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# Interaction between Nematode Inoculum Density and Plant Age on Growth and Yield of Cucumber and Reproduction of *Meloidogyne incognita*

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**MZK, TM and MAH designed the study, executed the experimental work and analyzed the data. TM supervised the work. MZK and TM helped in preparation of the manuscript.**

**Key words**
**Root-knot nematodes, Inoculum levels, *Cucumis sativus*, Reproductive factor, Pathogenicity.**

## ABSTRACT

The profitable cultivation of cucumber is seriously threatened by root-knot nematodes. As the damaging effects of *Meloidogyne incognita* have not been assessed on cucumber, therefore, the present studies report the effects of five initial population densities of *M. incognita* on growth and yield of cucumber and nematode reproduction. All inoculum densities caused significant reductions in growth and yield of cucumber and were found to be positively correlated with the latter. On the other hand, ages of plants at inoculation had negative correlations with reductions in these parameters at each inoculum density. The production of galls was found to be positively correlated with the inoculum densities and plant ages. However, rate of nematode build up decreased with an increase in inoculum density and appeared to be negatively correlated with inoculum densities and, on the contrary, was found to be positively correlated with plant ages. The results demonstrated that *M. incognita* has the potential to severely impair the growth of cucumber and by delaying early exposure of the latter to nematodes can significantly abate yield losses.

## INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are ubiquitous in distribution and infect a very wide range of hosts (Tariq-Khan *et al*., 2017; Karuri *et al*., 2017; Mukhtar *et al*., 2017a). Vegetables are among the most susceptible and severely affected by these nematodes (Mukhtar *et al*., 2014, 2017b; Hussain *et al*., 2016; Gómez-Rodríguez *et al*., 2017). Southern root-knot nematode, *Meloidogyne incognita*, is a damaging pathogen of vegetables and has been predominantly found infecting vegetable crops in warmer climates (Kayani *et al*., 2013; Hussain *et al*., 2014; Khan *et al*., 2017).

Cucumber is the most widely grown vegetable in Pakistan and is cultivated on an area of 3397 hectares with a total production of 142876 tons (Anonymous, 2013). The profitable cultivation of cucumber in the country is seriously threatened by viruses (Ashfaq *et al*., 2014a, b, 2015, 2017), fungi (Iqbal and Mukhtar, 2014; Iqbal *et al*., 2014), bacteria (Aslam *et al*., 2017a, b) and nematodes. Among nematodes, root-knot nematodes only can cause up to 52% infestation in cucumber (Khan *et al*., 2005). About 33% yield losses due to root-knot nematodes have been estimated in cucumber (Sasser, 1979). In Pakistan, *M. incognita* constituted 79% of all the root-knot nematode species associated with cucumber followed by *M. javanica* (19%) (Kayani *et al*., 2013). Root-knot nematodes have also been found associated with other soil borne plant pathogens resulting in increase in severity and incidence of wilt (Shahbaz *et al*., 2015).

Damage caused by nematodes is determined by relating pre-plant nematode densities to growth and yield of annual crops. The minimal density that causes a measurable reduction in plant growth or yield varies with nematode species, host plant, cultivar and environment (Barker and Olthof, 1976). The severity of plant damage by *Meloidogyne* spp. influenced by inoculum densities has been published for a number of crops. Similarly, the effects of initial population densities of *M. javanica* on cucumber have been reported (Charegani *et al*., 2012) but there is paucity of information on the relationships between initial density of *Meloidogyne* spp. and growth and yield of cucumber as influenced by plant age at the time of exposure to nematodes.

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It is generally assumed that young plants are more severely damaged by nematodes than are older plants. However, there are limited experimental data for supporting this notion. Therefore, the present studies were planned to investigate the relationship between initial population density of *M. incognita* and damage to cucumber. Also, the effects of plant age at the time of exposure to nematodes on reductions in growth and yield of cucumber and reproduction of *M. incognita* were studied under a range of inoculum densities.

## MATERIALS AND METHODS

*Nematode inoculum*

A purified culture of *M. incognita* initiated by a single egg mass and propagated on tomato cv. Money maker was used in the experiment. Twenty four hours old 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 cucumber plants.

*Effect of inoculum densities of* M. incognita *on cucumber*

The effects of different inoculum densities of *M. incognita* were assessed on a highly susceptible cultivar of cucumber (Royal Sluis). Three seeds of cucumber were sown in plastic pots containing 3 kg of sterilized soil (sand 70%, silt 21%, clay 8%, pH 7.5, organic matter, 1%) at weekly intervals in the greenhouse. After emergence one healthy seedling was maintained in each pot. When plants attained ages of 2, 3 and 4 weeks, these were inoculated with 500, 1000, 2000, 4000 and 8000 J2s of *M. incognita*. The plants which were not inoculated served as controls. Each treatment was replicated five times. The pots were maintained for nine weeks in a completely randomized design in a glasshouse at 25±2°C. The plants were watered when required. Nine weeks after inoculation, data were recorded regarding growth and yield parameters, number of galls and reproductive factor.

Root and shoot lengths were measured with the help of a meter scale. Fruits were harvested three times per week from the 30th day after inoculation till the termination of the experiment. At each harvest, the total number of fruits and their weights were recorded and total fruit yield was calculated.

Number of galls per root system was counted under stereomicroscope. Eggs extracted from roots of individual plants (Hussey and Barker, 1973) and juveniles extracted from soil of each pot (Whitehead and Hemming, 1965) after 48 h. made up the final population of the nematode. Reproductive factors were calculated by dividing the final populations by initial one.

*Statistical analysis*

The experiment was conducted twice. A completely randomized factorial design was used with three plant ages, each of which had five inoculum densities. Percent reductions in growth and yield parameters were calculated over control prior to statistical analysis (Fateh *et al*., 2017; Javed *et al*., 2017a, b; Kassi *et al*., 2018; Nabeel *et al*., 2018). All the data were found normally distributed and did not require transformation. Firstly, the data of both the experiments were analyzed to examine interaction between the experiments. As no significant interaction was observed between the data of both the experiments, so the two sets of data were combined (making ten replications) for final analysis. The combined data were subjected to two-way Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The differences among means were compared by Fisher's protected least significant difference test at ($P \leq 0.05$). Standard errors of means were calculated in Microsoft Excel 2007. Pearson correlation analysis (2-tailed) was performed to establish the relationships between inoculum densities and plant ages and growth parameters, yield and nematode reproduction. Data were also subjected to regression analysis. Plant growth variables and nematode reproduction were regressed as the dependent variable with the initial inoculum density as the independent variable at three inoculation ages.

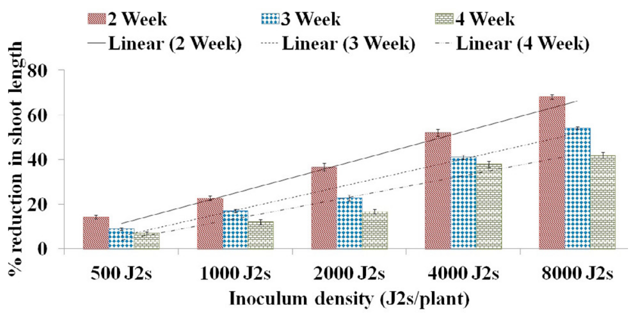


Fig. 1. Effect of *M. incognita* inoculum densities on reduction in shoot length. Data are means of ten replicates. Vertical error bars represent standard errors of differences of means.

## RESULTS

*Effect of inoculum densities on growth parameters and yield of cucumber*

All inoculum densities of *M. incognita* caused significant percent reductions in shoot and root lengths and fruit yield of cucumber at three plant ages (Supplementary Table I). Minimum reductions in these parameters were observed with the lowest inoculum density while the reductions were found to be the maximum with the highest density (Figs. 1-3). Inoculum densities were found to be positively correlated with these parameters (Supplementary Table II) indicating that with increase in inoculum densities, there were corresponding increases in percent reductions in these parameters. On the other hand, plant ages at inoculation had negative correlations with reductions in shoot and root lengths and fruit yield (Supplementary Table II) showing that the magnitude of damaging effects of nematode decreased as the age of plants increased at the time of inoculation.

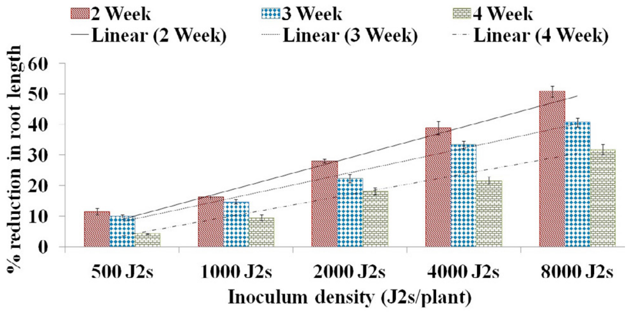


Fig. 2. Effect of *M. incognita* inoculum densities on reduction in root length. Data are means of ten replicates. Vertical error bars represent standard errors of differences of means.

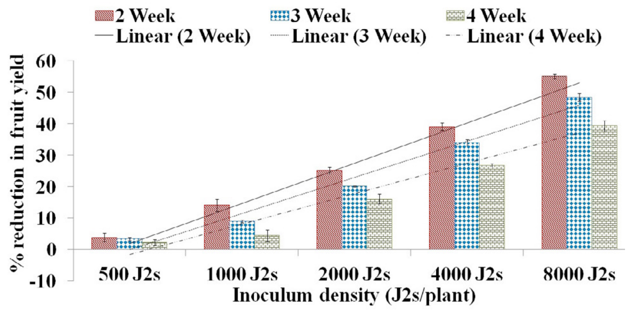


Fig. 3. Effect of *M. incognita* inoculum densities on reduction in fruit yield. Data are means of ten replicates. Vertical error bars represent standard errors of differences of means.

Linear models provided a good fit to the relationships between inoculum densities and reductions in shoot and root lengths and yield which have been shown by regression equations (Supplementary Table III) and trend lines in Figures 1, 2 and 3, respectively.

*Effect of inoculum densities on nematode reproduction*

Inoculum densities of *M. incognita*, plant ages and their interactions also had significant effects on number of galls and rate of nematode build up (Supplementary Table I). Minimum galls were observed in plants with the lowest inoculum density. As the density of *M. incognita* increased, the number of galls also increased significantly and reached to the maximum at the highest inoculum density. In other words, production of galls was found to be positively correlated with the inoculum densities and plant ages (Fig. 4; Supplementary Table II) and the relationships have been shown by regression equations given in Supplementary Table III. The numbers of galls on the roots inoculated after 2 and 3 weeks were not statistically different from each other at each inoculum density.

Rate of nematode build up decreased with an increase in the inoculum density and appeared to be negatively correlated with inoculum densities and on the contrary was found to be positively correlated with plant ages (Fig. 5; Supplementary Table II). The relationship has been shown by regression equations given in Supplementary Table III.

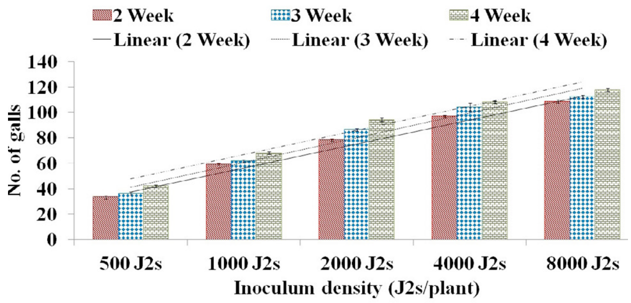


Fig. 4. Effect of *M. incognita* inoculum densities on number of galls. Data are means of ten replicates. Vertical error bars represent standard errors of differences of means.

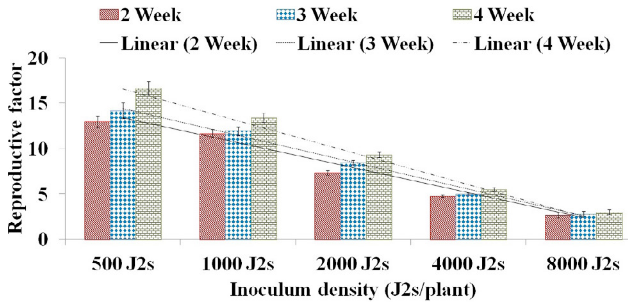


Fig. 5. Effect of *M. incognita* inoculum densities on reproductive factor. Data are means of ten replicates. Vertical error bars represent standard errors of differences of means.

## DISCUSSION

*M. incognita* caused significant reductions in shoot and root lengths and fruit yield of cucumber plants at all inoculum densities. However, the magnitude of these reductions decreased as the age of plants at the time of inoculation increased. Earlier studies have demonstrated a positive relationship between initial nematode density and reduction in growth and yield of host plants. On the other hand, these studies proved a negative relationship between initial inoculum density and nematode reproduction on the host plants (Seinhorst, 1970; Schomaker and Been, 2006; Neog and Bora, 2007; Greco and di Vito, 2009; Kayani *et al*., 2017). In the present study, inoculum densities of *M. incognita* greatly affected root length of cucumber. Reduction in root length was due to extensive damage of root system as a result of nematode feeding on giant cells, causing root growth to stop (Sasanelli *et al*., 2006; Hussain *et al*., 2011). This damage and shortening of roots affected growth and development of cucumber plants by limiting their ability to absorb water and nutrients from the soil, eventually resulting in stunting and reduction in fruit yield. Increases in nematode populations and subsequent corresponding reductions in growth and yield of crops or other manifestations of pathogenic effects have been reported by many workers (Vovlas *et al*., 2008; Azam *et al*., 2011) which corroborate present findings.

It is generally believed that young plants are more severely damaged by nematodes than the older ones, but there were limited experimental data to support this notion. The data presented in this study confirmed this belief. It was observed in the present study that the damaging effects of *M. incognita* population densities 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. Wong and Mai (1973) reported that high populations of *M. hapla* retarded the growth of young seedlings of lettuce more than that of older plants. 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. This suggested that maximum reduction in plant growth and yield by *M. incognita* was caused during the first two weeks after seeding (Ploeg and Phillips, 2001). It was also observed in the present study that as the age of plant at the time of inoculation increased, the number of galls also increased. On the other hand, reproductive factor decreased with an increase in inoculum density and increased with an increase in plant age. The positive correlation between increase in plant age and number of galls was probably due to the higher number of sites available for nematode penetration and feeding on the older plants. The lower rate of nematode build up at higher inoculum densities may be due to overcrowding of larvae which compete for food.

## CONCLUSIONS

The results demonstrated that *M. incognita* has the potential to severely impair growth of cucumber and can cause severe yield losses. It is also concluded that precluding instantaneous contact of 2-3 week old seedlings of cucumber with root knot nematodes may provide a way to avoid severe nematode damage. This can either be achieved by transplanting relatively older seedlings or by adopting control strategies *viz.* use of nematicides, soil amendments, soil solarization etc. (Shahzaman *et al*., 2015; Babaali *et al*., 2017; Jones *et al*., 2017; Julio *et al*., 2017; Rahoo *et al*., 2017).

*Supplementary material*

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.3.897.902

*Statement of conflict of interest*

The authors declare that there is no conflict of interests regarding the publication of this article.

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