

Original Research Article

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Quantifying the Interaction between Root-knot Nematode (*Meloidogyne incognita*) and Rhizobium on Moong (*Vigna radiata*)

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ABSTRACT

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Interaction between Root-knot Nematode and Rhizobia was evaluated in the green house on moong variety (SML-668). Seeds of the moong variety were sown in sterilized potted sandy soil and inoculated with 1000 freshly hatched juveniles of root knot nematode and 2ml rhizobia per pot (purchased from market). Treatments were kept as T₁ (Pot without nematode), T₂ (with 1000 J₂ inoculated/pot), T₃ (with 2ml of rhizobia/pot), T₄ (pot with 2ml of rhizobia +1000 nematode inoculated simultaneously), T₅ (with 1000 j₂ inoculated one week before rhizobia) and T₆ (with 2ml of rhizobia inoculated one week before nematode) and these pots were maintained for one month. Results revealed that the number of nodules developing on root-knot-infected roots (nodules/seedling) was lower than on healthy roots. When we added nematode simultaneously with rhizobia, singly or in combination reduced the number of nodules on moongbean as compared to check plants whereas nodulation is more in plants treated with rhizobia culture.

Introduction

Pulses are one of the major sources of protein in predominantly vegetarian diet of the people of India. They have a unique feature in plant kingdom as they are extremely endowed with the inherent ability to substantiate nitrogen enrichment of soil through root nodules from the atmospheric nitrogen. Nearly 11,000 species of legumes are known and many are important for industrial and medicinal purpose. Green gram is scientifically known as *Vigna radiata* and it is commonly known as mung in India. Green gram is an important pulse crop in India next to chickpea and pigeonpea and believed to be originated in

India. Green gram occupies a unique place for its use as a grain in various ways in our dietary system. Nematodes are microscopic, highly diverse biotrophic parasites. They use their stylet to feed on root as well as shoot of the plants. An estimated loss of 14.6% in developing countries and 8.8% in developed tropical and subtropical countries makes them major global challenge to ensure food security to global increasing population.

Among all Plant parasitic nematodes, Root knot nematodes are the most widely spread pests and a major threat to crops in tropical and sub-tropical regions throughout the world. Their cosmopolitan nature and

complex strategy of parasitism makes them one of the most specialized pests. Second stage juveniles (J2s) pierce root and feed on vascular cells of the plant. Mature female secretes gelatinous matrix and lays eggs partheno genetically within egg mass. Egg mass hatches upon receiving moisture and new J2s moves in soil towards the new root to parasitize. *M.incognita* induces below ground symptom i.e. knot or gall-like structure on host roots. This reflects as a above ground symptoms like wilting, yellowing, stunting in patches and reduced fruit sizes.

The nematodes adversely affect nodulation nitrogen fixation and yield. *Meloidogyne* infection, which primarily impairs water and nutrient uptake, and upward translocation by the root system. Disease complexes involving nematodes and fungal pathogens may cause significantly more crop losses than individually. Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize the rhizosphere and plant roots resulting in enhancement of plant growth or protection against certain plant pathogens. Studies were conducted to test the effect of *Meloidogyne incognita* and rhizobacterial inoculant to nodulation and plant growth parameters.

Materials and Methods

Culture maintenance of nematode

Seeds of Tomato cultivar Pusa Ruby were surface sterilized with 70% ethanol, solution of 0.1% SDS + 0.1% HgCl₂ and subsequently four-time washed with water. Ten pots of 15 cm diameter were filled with pot mixtures and used for seed sowing. Twenty days old plants were inoculated with surface sterilized 500 J2s/plant. Egg-masses were harvested 60 days post inoculation and placed over modified Baermann funnel for hatching.

Preparation of pure culture of rhizobia

The culture of rhizobia was purchased from the Department of Microbiology, CCSHAU, Hisar.

Soil sterilization

Sand brought to nematology laboratory and sterilized in autoclave at 15 lbs pressure with 121±1^oC for 1 hour. Sand allowed to dry for one day and then filled in 15 cm diameter earthen pots (1 kg capacity).

Sowing of seed

Seeds of the Moong variety soaked in tap water for 24 hours and the sprouted seeds were sown in sterilized potted sandy soil. Seven days after sowing, one plant was retained per pot. The pots were inoculated with freshly hatched of 1000 second-stage juveniles (j2), 2ml of rhizobia, nematode +rhizobia and maintained for one month. Inoculation of second stage juveniles of root-knot nematode, *M. incognita*

Nematode inoculation

Inoculation of nematodes was done by carefully removing the soil around the roots of plants in each pot to ensure direct and easy approach of juveniles to root system. The nematode inoculum in the form of suspension was vigorously bubbled first and then poured on exposed roots with pipette. The roots were immediately covered with soil and light watering was done in the pots.

Inoculation of rhizobia

Rhizobia inoculation was done by carefully removing the soil around the roots of plants in each pot to root system. 2 ml of inoculum per pot in the form of suspension was vigorously bubbled first and then poured on exposed roots with pipette.

Observation recorded

Thirty days after inoculation each plant was uprooted carefully from soil. The roots were retrieved carefully and kept in a basin of water to clear it from adhering soil particles and recorded the following observations: plant growth characteristics (shoot length, Root length, plant weight (wt), and also on nematode multiplication such as number of galls, number of eggs/plant and number of $j_2/200$ cc of soil. Roots were spread in the big sized. Petri plate which contained water and recorded the observations on number of galls with the help of the hand lens. Each treatment was replicated four times. Observations on the following growth parameters and nematode reproduction factors were recorded and the data were analysed statistically

Results and Discussion

The present studies were done to explore the competition between *M. incognita* and Rhizobia on Moongbean and its effects on nodulation. The reciprocal effects of prior invasion by either nematode or bacterial inoculant on Moong were evaluated. Data in table 1 revealed that competition between *M. incognita* larvae and root-nodule bacteria was postulated as a cause of reduced nodulation on moong. Root knot Nematode forms feeding cell on the moong roots and acts as source of sink & thus plant growth parameters affected. It is evident from the data that the plant height of mungbean declined as inoculated with the nematode. Maximum (12.41 cm) plant height was noted in treatment with rhizobia followed by control (11.56 cm) and minimum (12.22 cm) plant height was recorded with *M. incognita*, plants inoculated with nematodes were severely stunted, devitalized and looked sick. Similar trend was noticed with the root length of crop. Maximum (14.41 cm) root length was recorded in bacterial inoculants followed by check plant (12.83) and

minimum (9.06 cm) in highest level. On fresh plant weight basis maximum shoot weight was recorded with rhizobial inoculant (7.33g) and minimum (3.73g) with nematode. Significant and progressive decline in the plant growth parameters was noticed with inoculation of root knot nematode. Results also revealed that the number of nodules developing on root-knot-infected roots (nodules/seedling) was lower than on healthy roots. Our finding is with conformity with Taha and Raski observed that nodule formation took place either before or after the addition of larvae of *M. javanica* or *Heterodera trifolii* to white clover. When we added nematode simultaneously with rhizobia, singly or in combination reduced the number of nodules on moongbean as compared to check plants whereas nodulation is more in plants treated with rhizobia culture. Nutman *et al.*, concluded that reduced nodulation occurred only as a result of overall reduction of the root system. The sites of nodule initiation are present in the cortex of the root, and more lateral roots augment the infectable potential of the root. Results also revealed that when we added nematode along nodulation and plant growth parameters reduced due to gall formation on the roots by *M. incognita*, by occupying space on the root system. When *M. incognita* inoculation was delayed one week prior to rhizobia there is the significant increases in nodule formation (12.6) and reduction in the number of galls and egg masses to *M. incognita* and where the nematode and bacteria was inoculated simultaneously, competition occurs, prior invasion and occupation of root surface by Root knot nematode inhibits bacteria to infect roots and there is overall hamper of process of nodulation. Similar results were found by Kumar *et al* 2018 that the root knot nematode caused significant reduction in plant height, root length fresh and dry weights of shoots and roots.

Table.1

Treatments	Plant wt.(gm)	Shoot length(cm)	Root length(cm)	No. of egg mass	No. of galls	Final nematode	No of Nodules
Check	6.76	11.56	12.83	-	0.00	0.00	0.0
with 1000 J ₂ inoculated /pot	3.73	7.83	8.83	19.66	87.5	266.23	0.0
with 2ml of rhizobia /pot	7.33	12.41	14.41	-	0.00	0.00	18.0
rhizobia + nematode simultaneously	5.33	9.84	9.93	9.03	25.3	89.12	8.5
nematode inoculated one week prior tonematode	4.96	8.13	8.13	13.3	45.6	160.15	6.3
rhizobia inoculated one week before nematode	5.06	10.81	11.80	5.33	10.77	78.03	12.6
CD @ 5%	1.36	2.33	3.33	4.54	7.54	30.43	4.8

Highest level of nematode inoculum indicated inhibitory and damaging potential on plant growth parameters on blackgram Siddiqui *et al.*, (1993) conducted experiment on bengalgram and recorded significant decrease in rhizobial nodulation with the increase of *M. incognita* population. Jain and Trivedi (1995) also noticed that *Cicer arietinum* highly susceptible to *M. incognita* leading to poor growth and nodulation and adversely affecting the quality of the produce.

They also observed that great reduction in length and weight of shoots and roots, number of nodules in plants treated with nematode prior to Rhizobium inoculation. However, significant decrease in number of galls was observed in plants treated with Rhizobium and nematode inoculation. Nayak (2006) also reported that plant height of brinjal varieties due to root-knot nematode infection were decreased by 9.04, 18.48, 23.50 per cent in varieties Pusa Kranti, Kantabaigan and Pusa Purple Long, respectively over uninoculated control. Abd-Allah *et al.*, (2010) also revealed significant reduction in nitrogen, phosphorus and potassium contents of the plant parts caused by heavy nematode infestations. These

elements are needed for growth and development and ultimately for better yield.

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