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EFFECT OF *Meloidogyne graminicola* ON GROWTH AND NEMATODE MULTIPLICATION IN RICE

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ABSTRACT

The cultivation of rice is seriously affected by rice root knot nematode in nursery, transplanted crop as well as in direct seeded rice. Studies were conducted in pot house, Department of Plant Pathology to assess the damaging effects of *Meloidogyne graminicola* in two different rice varieties *viz*. PR 126 and Pusa Basmati 1121 at five initial inoculum levels on growth variables and nematode population. Sterilized soil was inoculated with second stage juveniles (J2) of *M. graminicola* to give 1000, 2000, 3000 and 4000 J2/g soil along with control where no inoculation of nematode was done. Amongst nematode variables, there was increase in soil nematode population and root galling index (RGI) with increase in inoculum levels. All inoculum levels caused significant reduction in growth variables of rice. With each increase in inoculum levels from 1000-4000 juveniles, nematode multiplication was increased. The extent of reduction in plant height, root length, and dry biomass was more pronounced in Pusa Basmati 1121 than in PR 126 which indicates varietal differences in susceptibility. The highest RGI and nematode population were recorded at 4000 J2/g soil which suggests that higher initial inoculums densities lead to greater nematode reproduction and damage. The study highlights the potential yield loss risk posed by *M. graminicola* even at low initial populations. This highlights the importance of selecting tolerant varieties and implementing effective nematode management strategies for sustainable rice production..

Keywords: Build up, Inoculum levels, *Meloidogyne graminicola*, Rice, Rice root knot nematode

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Plant pathogens have a qualitative and quantitative impact on crops which negatively affects the produce and economic gains. These biotic constraints along with abiotic ones are major hindrance to food security and sustainable productions. Plant parasitic nematodes have been reported to cause yield losses to the tune of 10% globally estimating to total USD 173 billion annually (Elling, 2013). Rice is an economically important crop grown worldwide. The rice root knot nematode, *Meloidogyne graminicola*, is an important pest of rice (Plowright and Bridge, 1990). *M. graminicola* can cause economic loss in upland, lowland and deep-water rice and rice nurseries, rainfed and direct seeded rice (Prasad *et al*., 1987; Bridge *et al*., 1990; Lorenzana *et al*., 1998). It is mainly distributed in the Southeast Asian countries, Burma, Bangladesh, Laos, Thailand, Vietnam, India and Indonesia and is likely to occur in other countries of the region (Bridge *et al*., 1990). The losses due to *M. graminicola* vary from 16-80% in India (Ravindra *et al.,* 2015). In Punjab, surveys conducted revealed 41.92 per cent of frequency of occurrence of rice root-knot nematode in nursery beds of rice and 22.2 per cent in rice field crop (Singh, 2017). Rice cultivation in Punjab continues to rely on irrigation till date. However, as water tables decline and water-stressed conditions become increasingly common in Punjab, there is transition from anaerobic to aerobic rice cultivation which has resulted in a rise in rice root knot nematodes. The nematode population is anticipated to be higher in direct seeded rice, primarily due to water stress conditions. The infested plants manifest symptoms of chlorosis, stunting and unthrifty growth (Archana and Saxena, 2012) with typical symptoms of hook shaped galls on roots. The root knot nematode species enter the root system of the plants, make a permanent feeding site and hamper the uptake of nutrients (Hunt and Handoo, 2009).

The damage caused by nematodes to crop is often assessed by establishing relationships between pre-plant or initial nematode numbers and growth and yield of plants. The knowledge of this relationship of initial population density (Pi) (Pi = initial inoculum level in experiments) to the damage caused allows the estimation of the threshold levels or the tolerance limit, which is defined as the nematode population

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density at planting up to which no measurable yield loss of a host occurs. Estimation of these critical levels is basic to the design of nematode management programmes (Ferris, 1978; Korayem, 2006). However, the minimum nematode levels that can inflict a considerable reduction in growth or yield of plants may depend further on the kind of host, nematode species, cultivars and environmental factors (Barker and Olthof, 1976).

Reduction in rice yield may depend upon the initial population level (Pi) of *M. graminicola* (Prasad *et al*., 1990; Plowright and Bridge, 1990; Prot *et al*., 1994; Main and Khan, 1995). High population density of *M. graminicola* second stage juveniles (J2) can cause seedling mortality, reduction in plant height, tiller number and grain yield losses up to 98% (Plowright and Bridge, 1990; Prasad *et al*., 1990). However, the relation of the initial populations and their threshold levels needs to be known. Therefore, the objectives of this study were to determine the effect of *M. graminicola* juvenile's inoculum levels on growth of rice and nematode reproduction.

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MATERIALS AND METHODS

Nematode inoculum production

For pure culture, the root-knot nematode species used for pure culture was initially identified from galled rice roots collected from infested fields. Galls were teased under a stereoscopic binocular microscope and adult females were extracted by teasing galls under a stereoscopic binocular microscope. Posterior part of females was dissected and the perennial pattern was observed under microscope. The identification was done according to key given by Yik and Birchfield (1979). Juveniles hatched from single egg mass were inoculated into small pots (8 inch) containing sterilized soil planted with rice seedlings. In the next season the culture was maintained in the pots planted with wheat (var. HD 2967) as it is susceptible host. Root-knot species was reconfirmed and the culture was maintained seasonally on either rice or wheat depending on crop suitability. The experiment was conducted over two years in *kharif* season (2018 and 2019) using two susceptible rice cultivars, PR 126 and Pusa Basmati 1121, to assess the effect of different inoculum densities of *Meloidogyne graminicola* under pothouse conditions at the Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India.

Ten seeds of rice were sown in pots containing loamy sand soil (Sand 75.5%, Silt 19.3, Clay 5.2, pH 7.6, EC 0.24 dS m^{-1}, Organic matter 0.672%). Temperature from June to October ranged from 24.2°C to 32.4°C (2018) and 24.5°C to 33.6°C (2019) while the relative humidity ranged from 52% to 75% (2019) and 42% to 77% (2019) during crop season. After 10 days, the plants were inoculated with 1000, 2000, 3000 and 4000 juveniles. These juveniles were concentrated in 1 ml of water and added to soil by making 4-5 holes around the base of the plant by using pipette. The holes were covered with soil after nematode inoculation. The un-inoculated plants served as control. Each treatment was replicated three times. The inoculated and un-inoculated pots were arranged in pothouse of Department of Plant Pathology. The general agronomic practices were followed for care of plants. Observations on soil nematode population and growth parameters were taken. The plants were uprooted from soil after sixty days of sowing. The roots were washed carefully with water to remove all the debris. The length (cm) and fresh weight (g) of shoot and root (g) were measured.

Nematode population in soil as well as roots

The roots of the harvested rice seedlings of different inoculum levels were each washed separately and dabbed dry with tissue paper. Soil population was estimated after extraction by Cobb's sieving and decantation technique (Cobb, 1918). Reproduction factor (Rf) which is the final population divided by the initial population was calculated. A Rf > 1, connotes reproduction, while Rf < 1, implies no reproduction. Galling was scored on scale of 1-5 rating chart by Bhatti and Jain (1994) where 1 = 0 galls; 2 = 0.1–10 galls; 3 = 10.1–30 galls; 4 = 30.1-100 galls; and 5 = more than 100 galls.

Statistical analysis

The experiment was conducted for two varieties (PR 126 & Pusa Basmati 1121) for two years. A completely randomized factorial design was used with two varieties, each of which had five inoculum levels. The data of the experiment during both years showed no significant difference, so the two sets of data (2018 & 2019) were combined for final analysis. The differences among means were compared by Tukey's method at ($p \leq 0.05$) using software program IBM SPSS 27.0. The standard error of means were calculated in Microsoft excel 2010.

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RESULTS AND DISCUSSION

Effect of different inoculum levels of *Meloidogyne graminicola* (PR126 and Pusa Basmati 1121) on nematode population in soil

A significant increase in soil nematode population was observed with increasing inoculum levels across both rice varieties. Increasing inoculum levels of *M. graminicola* led to a corresponding rise in soil nematode population in PR126, compared to the control (Fig. 1a). Minimum soil nematode population (1576.66 nem/250 cc soil) was recorded in treatment T_2 (1000 juveniles) followed by T_3 treatment (2000 juveniles) where 2833.33 nem/250 cc soil were observed. The multiplication rate (Rf-1.57) of *M. graminicola* was highest at the lowest inoculum level (T_2). There was significant increase in rice root knot nematode population in T_4 treatment as compared to T_2 and T_3 treatments. T_5 (4000 juveniles) showed the maximum soil nematode population (4600 nem/250 cc soil). Minimum reproduction factor was observed at inoculum level 4000 juveniles (Rf-1.15) (Table 1) while the maximum was observed at inoculum level of 1000 juveniles. Similar trends were observed in Pusa Basmati 1121. There was increase in *M. graminicola* population with increase in inoculum levels (Fig. 2a). Maximum soil nematode population was recorded in T_5 treatment (42.33 nem/250 cc soil) while minimum in T_2 treatment (1233.33 nem/250 cc soil) (Table 1).

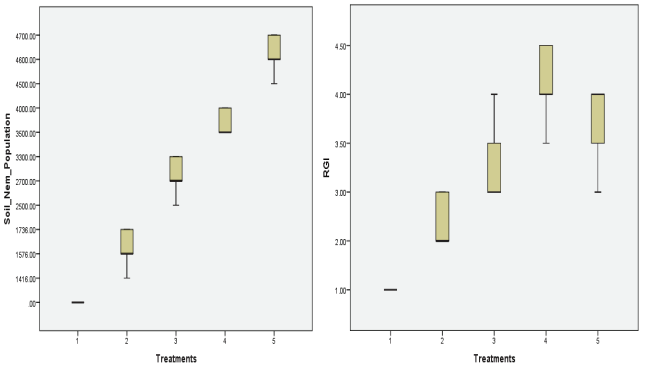


Fig. 1. Effect of different inoculum levels on a) soil nematode population/250cc soil and b) root galling index (RGI) of rice plant (var. PR126)

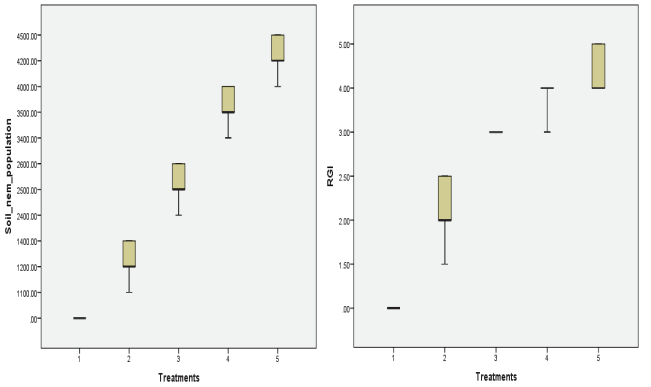


Fig. 2. Effect of different inoculum levels on a) soil nematode population/250cc soil and b) root galling index (RGI) of rice plant (var. PUSA Basmati 1121)

Effect of different inoculum levels of *M. graminicola* on nematode infestation in roots of rice (RGI)

The belowground symptoms of *M. graminicola* infection were formation of galls, excessive lateral branching in the root and reduced root length in both the varieties of rice. All inoculated PR126 plants exhibited gall formation. The root galling index was found to be lowest in un-inoculated plants (T_1) (Control). The root galling index was observed to be lower in T_2 treatment (RGI-2.33) and increased with increasing inoculum levels. The highest RGI was observed in T_5 (4.66), followed by T_4 (4.00) (Table 1). Similar trends were observed in Pusa Basmati 1121 (Figure 2b). The root galling index was observed to increase significantly with increasing inoculum levels

Table 1. Build-up of rice root knot nematode at different inoculum levels in two varieties of rice

Inoculum level	Variety: PR126			Variety: Pusa Basmati 1121		
	Soil nematode population/250 cc soil	Rf	RGI	Soil nematode population/250 cc soil	Rf	RGI
T_1: 0 juveniles (Control)	$0.00^{e}\pm0.00$	0.00	$1.00^{c}\pm0.00$	$0.00^{e}\pm0.00$	0.00	$1.00^{d}\pm0.00$
T_2: 1000 juveniles	$1576.66^{d}\pm92.37$	1.57	$2.33^{bc}\pm0.50$	$1233.33^{d}\pm107.15$	1.23	$2.00^{e}\pm0.28$
T_3: 2000 juveniles	$2833.33^{c}\pm315.05$	1.41	$3.33^{ab}\pm0.50$	$2500.00^{c}\pm57.73$	1.25	$3.00^{bc}\pm0.00$
T_4: 3000 juveniles	$3666.66^{b}\pm254.58$	1.22	$4.00^{ab}\pm0.28$	$3633.33^{b}\pm302.45$	1.21	$3.66^{ab}\pm0.19$
T_5: 4000 juveniles	$4600.00^{a}\pm57.73$	1.15	$4.66^{a}\pm0.50$	$4233.33^{a}\pm164.42$	1.05	$4.33^{a}\pm0.50$

Within a column means followed by different alphabets differ significantly at $p \leq 0.05$

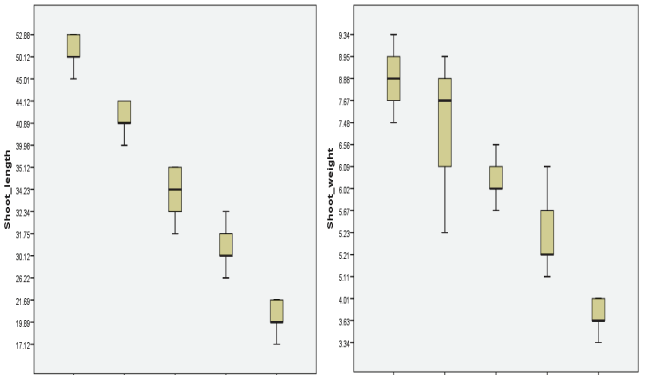


Fig. 3. Effect of different inoculum levels on a) Shoot length (cm) and b) Shoot weight (g) of rice plant (var. PR126)

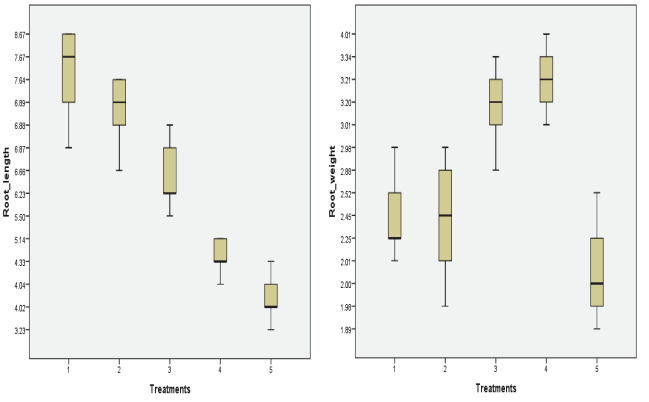


Fig. 4 Effect of different inoculum levels on a) Root length (cm) and b) Root weight (g) of rice plant (var. PR126)

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of rice root knot nematode. The lowest RGI was observed in T_2 treatment (1.23) and maximum was observed in T_5 treatment (1.05) where 4000 juveniles were inoculated.

Effect of different inoculum levels of *M. graminicola* on growth parameters of rice

Growth parameters such as shoot length, shoot weight, root length, and root weight were recorded in both rice varieties under different *M. graminicola* inoculum levels. It was observed that in variety PR 126 there was gradual stunting of plants when different levels of rice root knot nematode were inoculated as compared to control. In the highest inoculum treatment (T_5), plants exhibited leaf chlorosis. Plants appeared chlorotic, stunted, and showed reduced vigor. The highest reductions in shoot length (60.34%) and weight (57.24%) were recorded in T_5, compared to the control. The minimum reduction in shoot length (15.54%) and weight (14.95%) was observed in T_2 treatment (1000 juveniles) followed by T_3 treatment. It was observed that shoot length and weight had indirect relation with inoculum levels of *M. graminicola i.e.* with increase in inoculum levels, the shoot length and weight tend to decrease (Fig. 3).

Root length and weight (var. PR 126) were also adversely affected by inoculum levels of *M. graminicola* (Fig. 4). There was significant decrease in root length with increase in inoculum levels. The maximum root length (7.73 cm) was observed in T_1 treatment while the minimum was observed in T_5 treatment (3.86 cm). The reduction in root length was observed to be maximum (50.06%) in T_5 treatment and minimum in T_2 treatment. The direct relationship was observed between inoculum levels and root weight. Root weight increased with inoculum levels up to T_4, after which it declined. Highest root weight was observed in T_4 treatment and lowest was observed in T_5 treatment. The increase in root weight was recorded to be 2.48% in T_2 treatment and 30.29% in T_3 treatment. Maximum increase was found in T_4 treatment (41.49%). In contrast to this, reduction of about 11.61% was recorded in T_5 treatment as compared to control.

In the second variety (Pusa Basmati 1121), similar results were obtained. The shoot length, shoot weight and root length were found to decrease with advancement in inoculum levels of *M. graminicola*. The maximum reduction was observed in T_5 treatment (4000 juveniles) while the minimum reduction was recorded in T_2 treatment (1000 juveniles). The root weight was observed to increase up to inoculum level of 3000 juveniles and decreased at inoculum level of 4000 juveniles (Fig. 5 & 6).

The present findings revealed that both the cultivars of rice fostered build-up of rice root knot nematode (*M. graminicola*) population and reduced the growth parameters of rice. The damaging effects of nematode on rice were found to be increased as the inoculum levels increased. Different studies were carried out by various workers to check the effect of various inoculum densities of different *Meloidogyne* species on various hosts (El-Sherif *et al.,* 2007; Neog and Bora, 2007; Jiskani *et al.,* 2008; Hussain *et al.,* 2011; Irshad *et al.,* 2012). Present results are in conformity with Poudyal *et al*. (2005) who studied the effect of different inoculum densities of *M. graminicola* on growth of rice and nematode development. He concluded that with increase in inoculum densities of *M. graminicola* there was decrease in growth parameters. Abbasi and Hisamuddin (2014) also reported that reduction in growth parameters was

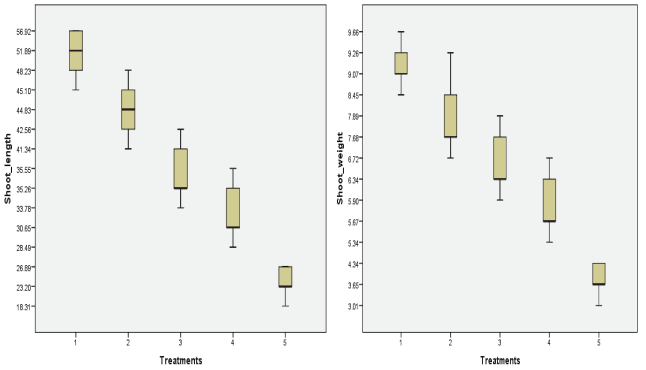


Fig. 5. Effect of different inoculum levels on a) Shoot length (cm) and b) Shoot weight (g) of rice plant (var. PUSA Basmati 1121)

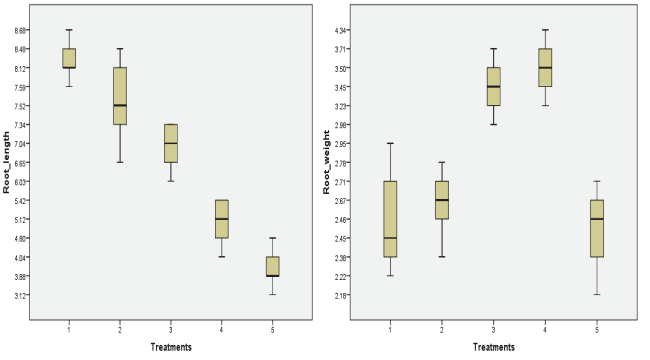


Fig. 6. Effect of different inoculum levels on a) root length (cm) and b) root weight (g) of rice plant (var. Pusa Basmati 1121)

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more at higher inoculum level of *M. incognita* in *Vigna radiata*. Lal *et al* (2015) also studied the effect of different inoculum levels of *M. graminicola* on growth and biochemical parameters of rice. He observed that with increase in inoculum levels there was reduction in growth parameters of rice. Kumar *et al* (2017) also reported that damaging threshold level of *graminicola* on scented (var. Pusa 1121) and non-scented rice (PR 114) was 100 J2/kg soil and he concluded that with increase in inoculum levels of *M. graminicola,* there was reduction in growth parameters of rice. Devi *et al.* (2017) studied the pathogenecity of five inoculum levels *viz.* 0, 1, 2, 3, 4 J2/g soil of *M. graminicola* in rice nursery in three types of soil (sandy loam, clay loam and loam). Inoculum level of *M. graminicola* was found at 1 J2/g soil was found to be pathogenic. The growth parameters of rice seedlings were significantly reduced at higher pathogenicity levels and were found to be more in clay loam soil followed by loam and sandy loam irrespective of inoculum levels.

The nematode infested plants exhibited shoot length, shoot weight and root length reduction as compared to no-infested plants. This could probably be due to damage caused by increasing numbers of nematodes with increase in inoculum levels that invaded roots and this may be due to interruption in nutrient and water uptake (Karssen and Moens, 2006). After a few days of development, root knot nematode juveniles penetrated roots, causing gall formation and large cells in the stellar area, as well as significant xylem tissue disruption. The upward absorption of water and nutrients was severely limited due to extensive damage of xylem arteries. The root-knot infection also had a significant impact on root water permeability. The females of root knot nematodes continued to feed on infected roots, causing higher transfer of photosynthates to these infection sites while the aboveground sections suffered acute nutritional deprivation (Wyss *et al.*, 1992). The formation and expansion of leaf tissues and their important elements, particularly chlorophyll pigments, were substantially impeded as the infected plants faced limited supplies of nutrients, photosynthates, energy, water, and other factors (Khan and Khan, 1997).

It was also observed that the growth parameters were more affected at higher inoculum levels. Wallace (1973) and Haseeb *et al.* (1990) have also observed that the initial density of nematodes in soil is also directly related to the concentration of nutrient compounds viz., potassium, iron, copper, sodium and zinc which was reduced at higher inoculum levels. It was observed in the findings of these workers that reduced crop yield, physiological responses and other manifestations of pathogenic effects are directly proportional to increase in population of nematodes thus having direct effect on the growth of plants.

The present study revealed that the multiplication of nematode is also related to initial inoculum densities of nematodes. The more nematode population was found at higher inoculum levels. This could be attributed to the nematode's high density in a small amount of soil. The occurrence of these conditions may have resulted in nematode competition for space, nourishment, and other requirements (Devi *et al.,* 2017). Rao and Israel (1972) also reported a high rate of *M. graminicola* reproduction in rice at low levels of inoculum, which could be attributable to favorable variables such as abundance of food, absence of competition, and the host's ability to support these population levels. The reduction in nematode developmental parameters at

large levels of inoculum, on the other hand, is thought to be attributable to a negative density factor, such as endoparasite crowding in the roots.

Root galling index was directly proportionate to the amount of inoculum level. At lower inoculum levels fewer galling was formed; on the other hand, at higher inoculum levels maximum galling was recorded. Similar findings have also been reported earlier by Parveen (2006) and Niyaz and Hisamuddin (2008).

CONCLUSION

It can be concluded from this study that increasing inoculum levels of *M. graminicola* significantly elevate soil and root nematode populations while severely reducing growth parameters in both PR126 and Pusa Basmati 1121 rice varieties. Based on observed damage and nematode multiplication, the inoculum level of 1000 juveniles was found to be the economic threshold level. Beyond this, losses in plant vigor and yield potential become economically significant, emphasizing the need for early detection and management in farmer fields.

Conflict of interests

The authors declare they have no conflict of interests.

Author's contribution

Conceptualization and designing of the research work (NKD); Execution of field/lab experiments and data collection (A); Analysis of data and interpretation (A); Preparation of manuscript (A).

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