drying. The plants were placed in glass house at 25°C ± 2. The plants were watered according to need. Eight weeks after inoculation, the plants were removed from the pots carefully and data regarding plant height, fresh shoot and root weights, number of galls, egg masses per plant, final nematodes populations and reproduction factor were recorded. Galls and egg masses were counted under a dissecting microscope at a magnification of 35×. After counting egg masses on the roots, eggs were obtained (Hussey & Barker, 1973) and counted as mentioned above. The nematodes were also extracted from soil using Whitehead and Hemming tray method (Whitehead & Hemming, 1965). The eggs and nematodes extracted from soil formed the final nematodes population (Pf). The reproduction factor (Rf) was calculated by dividing the final nematode population by initial nematode population.

Statistical analysis: The percent increases and reductions in various parameters were calculated over control (Hussain *et al*., 2011b; Kayani *et al*., 2012c; Mukhtar *et al*., 2013b) prior to statistical analysis. The experiment was repeated twice. Since there were no discrepancies in the mean values of all the corresponding treatments of the repeated experiments, the data of both the trials were amalgamated before statistical analysis. All the data were subjected to analysis of variance using statistical software Genstat 12th edition. Means were compared by Duncan's Multiple Range Test. A significant level of $p \leq 0.05$ was used in statistical analyses. The regression equations were done in Microsoft Excel, 2003 for windows.

Results and Discussion

All the inoculum levels of *M. incognita* caused significant reductions over control in plant height ($F = 26.36$; $df = 2, 36$; $p<0.00$) and fresh shoot weight ($F = 23.01$; $df= 2, 36$; $p<0.001$) at all plant ages. Highest reduction in plant height (55.72%) and shoot weight (48.37%) were observed when plants were inoculated at the age of 2 weeks with 4000 juveniles/plant and the lowest of 23.23 and 21.78% respectively at 1000 juveniles/plant inoculated after 5 weeks (Table 1 and 2). The reductions in these parameters increased significantly with an increase in the inoculum level. However, the magnitude of these reductions at each level diminished as the age of the plants increased at the time of inoculation. The relationships between different inocula and reductions in plant height and shoot weight at 4 plant ages have been shown by regression equations in Table 1 and 2.

Table 1. Effect of *Meloidogyne incognita* on reduction in plant height of *A. esculentus*.

Plant age at inoculation	Inoculum level			Regression equation	R^2
	1000	2000	4000		
2nd week	43.02 cde	48.48 abc	55.72 a	y = 0.0041x + 39.4	0.9881
3rd week	37.29 de	42.40 cde	51.76 ab	y = 0.0048x + 32.61	0.9995
4th week	34.25 e	38.26 de	44.92 bcd	y = 0.0035x + 30.92	0.9977
5th week	23.23 f	34.45 e	41.45 cde	y = 0.0057 + 19.73	0.8992

Data are means of four replicates. Means sharing common letters do not differ significantly ($p<0.005$)

Table 2. Effect of *Meloidogyne incognita* on reduction in fresh shoot weight of *A. esculentus*.

Plant age at inoculation	Inoculum level			Regression equation	R^2
	1000	2000	4000		
2nd week	35.70 bcd	41.52 ab	48.37 a	y = 0.0041x + 32.275	0.9796
3rd week	32.83 cde	37.04 bcd	43.40 ab	y = 0.0035x + 29.65	0.9946
4th week	24.89 ef	31.45 de	39.77 bc	y = 0.0048x + 20.73	0.9852
5th week	21.78 f	26.75 ef	31.88 cde	y = 0.0033 + 19.215	0.9676

Data are means of four replicates. Means sharing common letters do not differ significantly ($p<0.005$)

On the other hand, nematode inocula brought about increases in fresh root weights ($F = 9.90$; $df = 2, 36$; $p<0.001$) over control. Highest increases were recorded at an inoculum level of 4000 juveniles/plant at all plant ages (Table 3). The increase in fresh root weight was found to be directly proportional to inoculum levels. These relationships between nematode densities and increases in root weights have been described by regression equations in Table 3.

Similarly, nematode densities significantly affected number of galls ($F = 160.99$; $df = 2, 36$: $p<0.001$), egg masses ($F = 152.12$; $df = 2, 36$; $p<0.001$) and rate of nematode build up (Pf/Pi) ($F = 985.80$; $df = 2, 36$; $p<0.001$).

Maximum galls and egg masses were observed in plants inoculated with 4000 larvae per plant after 2 weeks while minimum with an inoculation of 1000 juveniles after 5weeks (Tables 4 and 5).The production of galls and egg masses was found to be directly proportional to inoculum levels and inversely proportional to plant ages. These relationships are shown by regression equations given in Table 4 and 5. On the other hand rate of nematode build up (Rf) decreased with an increase in the inoculum density, being higher at lowest levels. Highest reproduction factor of 12.09 fold was observed in plants inoculated with 1000 larvae/plant after 2 weeks. Plants inoculated with highest level of nematodes after 5 weeks, showed the lowest rate of nematode build up of 1.72-fold (Table 6). Rate of nematode build up appeared to be inversely proportional to the inoculum densities and plant ages. These relationships have been shown by regression equations in Table 6.

Table 3. Effect of *Meloidogyne incognita* on increase in fresh root weight of *A. esculentus*.

Plant age at inoculation	Inoculum level			Regression equation	R^2
	1000	2000	4000		
2nd week	16.03 b	24.62 ab	38.32 a	y = 0.0073x + 9.18	0.9966
3rd week	17.03 b	25.60 ab	38.38 a	y = 0.007x + 10.64	0.9941
4th week	17.92 b	24.57 ab	33.18 ab	y = 0.005x + 13.615	0.9866
5th week	14.34 b	20.80 ab	27.94 ab	y = 0.0044 + 10.77	0.9742

Data are means of four replicates. Means sharing common letters do not differ significantly ($p<0.005$).

Table 4. Effect of *Meloidogyne incognita* on number of galls on *A. esculentus*.

Plant age at inoculation	Inoculum level			Regression equation	R^2
	1000	2000	4000		
2nd week	71.75 d	84.75 c	110.25 a	y = 0.0128x + 59	1
3rd week	62.75 e	72.50 d	94.00 b	y = 0.0105x + 52	0.9994
4th week	52.75 fg	61.75 e	83.00 c	y = 0.0102x + 42.125	0.9984
5th week	45.75 g	55.50 ef	71.75 d	y = 0.0086 + 37.625	0.9978

Data are means of four replicates. Means sharing common letters do not differ significantly ($p<0.005$).

Table 5. Effect of *Meloidogyne incognita* on number of egg masses on *A. esculentus*.

Plant age at inoculation	Inoculum level			Regression equation	R^2
	1000	2000	4000		
2nd week	66.50 c	78.25 b	99.00 a	y = 0.0108x + 56.125	0.999
3rd week	58.25 de	66.00 c	84.50 b	y = 0.0088x + 49	0.9982
4th week	48.25 gh	56.00 ef	77.75 b	y = 0.01x + 37.375	0.994
5th week	42.50 h	50.50 fg	64.00 cd	y = 0.0071 + 35.75	0.9981

Data are means of four replicates. Means sharing common letters do not differ significantly ($p<0.005$).

Table 6. Effect of *Meloidogyne incognita* on rate of nematode build up (Rf = Pf/Pi).

Plant age at inoculation	Inoculum level			Regression equation	R^2
	1000	2000	4000		
2nd week	12.09 a	6.90 c	4.39 ef	y = 0.0024x + 13.343	0.8571
3rd week	8.21 b	5.36 d	3.15 g	y = 0.0016x + 9.311	0.9329
4th week	6.47 c	3.93 f	2.28 h	y = 0.0013x + 7.2895	0.9061
5th week	4.67 e	2.86 g	1.72 i	y = 0.0009 + 5.2375	0.9022

Data are means of four replicates. Means sharing common letters do not differ significantly ($p<0.005$).

The progressive impairment in growth confirms the great damage potential of *M. incognita* on okra. The effects of different inoculum densities of different *Meloidogyne* species have also been studied by different workers on different hosts (Akhtar *et al*., 2005; Sasanelli *et al*., 2006; Neog & Bora, 2007; Irshad *et al*., 2012; Maleita *et al*., 2012). 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.The damaging effects of *M. incognita* population levels were higher on younger plants as compared to older ones. This was due to the tenderness and succulence of tissues of younger plants being more attractive and susceptible for large number of nematodes. The older plants being harder and stronger, suffered less. Choudhury (1985) reported that one week old seedlings of tomato cv. Money maker did not tolerate the attack of *M. incognita* larvae, while 3 and 5 week old seedlings did. Salares and Capasin (1988) found that percent yield reduction of amplaya (*Momordica charania*) was lower on 8-week old plants compared with 2-, 4-and 6-week old plants when inoculated with different inoculum densities of *M. incognita*. Initial densities of *M. incognita* affected multiplication rate, lower initial densities resulting into higher multiplication. This may be explained due to destruction of root system and also due to the inability of the larvae to locate the new infection sites of subsequent generations. It is concluded that *M. incognita* is pathogenic to *A. esculentus* at all population levels and more damaging on younger plants compared to older ones. This emphasizes the importance of the level of soil nematode populations at the time of sowing or nursery transplantation and the need to reduce high populations during the beginning stages of plant growth which can be accomplished by adopting various control strategies (Ahmad *et al*., 2004; Cavoski *et al*., 2012; Dallemole-Giaretta *et al*., 2012; Klein *et al*., 2012; Mashela & Pofu, 2012; Singh *et al*., 2012a; 2012b; Vagelas *et al*., 2012; Verdejo-Lucas *et al*., 2012; 2013).

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References

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

Ahmad, Z., Saifullah, F. Raziq, H. Khan and M. Idrees. 2012. Chemical and biological control of *Fusarium* root rot of okra. *Pak. J. Bot*., 44(1): 453-457.

Akhtar, H., S. Anita, and P.K. Shukla. 2005. Effects of initial inoculum levels of *Meloidogyne incognita* on root-knot development and growth of *Vigna radiata* cv. ML-1108. *Ind. J. Nematol*., 35: 93-94.

Arain, A.R., M.M. Jiskani, K.H. Wagan, S.N. Khuhro and M.I. Khaskheli. 2012. Incidence and chemical control of okra leaf spot disease. *Pak. J. Bot*., 44(5): 1769-1774.

Archana, B. and R. Saxena. 2012. Nematicidal effect of root extract of certain medicinal plants in control of *Meloidogyne incognita in vitro* and *in vivo* conditions. *Pak. J. Nematol*., 30 (2): 179-187.

Cavoski, I., Z.Al. Chami, F. Bouzebboudja, N. Sasanelli, V. Simeone, D. Mondelli, T. Miano, G. Sarais, N.G. Ntalli and P. Caboni. 2012. *Melia azedarach* controls *Meloidogyne incognita* and triggers plant defense mechanisms on cucumber. *Crop Prot*., 35: 85-90.

Choudhury, B.C. 1985. Effect of a standard inoculum level of *Meloidogyne incognita* on tomatoes of different ages. *Int. Nematol. Network Newsl*., 2: 4-5.

Dallemole-Giaretta, R., L.G. Freitas, E.A. Lopes, O.L. Pereira, R.J.F. Zooca and S. Ferraz. 2012. Screening of *Pochonia chlamydosporia* Brazilian isolates as biocontrol agents of *Meloidogyne javanica*. *Crop Prot*., 42: 102-107.

Dong, L., C. Huang, L. Huang, X. Li and Y. Zuo. 2012. Screening plants resistant against *Meloidogyne incognita* and integrated management of plant resources for nematode control. *Crop Prot*., 33: 34-39.

Fabiyi, O.A., G.A. Olatunji and O. Atolani. 2012. Nematicidal activities of chromatographic fractions from *Alstonia boonei* and *Bridelia ferruginea* on *Meloidogyne incognita*. *Pak. J. Nematol*., 30 (2): 189-198.

Goswami, B.K., C. Bhattacharya, R. Paul and T.A. Khan. 2012. Performance of pesticide and biopesticide on growth, yield and Forskolin content in *Coleus forskohlii* infected with *Meloidogyne incognita*. *Pak. J. Nematol.*, 30 (1): 49-56.

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

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

Hussain, M.A., T. Mukhtar, M.Z. Kayani, M.N. Aslam and M.I. Haque. 2012. A survey of okra (*Abelmoschus esculentus*) in the Punjab province of Pakistan for the determination of prevalence, incidence and severity of root-knot disease caused by *Meloidogyne* spp. *Pak. J. Bot*., 44(6): 2071-2075.

Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rep*., 57: 1025-1028.

Iqbal, J., M. Sagheer, Mansoor-ul-Hasan and M. Nadeem. 2012. Management of *Amrasca biguttula biguttula* (Ishida) on okra, *Abelmoschus esculentus* (L.) Monech. *Pak. J. Agri. Sci*., 49: 179-184.

Irshad, U., T. Mukhtar, M. Ashfaq, M.Z. Kayani, S.B. Kayani, M. Hanif and S. Aslam. 2012. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on *Citrus jambhiri*. *J. Anim. Plant Sci*., 22(4): 1014-1018.

Ismail, A.E. and M.M. Mohamed. 2012. Nematicidal potentiality of some animal manures combined with urea against *Meloidogyne arenaria* and growth and productivity of sugar beet under field conditions. *Pak. J. Nematol.*, 30 (1): 57-65.

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. 2012c. Evaluation of nematicidal effects of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against root-knot nematodes, *Meloidogyne incognita*. *Crop Prot*., 39: 52-56.

Kayani, M.Z., T. Mukhtar, M.A. Hussain and M.I. Haque. 2013. Infestation assessment of root-knot nematodes (*Meloidogyne* spp.) associated with cucumber in the Pothowar region of Pakistan. *Crop Prot*., 47: 49-54.

Kayani, M.Z., T. Mukhtar, M.A. Hussain, M.I. Haque and R. Perveen. 2012b. Incidence and severity of root-knot nematodes (*Meloidogyne* spp.) on cucumber in district Rawalpindi. *Pak. J. Phytopathol*., 24(2): 122-128.

Khan, A., B. Nawab, S.S. Shaukat, M.A. Samad and J.K. Tareen. 2012a. Management of nematodes associated with chilli using fertinemakil-plus and carbofuran. *Pak. J. Nematol*., 30 (2): 151-155.

Khan, M.A., M. Sajid, Z. Hussain, A. Rab, K.B. Marwat, Fazal-i-Wahid and S. Bibi. 2013. How nitrogen and phosphorus influence the phenology of okra. *Pak. J. Bot*., 45(2): 479-482.

Khan, M.R., B. Zaidi and Z. Haque. 2012b. Nematicides control rice root-knot, caused by *Meloidogyne graminicola*. *Phytopathol. Mediterr*., 51: 298-306.

Klein, E., J. Katan and A. Gamliel. 2012. Soil suppressiveness to *Meloidogyne javanica* as induced by organic amendments and solarization in greenhouse crops. *Crop Prot*., 39: 26-32.

Maleita, C.M.N., R.H.C. Curtis, S.J. Powers and, I.M.de O. Abrantes. 2012. Inoculum levels of *Meloidogyne hispanica* and *M. javanica* affect nematode reproduction, and growth of tomato genotypes. *Phytopathol. Mediterr*., 51: 566-576.

Mashela, P.W. and K.M. Pofu. 2012. Interactive effects of *Meloidogyne incognita* race 2, *Bradyrhizobium japonicum* and crude extracts of *Cucumis myriocarpus* fruit on *Vigna unguiculata*. *Crop Prot*., 40: 69-72.

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

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

Neog, P.P. and B.C. Bora. 2007. Effect of inoculum levels of *Meloidogyne incognita* on Patchouli. *Ann. Plant Prot. Sci*., 15: 276-277.

Osman, H.A., M.M.A. Youssef, A.Y. El-Gindi, H.H. Ameen, N.A. Abd-Elbary and A.M.S. Lashein. 2012. Effect of salicylic acid and *Pseudomonas fluorescens* against *Meloidogyne incognita* in eggplant using split-root technique. *Pak. J. Nematol*., 30 (2): 101-113.

Qureshi, S.A., Ruqqia, V. Sultana, J. Ara and S. Ehteshamul-Haque. 2012. Nematicidal potential of culture filtrates of soil fungi associated with rhizosphere and rhizoplane of cultivated and wild plants. *Pak. J. Bot*., 44(3): 1041-1046.

Radwan, M.A., S.A.A. Farrag, M.M. Abu-Elamayem and N.S. Ahmed. 2012. Efficacy of some granular nematicides against root-knot nematode, *Meloidogyne incognita* associated with tomato. *Pak. J. Nematol.*, 30 (1): 41-47.

Rahoo, A.M., T. Mukhtar, S.R. Gowen and B. Pembroke. 2011. Virulence of Entomopathogenic Bacteria *Xenorhabdus bovienii* and *Photorhabdus luminescens* against *Galleria mellonella* Larvae. *Pak. J. Zool*., 43: 543-548.

Rizvi, Z.F., R. Mukhtar, M.F. Chaudhary and M. Zia. 2013. Antibacterial and antifungal activities of *Lawsonia inermis*, *Lantana camara* and *Swertia angustifolia*. *Pak. J. Bot*., 45(1): 275-278.

Salares, F.G. and R.M. Gapasin. 1988. Influence of inoculum density of root-knot nematode (*Meloidogyne incognita*) and plant age on yield of ampalaya (*Momordica charantia* L.). *Philipp. J. Crop Sci*., 13: 29.

Sasanelli, N., T. D'addabbo and M. Liskova. 2006. Influence of the root-knot nematode *Meloidogyne incognita* r. 1 on growth of grapevine. *Helminthologia*, 43: 168-170.

Sasser, J.N. 1979. Economic importance of *Meloidogyne* in tropical countries. In: Root-knot nematodes (*Meloidogyne* spp.): Systematics, Biology and Control (eds. F. Lamberti and C.E Taylor) pp. 359-374. Academic press, New York.

Shinwari, Z.K., N. Ahmad, J. Hussain and N.U. Rehman. 2013. Antimicrobial evaluation and proximate profile of *Nepeta leavigata*, *Nepeta kurramensis* and *Rhynchosia reniformis*. *Pak. J. Bot*., 45(1): 253-259.

Singh, U.B., A. Sahu, N. Sahu, R.K. Singh, Renu, R. Prabha, D.P. Singh, B.K. Sarma and M.C. Manna. 2012b. Co-inoculation of *Dactylaria brochopaga* and *Monacrosporium eudermatum* affects disease dynamics and biochemical responses in tomato (*Lycopersicon esculentum* Mill.) to enhance bio-protection against *Meloidogyne incognita*. Crop Prot., 35: 102-109.

Singh, V., R. Mawar and S. Lodha. 2012a. Combined effects of biocontrol agents and soil amendments on soil microbial populations, plant growth and incidence of charcoal rot of cowpea and wilt of cumin. *Phytopathol. Mediterr*., 51: 307-316.

Srivastava, D.S., M. Sehgal, A. Kumar, S. Verma, B.K. Dwivedi and S.P. Singh. 2012. Plant-parasitic nematodes associated with tomato and okra fields of Allahabad, Uttar Pradesh, India. *Pak. J. Nematol*., 30 (2): 157-167.

Taylor, D.P. and C. Nestscher. 1974. An improved technique for preparing perennial pattern of *Meloidogyne* spp. *Nematologica*, 20: 268-269.

Vagelas, I., M.D. Dennett, B. Pembroke and S.R. Gowen. 2012. Adhering *Pasteuria penetrans* endospores affect movements of root-knot nematode juveniles. *Phytopathol. Mediterr*., 51: 618-624.

Verdejo-Lucas, S., M. Blanco, L. Cortada and F.J. Sorribas. 2013. Resistance of tomato rootstocks to *Meloidogyne arenaria* and *Meloidogyne javanica* under intermittent elevated soil temperatures above 28°C. *Crop Prot*., 46: 57-62.

Verdejo-Lucas, S., M. Talavera and M.F. Andrés. 2012. Virulence response to the Mi.1 gene of *Meloidogyne* populations from tomato in greenhouses. *Crop Prot*., 39: 97-105.

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.

Wonang, D.L. and C.O. Akueshi. 1990. Relationship between population densities of *Meloidogyne incognita* and crop yield in tomato. *Int. Nematol. Network Newsl*., 7: 38-41.

Youssef, M.M.A. and W.M.A. Al-Nagdi. 2004. Assessment of faba bean damage caused by *Meloidogyne incognita* root knot nematode infection. *Pak. J. Nematol*., 22: 47-53.

Youssef, M.M.A., M.M.M. Mohammed and A.M. Korayam. 2012. Effect of organic and inorganic fertilizers on *Meloidogyne incognita* infesting sugar beet. *Pak. J. Nematol*., 30 (2): 143-149.

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