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# Effects of management practices of root-knot nematode, *Meloidogyne incognita* on growth of mungbean [*Vigna radiata* (L.) Wilczek]

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## ABSTRACT

In glass house, we studied the effects of different management practices [carbofuran @ 1 and 2 kg a.i./ha$^{-1}$, *Trichoderma harzianum* @1 and 2 % w/w and neem (*Azadirachta indica* L.) oil @ 1 and 2 % w/v (1500 ppm)] as seed treatment on root-knot nematode (RKN) and their impact on plant growth of mungbean. The neem oil (i). stimulated the nodule formation and functioning, (ii). improved the uptake of major nutrients [N (1.65 %), P (0.39 %), K (2.58 %), Ca (4.28 %) and Mg (0.58 %)] and (iii). increased shoot length (32.59 %), root length (12.89 %), fresh and dry weight of shoot (57.07 % and 75.41 % resp.) than untreated inoculated control plants. However, it decreased the root fresh (11.83 %) and dry weight (31.26 %) over control. While carbofuran treatment, showed decreased plant growth than control. The nematode incidence [root galls/plant, egg masses/plant and nematode population] was least with carbofuran followed by neem oil and *T. harzianum*. Neem oil significantly enhanced plant yield (number and weight of pods per plant) by 70% and 108%, respectively, compared to other treatments including carbofuran, *T. harzianum*, and control. The neem oil and *T. harzianum* not only provided good biocontrol of root-knot nematode but also enhanced to vegetative growth and nodulation in mungbean.

**Keywords:** Carbofuran, *Meloidogyne incognita*, mungbean, neem oil, nodulation, rhizobium, root-knot nematode, *Trichoderma harzianum*, *Vigna radiata*

## INTRODUCTION

Pulses are an integral component of Indian cropping systems due to their low water requirement and improving the soil nitrogen status (34). Mungbean [*Vigna radiata* (L.) Wilczek] is one of the major leguminous crop for protein supply to majority of Indians rely (2). The crop is grown in loamy soil, which favours the growth and multiplication of Plant parasitic nematodes (PPNs) (41). Among the PPNs, the root-knot nematode (RKN), *Meloidogyne incognita* is major biotic constraint of mungbean. In India, this nematode causes 19-40 % economic loss (1), but, limited research has been conducted on the effects of RKN on the growth, yield and rhizobial nodule formation in mungbeans. Moreover, the relationship between age of mungbean crop and RKN infection (gall size and giant cell formation) is poorly understood (25,40). The root-knot disease of mungbean is controlled by chemical, cultural, biological and integrated methods. Chemical management mostly with systemic nematicides like carbofuran is effective against disease affecting the nodulation (38). However, the effects of pesticides on the physiological functions of plants and on the development and function of nodules needs to be known (8). Neem oil have numerous uses in plant disease management and nematode control (7), because the neem products and their by-products directly affect the plants microbial interactions. Advances in biological disease control and the search for novel, distinctive natural microbial antagonists

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of plant pathogens have become emerging areas of research in agricultural sciences (12) since past two decades (3,23,24). Biological pest management is considered a natural control method and plays an important role in integrated pest management (11,43). The use of *T. harzianum* to control root-knot nematodes (5,30,45) and other PPNs (29) is promising control under natural system.

This study aimed to assess the relative effectiveness of carbofuran, neem oil and fungal bioagent (*T. harzianum*) against the mungbean root-knot nematode and impacts on nodule growth and function. Besides plant growth parameters, the nutritional status of plant's macronutrients due to their application and the role of treatments as tolerance inducer have also been investigated.

## MATERIALS AND METHODS

### Seed and soil

The pot experiment was carried out at ICAR – Indian Agricultural Research Institute (ICAR-IARI), New Delhi during 2020-21. The experimental soil was collected from fields of (IARI) New Delhi. It was autoclaved at 103.4 kPa for 20 min. for experimental purposes. Earthen pots (15 cm dia) were filled with a mixture of 1 kg sterilised soil, sand, and organic manure (FYM). Stones and other debris were removed from soil-sand mixture by passing it through a coarse sieve. Fresh seeds of mungbean cv. 'Pusa Vishal' were procured from National Seed Corporation, IARI, New Delhi, India.

### Nematode culture and inoculation

Root-knot nematode, *M. incognita* was cultured in susceptible brinjal variety (Pusa Vishal). The species was identified as per perineal pattern (13). At the time of requirement of nematodes, the infested plants were pulled out gently and washed in tap water to remove the soil particles. The egg masses were carefully removed under a microscope and placed on modified Baermann funnel assembly. The second-stage infective juveniles (J2s) were extracted (after 48 h), quantified and calibrated. The plants were inoculated with 2000 J2s/1000cc of soil when they were 15 days old. All treatments were replicated thrice in complete randomised design. Holes were made around the mungbean stem to apply the nematode suspension in root rhizosphere.

### Rhizobium culture

*Rhizobium* strain (*Rhizobium leguminosarum* COG 15) infecting mungbean was obtained from the culture collection, Division of Microbiology, Indian Agricultural Research Institute, New Delhi. This was multiplied on yeast extract mannitol medium on a rotary shaker (32). A 72-h old broth culture was used in measured quantities for the experiment. Prior to sowing, the mungbean seeds were inoculated with carrier- based culture of *R. leguminosarum* strain COG 15 as per the standard method (46).

### Coating of mungbean seed and soil application of carbofuran

The seeds of mungbean were coated with neem oil, RD-9 Repelin® (Manufacturer-ITC's India) @1 and 2 % w/v (1500 ppm) as seed treatment. The required quantity of seeds was mixed thoroughly with required quantity of neem oil (1or 2 ml/100g seed) and later dried in shade. Seeds were treated with *T. harzianum*, Nemastin-K 1 % W.P (Kan Biosys Private Limited) @1 and 2 % w/w (CFU count @ $2x10^6$/g) as seed treatment. The seeds

were weighed & mixed with gum arabic as adhesive. The required dose and quantity of *T. harzianum* was weighed and sprinkled on gum coated seeds. Again chalk powder was sprinkled and thoroughly mixed till the seeds were separated individually. Carbofuran 3G (Furadan 3G) @1 and 2 kg a.i./ha$^{-1}$ (Canary Agro Chemicals Pvt. Ltd.) were applied at 300 and 600 mg L$^{-1}$ /pot mixed into the soil.

Table 1. Treatments applied for management of root knot nematode, *Meloidogyne incognita* on mungbean

| Treatment ID | Treatment Description |
|---|---|
| T1 | Carbofuran 3G 1 kg a.i/ha$^{-1}$ + N + R |
| T2 | Carbofuran 3G 2 kg a.i./ha$^{-1}$ + N + R |
| T3 | *T. harzianum* 1 % w/w + N + R |
| T4 | *T. harzianum* 2 % w/w + N + R |
| T5 | Neem oil 1 % v/w + N + R |
| T6 | Neem oil 2 % v/w + N + R |
| T7 | Untreated inoculated control + N + R |
| T8 | Untreated uninoculated + R |
| T9 | Untreated inoculated + N |
| T10 | Untreated uninoculated |
| T11 | Carbofuran 3G 1 kg a.i/ha$^{-1}$ + R |
| T12 | Carbofuran 3G 2 kg a.i./ha$^{-1}$ + R |
| T13 | *T. harzianum* 1 % w/w + R |
| T14 | *T. harzianum* 2 % w/w + R |
| T15 | Neem oil 1 % v/w + R |
| T16 | Neem oil 2 % v/w + R |

Treatments (Abbreviations): T11-T16 = Treated uninoculated control (T11 = Carbofuran 3G 1 kg a.i/ha$^{-1}$ + R; T12 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + R; T13 = *T. harzianum* 1 % w/w + R; T14 = *T. harzianum* 2 % w/w + R; T15 =Neem oil 1 % v/w + R; T16 = Neem oil 2 % v/w + R. (Where, N: Nematode and R: *Rhizobium*).

**Raising seedlings of mungbean**

Five seeds per pot were sown in earthen pots. After germination, at 3-leaf stage they were thinned to one seedling per pot.

**Root staining**

For various treatments, the entire soil was depotted and submerged in water in a shallow bowl. After about 5 min. the loosened soil was gently rubbed off in water and seedlings were taken out. The roots were then immersed in 1.5 % NaOCl solution for 4.0 min and again washed in 2-3 changes of water. The root system was stained as per the method of Byrd *et al.* (6) to study the penetration of 2$^{nd}$ stage juvenile (J2s) into the roots. Later, differentially stained J2s and females inside the root system were counted under a stereoscopic microscope.

**Observations on plant growth characters and nematode development**

The effect of root-knot nematode, *M. incognita* on mungbean was observed 35 days after nematode inoculation, by taking observations on nematode development [nematode penetration, number of root-knot galls/plant, number of egg masses/plant, number of eggs/egg mass per plant and nematode population in soil/pot] and plant growth parameters [shoot and root length (cm), fresh and dry shoot weight (g), fresh and dry root weight (g),

pod weight and number of pods/plant]. The number of bacterial nodules and root-knot galls/plant were counted after washing the roots thoroughly in tap water under the stereoscopic microscope. Dry shoot and root weights were recorded, after 4-days oven drying at 50 $^{o}$C.

**Observations on nematode population in soil**

A composite sample of 100 g of soil from each treatment was taken from the experimental pots and washed by Cobb's washing, decanting and sieving technique (9), followed by Baermann funnel method (4). The volume of the suspension thus obtained was measured. One ml of this suspension was placed in a counting dish and the number of nematodes present in each treatment were counted using stereoscopic binocular microscope (Carl Zeiss, Germany).

**Observations on plant root nodule parameters**

The effects of root-knot nematode on nodulation of mungbean was observed 35 days after nematode inoculation, by taking observations [weight of fresh nodules/plant (g), number of nodules/plant, fresh weight of one nodule (g), Acetylene Reduction Assay (ARA) - nitrogenase enzyme activity (in 'n' moles of ethylene produced/ g nodule fresh weight), bacterial population (CFU count/ml) and leghemoglobin (Lb) content from fresh root nodules (mM)].

**Estimation of bacterial population and nitrogenase enzyme activity**

Bacterial populations from root nodules were recorded by dilution plating method (36). 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 were serially diluted. Appropriate dilutions were spread plated on solidified CRYEMA media on Petri plates and these petri plates were incubated in an incubator at 30$\pm$2°C for 5 days and then the number of colonies, morphologically similar to rhizobial colonies developed was counted. The bacterial population was expressed as CFU count/mg fresh weight of nodules. Nitrogenase enzyme activities were observed by ARA technique (17,18). After incubation in 10 % acetylene 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 hr per g fresh weight nodules (36).

**ARA activity was calculated as under**

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

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 Leghemoglobin content**

The 0.5 g of fresh root nodules were washed in sterile distilled water (SDW) and crushed in a sterile mortar and pestle in phosphate buffer (50 mM, pH 7.0). These were then transferred to round bottom test tubes. The mixture was centrifuged to throw down the large particles of nodule tissue (15 min at 500 x g) and transferred the supernatant to a 10 ml volumetric flask. The haemochrome was measured at 556 nm. Leghemoglobin content of nodules was estimated using the following formula:

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

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

**Laboratory analysis of plant macro nutrients (total N, P, K, Ca and Mg)**

Total nitrogen of inoculated and uninoculated plants was estimated taking 0.5 & 0.2 g material of oven dried shoot and root, respectively, by Kjeldahl method (33). For Phosphorous, the same materials (roots and shoots) digested by Tri-acid mixture ($HNO_3$:$H_2SO_4$: HCL: 9:4:1) and the digested materials were transferred to a volumetric flask by diluting with distilled water. After making the volume, the samples were filtered. Phosphorous was estimated colorimetrically by yellow color method (19) and expressed as a percentage of dry matter. The same digested sample as prepared for phosphorous was used to estimate total potassium measured by flame photometric technique using Corning flame photometer, which is a direct reading type instrument (19).

For Ca and Mg also, same digested sample as prepared for phosphorous was used to estimate the total calcium and magnesium and were measured by Atomic absorption spectrophotometer (AAS).

**Observations on chlorophyll analysis (chl. 'a', chl. 'b' and total chlorophyll)**

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

The formula for the amount of chlorophyll present in the extract mg chlorophyll/g tissue was as under:

$$\text{Chlorophyll a (mg/g) tissue} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times V/1000xW$$
$$\text{Chlorophyll b (mg/g) tissue} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times V/1000xW$$

$$\text{Total chlorophyll (m/g) tissue} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times V/1000xW$$

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

**Statistical analysis**

The data was recorded in triplicate and analyzed, using the SAS software version 9.2. Analysis of variance was calculated to find the critical difference amongst treatment and the mean values were compared by Fisher's least significance difference (LSD) test at $P < 0.05$.

# RESULTS AND DISCUSION

## Plant growth

### Shoot and root length

A perusal of data in Table 1 showed that shoot length was improved significantly by 33-37 % in neem oil treated inoculated plant in comparison to untreated inoculated control i.e. 17 %, while other treatments were similar to control. However, treatments amongst themselves were not significantly different. Treated uninoculated plants showed significant effect amongst themselves with a maximum shoot length recorded with neem treated plant (15.20-16.31 %) and minimum was observed with carbofuran (4.0-8.5 %). Treated uninoculated plants had better shoot length than the inoculated treated plants. The root length was not significantly affected in treated uninoculated plants or inoculated treated plants.

Table 1. Effects of treatments on plant growth of Mungbean (cv. Pusa Vishal)

| | Shoot | | Root | | Pods | |
|---|---|---|---|---|---|---|
| Treatment | Length (cm) | Dry weight (g) | Length (cm) | Dry weight (g) | No. of pods | Weight (g)/pod |
| | Pots with nematodes | | | | | |
| T1 | 49.67$^{bcd}$ (10.37 %) | 6.38$^{ab}$ (51.52 %) | 14.27$^{c}$ (2.15 %) | 0.65$^{cd}$ (-37.93 %) | 6.33$^{ab}$ (90.00 %) | 3.53$^{bcd}$ (49.30) |
| T2 | 50.33$^{abcd}$ (11.85 %) | 6.08$^{ab}$ (44.38 %) | 14.73$^{c}$ (5.49 %) | 0.60$^{d}$ (-42.60 %) | 5.00$^{abcd}$ (50.00 %) | 3.40$^{bcde}$ (43.66) |
| T3 | 54.00$^{abc}$ (20.00 %) | 6.38$^{ab}$ (51.50 %) | 14.57$^{c}$ (4.30 %) | 0.71$^{bcd}$ (-32.72 %) | 5.33$^{abc}$ (60.00 %) | 3.77$^{abc}$ (59.15) |
| T4 | 55.67$^{abc}$ (23.70 %) | 6.74$^{ab}$ (59.90 %) | 14.77$^{c}$ (5.73 %) | 0.73$^{bc}$ (-30.34 %) | 7.00$^{a}$ (110.00 %) | 4.03$^{ab}$ (70.42) |
| T5 | 61.53$^{a}$ (36.74 %) | 6.39$^{ab}$ (51.55 %) | 15.10$^{c}$ (8.11 %) | 0.78$^{b}$ (-25.70 %) | 6.67$^{a}$ (100.00 %) | 4.23$^{ab}$ (78.87) |
| T6 | 59.67$^{ab}$ (32.59 %) | 7.39$^{a}$ (75.41 %) | 15.77$^{c}$ (12.89 %) | 0.72$^{bc}$ (-31.26 %) | 5.67$^{ab}$ (70.00 %) | 4.93$^{a}$ (108.45) |
| T7 | 45.00$^{cd}$ (-16.92 %) | 4.21$^{c}$ (-25.41 %) | 13.97$^{c}$ (34.43 %) | 1.05$^{a}$ (55.07 %) | 3.33$^{cd}$ (-35.06 %) | 2.37$^{de}$ (-13.41) |
| T8 | 54.17$^{abc}$ (20.37 %) | 5.65$^{b}$ (34.06 %) | 21.30$^{b}$ (52.51 %) | 0.68$^{bcd}$ (-35.51 %) | 5.13$^{abcd}$ (54.00 %) | 2.73$^{cde}$ (15.49) |
| T9 | 40.00$^{d}$ (-11.11 %) | 4.07$^{c}$ (-3.47 %) | 11.43$^{d}$ (-18.14 %) | 1.07$^{a}$ (1.59 %) | 3.13$^{d}$ (6.00 %) | 2.30$^{e}$ (-2.82) |
| T10 | 53.00$^{abc}$ (17.78 %) | 6.17$^{ab}$ (46.45 %) | 23.40$^{a}$ (67.54 %) | 0.36$^{e}$ (-65.60 %) | 4.33$^{bcd}$ (30.00 %) | 3.80$^{abc}$ (60.56) |
| CD (P= 0.05) | 11.53 | 1.41 | 1.88 | 0.10 | 2.11 | 1.17 |
| | Pots without nematodes | | | | | |
| T11 | 52.00$^{bc}$ (-4.00 %) | 6.41 (13.49 %) | 15.10 (-29.11 %) | 0.67 (1.23 %) | 6.00$^{c}$ (26.62 %) | 4.17$^{bc}$ (57.32 %) |
| T12 | 51.00$^{c}$ (-5.85 %) | 6.39 (13.05 %) | 15.90 (-25.35 %) | 0.58 (-10.30 %) | 6.33$^{bc}$ (36.36 %) | 3.53$^{c}$ (37.20 %) |
| T13 | 55.50$^{b}$ (2.46 %) | 6.39 (13.05 %) | 14.60 (-31.46 %) | 0.69 (1.75 %) | 6.00$^{c}$ (7.14 %) | 3.97$^{c}$ (40.85 %) |
| T14 | 56.00$^{b}$ (3.38 %) | 7.09 (25.44 %) | 15.20 (-28.64 %) | 0.69 (-8.30 %) | 8.33$^{a}$ (65.58 %) | 4.30$^{abc}$ (53.66 %) |
| T15 | 62.40$^{a}$ (15.20 %) | 7.19 (27.21 %) | 15.40 (-27.70 %) | 0.74 (12.39 %) | 7.33$^{abc}$ (46.10 %) | 4.83$^{ab}$ (73.78 %) |

| | | | | | | |
|---|---|---|---|---|---|---|
| T16 | 63.00[a] (16.31 %) | 7.49 (32.52 %) | 15.79 (-25.87 %) | 0.74 (12.17 %) | 8.00[ab] (65.58 %) | 5.07[a] (86.59 %) |
| CD (P= 0.05) | 4.48 | 1.53 (NS) | 3.38 (NS) | 0.14 (NS) | 1.77 | 0.79 |

[Figures in parenthesis () indicate percent stimulation over control. Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Fisher's Least Significant Difference (LSD)] ]
N: Nematode & R: Rhizobium, NS: Non significant, CD: Critical difference, CV %: Coefficient of variation, SE(d): Standard error.

N: Nematode & R: Rhizobium, NS: Non significant, CD: Critical difference
T1 = Carbofuran 3G 1 kg a.i/ha$^{-1}$ + N + R; T2 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + N + R; T3 = *Trichoderma harzianum* 1 % w/w + N + R; T4 = *Trichoderma harzianum* 2 % w/w + N + R; T5 = Neem oil 1 % v/w + N + R; T6 = Neem oil 2 % v/w + N + R; T7 = Untreated inoculated control + N + R; T8 = Untreated uninoculated + R; T9 = Untreated inoculated + N; T10 = Untreated uninoculated.

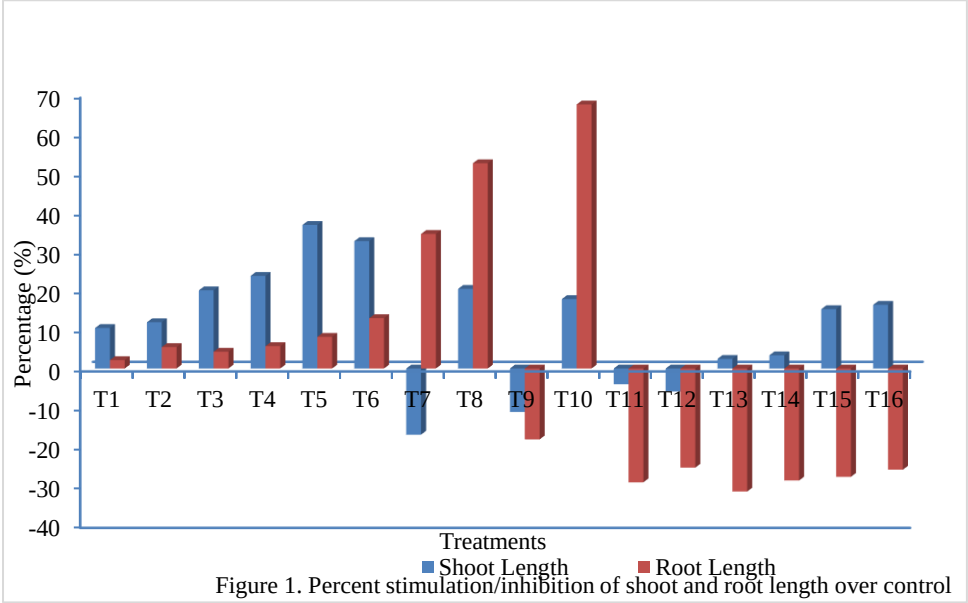

Figure 1. Percent stimulation/inhibition of shoot and root length over control

**Fresh root & shoot weight**

Fresh root weight was higher in treated inoculated plants (14.81 %) than the treated uninoculated. Root weight was observed to be maximum in neem oil treated inoculated plant while lowest was observed with carbofuran at low dose. All the treatments for fresh root weight were not significantly different from each other and were similarly affected, however it was lower than the inoculated control plant. Fresh shoot weight was improved significantly by 57.07 % in neem oil @ 2 % w/v treated inoculated compared to control (20.36 %) while in other treatments were at par with each other. Uninoculated treatments were not significantly different to each other.

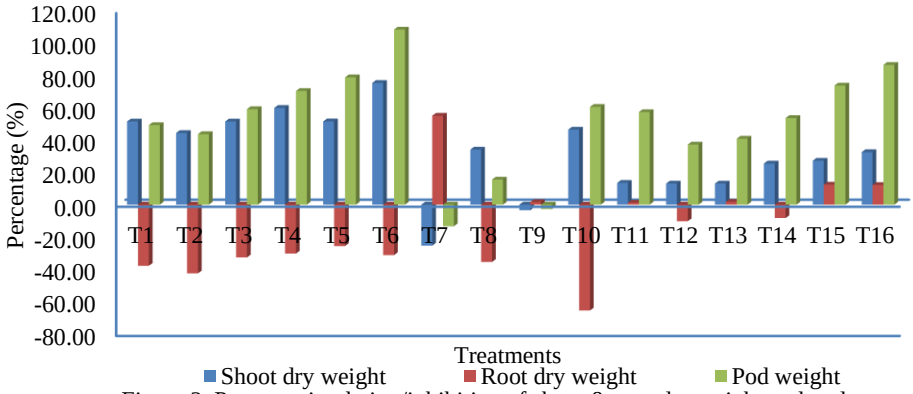


Figure 2. Percent stimulation/inhibition of shoot & root dry weight and pod weight over control

**Dry shoot weight**

Dry shoot weight was increased by 75.41 % in neem oil @ 2 % w/v treatment and was significantly better than the inoculated control (25.41 %), however it was not significantly different than the other treatments. In general, treated inoculated plants were significantly better in dry shoot weight in comparison to control although fresh shoot was not so.

**Pod number and pod weight**

Pod number and pod weight were significantly improved by 100 % and 78.87 %, respectively in the treatment with *T. harzianum* and neem oil @ 1 % w/v in comparison to inoculated control (35.06 % and 13.41 %, respectively). There was no significant differences in number of pods amongst inoculated treatments and thus were similarly affected. Numbers of pods were improved by 50 % by the application of various treatments. Numbers of pods were higher in the treated uninoculated in comparison to treated inoculated plants. Maximum numbers of pods were obtained with *T. harzianum* at higher dose in both inoculated and uninoculated treatments. Pod weight was observed to be higher for uninoculated treated than the inoculated treated plants. Pod weight was increased in the range of 48-100 % in various treatments. Neem oil treated plants showed maximum pod weight.

The above results correspond to the findings of the studies of Sumita and Das (47), as they have reported the efficacy of different bio-agents (*Trichoderma viride*, *T. harzianum* and neem cake) against root-knot nematode, *M. incognita* in green gram (*Vigna radiata*) revealed that soil application of neem cake and *T. viridae*, significantly increased the shoot length, fresh and dry weight of shoot of green gram as compared to untreated control. Bio-agents exhibited the best result in reducing the galls, egg masses and final soil nematode population followed by neem cake. Similarly, Sahu *et al.* (37) also observed the better efficacy of neem oil cakes for the management of root nematodes infecting tomatoes.

Bioagent like, *Trichoderma harzianum*, affect the nematode by more than one ways i.e. by root colonization, egg parasitism, and secondary metabolites which acts against nematodes (21). When nematode infection is low by these treatments then there is no cell

deformity of vascular tissues and plant then have better nutrient absorption and translocation as has been seen. The inhibition in nematode infection leads to better nodulation in term of quality and quantity. The colour and size of nodules were better that may have high in bacteroids content and thus higher nitrogen fixation. All these together have resulted in improved pod formation and hence better yield.

Mungbean growth was affected significantly by the application of neem products as well as by biological control agents by minimizing negative effects of root-knot nematodes. The better plant growth in response to neem seed product is due to the fact that it possesses several alkaloids such as, azadirachtin, salannin, nimbin and its derivative compound belonging to the group of triterpenoid, limonoid, etc. which has anti nematode property (22). It is also considered as a source of useful organic fertilizer. This kind of fertilizer not only provides nutrition for plants but also controls the root-knot nematodes (39). Neem products also promote microbial activity, which could also be good for plant growth and bad for nematodes, but does not affect the beneficial microorganisms in the soil (16,42,48).

**Nematode control**

**Number of galls**

The number of galls were significantly reduced by the treatments and maximum inhibition was achieved with carbofuran (55/plant) in comparison to control (209.67/plant) given in Table 2. Gall inhibition by all the treatments were not significantly different and was similarly affected (66-73 %).

Table 2. Effects of treatments on *Meloidogyne incognita* infection in Mungbean (cv. Pusa Vishal)

| Treatment | No. of galls/plant | No. of egg masses | No. of eggs/egg mass | Nematode population in soil |
|---|---|---|---|---|
| T1 | $58.33^{b}$ (7.70) | $54.67^{c}$ (7.46) | $160.00^{d}$ (12.68) | $48.33^{b}$ (7.02) |
| T2 | $55.33^{b}$ (7.50) | $45.33^{c}$ (6.78) | $157.33^{d}$ (12.57) | $45.00^{b}$ (6.78) |
| T3 | $69.00^{b}$ (8.33) | $65.00^{c}$ (8.10) | $265.00^{abc}$ (16.20) | $63.33^{b}$ (7.99) |
| T4 | $67.33^{b}$ (8.33) | $62.00^{c}$ (7.92) | $219.00^{b-d}$ (14.80) | $62.66^{b}$ (7.98) |
| T5 | $66.33^{b}$ (8.20) | $59.67^{c}$ (7.78) | $227.67^{a-d}$ (15.12) | $58.66^{b}$ (7.72) |
| T6 | $64.00^{b}$ (8.06) | $57.33^{c}$ (7.64) | $203.33^{cd}$ (14.29) | $57.00^{b}$ (7.60) |
| T7 | $209.67^{a}$ (14.48) | $216.33^{b}$ (14.72) | $295.00^{ab}$ (17.10) | $502.00^{a}$ (22.40) |
| T8 | 00 (1.00) | 00 (1.00) | 00 (1.00) | 00 (1.00) |
| T9 | $164.67^{a}$ (1.00) | $269.33^{a}$ (16.38) | $303.67^{a}$ (17.37) | $541.00^{a}$ (23.19) |
| T10 | 00 (1.00) | 00 (1.00) | 00 (1.00) | 00 (1.00) |
| CD (P= 0.05) | 73.68 (3.10) | 40.36 (1.52) | 84.43 (2.76) | 83.16 (2.10) |
| [Figures in parenthesis () are sqrt (x+1) transformed value. Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Fisher's Least Significant Difference (LSD)] | | | | |

N: Nematode & R: Rhizobium, NS: Non significant, CD: Critical difference

T1 = Carbofuran 3G 1 kg a.i/ha$^{-1}$ + N + R; T2 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + N + R; T3 = *Trichoderma harzianum* 1 % w/w + N + R; T4 = *Trichoderma harzianum* 2 % w/w + N + R; T5 = Neem oil 1 % v/w + N + R; T6 = Neem oil 2 % v/w + N + R; T7 = Untreated inoculated control + N + R; T8 = Untreated uninoculated + R; T9 = Untreated inoculated + N; T10 = Untreated uninoculated.

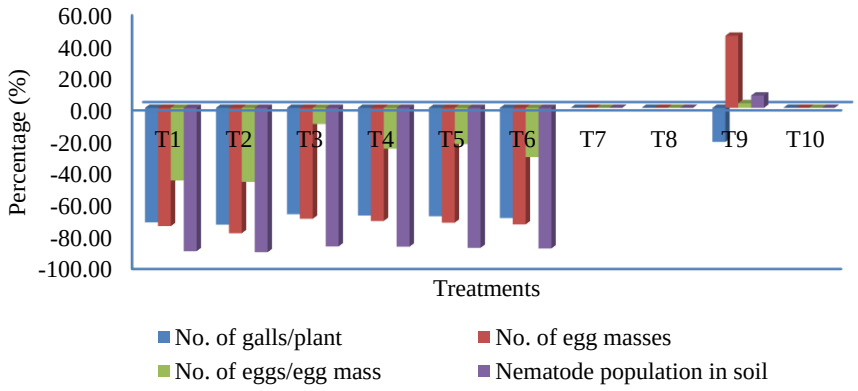


Figure 3. Percent stimulation/inhibition of nematode parameters over control

**Number of egg masses, Number of eggs per egg mass**

Similar observations were recorded for number of egg masses which were decreased by 79 % without any significant variation amongst treatments. Number of eggs per egg mass were lowest with the application of carbofuran (157) followed by neem oil (203) and *T. harzianum* (at their respective higher doses).

**Reproduction factor**

The reproduction factor was lowest (3.57-4.75) with carbofuran, followed by (6.79-8.61) in *T. harzianum* and 5.8-6.8 with neem oil.

Correspondingly, in the study of Dennis and Webster (1971), Elad *et al.* (1982) have shown the significant suppression of *M. incognita* by *T. harzianum.* The possible mechanism involved in *Trichoderma* antagonism had been studied intensively in terms of antibiotic and enzyme production as hyphal interactions. Production of chitinases may have direct significance in the parasitism of *Trichoderma* on *M. incognita* as these enzymes function by breaking down the polysaccharides, chitin and β-glucan. Additional investigations illustrated the effects of combining organic amendments with the fungal bio-agent, *T. harzianum* on nematode inhibition in cases of numbers of galls, egg masses, and second stage infective juveniles (J2s) per root system in pots. Additionally, the treatments demonstrated significantly increased growth and yield criteria for peas as expressed on shoot length and fresh and dry weights and root fresh weight, number, and fresh and dry weights of pods compared to a nematicide, carbofuran 10G, and untreated check (15).

Carbofuran, a synthetic carbamate, has a systemic effect. Therefore, besides affecting the plant growth and nematode multiplication also plays a further role in interactions with the mungbean rhizosphere. To summarise on nematode multiplication, it was found that maximum inhibition in nematode count was obtained with carbofuran treated plants followed by neem oil, and *T. harzianum*. Lowest reproduction factor of root-knot nematode was recorded for carbofuran treated plants. In plants treated with carbofuran, the number of eggs per egg mass was also noted to be the lowest; however, this was not significantly superior to the other treatments. This proves that despite having minimal nematode damage, carbofuran did not have a correspondingly improved growth.

Table 3. Effects of management options on the growth and nodulation of Mungbean (cv. Pusa Vishal) (Mean of three replications)

| | Nodules | | Nodules | | Nodules | | Acetylene reduction activity | | Leghaemoglobin | | Bacterial population | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | No. per plant | Inhibition/ increase (%) over control | Weight per plant(g) | Inhibition/ increase (%) over control | Weight per nodule (g) | Inhibition/ increase (%) over control | 'n' moles of ethylene produced/g FW of nodules | Inhibition/ increase (%) over control | (mM)/g FW of nodules | Inhibition/ increase (%) over control | ($10^6$/g FW nodules) | Inhibition/ increase (%) over control |
| | Pots with nematodes | | | | | | | | | | | |
| T1 | $38.67^{de}$ | 34.88 | $0.40^{b}$ | 250.14 | $0.010^{de}$ | 56.45 | $888.80^{bcd}$ | 65.75 | $2.10^{cd}$ | 30.57 | $7.34^{b}$ | 260.39 |
| T2 | $37.67^{de}$ | 31.40 | $0.30^{bc}$ | 160.29 | $0.007^{e}$ | 41.94 | $662.81^{cd}$ | 25.09 | $1.90^{cde}$ | 17.83 | $7.17^{b}$ | 251.88 |
| T3 | $52.67^{bc}$ | 83.72 | $0.80^{a}$ | 545.22 | $0.015^{a}$ | 114.52 | $903.22^{bcd}$ | 70.47 | $2.18^{bc}$ | 35.63 | $7.44^{b}$ | 265.30 |
| T4 | $59.33^{ab}$ | 106.98 | $0.87^{a}$ | 548.99 | $0.014^{ab}$ | 127.42 | $1183.07^{abc}$ | 123.29 | $2.64^{b}$ | 63.91 | $7.51^{b}$ | 268.74 |
| T5 | $62.67^{ab}$ | 118.60 | $0.85^{a}$ | 640.29 | $0.013^{bc}$ | 140.32 | $1308.31^{ab}$ | 146.92 | $3.54^{a}$ | 119.57 | $8.21^{b}$ | 303.11 |
| T6 | $63.67^{ab}$ | 122.09 | $0.86^{a}$ | 651.88 | $0.014^{ab}$ | 145.16 | $1486.04^{a}$ | 180.47 | $3.81^{a}$ | 136.38 | $9.95^{a}$ | 388.71 |
| T7 | $28.67^{ef}$ | | $0.12^{c}$ | | $0.003^{g}$ | | $529.85^{d}$ | | $1.61^{e}$ | | $2.04^{d}$ | |
| T8 | $65.67^{a}$ | 129.07 | $0.82^{a}$ | 610.72 | $0.012^{cd}$ | 162.90 | $1745.75^{a}$ | 229.48 | $3.92^{a}$ | 143.45 | $8.28^{b}$ | 306.55 |
| T9 | $22.67^{f}$ | -20.93 | $0.10^{c}$ | -12.17 | $0.004^{fg}$ | -11.29 | $443.60^{d}$ | -16.28 | $1.61^{e}$ | -0.21 | $1.68^{d}$ | -17.51 |
| T10 | $41.67^{cd}$ | 45.35 | $0.27^{bc}$ | 137.10 | $0.006^{f}$ | 6.45 | $481.82^{d}$ | -9.06 | $1.68^{de}$ | 4.10 | $3.53^{c}$ | 73.47 |
| CD(P=0.05) | 12.78 | | 0.25 | | 0.002 | | 565.2 | | 0.49 | | 1.27 | |
| | Pots without nematode | | | | | | | | | | | |
| T11 | $40.00^{c}$ | -39.08 | $0.62^{b}$ | -24.02 | 0.015 | -26.38 | $992.35^{b}$ | -43.12 | $3.26^{ab}$ | -16.72 | $7.52^{c}$ | -9.18 |
| T12 | $39.33^{c}$ | -39.84 | $0.53^{b}$ | -34.79 | 0.013 | -44.79 | $904.97^{b}$ | -48.16 | $2.71^{bc}$ | -30.43 | $7.26^{c}$ | -12.26 |
| T13 | $58.00^{b}$ | -11.67 | $0.80^{ab}$ | -1.69 | 0.012 | -9.82 | $1518.23^{a}$ | -13.03 | $2.21^{c}$ | -45.53 | $7.55^{c}$ | -8.45 |
| T14 | $60.00^{ab}$ | -8.62 | $0.88^{ab}$ | 108.10 | 0.014 | -5.21 | $1531.89^{a}$ | -12.25 | $3.35^{ab}$ | -14.75 | $7.73^{c}$ | -6.76 |
| T15 | $67.00^{a}$ | 2.03 | $1.00^{a}$ | 22.65 | 0.014 | 1.23 | $1818.18^{a}$ | 4.15 | $3.60^{a}$ | -7.99 | $8.69^{b}$ | 0.57 |
| T16 | $65.00^{ab}$ | -1.01 | $1.09^{a}$ | 34.16 | 0.016 | 3.99 | $1830.14^{a}$ | 4.83 | $3.68^{a}$ | -6.03 | $10.06^{a}$ | 21.74 |
| CD(P=0.05) | 8.69 | | 0.35 | | NS | | 495.9 | | 0.83 | | 0.64 | |
| [Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Fisher's Least Significant Difference (LSD)] | | | | | | | | | | | | |

N: Nematode & R: Rhizobium, NS: Non significant, CD: Critical difference

T1 = carbofuran 3G 1 kg a.i/ha$^{-1}$ + N + R; T2 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + N + R; T3 = *Trichoderma harzianum* 1 % w/w + N + R; T4 = *Trichoderma harzianum* 2 % w/w + N + R; T5 = Neem oil 1 % v/w + N + R; T6 = Neem oil 2 % v/w + N + R; T7 = Untreated inoculated control + N + R; T8 = Untreated uninoculated + R; T9 = Untreated inoculated + N; T10 = Untreated uninoculated; T11 = carbofuran 3G 1 kg a.i/ha$^{-1}$ + R; T12 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + R; T13 = *Trichoderma harzianum* 1 % w/w + R; T14 = *Trichoderma harzianum* 2 % w/w + R; T15 = Neem oil 1 % v/w + R; T16 = Neem oil 2 % v/w + R

**Nodulation**

**Number of nodules**

The number of nodules were significantly higher in the treated inoculated plants compared to untreated inoculated plants, as shown in Table 3. Amongst the treated plants neem oil and *T. harzianum* improved the nodule number significantly over carbofuran and control inoculated. The number of nodules was increased by 122 % with neem oil treatment. Number of nodules were significantly higher in neem oil treated plant (63) over control (30). There were increases in number of nodules with the increase in dose of *T. harzianum* and neem oil.

In contrast number of nodules decreased with increase of concentration of carbofuran. Nodule number was increased in the range of 31-122 % in various treatments.

**Weight of nodules per plant**

Weight of nodules per plant was in the range of 0.30-0.8 g in various treatments in comparison to control (0.12). *T. harzianum* treated plants were having maximum nodule weight. The decrease in the weight of nodule is related to concentration of carbofuran. Invariably treated uninoculated had higher weight of nodules per plant than the treated inoculated. There was a significant improvement in ARA activity with neem oil (180 %) over control set out in Table 3. Carbofuran and *T. harzianum* 1 % were not significantly better than the control. Generally, uninoculated treatment had higher nitrogenase content than the treated inoculated treatments.

**Leg-hemoglobin**

Leg-hemoglobin content followed similar pattern as that of ARA activity which was improved in the range of 30-136 % in various treatments. Bacteroid population was found to be highest with neem oil treated plants. Similarly treated uninoculated neem oil also had highest bacteroid content. Bacteroid content was observed to be improved by more than 200 % in treated plants. Effect of rhizobia on root-knot nematode multiplication can be observed if the comparison is made between T7 (inoculated with rhizobia) and T9 (inoculated without rhizobia). It showed that the number of galls were less in without rhizobia treatment. This points out that rhizobia has to some extent enhanced multiplication of root-knot nematode as also indicated from reproduction factor, this could be because of rhizobial Nod factors as they are some regards, similar to signals generated by plant parasitic RKN in vascular root tissue induction (51).

Effect of different treatments on nodulation can be viewed if the comparison is made between T8, T11-T16. It showed that the treatments had a negative impact on the formation of nodules in terms of nodule number. This is also true for weight of nodules per plant and weight of one nodule particularly with carbofuran and *T. harzianum.* Similarly, nitrogenase, leghemoglobin and bacterial population was negatively affected by treatments. The application of rhizobium has enhanced the formation of nodules and the function is clear from T8 and T10 treatments. In the presence of nematode whether these parameters affected can be observed if the comparison is made T7 and T8. Nematodes had a significant effect on the number of nodules, their weight and function. To substantiate, studies conducted on the impact of root-knot nematode infection on mungbean nodulation character and growth parameters can be provided (26,44,49).

It could be analyzed that nematode damage was mitigated by different treatments to a varying degree. The Inhibition in root-knot nematode count does not necessarily mean improvement in nodule formation and functioning. Though, the nematode was best managed by carbofuran (70 %) but the nodulation was not so much improved (20 %) compared to the use of the bioagent, *T. harzianum* which reduced the number of galls by 70 % and improved the nodulation by 80 %, while neem oil reduced 70 % galling and improved the nodulation by 100 % over the control. In this regard, Jada *et al.* (2011), while working on groundnut with carbofuran 4 kg a.i./ha$^{-1}$ against root-knot nematode *M. javanica*, observed that number of nodules were marginally affected by application of pesticide at 3 weeks after planting while at planting there was little increase in nodule number. Similar conclusions were reached in one of study to control the cowpea root knot-nematode and found that compared to untreated, uninoculated plants, treated plants formed bacterial nodules at a lower rate. The nodules were reduced by 10 % and 30 %.in carbofuran treated plants and in *T. viride* treated plants respectively (27).

**Chlorophyll contents**

Chlorophyll 'a' content was observed to be improved in the range of 30-83 % in various treatments compared to control as indicated in Table 4. Neem oil @ 2 % w/v showed highest chlorophyll 'a' content. While, with the exception of plants treated with carbofuran, chlorophyll "a" was higher in untreated plants than in treated plants. Chlorophyll b was generally reduced in treatments and it was reduced by 21.9 % in carbofuran treated plants. Chlorophyll b was not significantly changed in treated inoculated plants in comparison to control. Total chlorophyll content was improved in treated inoculated plants (16-64 %). However, treated plants do not differ significantly among them as well as with control (1.6 %) with regards to total chlorophyll content and thus were similarly affected except for neem oil (2.7 %).

Table 4. Effects of treatments on the chlorophyll a, chlorophyll b and total chlorophyll contents in Mungbean (cv. Pusa Vishal)

| Treatment | Chlorophyll-a | | Chlorophyll-b | | Total chlorophyll | |
|---|---|---|---|---|---|---|
| | (µg/ml) | Inhibition (%) over check | (µg/ml) | Inhibition (%) over check | (µg/ml) | Inhibition (%) over check |
| | Pots with nematodes | | | | | |
| T1 | 1.74 | 29.07 | 0.38 | -15.77 | 2.12 | 21.08 |
| T2 | 1.61 | 23.15 | 0.34 | -28.06 | 1.95 | 14.18 |
| T3 | 1.85 | 33.29 | 0.44 | -0.21 | 2.29 | 26.90 |
| T4 | 1.84 | 32.95 | 0.42 | -4.00 | 2.26 | 26.09 |
| T5 | 2.04 | 39.42 | 0.44 | 0.98 | 2.48 | 32.57 |
| T6 | 2.27 | 45.62 | 0.48 | 8.28 | 2.75 | 39.14 |
| T7 | 1.24 | | 0.44 | | 1.67 | |
| T8 | 2.07 | 40.29 | 0.31 | -40.00 | 2.38 | 29.76 |
| T9 | 1.21 | -1.99 | 0.37 | -18.88 | 1.58 | -5.92 |
| T10 | 1.53 | 19.02 | 0.33 | -30.55 | 1.86 | 10.10 |
| CD (P=0.05) | 0.70 (NS) | | 0.15 (NS) | | 0.77 (NS) | |
| Pots without nematode | | | | | | |
| T11 | 1.48$^{c}$ | 16.92 | 0.40$^{b}$ | -6.78 | 1.90$^{d}$ | 11.80 |
| T12 | 1.74$^{b}$ | 29.02 | 0.34$^{c}$ | -25.94 | 2.09$^{c}$ | 19.88 |

| | | | | | | |
|---|---|---|---|---|---|---|
| T13 | 1.88[b] | 34.44 | 0.44[ab] | 1.38 | 2.33[b] | 28.14 |
| T14 | 1.86[b] | 33.87 | 0.41[b] | -5.45 | 2.28[b] | 26.73 |
| T15 | 2.15[a] | 42.81 | 0.44[ab] | 2.38 | 2.61[a] | 35.87 |
| T16 | 2.18[a] | 43.57 | 0.45[a] | 4.92 | 2.65[a] | 36.86 |
| CD (0.05) | 0.30 | | 0.04 | | 0.30 | |
| [Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Fisher's Least Significant Difference (LSD)] | | | | | | |

N: Nematode & R: Rhizobium, NS: Non significant, CD: Critical difference
T1 = carbofuran 3G 1 kg a.i/ha$^{-1}$ + N + R; T2 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + N + R; T3 = *Trichoderma harzianum* 1 % w/w + N + R; T4 = *Trichoderma harzianum* 2 % w/w + N + R; T5 = Neem oil 1 % v/w + N + R; T6 = neem oil 2 % v/w + N + R; T7 = Untreated inoculated control + N + R; T8 = Untreated uninoculated + R; T9 = Untreated inoculated + N; T10 = Untreated uninoculated; T11 = Carbofuran 3G 1 kg a.i/ha$^{-1}$ + R; T12 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + R; T13 = *Trichoderma harzianum* 1 % w/w + R; T14 = *Trichoderma harzianum* 2 % w/w + R; T15 = Neem oil 1 % v/w + R; T16 = Neem oil 2 % v/w + R.

Consistently good plant growth in terms of chlorophyll content, nutrient status and nodulation were observed with neem treated plant, although nematode control was less in comparison to carbofuran. This suggests that neem oil had played a greater role in overall boosting of the plant health system which has been translated in the form of pod yields. Also, the better chlorophyll content has also been shown on lentil infected with root knot nematode by the use of bioagents (50).

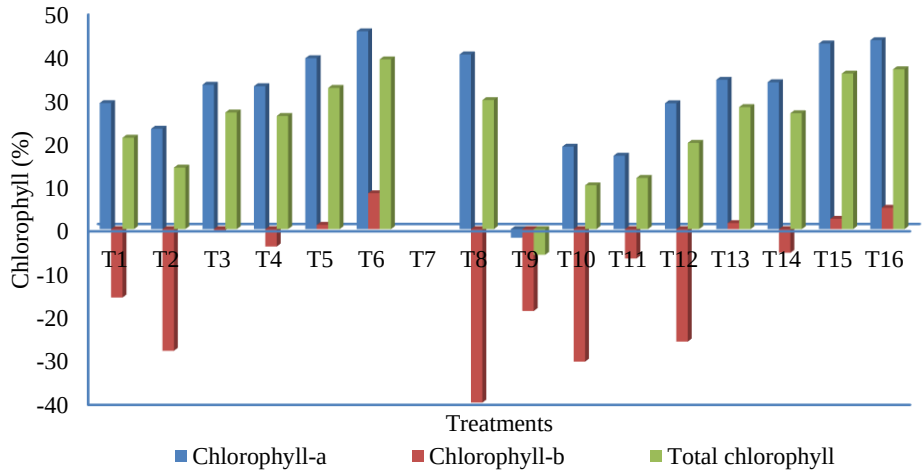


Figure 4. Percent stimulation/inhibition of chlorophyll a, chlorophyll b and total chlorophyll over control

**Nutrient status in plant**

**Impact on Nitrogen**

Major nutrients in shoot and root showed that nitrogen content in shoot was significantly higher in all the treatments over the control shown in Table 5. The nitrogen content was lower in carbofuran treated plants in comparison to other treatments. The level

of nitrogen in shoot was observed to be highest with neem oil treated plants. The nitrogen content of shoot was also better in untreated uninoculated plants than the treated inoculated plants. In root, the nitrogen content was better in the carbofuran treated plant especially at lower dose. Neem oil treated uninoculated plants had lower level of nitrogen than the inoculated treated plant. The level of nitrogen in the root treated inoculated plants was lower than the inoculated control (1.2 %).

Table 5. Effects of treatments on the total shoot and root N, P, K and Ca in Mungbean (cv. Pusa Vishal)

| Treatment | Nitrogen (%) | | Phosphorous (%) | | Potassium (%) | | Calcium (%) | | Magnesium (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| | Pots with nematodes | | | | | | | | | |
| T1 | $1.31^{c}$ | $0.74^{bc}$ | $0.33^{a}$ | $0.25^{bc}$ | $2.23^{ab}$ | $1.14^{b}$ | 4.20 | $1.39^{cd}$ | $0.92^{a}$ | $0.63^{b}$ |
| T2 | $1.36^{bc}$ | $0.62^{c}$ | $0.35^{a}$ | $0.24^{c}$ | $2.21^{abc}$ | $1.08^{bc}$ | 4.72 | $1.48^{cd}$ | $0.86^{ab}$ | $0.61^{b}$ |
| T3 | $1.41^{bc}$ | $0.69^{bc}$ | $0.32^{a}$ | $0.23^{c}$ | $2.24^{ab}$ | $1.20^{b}$ | 6.20 | $1.57^{cd}$ | $0.75^{ab}$ | $0.57^{b}$ |
| T4 | $1.53^{ab}$ | $0.55^{c}$ | $0.36^{a}$ | $0.21^{c}$ | $2.05^{abcd}$ | $1.14^{b}$ | 4.83 | $1.49^{cd}$ | $0.76^{ab}$ | $0.35^{b}$ |
| T5 | $1.52^{ab}$ | $0.75^{bc}$ | $0.38^{a}$ | $0.23^{c}$ | $2.45^{a}$ | $0.96^{bc}$ | 4.67 | $1.75^{c}$ | $0.80^{ab}$ | $0.66^{b}$ |
| T6 | $1.65^{a}$ | $0.62^{bc}$ | $0.39^{a}$ | $0.20^{c}$ | $2.58^{a}$ | $0.75^{c}$ | 4.28 | $1.72^{cd}$ | $0.58^{bc}$ | $0.76^{b}$ |
| T7 | $0.87^{d}$ | $1.21^{a}$ | $0.16^{c}$ | $0.32^{ab}$ | $1.53^{cd}$ | $2.07^{a}$ | 2.25 | $2.93^{b}$ | $0.35^{c}$ | $1.84^{a}$ |
| T8 | $1.39^{bc}$ | $0.63^{c}$ | $0.30^{ab}$ | $0.22^{c}$ | $1.98^{abcd}$ | $1.05^{bc}$ | 5.09 | $1.09^{d}$ | $0.91^{a}$ | $0.84^{b}$ |
| T9 | $0.71^{d}$ | $1.32^{a}$ | $0.16^{c}$ | $0.33^{a}$ | $1.47^{d}$ | $2.22^{a}$ | 2.06 | $3.72^{a}$ | $0.27^{c}$ | $1.88^{a}$ |
| T10 | $1.28^{c}$ | $0.90^{b}$ | $0.23^{bc}$ | $0.23^{c}$ | $1.66^{bcd}$ | $1.06^{bc}$ | 4.26 | $1.25^{cd}$ | $0.67^{ab}$ | $0.68^{b}$ |
| CD (P= 0.05) | 0.19 | 0.26 | 0.09 | 0.07 | 0.69 | 0.36 | 2.15 (NS) | 0.63 | 0.32 | 0.49 |
| | Pots without nematode | | | | | | | | | |
| T11 | $1.40^{b}$ | 0.67 | 0.35 | 0.19 | $2.54^{b}$ | $0.90^{a}$ | $4.19^{b}$ | 0.76 | $1.00^{a}$ | 0.57 |
| T12 | $1.44^{b}$ | 0.60 | 0.36 | 0.19 | $2.78^{b}$ | $0.94^{a}$ | $5.71^{ab}$ | 0.66 | $0.69^{c}$ | 0.52 |
| T13 | $1.51^{b}$ | 0.72 | 0.37 | 0.19 | $3.40^{a}$ | $0.96^{a}$ | $4.56^{b}$ | 0.73 | $0.75^{c}$ | 0.73 |
| T14 | $1.55^{b}$ | 0.67 | 0.40 | 0.20 | $2.61^{b}$ | $0.93^{a}$ | $5.53^{ab}$ | 1.21 | $0.61^{d}$ | 0.76 |
| T15 | $1.55^{b}$ | 0.58 | 0.40 | 0.20 | $2.61^{b}$ | $0.79^{b}$ | $7.34^{a}$ | 0.75 | $0.89^{b}$ | 0.70 |
| T16 | $1.72^{a}$ | 0.51 | 0.41 | 0.20 | $3.44^{a}$ | $0.72^{c}$ | $7.68^{a}$ | 0.62 | $0.84^{b}$ | 0.69 |
| CD (P= 0.05) | 0.15 | 0.22 (NS) | 0.20 (NS) | 0.04 (NS) | 0.49 | 0.05 | 2.30 | 0.48 (NS) | 0.10 | 0.25 (NS) |
| [Different letters on each column indicate statistically significant difference between treatments at ($P \leq 0.05$) using Fisher's Least Significant Difference (LSD)] | | | | | | | | | | |

N: Nematode & R: Rhizobium, NS: Non significant, CD: Critical difference
T1 = carbofuran 3G 1 kg a.i/ha$^{-1}$ + N + R; T2 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + N + R; T3 = *Trichoderma harzianum* 1 % w/w + N + R; T4 = *Trichoderma harzianum* 2 % w/w + N + R; T5 = neem oil 1 % v/w + N + R; T6 = neem oil 2 % v/w + N + R; T7 = Untreated inoculated control + N + R; T8 = Untreated uninoculated + R; T9 = Untreated inoculated + N; T10 = Untreated uninoculated; T11 = Carbofuran 3G 1 kg a.i/ha$^{-1}$ + R; T12 = Carbofuran 3G 2 kg a.i./ha$^{-1}$ + R; T13 = *Trichoderma harzianum* 1 % w/w + R; T14 = *Trichoderma harzianum* 2 % w/w + R; T15 = Neem oil 1 % v/w + R; T16 = Neem oil 2 % v/w + R .

**Impact on Phosphorus**

Phosphorus content was observed to be in the range of 0.32-0.39 % and thus it was similarly affected by the treatments in shoot however significantly higher than the control. The phosphorus content in the uninoculated treated plants was higher than the inoculated treated plants. In case of root phosphorus trend was opposite.

**Impact on Potassium**

Potassium content was highest in neem oil treated plant in shoot while in roots it was low. Potash level was in the range of 2.2-2.5 % in shoot amongst various treatments was similar based on critical difference value. Potassium content of shoot was higher in uninoculated treated plants than the inoculated treated plants while in case of root the trend was opposite.

**Impact on Calcium and magnesium**

Calcium content was in the range of 4.2-6.2 % in shoot in various treatments in comparison to control (2.2 %) and was significantly higher in treatments. The level of calcium was higher in shoot than the root. With regards to magnesium it was improved maximum with carbofuran treated plant in shoot (0.9). The different treatments do not show significant difference with respect to nutrient content among themselves however these were significantly better than the control (0.35 %). In general, there were increased level of nutrients in treated inoculated plants in comparison to control however, in root the trend was opposite i.e. control had higher level of nutrient than the treated.

As regards to nutrients N, P, K, Ca and Mg in the shoot improved significantly with the application of treatments in comparison to control. Thus, content of nutrients was better in shoot in treated plants than the untreated plants and the trends were reversed in case of root. In general, treated inoculated had less nutrient content than treated uninoculated showed the effect of nematode particularly in shoot, however in root, the trend was opposite i.e. treated inoculated (T1-T6) had higher content of nutrients than the uninoculated treated (T11-116) ones. Likewise, Rose *et al.* (2018), studied individual and interactive effects of different plant symbionts (*Glomus fasciculatum*, *Mesorhizobium ciceri*), bio-organic waste (Avena sativa straw) and antagonistic fungi, *T. harzianum* in all possible combinations for biocontrol of root-knot nematode, *M. incognita* infecting chickpea var. Avrodhi and revealed combined treatments resulted in higher plant growth, biomass, chlorophyll and nutrient status (N, P and K). Antagonistic fungi, *T. harzianum* @ $10^6$ spores plant$^{-1}$ proved to be most effective of all the plant symbionts (*G. fasciculatum*, *M. ciceri*) and organic waste in suppressing the nematode-related parameters in chickpea plants. Application of the combined treatment of yeast plus *T. harzianum* gave the best results in improving peanut production, plant growth parameters, and seed nutrient contents such as N, P, K, Zn, Mg and Cu compared to the untreated control (31). It is thus emanated from the present investigation that controlling nematode on mungbean can translate into better yield.

## CONCLUSIONS

In conclusion, the study presents a complex scenario for root knot nematode control in mungbean cultivation. The tested control methods, carbofuran (2 kg a.i. ha$^{-1}$), *T. harzianum* (2 % w/w), and neem oil (2 % w/v), exhibit varying impacts on different aspects of mungbean growth and nematode control. While carbofuran (2 kg a.i. ha$^{-1}$) effectively suppresses nematode reproduction, it falls short in promoting plant growth and grain yield. On the other hand, neem oil (2 % w/v), with its eco-friendly properties, emerges as a promising option. Neem oil not only demonstrates good nematode control but also positively influences plant growth parameters, nodule formation, and ultimately, grain yield. *T. harzianum* (2 % w/w) also shows promise but is slightly less effective than neem oil. The findings underscore the multifaceted nature of pest control in agriculture, emphasizing the

ecological advantages of neem oil in improving mungbean productivity. Further research is needed to optimize the use of these methods in integrated pest management strategies.

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## DECLARATION

We declare that all authors of this manuscript made a significant contribution, and we have not excluded any author that substantially contributed. We have followed the ethical norms established by our respective institutions.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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## PUBLISHER NOTE