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Pathogenic Potential of Javanese Root-knot Nematode on Susceptible and Resistant Okra Cultivars

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Authors' Contribution
TM and MAH designed the study, conducted the surveys, executed experimental work, analyzed the data and prepared the manuscript. TM supervised the work.

Key words
***Meloidogyne javanica*, reproductive factor, inoculum densities, *Abelmoschus esculentus*, pathogenicity**

ABSTRACT

The Javanese root-knot nematode (*Meloidogyne javanica*) is becoming a serious threat to okra production in Pakistan. The damaging potential of this nematode has not been studied on resistant and susceptible okra cultivars, therefore, in the present study, the effects of six inoculum levels of *M. javanica* were compared on a highly susceptible cultivar of okra 'Sharmeeli' and a moderately resistant one 'Sanam'. All the inoculum levels of *M. javanica* resulted in significant reductions in growth variables and increases in nematode infestations of both the cultivars over their controls. With an increase in inoculum level, the magnitudes of reductions in shoot weight, root and shoot lengths and increase in root weight also increased and were found to be positively correlated with the inoculum levels. Likewise, the numbers of galls and egg masses also showed positive correlations with the inoculum levels. Contrarily, a gradual decline in reproductive factors was observed with an increase in the inoculum level and therefore, appeared to be negatively correlated with the latter. It was also observed that the reductions in moderately resistant cultivar were significantly lower as compared to the highly susceptible at all inoculum levels. It is concluded that the plants of moderately resistant cultivar Sanam suffered less damage and suppressed nematode infections at all inoculum levels and therefore, recommended for cultivation in root-knot nematode infested fields to abate yield losses and repress the nematode from further multiplication.

INTRODUCTION

Okra is vulnerable to many biotic and abiotic perturbations that cause reductions in yield. The biotic factors include insect pests (Javed *et al*., 2017a, b; Iftikhar *et al*., 2018; Kassi *et al*., 2018, 2019a,b; Nabeel *et al*., 2018; Aslam *et al*., 2019a) and disease inciting agents like fungi (Fateh *et al*., 2017), viruses (Ashfaq *et al*., 2017), bacteria (Aslam *et al*., 2017a, b, 2019b) and nematodes (Mukhtar *et al*., 2017a,b, 2018). Among nematodes, root-knot nematodes of the genus *Meloidogyne* spp. are the most widespread and economically important (Mukhtar, 2018). These nematodes complete their life cycles in 25 days at a temperature of 27°C and at lower or higher temperatures, the life cycle is delayed. The short life cycle enables root-knot nematodes to thrive well in the presence of a suitable host and their populations pullulate to the maximum as crops attain maturity. Root-knot nematodes infect a wide range of important crop plants and are particularly damaging the vegetable crops in tropical and subtropical countries (Tariq-Khan *et al*., 2017). The infested plants manifest symptoms of chlorosis, stunting and unthrifty growth (Archana and Saxena, 2012). There are over 100 described species of *Meloidogyne*, but the four most commonly occuring species are *Meloidogyne incognita*, *M. javanica, M. arenaria* and *M. hapla*. The species of root-knot nematode attack about 3000 species of plants including almost all the 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* and *M. javanica* are the most destructive ones and hence are of economic importance (Hussain *et al*., 2016). Root-knot nematodes have been found to prevail in 85% of okra fields with an average incidence of 39% and of the four most common root-knot species, *M. incognita* constituted 74.74%, *M. javanica* 24.02%, *M. arenaria* 1.57% and *M. hapla* 0.78% (Hussain and Mukhtar, 2019).

Losses in Pakistan due to nematodes to crops have been found more serious and complex as compared to the developed countries owing to numerous causes. The cultivation of susceptible crops year after year in the same piece of land permits rapid multiplication of nematodes which results in severe infections and damage. On the other hand, antagonistic fungi and entomopathogenic nematodes can reduce the incidence and severity of root-knot nematodes (Khan *et al*., 2017; Rahoo *et al*., 2017, 2018a, b, 2019). Root-knot nematodes have also been found associated with fungal and bacterial pathogens resulting in disease complexes and aggravate the severity of the latter (Kayani and Mukhtar, 2018).

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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 and Akueshi, 1990). The effect of different inoculum levels of *Meloidogyne* spp. on different crops have been studied by different workers (Kayani *et al*., 2017, 2018) and such information is lacking on resistant and susceptible okra cultivars. Keeping in view the economic importance of root-knot nematodes in reducing the quality and quantity of crops, the present study was designed to compare the effects of different inoculum densities of Javanese root-knot nematode (*M. javanica*) on resistant and susceptible okra cultivars which will help in the determination of economic threshold level.

MATERIALS AND METHODS

The nematode, *M. javanica*, used in the study was multiplied as described by Mukhtar *et al*. (2017a). Second stage juveniles (J2s) of the nematode were extracted by following the method described by Whitehead and Hemming (1965), standardized, concentrated and used for inoculation of okra plants.

The effect of different inoculum densities of *M. javanica* was evaluated on a moderately resistant cultivar viz. Sanam and a susceptible one viz. Sharmeeli (Mukhtar *et al*., 2014). The seeds of these two cultivars were separately sown in pots containing formalin sterilized soil. After germination, one healthy seedling was maintained in each pot. Ten days after emergence, each plant in the pots was inoculated with freshly hatched J2s of *M. javanica* at the rates of 250, 500, 1000, 2000, 4000 and 8000 by drenching around the stem. The untreated plants were kept as control. Each treatment was replicated five times. The pots were arranged in a completely randomized design in the greenhouse at 25±2°C. The pots were watered when required. Forty five days after inoculation, the plants of both the cultivars inoculated with different levels were gently uprooted from their respective pots and the data were recorded regarding shoot and root lengths and weights, number of galls, egg masses and reproductive factor. Percentage decreases or increases over control in growth variables were calculated.

Galls and egg masses were counted under a stereomicroscope at a magnification of 35×. After counting egg masses on the roots, eggs were extracted from the roots (Hussey and Barker, 1973) and counted. The nematodes were also extracted from soil of each pot using Whitehead and Hemming tray method (Whitehead and Hemming, 1965). The eggs and nematodes extracted from soil formed the final nematodes population. The reproduction factors were calculated by dividing the final nematode populations by the initial ones.

Two factorial completely randomized design was used in the experiment. All the data were subjected to analysis of variance using statistical software Genstat 12th edition. Means were compared by Fisher's Protected least significant difference test. A significant level of $p \leq 0.05$ was used in statistical analyses. The linear relationships between inoculum densities as independent variable (x) and growth parameters and nematode infestations as dependent variables (y) were calculated in Microsoft Excel 2007 to draw a "best-fit" straight line. Regression equations and correlation coefficients (R^2) were also calculated in Microsoft Excel 2007. The closer the R^2 is to 1.00, the better the fit.

RESULTS

The analysis of variance showed highly significant results regarding effects of inoculum densities on growth parameters and nematode infestations. All the inoculum densities of *M. javanica* resulted in significant reductions in growth variables of both the cultivars over their controls. The reductions in moderately resistant cultivar were significantly lower as compared to the highly susceptible cultivar at all inoculum levels. The highest inoculum level of 8000 J2s caused the maximum reductions in shoot weight and shoot and root lengths followed by levels of 4000 and 2000 J2s. Similarly, the lowest inoculum level of 250 J2s resulted in the minimum reduction followed by 500 and 1000 J2s as shown in Figure 1. It was observed that the reductions in these growth variables increased with an increase in the inoculum density showing a positive direct relationship and these relationships have been shown by regression equations (Table I). On the other hand, the inoculum levels caused an increase in root weight. The higher inoculum levels caused higher increases while at lower inoculum levels, the increases were lower. The increases in root weights were significantly lower in case of moderately resistant cultivar as compared to the highly susceptible one (Fig. 1). A direct relationship was found between the increase in root weight and inoculum levels and has been shown by regression equation as given in Table I.

In the same way, statistically significant increases in number of galls and egg masses were observed at all inoculum levels. Significant differences in number of galls and egg masses were noticed between the moderately resistant and highly susceptible cultivars at all inoculum levels.

Table I.- Regression equations and correlation coefficients of growth variables and nematode infestations.

Parameter	Regression equation		R^2	
	Sanam	Sharmeeli	Sanam	Sharmeeli
Shoot weight	y = 2.72x - 3.41	y = 5.85x - 6.06	0.9459	0.9453
Root weight	y = 2.12x - 2.38	y = 6.24x - 8.03	0.9730	0.9627
Shoot length	y = 1.79x - 2.15	y = 4.56x - 6.50	0.9174	0.9095
Root length	y = 1.29x - 1.77	y = 5.82x - 9.18	0.9352	0.9222
Number of galls	Y = 5.26x - 3.43	y = 43.78x - 51.32	0.9454	0.9589
Number of egg masses	y = 5.17x - 3.57	y = 42.54x - 50.77	0.9478	0.9586
Reproductive factor	y = -0.59x + 4.49	y = -1.59x + 20.20	0.9561	0.7210

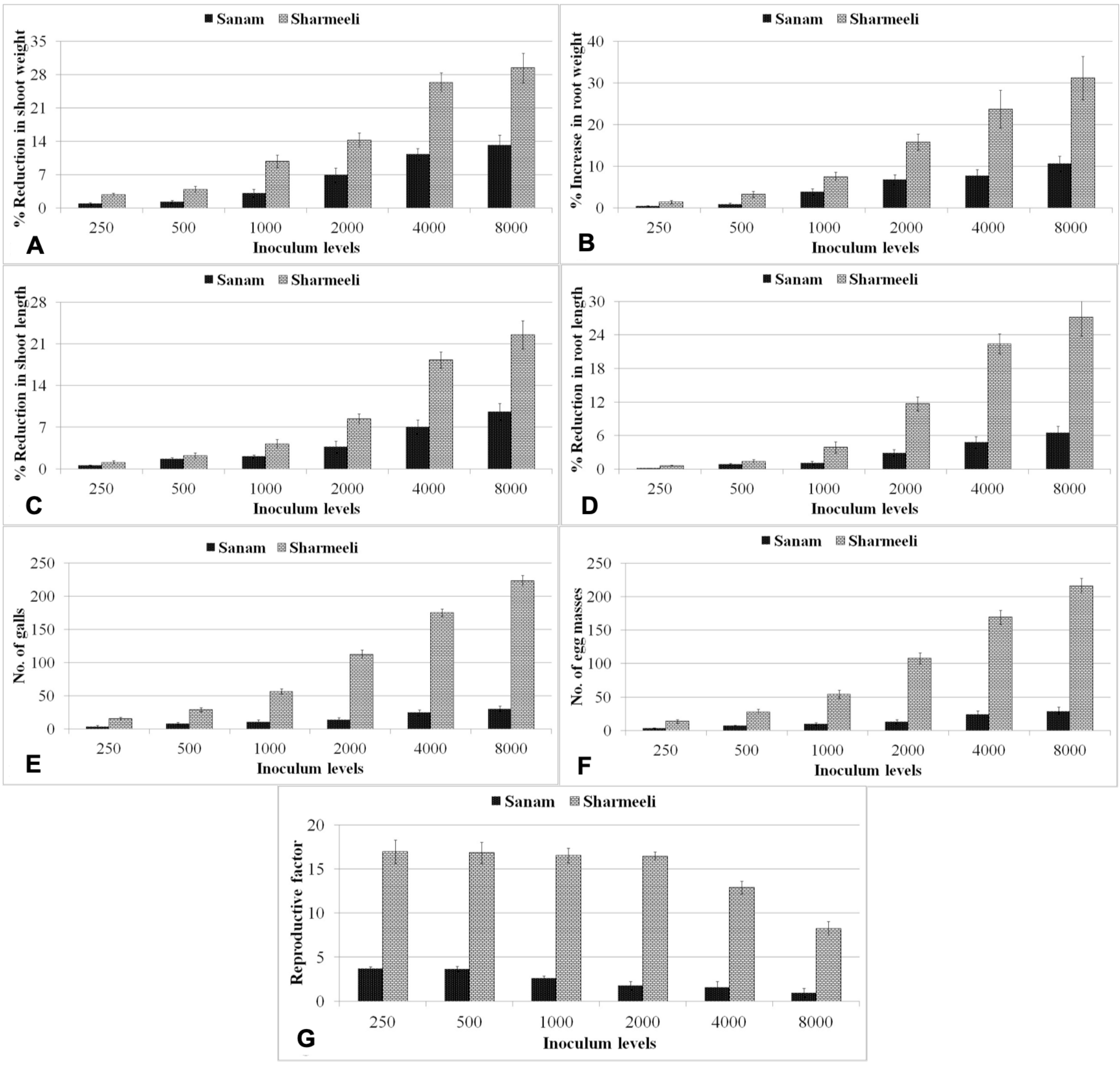

Fig. 1. Effect of inoculum levels of *Meloidogyne javanica* on shoot weight (A), root weight (B), shoot length (C), root length (D), number of galls (E), egg masses (F) and reproductive factor (G) of okra cultivars

The nematode produced the maximum galls on the roots of okra plants at a level of 8000 J2s followed by 4000 and 2000 J2s inoculum levels. On the contrary, the minimum galls and egg masses were observed at the lowest inoculum level of 250 J2s followed by the densities of 500 and 1000 J2s (Fig. 1). Again direct relationships were observed between inoculum levels and number of galls and egg masses as represented by regression equations in Table I.

All the inoculum levels varied significantly regarding reproductive factor. The maximum reproductive factor of 19-fold was observed at the lowest inoculum level in case of highly susceptible cultivar while in case of moderately resistant cultivar the maximum reproductive factor of 3.7-fold was found at the same inoculum level. On the other hand, the highest inoculum level of 8000 J2s gave the minimum reproductive factors of 1 and 8-folds in case of both the cultivars as shown in Figure 1. It was observed that with an increase in inoculum level there was a corresponding decrease in reproductive factor in case of both the cultivars showing an inverse relationship and has been shown by regression equation in Table I.

DISCUSSION

In the present study, the effects of six inoculum levels of *M. javanica* were compared on a highly susceptible cultivar 'Sharmeeli' and a moderately resistant one 'Sanam'. All the inoculum densities of *M. javanica* resulted in significant reductions in growth variables and increases in nematode infestations of both the cultivars over their controls. The reductions in moderately resistant cultivar were significantly lower as compared to the highly susceptible cultivar at all inoculum levels.

The progressive destruction in plant growth confirmed the damaging potential of *M. javanica* on okra cultivars. Previously many studies have been conducted by various researchers to assess the effects of different inoculum levels of different *Meloidogyne* species on different crops (El-Sherif *et al*., 2007; Neog and Bora, 2007; Jiskani *et al*., 2008). The findings of these researchers showed that reduced crop yield, physiological responses and other manifestations of pathogenic effects were directly proportional to increase in population of nematodes. It was further proved in these studies that concentrations of potassium, iron, copper, sodium and zinc were also directly related to initial densities of nematodes in the soil (Wallace, 1973; Haseeb *et al*., 1990).

It was also noticed in the current study that the plants of highly susceptible cultivar 'Sharmeeli' were affected more than those of moderately resistant one. The nematode produced more galls and egg masses on the roots of Sarmeeli as compared to Sanam at all inoculum levels. This was due to the fact that Sharmeeli being the highly susceptible, allowed the maximum juveniles to penetrate the roots and complete their life cycles successfully. On the other hand, lesser number of juveniles led to maturity in case of Saman as it allowed only a limited number of juveniles of *M. javanica* to enter the roots which is evident by the number of galls and egg masses on its roots.

High rate of multiplication of nematodes with low level of inocula might be due to encouraging factors like plenty of food, reduced competition level and the ability of hosts to support these populations (Haynes and Jones, 1976; Bendezu and Starr, 2003). Initial densities of *M. javanica* affected the rate of nematode multiplication; higher reproduction rates were observed where initial densities were lower. This might be due to destruction of root system by the nematodes. As root-knot nematodes are more pathogenic and damaging at higher densities, the larvae of subsequent generations fail to locate new infectious sites (Ogunfowora, 1977). According to Oostenbrink (1966), initial density of nematodes is responsible for subsequent reduction in yield of crops and increase in nematode populations. In the present studies final nematode populations and gall formations proportionally affected plant growth variables which corroborated the findings of Oostenbrink (1966). Differences in multiplication rates between resistant and susceptible cultivars might be in part, due to genetic factor in the host which confers susceptibility or resistance as well as genetic differences between nematode populations (Griffin, 1982; Jacquet *et al*., 2005; Castagnone- Sereno, 2006). Various stages in the life cycle of the nematode could be affected by host differences. The juveniles in a resistant plant are either incapable of penetrating the roots or their death may result ensuing penetration, or they fail to develop or females cannot reproduce. The differences in the susceptibility to *M. javanica* in okra cultivars are due to differences in their genetic makeup which can be explained in terms of number of galls.

CONCLUSIONS

In the present study, significant differences in growth reductions and increase in nematode infections were observed between the moderately resistant and highly susceptible okra cultivars at all inoculum levels. The plants of moderately resistant cultivar Sanam suffered less damage and suppressed nematode infection at all inoculum levels and therefore, recommended for cultivation in root-knot nematode infested fields to abate yield losses and repress the nematode from further multiplication.

Statement of conflict of interest

The authors declare no conflict of interest.

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