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Influence of root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood infection on different plant growth parameters in Mungbean, *Vigna radiata* (L.) Wilczek

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Root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is a major threat to mungbean cultivation. The pest causes a significant reduction in plant growth parameters that ultimately results in loss of grain yield. The present study was carried out under glass house condition to study the effect of different inoculum load of root-knot nematode *M. incognita* on plant growth, nodulation and nematode development and nutrients status of Mungbean. The results revealed a progressive decline in plant growth parameters *viz*., fresh and dry shoot weight and shoot length with respect to increase in inoculum level. However, fresh and dry root weight showed the opposite trend. The fresh and dry shoot weight was decreased by 44% and 66%, respectively at 4 J_2s/g soil. The chlorophyll content in the leaves also decreased with the increase of inoculum level from 100-6000 J2s/pot. Nutrients contents of the plant *viz.* N, P, K, Ca and Mg were significantly reduced in shoots while in roots these was increased with an increase of inoculum levels. Nodulation was affected by 80% at the highest inoculum level i.e. 6000 J2s/pot. Also leghaemoglobin, bacteroid content and nitrogenase activity was reduced progressively with increased levels of nematode inoculum. Thus, the root-knot nematode, *M. incognita* interferes with the process of symbiotic nitrogen fixation between mungbean host and rhizobium and that can affect the quality of produce.

Keywords: Chlorophyll, Green gram, Nematode inoculum, Nitrogenase, Southern root-knot nematode

Root-knot nematodes (RKN), *Meloidogyne* spp. affect food grain production globally and have therefore been one of the most destructive plant pathogens[1]. These soil pathogens cause typical root galls that interfere with nutrients and water absorption resulting in nutritional deficiencies reflected in leaf yellowing and stunted growth which affect the yield of crops both qualitatively and quantitatively[2]. Root knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is the most important nematode species with worldwide distribution in tropical and subtropical climate. It is also having diversity of sub-species in the form of races which creates the population diversity in terms of variation in pathogenicity[3]. Among the several species of root-knot nematode, *M. incognita* constitute more than 40% population and has wide host range including pulse crop[4]. It is well known that its threshold limit on mungbean is 2 J_2s/g of soil and it may differ with variety of crops[1].

The mungbean (Mung or green gram or golden gram) an important short duration pulse crop is widely cultivated throughout the tropical countries South-East Asia particularly in India. Among the various pests and diseases associated with mungbean, plant parasitic nematodes particularly root-knot nematode (*M. incognita*) is an important limiting factor in the successful cultivation of the crop[5-7]. The pathogenic effect of root-knot nematodes on growth parameters, yield and nutrient uptake of leguminous crops have been reported by several workers and it is documented as potential threat to various leguminous plants[8-10.] The damage has been observed in crop in terms of the plant growth parameters however little information is available on how the plant growth is affected. Since the nodulation is an important feature for the sustenance of crop and root-knot nematodes which is root bound may have adverse effect on nodules and absorption of nutrients[11] in Peas and leghemoglobin content in mungbean[12]. Nematode also impairs root tissues which affect the absorption and translocation of minerals in root. It has implication on the chlorophyll content[13,14] of the plant thus, affecting the photosynthesis and ultimately the yield potential of the crop plant. Therefore, it is important to study the role of varying levels of root-knot nematode

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(*M. incognita*) on mungbean its plant nodule efficiency and nutritional content beside overall growth of the plant.

Materials and Methods

Seed coating with rhizobia and sowing

The experiment was conducted in 15 cm earthen pots in completely randomized design (CRD) under glasshouse situation with four replications. Field soil was collected and autoclaved from the fields of the Indian Agricultural Research Institute, New Delhi and used for experimental purpose and were later mixed with sand in the ratio of 3:1. Seeds of mungbean (*V. radiata*) cv. Pusa vishal was procured from the National Seed Corporation, IARI, New Delhi, India. Seeds coated with *Rhizobium leguminosarum* (Mungbean strain) at 2% as seed coat. Rhizobium strain of Mungbean was obtained from the culture collection of the Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi. This was multiplied on yeast extract mannitol medium on a rotary incubator shaker. A 72 h old broth culture was used in measured quantities for the experiment. Each pot was sown with five seeds and after germination at three leaf stage; these were thinned to one seedling per pot.

Root knot culture preparation and inoculation

Root knot nematodes, *M. incognita* was cultured in susceptible brinjal variety (Pusa vishal). The species was identified based on perineal pattern as *M. incognita*[15]. At the time of requirement of nematodes, the infested plants pulled out gently and washed in tap water to remove soil particles. The egg masses carefully removed under microscope and placed on modified Baermann funnel assembly. The infective juveniles 2nd stage (IJ_2s) were extracted (after 48 h), quantified and calibrated[16]. Inoculation was done in the 15 days old plants @ 0, 100, 1000, 2000, 4000, 6000 J2s/1000cc soil. All the treatments were replicated 4 times including control. The nematode suspension was poured in the root rhizosphere by making holes around the stem.

Mungbean growth *Vis-a-Vis* to nematode multiplication

The effect of root-knot nematode, *M. incognita* on plant growth parameters *viz.* shoot and root length (cm), fresh and dry shoot weight (g), fresh and dry root weight (g), pod weight and number of pods/plant of mungbean was observed after 35 days of nematode inoculation. Nematode development *viz*. number of root-knot gall/plant, number of egg masses/plant, number of eggs/egg mass and nematode population in soil/pot was also observed. The observations on various plant growth parameters *viz*. shoot and root length and fresh shoot and root weight was taken immediately after harvest of the experiment while dry shoot and root weight, were taken after keeping the shoot and root portions in oven at 40°C for four days. The number of bacterial nodules and root-knot galls/plant was counted after washing roots thoroughly and carefully in tap water. After 35 days of inoculation, an observation on nematode population was observed both in soil and root. The seedling along with clinging soil was kept erect depotted and immersed in water in a small bowl. After about 5 min, the loosened soil was gently rubbed off into the water and the seedling was taken out. The root system of the seedling was further washed in clean water to remove any soil debris adhering on roots. The plant from each replication were carefully uprooted, thoroughly washed and processed through sodium hypochlorite acid fuchsin glycerin method[16]. The roots were pressed between 2-3 folds of blotting paper to absorb the extra water and finally kept in glass Petri plates containing glycerin.

A composite sample of 100 g soil from each treatment was taken from the experimental pots and washed by Cobb's washing, decanting and sieving technique (1918) followed by Baermann funnel method (1917). The volume of the suspension thus obtained was measured. One mL of this suspension was taken in a counting dish to count the number of nematodes present in each treatment with the help of stereoscopic binocular microscope (Carl Zeiss, Germany).

The effect of root-knot nematode on nodulation of mungbean was observed after 35 days of nematode inoculation by taking observations *viz*. weight of fresh nodules/plant (g), number of nodules/plant, fresh weight of one nodule (g), nitrogenase enzyme activity (in 'n' moles of ethylene produced/ g nodule fresh weight), bacterial population (cfu/mL) and leghaemoglobin (Lb) content from fresh nodule.

Estimation of Bacterial population and nitrogenase enzyme activity

Bacterial population from root nodules was observed by dilution plating method[1]. Fresh nodules were detached from both inoculated and uninoculated mungbean plant roots and exact quantity of nodules were taken and sterilized by 0.1% mercuric chloride.

Then all nodules were washed 10 times by distilled water and different treatments of root nodules crushed in small beaker by adding some quantity of distilled water and serially diluted. Appropriate dilutions of CRYEMA media was prepared and spread on Petri plates and these Petri plates was incubated at 30 ± 2°C for 5 days and then number of colonies, morphologically similar to rhizobial colonies developed was counted. The bacterial population was expressed as cfu/mg fresh weight of nodules.

Nitrogenase enzyme activity was observed by Acetylene reduction assay technique (ARA) [17,-18]. After incubation in 10% acetylene atmosphere for 1 h, ethylene produced by the nodules was measured using gas chromatograph (Nucon model 5765) and the activity was expressed as 'n' moles ethylene produced per hour per g fresh nodules[19].

ARA activity was calculated by the formula:

'n' moles of C_2H_4 produced hr^{-1} mg^{-1} protein/mg nodule fresh wt. = $\frac{C \times Ps \times As \times V}{Pstd \times Astd. \times T \times P}$

where: C = concentration of ethylene in the standard in 'n' moles; Ps = Peak area of sample; As = Attenuation used for sample; P std = Peak area of standard; A std = Attenuation used for standard; T = time of incubation in hrs.; P = Protein content of bacterial growth on slant in mg/ mg nodule fresh wt.; V = Volume of air space in the assay vial

Estimation of Leghaemoglobin content

Fresh root nodules weighing 0.5 g were washed in sterile distilled water (SDW) and crushed in sterile pestle mortar and in phosphate buffer (50 mM, pH 7.0). These were then transferred in test tubes. The mixture was centrifuged (15 min at 500 ×g) and transferred supernatant into 10 mL volumetric flask. The haemochrome was measured at 556 nm. Leghemoglobin content of nodules was estimated using the formula as given below:

$$Lb \text{ concentration (mM)} = A_{556} - A_{539} \times 2D/23.4$$

where D is the initial dilution. (The calculation is based upon the equation, $E = 23.4 \times 10^3$ mol^{-1} cm^{-1})

Laboratory analysis of plant samples (estimation of total N, P, K, Ca and Mg)

Total nitrogen of inoculated and uninoculated plants was estimated for shoot and root separately by the Kjeldahl method[20]. About 0.5 & 0.2 g material of the oven dried shoot and root respectively was used for this experiment. For Phosphorous, the same material (roots and shoots) digested by Tri-acid mixture (HNO_3:H_2SO_4: HCL: 9:4:1) and then transferred to a volumetric flask and further diluted with distilled water. After making the requisite volume, samples was filtered. Phosphorous was estimated colorimetrically by yellow colour method[21] and expressed as percentage of dry matter. The same digested sample as prepared for phosphorous was used to estimate total potassium and was measured by flame photometric technique using Corning flame photometer which is a direct reading type instrument[21].

For calcium and magnesium also, same digested sample as prepared for phosphorous was used to estimate the total calcium and magnesium and was measured by Atomic absorption spectrophotometer (AAS) which is also a direct reading type instrument.

Observations on chlorophyll analysis

Chlorophyll analysis experiment was observed by Dimethoxide method (DMSO). 0.050 g of fresh leaf samples of different treatments (both inoculated and uninoculated nematode) was added in 10 mL of DMSO solution in glass tubes and all samples were kept in oven dried at 55-60°C for 4 h. Then sample reading for chlorophyll analysis was observed by using spectrophotometer at 645 and 663 nm frequencies.

The formula for the amount of chlorophyll present in the extract is:

mg chlorophyll a/g tissue = 12.7 (A_{663}) –2.69 (A_{645}) × V/1000XW; mg chlorophyll b/g tissue = 22.9 (A_{645}) - 4.68 (A_{663}) × V/1000XW; mg total chlorophyll/g tissue = 20.2 (A_{645}) +8.02 (A_{663}) × V/1000XW

where A= absorbance at specific wavelengths, V= final value of chlorophyll extracts in DMSO, and W= fresh weight of tissue extracted.

Results

Effect of varying levels of RKN on plant growth of Mungbean

The data (Table 1) revealed that plant growth was decreased progressively with the increase of inoculum levels. Shoot length was observed to be significantly ($P \leq 0.05$) low at or above 2000 J_2s/plant in comparison to uninoculated control. However, shoot length was also affected significantly in nematode inoculum range (2000-6000 J_2s/plant) and these were statistically similar amongst themselves. The shoot length was not affected significantly up to 1000 J_2s/plant. The percent reduction in shoot length was nearly 8% with nematode load of 1000 J_2s/plant which is further reduced to more than 40% at or above 2000 J_2s/plant.

Table 1 — Effect of different inoculum level of root knot-nematode, *Meloidogyne incognita* on plant growth parameters of Mungbean (cv. Pusa vishal) (Mean of four replications)

Inoculum level (J_2s/pot)	Shoot length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Root length (cm)	Dry shoot weight (g)	Dry root weight (g)	No. of pods/plant	Pod weight/plant (g)
0	50.00^{a}	18.20^{a}	2.25^{cd}	19.35^{a}	7.23^{a}	0.65^{c}	3.50^{a}	4.03^{a}
100	43.00^{a} (–14%)	15.40^{ab} (–15.38%)	3.30^{bc} (46.67%)	16.73^{b} (–13.57%)	6.05^{b} (–16.35%)	0.75^{b} (14.35%)	2.75^{ab} (–21.43%)	3.43^{ab} (–14.91%)
1000	42.75^{a} (–14.50%)	14.18^{abc} (–22.12%)	3.55^{ab} (57.78%)	14.65^{c} (–24.29%)	3.58^{c} (–50.49%)	0.83^{b} (26.32%)	1.25^{bc} (–64.29%)	1.28^{cd} (–68.32%)
2000	31.50^{b} (–37%)	11.75^{bc} (–35.44%)	3.83^{ab} (70%)	11.45^{d} (–40.83%)	3.20^{c} (–55.79%)	1.03^{a} (58.26%)	1.00^{c} (–71.43%)	0.73^{cd} (–81.99%)
4000	28.00^{b} (–44%)	10.18^{c} (–44.09%)	4.50^{ab} (100%)	10.05^{e} (–48.06%)	2.74^{c} (–62.07%)	0.98^{a} (49.66%)	0.75^{c} (–78.57%)	0.38^{d} (–90.68%)
6000	26.25^{b} (47.50%)	11.93^{bc} (–34.48%)	4.75^{a} (111.11%)	10.33^{e} (–46.64%)	2.53^{c} (–65.02%)	0.95^{a} (46.06%)	0.50^{c} (–85.71%)	0.22^{d} (–94.41%)
Without rhizobium & nematode	48.00^{a} (–4%)	14.15^{abc} (–22.25%)	1.60^{d} (–28.89%)	17.50^{b} (–9.56%)	3.59^{c} (–50.34%)	0.42^{d} (–35.62%)	1.75^{bc} (–50%)	2.18^{bc} (–45.96%)
CV (%)	16.80	20.72	24.78	5.27	18.31	7.30	71.53	64.17
SE(d)	4.57	2.0	0.59	0.53	0.53	0.04	0.83	0.79
CD (P= 0.05)	9.51	4.1	1.2	1.10	1.11	0.08	1.72	1.64

[Figures in parenthesis () indicate percent gain or reduction over control. Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Tukey's HSD test]

Similarly, the fresh shoot weight was decreased with increase in inoculum levels. At highest inoculum level fresh shoot weight was decreased by 44% over control. Shoot weight declined in the range of 15-44% in various treatments. However, dry shoot weight reduced in the range of 49-75% in various treatments. In contrast, fresh and dry root weight was increased with the increase in inoculum levels. Based on critical difference value, root weight was similar for 100 J_2s/plant and 1000 J_2s/plant and also for 2000, 4000 and 6000 J_2s/plants however, it was significantly different in treatments 100-1000 J_2s/plant and 2000 and above J_2s/plant.

There were progressive reduction in both the number of pods and pod weight compared to the uninoculated check plant with the increase in inoculum levels. The weight and number of pods per plant reduced in the range of 14-95% and 21-85% respectively in various treatments over untreated control. The maximum and minimum reduction had been in the highest inoculum and lowest inoculum level respectively. The number of pods was not significantly ($P \leq 0.05$) altered up to 4000 J_2s/plant while pod weight was significantly altered even at 1000 J_2s/plant. Thus, there was all possibility that nematode had potential to affect yield of crop even at $1J_2$/g soil. The treatments above 1000 J_2s/plant, pod weight was similar.

Plant growth parameter was improved in the presence of rhizobium in comparison to non-rhizobia treatment. The comparative reduction in shoot weight of fresh and dry biomass indicated that percent reduction was more in dry mass with the increase in inoculum levels. However, fresh root and dry root weight increased among treatment.

There was significantly less number of nodules in all treatments where nematodes were inoculated. The number of nodules was reduced up to 87% at the highest inoculum level. Similarly, weight of nodules/plant and weight of one nodule/plant reduced significantly ($P \leq 0.05$) due to increase in nematode population density. Weight of nodules per plant and average weight of one nodule was observed at 2000, 4000 and 6000 J_2s/pot as 0.17, 0.13, 0.10 g and 0.004, 0.007 and 0.008 g, respectively compared to the uninoculated check (nodules weight/plant 0.74 g and one nodule weight/plant 0.008 g). More than 60% reduction in both the parameters at highest inoculum level was observed. Rhizobium inoculated plants without nematodes had conspicuously large and pink coloured nodules whereas nodules on nematode infested plants were brownish in colour.

Nematode development, multiplication and chlorophyll content

The number of galls (Table 2) increased with the increase of the initial inoculum level up to 2000 J2s per plant and above this there was reduction in the number of galls. Similar trend was observed with number of egg masses and eggs per egg mass. The number of eggs/egg mass were 426.50 at 2000 J_2s inoculum level, which reduced to 195.50 at the inoculum level of 6000 J_2s per plant. Reproduction factor decreased with the increase of inoculum level.

Nematode infection reduced chlorophyll a, b and total chlorophyll (a+b) contents in the leaves at all levels of inoculum in comparison to uninoculated control (Table 2). Chlorophyll 'a' was observed to be affected by 20-40% amongst various treatments and this was also statistically reduced between the treatments. Chlorophyll 'b' was also affected similarly amongst treatments. The significant ($P \leq 0.05$) reduction in chlorophyll level was recorded even at the lowest nematode load i.e. 100 J_2s/plant. The total chlorophyll count was reduced in the range of 20-54% amongst various treatments. Chlorophyll 'a' and 'b' do not differ significantly ($P \leq 0.05$) above 2000 J2s/plant treatments.

Nitrogenase activity, leghaemoglobin and bacterial contents of nodules

Acetylene reductase activity (*ARA*)

Nitrogenase enzyme, leghaemoglobin and bacterial contents (Table 3) of nodules were significantly ($P \leq 0.05$) reduced by nematode infection. Inoculum levels of 6000 J2s/pot caused maximum reduction of ARA activity (430.85 moles ethylene produced /g

Table 2— Effect of different inoculum level of root-knot nematode, *Meloidogyne incognita* on nematode multiplication and chlorophyll content in Mungbean (cv. Pusa vishal) (Mean of four replications)

Inoculum level (J_2s/pot)	No. of galls	No. of egg mass	Eggs/ egg mass	Soil nématode population	RF	Chl a (µg/ mL)	Chl b (µg/ mL)	Total chlorophyll (µg/ mL)
0	0^{c} (1.0)	0^{e} (1.0)	0^{d} (1.0)	0^{d} (1.0)	0	2.35^{a}	0.65^{a}	3.00^{a}
100	58.75^{c} (8.29)	43.75^{d} (6.63)	224.25^{c} (15.0)	356.75^{c} (18.79)	178	1.87^{b} (–20.54%)	0.55^{b} (–16.44%)	2.41^{b} (–19.65%)
1000	257.50^{b}(16.07)	67.75^{c} (8.27)	373.25^{ab}(19.36)	492.50^{b} (22.04)	24.6	1.51^{c} (–35.65%)	0.41^{cd} (–36.66%)	1.93^{c} (–35.87%)
2000	479.75^{a}(21.9)	197.25^{a} (14.07)	426.50^{a} (20.6)	742.50^{a} (27.24)	18.1	1.27^{d} (–45.83%)	0.34^{de} (–47.74%)	1.6^{d} (–46.24%)
4000	265.25^{b}(16.3)	145.50^{b} (12.08)	342.25^{b} (18.5)	357.50^{c} (18.86)	4.1	1.12^{d} (–51.41%)	0.30^{ef} (–54.30%)	1.42^{d} (–52.82%)
6000	215.75^{b}(14.7)	75.25^{c} (8.71)	195.50^{c} (14.0)	251.25^{c} (15.85.)	2.0	1.12^{d} (–52.42%)	0.25^{f} (–62.19%)	1.30^{d} (–54.55%)
Without rhizobium & nematode	0^{c} (1.0)	0^{e} (1.0)	0^{d} (1.0)	0^{d} (1.0)	0	2.15^{a} (–8.37%)	0.47^{c} (–28.64%)	2.62^{b} (–12.77%)
CV(%)	38.80	13.18	23.98	24.07		9.49	12.06	8.44
SE(d)	50.05	7.04	37.83	53.50		0.10	0.03	0.12
CD(P=0.05%)	(104.1)	(14.65)	(78.67)	(111.26)	?	0.22	0.07	0.25

[Figures in parenthesis () are sqrt (x+1) transformed value and percent (%) values in bracket indicate reduction over control. Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Turkey's HSD test]

Table 3 — Effect of different inoculum level of root-knot nematode, *Meloidogyne incognita* on nodulation in Mungbean (cv. Pusa vishal) (Mean of four replications)

Inoculum level (J_2s/pot)	No. of nodules per plant	Weight of nodules per plant (g)	Average wt. of one nodule (g)	Acetylene reduction activity 'n' moles of ethylene produced/g fresh wt. of nodules	Leghaemoglobin (mM)/g fresh wt. of nodules	Bacterial population (10^6 cfu/g fresh wt. nodules)
0	92.00^{a}	0.74^{a}	0.008^{a}	1782.50^{a}	3.54^{a}	9.46^{a}
100	75.50^{b} (–17.93%)	0.41^{b} (–45.04%)	0.005^{c} (–37.50%)	1672.09^{a} (–6.19%)	2.97^{ab} (–16.08%)	7.55^{b} (–20.20%)
1000	54.50^{c} (–40.76%)	0.33^{bc} (–56.21%)	0.006^{bc} (–25%)	848.27^{b} (–52.41%)	2.08^{bc} (–41.32%)	4.07^{c} (–57.02%)
2000	34.75^{d} (–62.23%)	0.17^{cd} (–76.69%)	0.004^{c} (–50%)	787.54^{b} (–55.82%)	1.82^{c} (–8.44%)	1.98^{d} (–79.04%)
4000	17.00^{e} (–81.52%)	0.13^{cd} (–83.05%)	0.007^{ab} (–12.50%)	582.16^{b} (–67.34%)	1.22^{cd} (–65.47%)	1.72^{d} (–81.79%)
6000	11.50^{e} (–87.50%)	0.10^{d} (–86.81%)	0.008^{a} (0%)	430.85^{b} (–75.83%)	1.13^{cd} (–68.01%)	1.36^{d} (–85.62%)
Without rhizobium & nematode	56.00^{c} (–39.13%)	0.28^{bcd} (–61.99%)	0.005^{bc} (–37.50%)	633.31^{b} (–64.47%)	0.81^{d} (–77.06%)	3.80^{c} (–59.85%)
CV (%)	8.56	46.92	21.71	43.89	33.52	13.30
SE(d)	2.95	0.10	0.001	298.6	0.46	0.40
CD ($P = 0.05$)	6.13	0.21	0.01	621.2	0.95	0.83

[Figures in parenthesis () indicate percent reduction over control. Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Tukey's HSD test]

Table 4 — Effect of different inoculum level of root-knot nematode, *Meloidogyne incognita* on nutrients in shoots and roots of Mungbean (cv. Pusa vishal) (Mean of four replications)

Inoculum level (J_2s/pot)	Nitrogen (%)		Phosphorous (%)		Potassium (%)		Calcium (%)		Magnesium (%)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	1.44^{a}	0.64^{d}	0.48^{a}	0.21^{c}	3.83^{a}	0.91^{cd}	6.24^{a}	1.54^{e}	0.98^{a}	0.74^{d}
100	0.91^{b}	0.80^{cd}	0.32^{b}	0.29^{b}	3.35^{b}	1.26^{c}	5.76^{a}	2.29^{d}	0.53^{b}	0.89^{cd}
1000	0.79^{c}	0.88^{bc}	0.22^{c}	0.32^{b}	3.15^{b}	2.02^{b}	3.74^{b}	3.18^{c}	0.40^{bc}	1.13^{bc}
2000	0.63^{d}	1.02^{ac}	0.12^{d}	0.40^{a}	3.00^{b}	2.69^{a}	2.73^{b}	3.78^{b}	0.40^{bc}	1.35^{b}
4000	0.53^{de}	1.14^{a}	0.11^{d}	0.41^{a}	2.53^{c}	2.73^{a}	0.99^{c}	4.49^{a}	0.27^{c}	1.71^{a}
6000	0.42^{e}	1.07^{ab}	0.10^{d}	0.41^{a}	2.38^{c}	2.76^{a}	0.83^{c}	4.58^{a}	0.22^{c}	1.82^{a}
No *Rhizobium*	0.98^{b}	0.62^{d}	0.25^{c}	0.20^{c}	3.14^{b}	0.85^{d}	3.31^{b}	1.35^{e}	0.90^{a}	0.94^{cd}
CV (%)	8.86	18.30	14.89	11.31	8.16	13.45	25.77	7.21	31.59	14.11
SE(d)	0.05	0.11	0.02	0.02	0.17	0.17	0.61	0.15	0.11	0.12
CD (P= 0.05)	0.10	0.23	0.04	0.54	0.36	0.37	1.27	0.32	0.24	0.25

[Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Tukey's HSD test]

fresh wt. of nodules) compared to the uninoculated check plant (1782.50 moles ethylene produced per /g fresh weight nodules). Similarly, leghaemoglobin was also observed minimum (1.13 mM/g fresh wt of nodules) compared to the check (3.54 mM/g fresh wt. of nodules) as compared to 0.81 mM/g fresh wt. of nodules at 6000 J_2s/pot. Bacteroid contents in nodule recorded were 1.98, 1.72 and 1.36 (10^6 cfu/g fresh wt nodules) at 2000, 4000 and 6000 J_2s/pot, respectively compared to check 9.46 (10^6 cfu/g fresh wt nodule). In an uninoculated check the plant with rhizobia, better ARA activity, leghaemoglobin and bacteroid content of nodules was observed than nematode inoculated ones. Thus, all these parameters were statistically significantly affected especially nodule number and weight with 100 J_2s/plant onward and ARA activity and leghemoglobin at 1000 J_2s/plant onward.

Nutrients status in plant (Shoot and Root)

A perusal of the data (Table 4) revealed that the concentration of macronutrients *viz.* N, P, K, Ca and Mg were significantly ($P \leq 0.05$) changed due to nematode infection as compared to the uninoculated plants with rhizobia in both root and shoot. Nitrogen level in the shoot decreased with the increase of the inoculum level while in root it was increased. The significant difference in nitrogen in shoot was not observed in treatments except at the highest level of nematode inoculum. Similar trend was observed with phosphorus as well as potassium which increased in roots by 90% and in the shoots, reduced by 80%. Even though phosphorus content reduction in shoot was significantly ($P \leq 0.05$) altered at the lowest inoculum level. Potassium level also altered similarly however no differences were found in treatments above 2000 J_2s/plant. Calcium and magnesium was affected more than 100% at the highest inoculum in shoot. If the accumulation of nutrients is noted in root it may also be following a similar trend as the concentration was increased to 100% in the treated plants comparison to uninoculated. As a result, the nutrient level was highest in the highest inoculum level in the root, whereas it was lowest in the shoot in the highest inoculum level. Therefore, it could be inferred that there was direct positive correlation between levels of nematode inoculum and nutrient content in root and nutrient contents were inversely related with inoculum level in shoot.

Discussion

Root-knot nematode damage to mungbean crop is linked to the level of nematode density in the soil. As the population density increases, the effect is visible in terms of reduction in plant growth of mungbean which might be due to number of factors such as reduced number of nodule formation and its efficiency. In contrast to the reduction in the length and weight of shoots, root weight increased in infected plant possibly due to the formation of giant cell and galls. Giant cells provide a nutrient sink on which the nematode feeds. As a result, the plant is no longer able to provide nutrients to its upper part. This limitation of nutrient elements in the plant is probably the first effect that nematode has on the physiology and metabolism of its host.

Likewise, Joshi *et al.*[22] reported reduction in shoot length, shoot weight (fresh and dry) and chlorophyll content of mungbean by more than 50% at various levels of *M. incognita* inoculation[12]. They also found reduction in number of nodules by more than 90%. Nitrogenase, leghemoglobin and bacterial contents were also affected severely. Similarly, pod weight, pod number, plant growth parameters along with number of nodules and weight were found to be

severely affected by different levels of *M. javanica* on Peanut as reported by Sheriff *et al*.[23] and Osman *et al*.[24].

In the present study, the levels of N, P, K, Ca and Mg were significantly ($P \leq 0.05$) changed due to the nematode infection. The nutrient contents were more in root tissues compared to the shoots. These indicate that nematodes have impaired the upward movement of the nutrients due to damage done by different levels of nematode inoculum in the vascular tissues of root. Similarly, in one study it was observed that the increasing level of *Heterodera cajani* inoculum was negatively correlated with N, P, K, Zn, Mn and Cu but positive correlation with those of Ca, Mg, and Fe in Urdbean (*Vigna mungo*)[6]. The reductions in level of nutrients also have low chlorophyll content. There has also been reduction in the growth and physiological function of French bean with an increase in initial inoculum level of *M. incognita*[25,26]. The increase in root weight as a result of infection by nematode could also be compensatory mechanism in plant. The effect of varying levels of root-knot, reniform and stunt nematode on Chickpea was studied that plant biomass, nodules number and chlorophyll content was significantly ($P \leq 0.05$) affected by varying levels of nematodes[27]. *M. incognita* invaded aseptic roots of *Pisum sativum* and *Phaseolus vulgaris* and found that juvenile nematodes invaded nodules initiated by rhizobium. *M. incognita* suppressed root and nodule growth. However, *M. incognita* stimulated the initiation of nodules which remained undeveloped[28]. Similar findings reported on French bean infested with *M incognita*[25].

The extensive studies on lentil infected with root-knot nematode revealed that nitrogen, potassium and chlorophyll contents are affected by duration and level of infestation. Besides the plant weight, most elements and yield component decreased significantly ($P \leq 0.05$) with increasing level of infestation[29].

The damage potential of root-knot nematode to mungbean is clear if we see the overall gap in nutrient level of plants infected *vs*. non-infected. The root-knot nematode infestation has multiple damaging effects on the plant growth, development and efficiency of root system of crop plants. With regards to the relation of nematode with nodulation it could be understood that nematode and bacteria compete for space in root-knot nematode modified physiology of plant and it may not suit well to bacterium for nodule formation as observed with reduction in nodulation[30]. However, cause and effect relationship are not clearly linked.

Ali *et al*.[31] observed that root-knot nematode induced giant cells formation inside the vascular bundles of the root in the nodules of *Vigna unguiculata*. They also observed a mature female inside the bacterial tissue inducing syncytia in the cortex. Wright *et al*.[2] have also reported that *M. incognita* developed and reproduced in the nodular tissues of *Trifolium alexandrinum*, *Vicia faba*, *Lupinus termis* and *Pisum sativum* and further observed that nematode did not alter structural details of nodules in spite of the presence of giant cells. In all the tested hosts, giant cells had unbroken walls, dense cytoplasm and clusters of nuclei[2]

Number of nodules was not completely inhibited by nematode though there was drastic reduction above 80% in nodule count in highest level of nematode inoculum. Almost similar reduction happened with 4000 J_2s/g soil. While at 2000 J_2s/g soil reduction in nodules was 62%. This showed that nodules were not proportionately reduced with the increase of inoculum level. In general, 60 nodules were reduced by inoculum of 2000 J_2s/plant. It means that 35 nematodes were responsible for the reduction of one nodule. Similarly, damage threshold limit was found to be 2000 J_2s/g soil based on fresh shoot length and shoot weight. However, chlorophyll content was significantly ($P \leq 0.05$) lower even at 100 J_2s/g soil. Khan *et al*.[32] reported significant reduction ($P \leq 0.05$) in plant growth parameters (Plant length, fresh and dry weight of plant, seed weight, and number of nodules/root system) at an initial inoculum level of 1000 J_2s of *M. incognita*, 2000 J_2s of *M. javanica* and *M. arenaria* of 4000 J_2/kg soil on Mungbean. It was also observed that with the increase in the level of inoculum there was progressive increase in host infestation as indicated by the number of galls as well as nematode multiplication[33]. Moreover, the trend in nematode multiplication seems to be negatively correlated with the inoculum of root-knot nematodes. It was concluded by them that the pathogenic level of *M. arenaria*, *M. incognita* and *M. javanica* on Mungbean were 4000, 2000 and 1000 J2s/kg soil, respectively.

Influence of phytoparasitic nematodes on symbiotic nitrogen fixation in tropical herbaceous legume cover crops Mucuna and Lablab showed that biomass yield of Mucuna was not significantly affected by either *Meloidogyne* spp. or the other

genera of phytoparasitic nematodes[34]. In contrast, the dry matter yield of lablab measured at 12 weeks was reduced by 16% in inoculated compared with fumigated soils. Inoculation with *Meloidogyne* spp. Significantly ($P \leq 0.05$) increased the number of nodules on Lablab roots compared with noninoculated treatments thus, nodulation in Mucuna was not negatively affected by soil treatment[34]. Vovlas *et al.*[35] revealed that root knot nematode infestation of Siratro (*Macroptilium atropurpureum*) can increase or decrease nodule functionality. The crops wherein nodule function is unaffected by the presence of nematode might be tolerant.

Conclusion

The root-knot nematode, *Meloidogyne incognita* affects symbiotic nitrogen fixation not only by reducing the number of nodules but also by disturbing the functioning of nodules due to decrease in the bacteroid and leghaemoglobin contents of nodules of Mungbean. Thus, nematode plays great role in the interaction between rhizobia and plant. Nematode has affected the major nutrient supply to plants which actually determine the plant growth and yields. However, damage to plant is nematode density dependent.

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Conflict of interest

Authors declare no competing interests.

References

1 Sasser JN & Freckman DW, A World Perspective on Nematology: The Role of the Society. In: *Vistas on Nematology* (Eds. JA Veech & DW Dickson Hyattsville, Maryland) 1987, 7.

2 Bernard GC, Egnin M & Bonsi C, The Impact of Plant-Parasitic Nematodes on Agriculture and Methods of Control. In: Nematology- Concepts, Diagnosis and Control. (Eds. MM Shah & M Mahamood; Intechopen, London), 2017, 121.

3 Caillaud MC, Dubreuil G, Quentin M, Perfus-Barbeoch L & Lecomte P, Root-knot nematodes manipulates plant cell functions during a compatible interaction. *J Plant Physiol*, 165 (2008) 104.

4 Karssen G & Moens M, Root-knot nematodes. In (R. N. Perry & M. Moens (Eds.), Plant nematology. Wallingford: CABI International) 2006, 59.

5 Chakrabarti U, Nageswari S & Mishra SD, In-vitro study on the effect of neem products on germination of mungbean seeds. *Current Nematology* 12 (2001) 35.

6 Verdejo S, Green CD & Podder AK, Influence of *Meloidogyne Incognita* on Nodulation and Growth of Pea and Black Bean. *Nematologica* 34 (1988) 88.

7 Vishwadhar, paper presented to the national symposium on Pulses for crop diversification and natural resource management. Indian Institute of Pulse Research, Kanpur, India, 20-22 December, 2003.

8 Gupta DC, Paruthi IJ & Verma KK, Reaction of mungbean germplasms and its pathogenicity against *Meloidogyne javanica. Indian J Nematol*, 16 (1986) 194.

9 Bhagwati B, Phukan PN, Pathogenicity of root-knot nematode, *Meloidogvne incognita* on pea. *Indian J Nematol*, 21(1991) 141.

10 Kalita DN & Phukan PN, Pathogenicity of *Meloidogyne incognita* on black gram. *Indian J Nematol* 23 (1993) 105.

11 Kumar R, Dhillon NK, Kaur S, Anupam & Srari A, Resistance in Mungbean against *Meloidogyne incognita* and its Impact on Nodulation. *Legum Res*, 43 (2020) 1.

12 Chahal PPK & Chahal VPS, Adverse effect of *Meloidogyne incognita* on the functioning of nodules of mungbean (*Vigna radiata*). *Nematol Mediterr*, 15 (1987) 13.

13 Ahmed NM, Waseem A, Shaukat SS & Zaki MJ, Physiological changes in leaves of mungbean plants infected with *Meloidogyne javanica*. *Phytopathol Mediterr*, 48 (2009) 262.

14 Perveen K, Haseeb A & Shukla PK, Pathogenic potential of *Meloidogyne incognita* on *Mentha arvensis* cv. Gomti. *Indian J Nematol*, 36 (2006) 157.

15 Eisenback JD, Hirschmann H, Sasser JN & Triantaphyllou AC, A Guide to the Four Most Common Species of Root-Knot Nematodes *(Meloidogyne* Spp.) with a Pictorial Key. Department of Plant Pathology North Carolina State University Raleigh, NC 27650 USA, 1981.

16 Byrd DW, Kirkpatrich Jr & Backer KR, An improved technique for cleaning and staining plant tissues for detection of nematodes. *J Nematol*, 15 (1983) 142.

17 Hardy RWF, Burns RC, Hebert RR, Holsten RD & Jackson EK, Biological nitrogen fixation: a key to world protein. *Plant Soil*, 35 (1971a) 561.

18 Hardy RWF, Burns RC, Hebert RR, Holsten RD & Jackson EK, *Applications of the acetylene-ethylene assay for measurement of nitrogen fixation*. Paper presented to the Symposium on Nitrogen Economy of Plant Communities. 12th Pacific Science Congress, Canberra, Australia, 1971b.

19 Sadasivam S & Manickam A, *Biochemical Methods for Agricultural Sciences.* (New age international (P) Publisher, Uttar Pradesh, India), 1992.

20 Piper CS, *Soil and Plant Analysis*, IVth Edn. (University of Adeleide, Australia), 1966, 135.

21 Jackson ML, *Soil Chemical Analysis*. (Prentice-Hall of India Pvt. Ltd., New Delhi, India), 1973.

22 Joshi V, Kumar S & Rawat S, Study on infection and development of Root-Knot Nematode, *Meloidogyne javanica* on mungbean. *J Entomol*, 8 (2020) 1621.

23 El-Sherif AG, Refaei AR & Gad SB, The role of different inoculum levels of *Meloidogyne javanica* juveniles on nematode reproduction and host response of peanut plant. *Pak J Biol Sci*, 12 (2009) 551.

24 Osman HA, Ameen HH, Mohamed M & Elkelany US, Efficacy of integrated microorganisms in controlling root-knot nematode *Meloidogyne javanica* infecting peanut plants under field conditions. *Bull Natl Res Cent*, 44 (2020) 134.

25 Singh DB & Reddy PP, Influence of *M. incognita* infestation on rhizobium nodule formation in French bean. *Nematol Mediterr*, 8 (1981) 1.
26 Sharf R & Hisamuddin, Effect of *Meloidogyne incognita* on the growth, physiology and expression of ME-1 gene and pathogenesis related proteins in *Phaseolus vulgaris. Acta Sci Agric*, 3 (2019) 111.
27 Tiyagi SA & Alam MM, Effect of root-knot, reniform and stunt nematodes on plant growth, water absorption capability and chlorophyll content of chickpea. Intern Chickpea. *Newsl*, 22 (1990) 40.
28 Tiwari SP, Interaction of *Heterodera cajani* and *Rhizoctonia bataticola* with *Vigna mungo. Ann Plant Protect Sci*, 6 (1998) 33.
29 Hisamuddin SS & Azam T, Pathogenicity of root-knot nematode, *Meloidogyne incognita* on *Lens culinaris* (Medik.). *Arch Phytopathol Pflanzenschutz*, 43 (2010) 1504.
30 Elhady A, Hallmann J & Heuer H, Symbiosis of soybean with nitrogen fixing bacteria affected by root lesion nematodes in a density-dependent manner. *Sci Rep*, 10 (2020) 1619.
31 Ali MA, Trabulsi, IY & ABD-Elsamea, M.E, Antagonistic interaction between *Meloidogyne incognita* and *Rhizobium leguminosarum* on Cowpea. *Plant Dis*, 65 (1982) 432.
32 Yousif GM, Histological responses of four leguminous crops infected with *Meloidogyne incognita. J Nematol*, 11 (1979) 395.
33 Siengchin K, Ruanpanun P, & Somta P, Damage potential of root-knot nematode (*Meloidogyne incognita* chitwood) population density on plant growth parameters related to plant age of mung bean (*Vigna radiata* (l.) wilczek), *J Int Soc Southeast Asian Agric Sci*, 26 (2020), 111.
34 Ibewiro B, Sanginga N, Vanlauwe B & Merckx R, Influence of phytoparasitic nematodes on symbiotic N2 fixation in tropical herbaceous legume cover crops. *Biol Fertil Soils*, 3 (2000) 254.
35 Vovlas N, Vovlas A, Leonetti P, Liebanas, G, Castillo P, Subbotin SA & Palomares Rius JE, Parasitism effects on white clover by root-knot and cyst nematodes and molecular separation of *Heterodera daverti* from *H. trifolii*. *Eur J Plant Pathol*, 143 (2015) 833.