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PLANT PROTECTION
Article

Comparison of the damaging effects of *Meloidogyne incognita* on a resistant and susceptible cultivar of cucumber

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ABSTRACT: In the present study, the damaging effects of six inoculum densities of *Meloidogyne incognita* were compared on a resistant (Long Green) and a highly susceptible (Mirage) cultivar of cucumber. All the inoculum densities of *M. incognita* resulted in significant reductions in growth and yield variables of both the cultivars over their controls. The reductions in resistant cultivar were significantly lower as compared to the highly susceptible cultivar at all inoculum levels. The highest inoculum level caused the maximum reductions in growth and yield variables while the lowest inoculum level resulted in the minimum reductions. The reductions in growth and yield variables increased with an increase in the inoculum density showing a positive relationship. 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 the resistant cultivar when compared to the highly susceptible one showing a direct relationship between the increase in root weight and inoculum levels. Similarly, significant differences in number of galls and egg masses were noticed between the resistant and highly susceptible cultivar at all inoculum levels. The galls produced on highly susceptible cultivar were significantly higher as compared to the resistant one. A direct relationship was observed between inoculum levels and number of galls and egg masses. On the other hand, all the inoculum levels varied significantly regarding reproductive factor on the resistant and highly susceptible cultivars.

Key words: root-knot nematode, inoculum level, reproductive factor, resistant and susceptible cultivars, damaging effects.

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INTRODUCTION

Like fungal, bacterial and viral pathogens, nematodes also have a qualitative and quantitative impact on crops and resultantly have profound deleterious effects on food security, particularly in poverty-stricken regions of the world. Plant parasitic nematodes have been reported to cause estimated crop yield losses of 14.6% in tropical and subtropical countries while losses of 8.8% have been observed in developed countries (Sasser and Freckman 1987). A 10% of yield loss is incurred by these nematodes globally, which total $173 billion annually (Elling 2013).

Similar to all other crops and vegetables, cucumber is also vulnerable to many biotic and abiotic perturbations that cause reductions in yield. Biotic factors include insect pests (Kassi et al. 2019 a, b; Aslam et al. 2019 a) and disease inciting agents (Aslam et al. 2019 b; Hussain and Mukhtar 2019; Saeed et al. 2019; Iqbal and Mukhtar 2020). Among nematodes, root-knot nematodes of the genus *Meloidogyne* are the most widespread and economically important (Khan et al. 2019; Mukhtar and Hussain 2019; Asghar et al. 2020; Azeem et al. 2020). These nematodes complete their life cycles in a relatively short period of about 25 days at a temperature of 27 °C. The comparatively short life cycle facilitates root-knot nematodes to prosper effectively when a suitable host is present and cause their populations to pullulate to the maximum as crops reach maturity. Root-knot nematodes invade an array of important crops and have been found more damaging to vegetables especially in the tropical and subtropical countries of the world (Tariq-Khan et al. 2017). So far more than 100 species of *Meloidogyne* have been described throughout the world and, among these, four species viz. *Meloidogyne incognita*, *M. javanica, M. arenaria* and *M. hapla* are commonly found. More than 3000 plant species, almost all cultivated plants, have been recorded as the hosts of root-knot nematodes. The infested plants manifest symptoms of chlorosis, stunting and unthrifty growth (Archana and Saxena 2012). Root-knot nematodes are reported to cause annual losses in the tropics up to 33% in cucumber (Sasser 1979). Among root-knot nematodes, *M. incognita* and *M. javanica* are of economic importance and the most devastating ones. Of all the root-knot species associated with cucumber, *M. incognita* constituted 79% and *M. javanica* 19% in Pakistan. As a single population, *M. incognita* was recorded in 30% of cucumber plantations and, as mixed population, it was predominantly found with M. javanica in 70% of cucumber fields (Kayani et al. 2013).

Crops losses in Pakistan due to nematodes have been found more serious and complex as compared to the developed countries owing to numerous causes. Firstly, the country is positioned in the tropical region where the environmental conditions are encouraging for infectivity, growth and reproduction of these nematodes all the year round. Secondly, the arid zone of the country being sandy in nature is favorable for the activities of these nematodes. Lastly, the cultivation of perennial or susceptible crops year after year in the same piece of land in irrigated plains permits rapid multiplication of nematodes which results in severe infections and damage. On the other hand, entomopathogenic fungi and nematodes can reduce the incidence and severity of root-knot nematodes (Javed et al. 2019 a, b; Rahoo et al. 2019 a, b). Root-knot nematodes have also been found associated with fungal and bacterial pathogens resulting in disease complexes and aggravate the severity of the latter (Mukhtar and Kayani 2019; Nazir et al. 2019).

Nematode damage to plants is often measured by establishing relationships between preplant or initial nematode numbers and growth and yield of plants. The minimum nematode density that can inflict a considerable reduction in growth and/or yield of plants depends on the kind of host, nematode species, cultivars and environmental factors (Barker and Olthof 1976). Infections caused by low numbers of root-knot nematodes on poor or good hosts may not affect the plants (Madamba et al. 1965) or can result in enhancement of growth and yield of plants (Madamba et al. 1965; Olthof and Potter 1972) or can damage crops severely (Barker and Olthof 1976). The influence of inoculum levels of *Meloidogyne* spp. have been assessed on the extent of damage of various crops and on plants of different ages (Kayani et al. 2018), but such effects of nematode densities have not been compared between resistant and susceptible cultivars of the same plant. Therefore, the present study was designed to compare the effects of six inoculum densities of *M. incognita* on resistant and susceptible cultivars of cucumber.

MATERIALS AND METHODS

The nematode

An indigenous population of root-knot nematode (*Meloidogyne incognita*) initially isolated from cucumber roots, identified on the basis of perineal pattern and maintained on the highly susceptible cultivar of tomato (Money Maker) was used in the assessment. The nematode was mass produced on the highly susceptible cultivar of tomato (Money Maker) as previously described (Mukhtar and Hussain 2019). Second stage juveniles (J2s) were extracted from the infected roots for inoculation of plants as described by Whitehead and Hemming (1965).

Damaging effects of *M. incognita* inoculum levels on resistant and susceptible cultivars

The damaging effects of inoculum levels of *M. incognita* were assessed on a resistant cultivar of cucumber viz. Long Green and a highly susceptible one viz. Mirage (Mukhtar et al. 2013). 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. incognita* at the rates of 250, 500, 1000, 2000, 4000 and 8000 by making four holes around the stem. The plants which were not inoculated with nematodes served as controls. There were five replications for each inoculum level. The pots were placed in a completely randomized design on a greenhouse bench at 25 ± 2 °C. The plants were watered when required.

Recording of data

Eight weeks after inoculation, the plants of both cultivars inoculated with different quantity of nematodes were gently uprooted from their respective pots and the data were recorded regarding shoot and root lengths and weights, fruit yield, number of galls, egg masses and reproductive factor. Root and shoot lengths were measured with the help of a meter scale. Fruits were harvested twice a week from the 30^{th} day after inoculation till the termination of the experiment. At each harvest, the total number of fruits and their weights were recorded and the total fruit yield was calculated. Data regarding numbers of galls and egg masses on the roots of each plant were taken with the aid of a stereomicroscope at a magnification of 35×. Eggs were extracted from the roots of each plant after recording egg masses as described by Hussey and Barker (1973) and their numbers were counted. Similarly, juveniles from the soil of each pot were also extracted by employing the method devised by Whitehead and Hemming (1965). The eggs extracted from the roots and juveniles from the soil were added up and the reproductive factor was calculated (Khan et al. 2019).

Statistical analysis

The test was repeated once. Before statistical analysis, percent of decreases in growth and yield components were determined over their controls. First, the data of both experiments were separately analyzed by two-way analysis of variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278, for significant interaction. As there was no significant interaction between the two experiments, the two sets of data were combined and again statistically analyzed. The means of each parameter were compared for significant differences by Fisher's protected least significant difference test at ($p \leq 0.05$). Taking nematode levels as dependent variables (X) and reductions or increases in plant growth parameters, yield components and nematode infections as dependent variables (Y), linear relationships were established in Microsoft Excel 2010 to represent best-fit straight lines.

RESULTS

Damaging effects of *M. incognita* on growth variables of resistant and susceptible cucumber cultivars

The analysis of variance showed highly significant results regarding effects of inoculum densities on growth and yield parameters and nematode infestations. All the inoculum densities of *M. incognita* resulted in significant reductions in growth variables of both cultivars over their controls. The reductions in the 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 and root lengths and shoot weight followed by levels of 4000 and 2000 J2s. Similarly, the lowest inoculum level of 250 J2s resulted in the minimum reductions followed by 500 and 1000 J2s as shown in Tables 1, 2 and 3. 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 in Table 4. 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 the moderately resistant cultivar as compared to the highly susceptible one (Table 5). A direct relationship was found between the increase in root weight and inoculum levels and has been shown by regression equation given in Table 4.

Table 1. Effect of inoculum levels of *Meloidogyne incognita* on % decrease in shoot length of resistant and highly susceptible cucumber cultivars.

Inoculum level	Cultivar	
	Mirage	Long Green
250	2.1 ± 0.26	0.5 ± 0.11
500	6.2 ± 0.58	0.7 ± 0.22
1000	8.1 ± 1.02	1.3 ± 0.43
2000	14.0 ± 1.13	3.9 ± 0.85
4000	17.4 ± 1.35	5.6 ± 0.72
8000	23.2 ± 1.65	7.4 ± 0.94

Data are means of ten replicates.

Table 2. Effect of inoculum levels of *Meloidogyne incognita* on % decrease in root length of resistant and highly susceptible cucumber cultivars.

Inoculum level	Cultivar	
	Mirage	Long Green
250	2.6 ± 0.33	0.2 ± 0.08
500	3.8 ± 0.68	1.3 ± 0.32
1000	7.9 ± 0.76	3.0 ± 0.54
2000	9.9 ± 1.01	4.3 ± 0.68
4000	16.0 ± 1.33	4.9 ± 0.87
8000	17.2 ± 1.65	5.4 ± 0.68

Data are means of ten replicates.

Table 3. Effect of inoculum levels of *Meloidogyne incognita* on % decrease in shoot weight of resistant and highly susceptible cucumber cultivars.

Inoculum level	Cultivar	
	Mirage	Long Green
250	3 ± 0.41	0.4 ± 0.29
500	5 ± 0.97	0.9 ± 0.37
1000	10 ± 1.29	1.3 ± 0.73
2000	12 ± 1.30	4.5 ± 0.85
4000	23 ± 1.51	6.3 ± 0.92
8000	27 ± 2.10	7.2 ± 1.10

Data are means of ten replicates.

Table 4. Regression equations of different growth and yield variables of resistant and highly susceptible cucumber cultivars.

Growth and yield variables	Cultivar			
	Mirage		Long Green	
	Regression equation	R^2	Regression equation	R^2
Shoot length	y = 4.1x - 2.6667	0.9825	y = 1.48x - 1.9467	0.9342
Root length	y = 3.1886x - 1.5933	0.9652	y = 1.0886x - 0.6267	0.9606
Shoot weight	y = 5.0286x - 4.2667	0.9429	y = 1.5257x - 1.9067	0.9319
Root weight	y = 4.0571x - 3.8667	0.9252	y = 1.5314x - 2.06	0.9396
Number of fruits	y = 5.1257x - 4.2067	0.9309	y = 1.3743x - 1.86	0.9439
Fruit weight	y = 5.34x - 5.44	0.9513	y = 1.4114x - 1.9733	0.8525
Fruit yield	y = 7.0257x - 8.0067	0.886	y = 2.1943x - 2.5467	0.9661

Table 5. Effect of inoculum levels of *Meloidogyne incognita* on % increase in root weight of resistant and highly susceptible cucumber cultivars.

Inoculum level	Cultivar	
	Mirage	Long Green
250	2 ± 0.24	0.2 ± 0.09
500	4 ± 0.77	0.6 ± 0.21
1000	7 ± 1.02	1.4 ± 0.47
2000	9 ± 1.13	4.4 ± 0.85
4000	19 ± 1.95	6.3 ± 0.92
8000	21 ± 1.81	6.9 ± 1.00

Data are means of ten replicates.

Damaging effects of *M. incognita* on yield variables of resistant and susceptible cucumber cultivars

Significant variations in yield components of both resistant and susceptible cultivars were observed as a result of nematode infection; the lowest inoculum levels resulted in the minimum decreases. In the highly susceptible cultivar, the reductions in yield variables were the maximum whereas the reductions in the resistant cultivar were found to be the minimum at all inoculum levels as shown in Tables 6, 7 and 8. It was also observed that the increases in these parameters occurred with the increase in inoculum levels and a direct relationship was found between inoculum levels and yield variables, as shown in Table 4.

Table 6. Effect of inoculum levels of *Meloidogyne incognita* on % decrease in number of fruits on resistant and highly susceptible cucumber cultivars.

Inoculum level	**Cultivar**	
	Mirage	**Long Green**
250	3.4 ± 0.42	0.2 ± 0.10
500	5.3 ± 0.97	0.9 ± 0.22
1000	6.7 ± 1.13	1.2 ± 0.35
2000	17.2 ± 1.35	3.3 ± 0.53
4000	24.0 ± 1.73	5.4 ± 0.54
8000	26.3 ± 1.87	6.7 ± 0.77

Data are means of ten replicates.

Table 7. Effect of inoculum levels of *Meloidogyne incognita* on % decrease in fruit weight of resistant and highly susceptible cucumber cultivars.

Inoculum level	**Cultivar**	
	Mirage	**Long Green**
250	2.1 ± 0.31	0.1 ± 0.05
500	5.4 ± 0.67	1.2 ± 0.32
1000	7.8 ± 1.24	2.0 ± 0.54
2000	15.2 ± 1.32	2.1 ± 0.57
4000	19.4 ± 1.33	4.5 ± 0.53
8000	29.6 ± 1.87	8.0 ± 0.87

Data are means of ten replicates.

Table 8. Effect of inoculum levels of *Meloidogyne incognita* on % decrease in fruit yield of resistant and highly susceptible cucumber cultivars.

Inoculum level	Cultivar	
	Mirage	Long Green
250	3.7 ± 0.43	0.7 ± 0.13
500	5.2 ± 0.67	1.1 ± 0.32
1000	11.3 ± 1.32	3.4 ± 0.45
2000	13.9 ± 1.43	6.3 ± 0.66
4000	24.8 ± 1.93	7.9 ± 0.75
8000	40.6 ± 2.23	11.4 ± 1.24

Data are means of ten replicates.

Nematode infestations on resistant and susceptible cucumber cultivars

Significant differences in number of galls and egg masses were noticed between the resistant and highly susceptible cultivars at all inoculum levels. The nematode produced the maximum galls on the roots of resistant and highly susceptible cultivars 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 (Figs. 1 and 2). The galls produced on the highly susceptible cultivar were significantly higher as compared to the resistant one. Again, direct relationships were observed between inoculum levels and number of galls and egg masses as represented by regression equations and trend lines in Figs. 1 and 2.

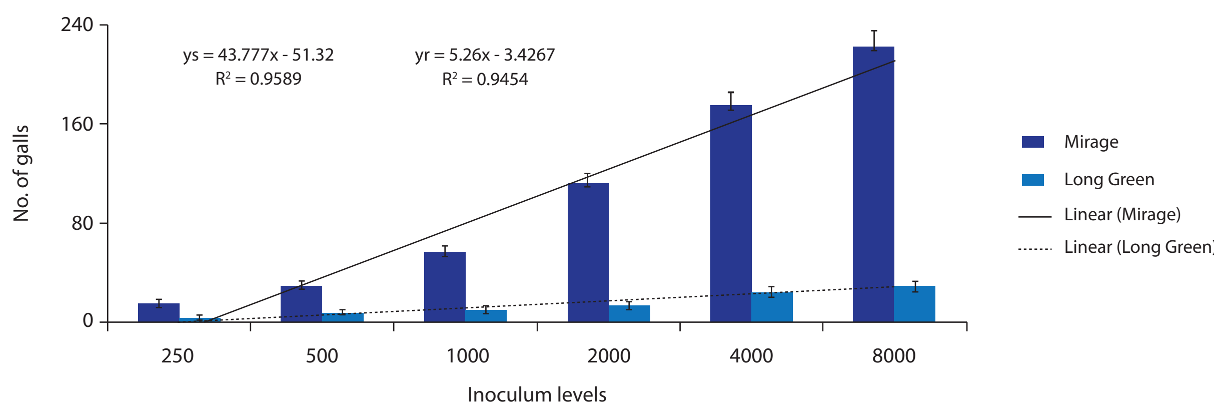

Figure 1. Number of galls on resistant and highly susceptible cucumber cultivars.

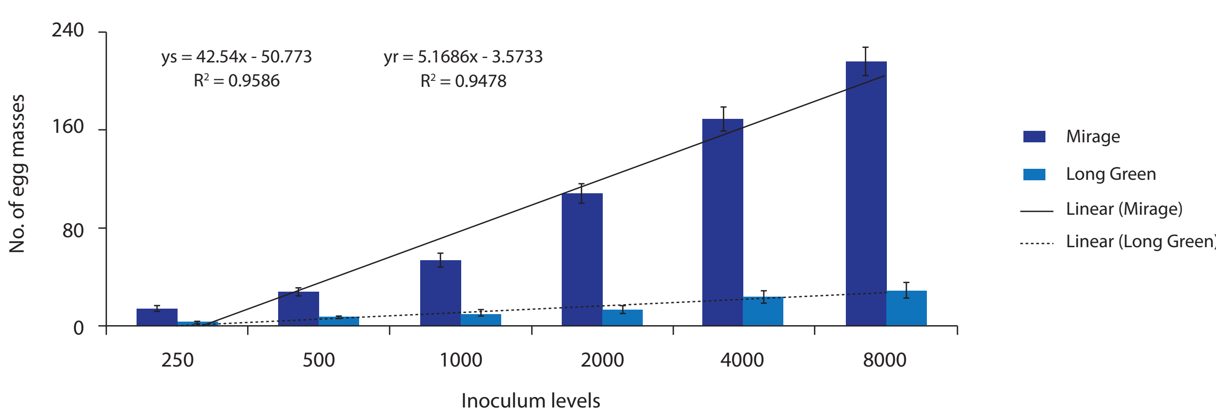

Figure 2. Number of egg masses on resistant and highly susceptible cucumber cultivars.

On the other hand, all the inoculum levels significantly varied regarding reproductive factor on the resistant and the highly susceptible cultivars. The maximum reproductive factor of 16.96-fold was observed at the lowest inoculum level in the highly susceptible cultivar while in the resistant cultivar the maximum reproductive factor of 3.72-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 0.96 and 8.23-folds in the resistant and the highly susceptible cultivars as shown in Fig. 3. It was found that there was a corresponding decrease in the reproductive factor for both cultivars with an increase in the level of inoculum, showing inverse relationships, which can be seen by regression equations and trend lines in Fig. 3.

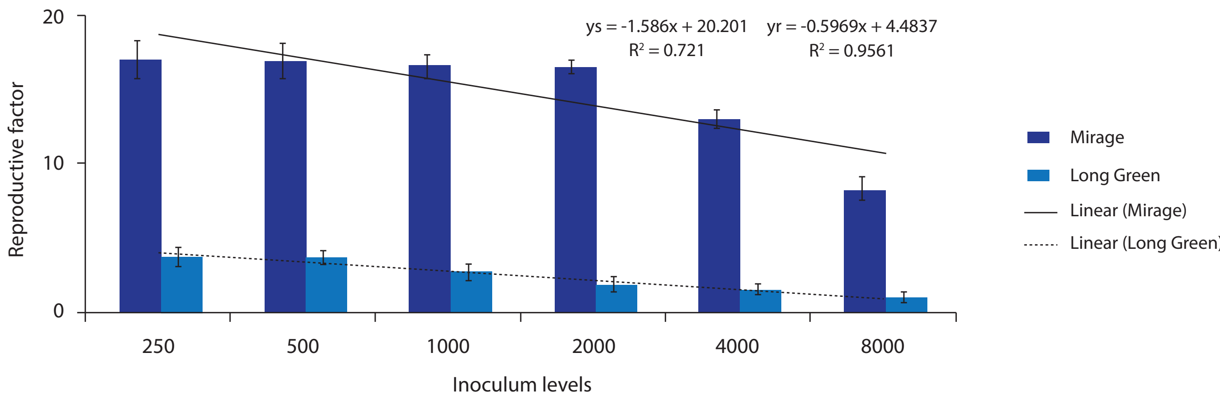

Figure 3. Reproductive factor of *Meloidogyne incognita* on resistant and highly susceptible cucumber cultivars.

DISCUSSION

In the present study, the effects of six inoculum levels of *M. incognita* were compared on a highly susceptible cultivar (Mirage) and a resistant one (Long Green) of cucumber. All the inoculum densities of *M. incognita* resulted in significant reductions in growth and yield variables and increases in nematode infestations of both cultivars over their controls. The reductions in the resistant cultivar were significantly lower when compared to the highly susceptible one at all inoculum levels.

The maximum reductions in growth and yield variables in the highly susceptible cultivar can be imputed to severe root damage due to penetration and/or feeding of maximum numbers of nematodes into the roots, which resulted in impairment and disruption of water absorption by the infected root systems. After penetration into roots, the root-knot females induced gall formation and giant cells in the stellar region and caused severe disruption of xylem tissues. Due to extensive disruption of xylem vessels, the upward uptake of water and nutrients was greatly reduced. The root-knot infection also greatly affected permeability of roots to water. Due to induction of nurse cell systems by the females of root-knot nematodes for incessant feeding in infected roots, there was a greater translocation of photosynthates towards these infection sites while the above ground parts experienced acute deficiency of nutrients (Wyss 2002; Di Vito et al. 2004). As the infected plants faced insufficient supply of nutrients, photosynthates, energy, water etc., therefore, the development and growth of leaf tissues and their essential constituents, particularly chlorophyll pigments, were greatly hampered (Khan and Khan 1997). The stunted and reduced growth of foliar parts subsequently results in reduced biomass and productivity (Khan et al. 2019). On the other hand, minimum numbers of nematodes entered into the roots of the resistant cultivar resulting in less root damage, reduced and/or poor induction of galls and giant cells causing no significant foliar damage. This resulted in insignificant foliar damage and growth and yield variables were least affected causing minimum reductions in these parameters on the resistant cultivar.

The successful parasitism by root-knot nematodes in vascular tissues of highly susceptible plants is characterized by the formation of giant cells. These nurse cells are highly specific and are induced and maintained by females of root-knot nematodes. On the other hand, in the resistant cultivar, the juveniles cannot induce the development of giant cells essential for successful parasitism and, consequently, the juveniles either die or leave the roots. As a result of unsuccessful parasitism, there is no infection of the host and the yield is not affected. The other major factors that determine the variations in growth and yield variables among cucumber cultivars are the multiplication of nematodes in the hosts and production of egg masses on the roots by females (Mukhtar and Hussain 2019). The variations in reproductive rates might partially be the result of genetic factors which impart resistance or susceptibility to the host or due to genetic variations in nematode populations (Griffin 1982; Jacquet et al. 2005; Castagnone-Sereno 2006). The variations in the hosts can influence various stages in the life cycle of the nematode. The juveniles either fail to enter the resistant hosts or they are killed after their penetration into the roots. If somehow the juveniles are successful in entering the roots of resistant cultivars, there will be no development and/or reproduction of the nematode.

The variations in reproduction and multiplication of *M. incognita* on cucumber cultivars are owing to variations in their genetic makeup which can be explained in terms of number of egg masses (Mukhtar et al. 2013). The production of maximum egg masses and eggs on the roots in the highly susceptible cultivar explains that maximum numbers of juveniles entered the roots and were successful in completing their life cycles in the host. On the contrary, only few juveniles made their way into the roots of resistant cultivar and got matured, which is obvious by the number of egg masses and their reproductive factors (Khan et al. 2019). Resistant cultivars contain a limited number of developed nematodes as compared to susceptible cultivars (Dropkin and Nelson 1960). The hindrance in invasion by second-stage juveniles of the nematode has been ascribed to failure to develop the maximum number of juveniles in the infected roots and/or hypersensitive reactions in the host (Dropkin 1969). In the case of susceptible hosts, the juveniles had the maximum potential to fully develop, as evident by their reproductive factors in the highly susceptible cultivar (Mukhtar and Hussain 2019). On the other hand, in the resistant cultivar, the development of the juveniles was either curtailed or delayed (Nelson et al. 1990).

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 study, final nematode populations and gall formations proportionally affected plant growth variables which corroborated the findings of Oostenbrink (1966).

CONCLUSION

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

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