

## **Inoculum levels for Sustainable Control of Root-Knot Nematodes in Tomato under polyhouse condition: A Study from North eastern part of India**

### **Abstract**

The present study aim to provide early predictions of root-knot nematode infestations which will help for acquainted with drastic losses as the losses increase with the increase in crop ages and also to guide farmers on sustainable management strategies, including biological treatments, to keep nematode populations below the economic threshold level. **Due to its polyphagous nature and adaptability to adverse conditions, the root-knot nematode is considered a serious agricultural issue in India.** It affects a wide range of crops, including vegetables, fruits, legumes, oilseeds, and ornamental plants, causing progressive yield declines over time. Root-knot nematodes primarily attack underground parts of plants, such as roots, stems, and pods, leading to persistent, slow-spreading diseases. Major tomato-growing areas in Nongpoh, East Khasi Hills, and West Jaintia Hills districts of Meghalaya were selected at random for soil sample collection. Using a hand shovel, samples were gathered from a depth of 15-20 cm at each collection location. In the present study, increasing the inoculum level from 500 to 8000 J2 resulted in a significant, progressive increase in root infection by the root knot nematode RKN, as indicated by the root gall index (on a 1-5 scale), the number of egg masses, and nematode population. The highest root gall index (5.00) and egg mass count (28.71) were observed at the 8000 J2 inoculum level, while the lowest (3.5 and 9.28, respectively) were recorded at 500 J2. This study underscores the importance of managing nematode populations to minimize crop losses and suggests that even low inoculum levels (500 J2) can cause significant damage, highlighting the need for effective control strategies.

**Keywords: inoculum level, polyphagous, sustainable management, seed quality**

### **Introduction:**

In India, around 58% of the population primarily depends on agriculture for their livelihood. The country is a leading global producer of various crops, such as wheat, rice, pulses, sugarcane, and cotton, and ranks second in fruit and vegetable production. Among the most significant vegetables is the tomato, which thrives in both temperate and tropical climates worldwide. Tomatoes play a vital role in nutrition, being rich in essential amino acids and minerals. However, tomato yield is often hindered by several factors, including poor seed quality and pest infestations, notably plant-parasitic nematodes. In north eastern region also, the tomato production is quite low as compare to mainland region. Therefore, it is mostly imported from other states of India and due to this the economic value of local tomato is very high.

Root-knot nematodes (*Meloidogyne* spp.) are among the most prevalent plant-parasitic nematodes, causing substantial economic losses to vegetable crops. Globally, these nematodes are responsible for an average yield loss of 12.3% in major crops (Sasser and Freckman, 1987). In India, an estimated annual loss of 19.6% is attributed to plant-parasitic nematodes. In the vegetable-growing regions of Meghalaya, *Meloidogyne incognita* (RKN) is the dominant species, followed by *Helicotylenchus* spp. (Firake et al., 2015; Jeevan et al., 2024). The tomato crop is particularly vulnerable to root-knot nematode infestation (Bhardwaj, 1972), and *Meloidogyne incognita* has been recognized as one of the most critical constraints to agricultural production globally (Khan and Pariari, 2013).

Due to its polyphagous nature and adaptability to adverse conditions, the root-knot nematode is considered a serious agricultural issue in India. It affects a wide range of crops, including vegetables,

fruits, legumes, oilseeds, and ornamental plants, causing progressive yield declines over time. Root-knot nematodes primarily attack underground parts of plants, such as roots, stems, and pods, leading to persistent, slow-spreading diseases.

Subjected to the importance of crop and hazardous economic losses due to this RKN, this research focuses on reproducing the pathogenic effects of different inoculum levels of native root-knot nematodes (*Meloidogyne* sp.) from Meghalaya on tomatoes under polyhouse pot conditions. Samples were collected from major tomato-growing areas in the region where root-knot nematode infestation was observed. Although no significant yield damage was initially noted, we conducted a pathogenesis experiment using various concentrations of the nematode inoculum to understand the gradient of yield loss corresponding to infestation levels. The findings aim to provide early predictions of root-knot nematode infestations which will help for acquainted with drastic losses as the losses increase with the increase in crop ages and also to guide farmers on sustainable management strategies, including biological treatments, to keep nematode populations below the economic threshold level.

## **Materials and methods:**

### **Soil and plant sample collection**

Major tomato-growing areas in Nongpoh, East Khasi Hills, and West Jaintia Hills districts of Meghalaya were selected at random for soil sample collection. Using a hand shovel, samples were gathered from a depth of 15-20 cm at each collection location. Each sample was made up of a composite of three to five randomly selected subsamples. After combining these subsamples to form a composite sample, 250 cc of soil were extracted for additional examination. The shovels were cleaned with 70% ethanol before proceeding to the next location. After being labelled with pertinent information and placed in plastic bags to keep them from drying out, the soil samples were taken to the lab to be processed.

### **Preparation of soil and pots**

For conducting the pot experiment, well pulverised soil free from plant debris and gravels was collected from the experimental field, School of crop protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya. Then the collected soil was mixed thoroughly with sand and FYM autoclaved for 20 minutes at 121°C. The sterilized soil was spread on a clean polythene sheet for 24 hours for renaturation of soil. In the meantime, pots of 15cm diameter were cleaned and surface sterilized using 4% formalin solution and were made air dry. Pots were then filled with aerated sterilized soil (1 kg).

### **Isolation and maintenance of pure culture of RKN**

The J<sub>2</sub> inoculum was obtained from the infested tomato plants maintained as pure culture of *M. incognita*. Infested plants were uprooted carefully, and then cleaned. From those roots, matured egg masses were collected with the help of forceps and placed in a glass beaker filled with sterile water. The egg masses were then transferred to a double-layered tissue paper stretched over an aluminium wire gauge and placed on a petri-dish filled with water in such a way so that bottom of the aluminium wire gauge should touch the water of petri dish and kept for 24 hours. Subsequent collection of freshly hatched juveniles (J<sub>2</sub>) of *M. incognita* was done for inoculation (Ozdemir and Gozel, 2018).

### **Sowing and thinning**

Pusa ruby which is a nematode susceptible variety of tomato was used for experiment. Then the seeds were sown in portrays containing the sterilized soil. Three seedlings of 25-30 days were transplanted in each pot filled with sterilized soil. Watering was then done at regular interval. Then, thinning of

seedlings was done after one week of transplantation and only one healthy seedling was kept in each pot.

#### **Standardizing nematode number in stock solution**

Freshly hatched second stage juveniles of *M. incognita* were isolated in a beaker. Then the nematode suspension with J<sub>2</sub> was thoroughly stirred before taking a 1ml suspension in rectangular counting dish to count the nematodes, under a stereoscopic microscope. The average of total number of nematodes present in the 1ml stock sample was determined. Nematode population was estimated by counting replicated aliquots of the suspension.

#### **To evaluate the pathogenicity of root-knot nematode.**

Pathogenicity experiment was conducted under poly house condition to test the pathogenic potential of root-knot nematode, *M. incognita* in tomato plants. Healthy seedlings of tomato were sown in each pot of size 15cm diameter containing autoclave sterilized soil mixture. After 25-30 days, the tomato plants in each pot were thinned out keeping only one healthy plant per pot. Regular care was taken for maintenance of the plants in the poly house.

#### **Inoculation of nematodes**

A measured volume of hatched juveniles' suspension at different levels (0, 500, 1000, 2000, 4000, 6000, 8000 per pot) was pipetted and put around the root zone by making small holes according to necessity. Holes were then filled with soil followed by light watering. Altogether, 7 treatments with 4 replications including an uninoculated check was arranged in completely Randomized Design. At 60 days after inoculation, the experiment was terminated and observations were recorded on plant growth parameters as well as on the nematode population development.

#### **Recording of observations**

At 60 days after inoculation, each plant was removed from the pots carefully. Roots were washed free of soil and adhering particles under slow stream of water and observation were recorded on different plant growth characters, number of galls and nematode population in soil as well as in root. Shoot length of each plant was measured from the plant up to the top most portions in meter scale. Root portion of each plant was cut, labelled and knots if any, were opened followed by straightening of roots. The root length of each plant was measured up to the tip in the meter scale. Fresh weight of shoot and root of individual plant were recorded in grams. These were further labelled and kept for recording of dry weights. Shoot and root of individual plant were air dried and kept in separate paper packets. Such packets were then placed in hot air oven at 70° C temperature for 48 hours after which, the dry weight was recorded in gram. Total number of galls on roots of each plant was counted with the help of hand tally counter before drying and recorded. Number of egg masses in roots were counted under stereoscopic microscope and recorded.

#### **Estimation of RKN population in the root system**

At the time of harvest, roots of Tomato plants inoculated with RKN were lifted carefully. Infected root measuring 5g from each replication of different treatments were tied separately with cotton threads

and labelled accordingly. Then nematodes in roots are stained by Byrd method (Byrd *et al.*, 1983). First infected roots were washed and placed in 150 ml water. Large roots were cut in to segments. Roots were rinsed for 45 seconds in running water and then soaked in tap water for 15 minute and then water was drained and all roots were transferred to a beaker with 50ml of tap water, in to which 1 ml of stock acid fuchsin stain solution (3.5 g acid fuchsin in 250 ml of acetic acid with 750 ml of distilled water) was added. The solution was then boiled for 30 seconds on a hot plate. After that the solution was cooled in room temperature and drained out leaving only roots. Roots were rinsed in running water. Then the roots were placed in 20-30 ml glycerine acidified with a few drops of 5N HCl and heated to boiling for destaining. After destaining, roots were examined under stereoscopic microscope.

### **Nematode population in soil**

Soil from each pot was mixed thoroughly and 200 ml sample from each pot was collected and screened by Cobbs' sieving technique (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1961) for estimation of nematode population in different treatments.

### **Reproduction Factor**

(Rf value) It is defined as the ratio of total nematodes present in soil and root after harvest to the total number of nematodes inoculated to each pot initially.

$$Rf = Pf / Pi$$

Where,

Rf= Rate of nematode multiplication

Pf = Final nematode population

Pi= Inoculated (initial) nematode population

### **Statistical analysis**

The experiments were carried out using completely randomized and randomized block designs in pots under polyhouse conditions. The experimental data obtained were statistically analysed using Web Agri Stat Package (WASP) version 2.0 (at 5%).

### **Results:**

#### **1. Effect of different inoculum level of *M. incognita* on plant growth parameters**

##### **Shoot and root length**

The data presented in Table 1 and Figure 1 reveal that the maximum plant height (80.00 cm) was observed in the uninoculated treatment (T1). This was followed by plant heights of 66.43 cm, 60.75 cm, 59.00 cm, 53.00 cm, and 51.00 cm at inoculum levels of 500, 1000, 2000, 4000, and 6000 J2 per plant, respectively. The shortest shoot length (44.5 cm) was recorded at the highest inoculum level of 8000 J2 per plant (T7). A direct correlation was found between increasing inoculum levels and decreasing plant height. Infected plants exhibited stunted stems, fewer leaves per plant, and smaller, chlorotic leaves with patches. The reduction in plant height ranged from 16.96% (500 J2/plant) to 44.37% (8000 J2/plant) compared to the control. A significant reduction in plant height was observed even at the lowest inoculum level.

The data presented in Table 2 and Figure 1 show a significant reduction in root weight across different inoculum levels compared to the control. The shortest root length (15.75 cm) was recorded at the highest inoculum level (T7, 8000 J2/plant), while the longest root length (30.25 cm) was observed in

the uninoculated control (T1). Following this, root lengths of 26.25 cm, 24.37 cm, 21.75 cm, and 19.25 cm were recorded at inoculum levels of 1000, 2000, 4000, and 6000 J2 per plant, respectively. The reduction in root length ranged from 15.33% (500 J2/plant) to 57.33% (8000 J2/plant) compared to the control. The inhibition in root growth led to the formation of numerous root knots, with a progressive decrease in root length as the initial nematode inoculum levels increased.

### Fresh and dry weight of shoot and root

Table 1 and Figure 1 illustrate a significant reduction in both fresh and dry shoot weight as the inoculum level of RKN in the soil increased. A progressive decrease in fresh and dry shoot weight was observed compared to the control, with all treatments showing significant differences. The highest fresh shoot weight (49.10 g) was recorded in the uninoculated treatment (T1), while the lowest (15.12 g) occurred in the treatment with the highest inoculum level (T7, 8000 J2 per plant). The reductions in fresh shoot weight were 40.12 g, 31.51 g, 27.45 g, and 23.58 g at inoculum levels of 1000, 2000, 4000, and 6000 J2 per plant, respectively. The reduction in fresh shoot weight ranged from 18.28% (500 J2/plant) to 69.20% (8000 J2/plant) compared to the control.

Similarly, dry shoot weight significantly decreased with increasing inoculum density. The highest dry shoot weight (28.02 g) was recorded in the uninoculated control (T1), and the lowest (11.62 g) in the treatment with the highest inoculum level (T7, 8000 J2 per plant). Reductions in dry shoot weight were 22.90 g, 21.38 g, 19.53 g, 17.82 g, and 14.41 g at inoculum levels of 1000, 2000, 4000, and 6000 J2 per plant, respectively. The reduction in dry shoot weight ranged from 18.27% (500 J2/plant) to 58.60% (8000 J2/plant) compared to the control.

As the inoculum level of RKN increased in the soil, there was a progressive decrease in both fresh and dry root weights. This reduction began at the lowest inoculum level. The highest fresh root weight (28.02 g) was recorded in the uninoculated control treatment (T1), followed by 22.90 g, 19.88 g, 18.53 g, 16.57 g, and 14.41 g at inoculum levels of 1000, 2000, 4000, and 6000 J2/plant, respectively. The lowest fresh root weight (11.62 g) was observed in the treatment with the highest inoculum density (T8, 8000 J2/plant), showing a 58.52% reduction compared to the control.

Similarly, the highest dry root weight (4.05 g) was recorded in the uninoculated treatment (T1), followed by 2.88 g, 2.66 g, 2.41 g, 2.26 g, and 2.10 g at inoculum levels of 1000, 2000, 4000, and 6000 J2/plant, respectively. The lowest dry root weight (1.7 g) was recorded in the treatment with the maximum inoculum density (T7, 8000 J2/plant), representing a 58.02% reduction compared to the control. The dry root weight consistently decreased as the inoculum density increased, as shown in Table 2 and Figure 1.

**Table 1: Effect of inoculum level of *M. incognita* on plant growth parameters (plant height, fresh and dry shoot weight) in tomato**

Treatments	Plant height (cm)	% decrease over control	Shoot fresh weight (g)	% decrease over control	Shoot dry weight (g)	% decrease over control
T <sub>1</sub> = uninoculated (control)	80.00± 14.32 <sup>a</sup>	-	49.10±13.35 <sup>a</sup>	-	28.02±1.42 <sup>a</sup>	-
T <sub>2</sub> = 500 J <sub>2</sub> /kg soil	66.43±5.97 <sup>b</sup>	16.96	40.12±2.89 <sup>ab</sup>	18.28	22.90±0.22 <sup>ab</sup>	18.27
T <sub>3</sub> = 1000 J <sub>2</sub> /kg soil	60.75±4.03 <sup>bc</sup>	24.06	31.51±10.21 <sup>bc</sup>	35.82	21.38±1.10 <sup>ab</sup>	23.69
T <sub>4</sub> = 2000 J <sub>2</sub> /kg soil	59.00±9.98 <sup>bc</sup>	26.25	30.49±6.72 <sup>bc</sup>	37.90	19.53±0.72 <sup>abc</sup>	30.29

<b>T<sub>5</sub> = 4000 J<sub>2</sub>/kg soil</b>	53.00±4.76 <sup>bcd</sup>	33.75	27.45±2.43 <sup>c</sup>	44.09	17.82±0.71 <sup>bc</sup>	36.40
<b>T<sub>6</sub> = 6000 J<sub>2</sub>/kg soil</b>	51.50±12.26 <sup>cd</sup>	35.62	23.58±4.77 <sup>cd</sup>	51.97	14.41±0.43 <sup>bc</sup>	48.57
<b>T<sub>7</sub> = 8000 J<sub>2</sub>/kg soil</b>	44.5±5.25 <sup>d</sup>	44.37	15.12±7.48 <sup>d</sup>	69.20	11.62±0.51 <sup>c</sup>	58.60
<b>SEm (±)</b>	<b>3.15</b>	-	<b>3.61</b>	-	<b>2.81</b>	-
<b>CD at 5%</b>	<b>9.26</b>	-	<b>10.63</b>	-	<b>8.28</b>	-

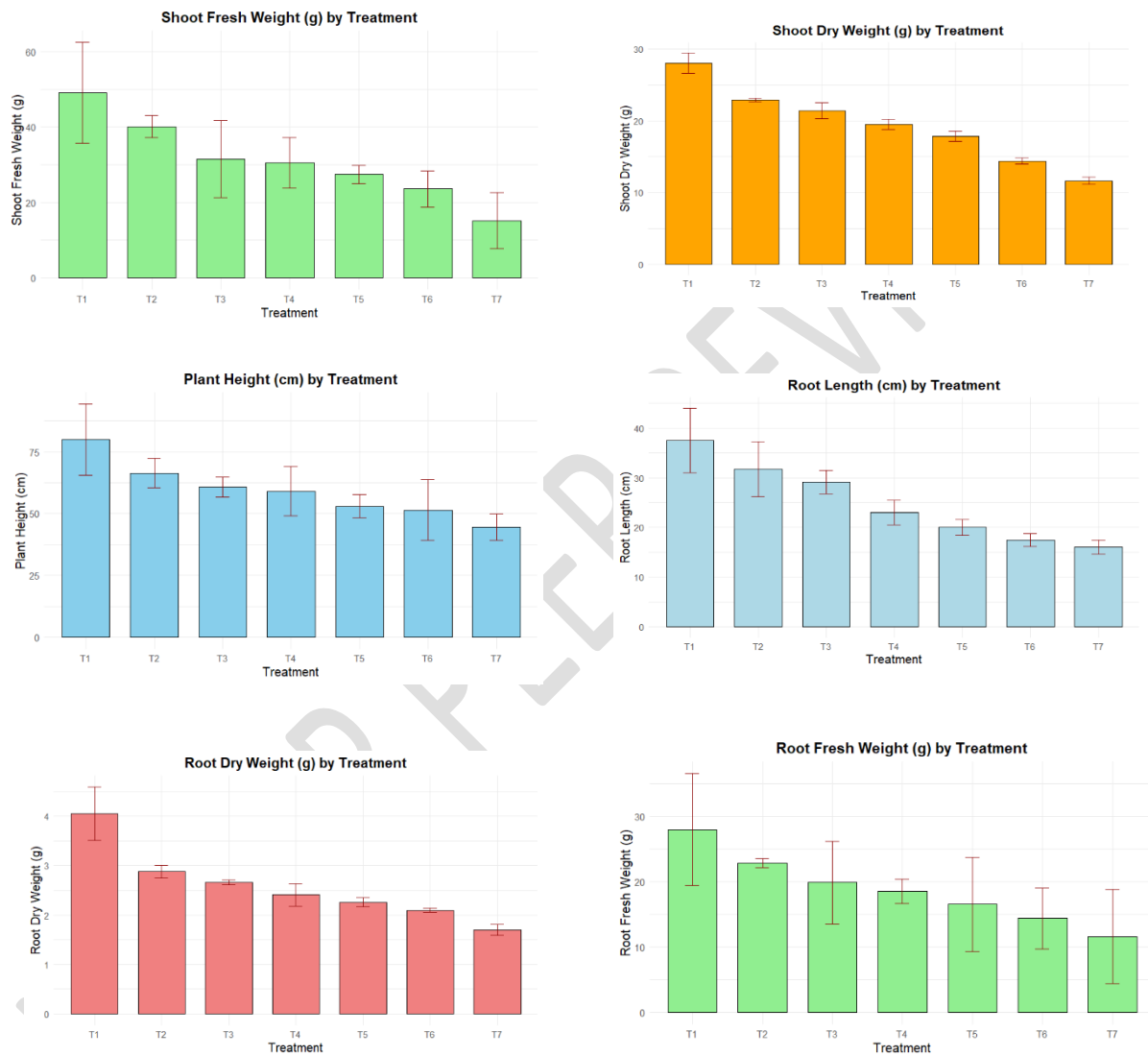
Data shown correspond to the mean of three replicates. Means with the same alphabet letters on each column are not significantly ( $P < 0.05$ ) different CD-critical difference, CV-coefficient of variation, SE - standard error.

**Table 2: Effect of inoculum level of *M. incognita* on plant growth parameters (root) in tomato**

Treatments	Root length (cm)	% decrease over control	Root fresh weight (g)	% decrease over control	Root dry weight (g)	% decrease over control
<b>T<sub>1</sub>= uninoculated (control)</b>	37.50±6.45 <sup>a</sup>	-	28.02±8.56 <sup>a</sup>	-	4.05±0.54 <sup>a</sup>	-
<b>T<sub>2</sub>=500 J<sub>2</sub>/kg soil</b>	31.75±5.56 <sup>b</sup>	15.33	22.90±0.69 <sup>ab</sup>	18.27	2.88±0.13 <sup>ab</sup>	28.88
<b>T<sub>3</sub>=1000 J<sub>2</sub>/kg soil</b>	29.13±2.39 <sup>b</sup>	22.32	19.88±6.34 <sup>abc</sup>	29.05	2.66±0.05 <sup>ab</sup>	29.10
<b>T<sub>4</sub>= 2000 J<sub>2</sub>/kg soil</b>	23.00±2.44 <sup>c</sup>	38.66	18.53±1.84 <sup>bc</sup>	33.86	2.41±0.23 <sup>abc</sup>	40.49
<b>T<sub>5</sub>= 4000 J<sub>2</sub>/kg soil</b>	20.00±1.63 <sup>cd</sup>	46.66	16.57±2.0 <sup>bc</sup>	40.86	2.26±0.09 <sup>bc</sup>	44.19
<b>T<sub>6</sub>= 6000 J<sub>2</sub>/kg soil</b>	17.50±1.29 <sup>cd</sup>	53.33	14.41±4.67 <sup>bc</sup>	48.57	2.10±0.04 <sup>bc</sup>	48.14
<b>T<sub>7</sub>= 8000 J<sub>2</sub>/kg soil</b>	16.00±1.41 <sup>d</sup>	57.33	11.62±7.21 <sup>c</sup>	58.52	1.7±0.11 <sup>c</sup>	58.02
<b>SEm (±)</b>	<b>1.71</b>	-	<b>2.60</b>	-	<b>0.10</b>	-

CD at 5%	5.05	-	7.67	-	0.30	-
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Data shown correspond to the mean of three replicates. Means with the same alphabet letters on each column are not significantly ( $P < 0.05$ ) different CD-critical difference, CV-coefficient of variation, SE - standard error.



**Figure.1** Bar graphs representing the effect of inoculum level of RKN on plant growth parameters

## 2. Effect of inoculum level of RKN on nematode population

### Gall Index/ plant

Data presented in Table 3 and Figure 2 revealed that there was significant increase in galls/ plant with different inoculum levels. As the inoculum level increased the galls/ plant also increased. The significantly minimum galls/ plant with gall index, 3.5 was recorded in the inoculum level of (T<sub>2</sub>) 500 J<sub>2</sub>

followed by 4.5 at (T<sub>3</sub>) 1000 J<sub>2</sub> which were at par with each other. However, highest galls/ plant (with gall index-5.00) was recorded in (T<sub>5</sub>) 4000, (T<sub>6</sub>) 6000 and (T<sub>7</sub>)8000 level of inoculums and they were at par with each other. This was also observed that there was no gall formation in roots of plant in (T<sub>1</sub>) control where the nematodes were not inoculated.

### **Number of egg masses/ plant**

Data presented in Table 3 and Figure.2 showed that number of egg masses/ plant significantly increased with different inoculum levels. As the inoculum level increased, the number of egg masses/ plant also increased. The significantly lowest egg masses/ plant (9.28) was recorded in the inoculum level of (T<sub>2</sub>) 500 J<sub>2</sub>/plant followed by 13.42, 14.85, 16.28 and 24.28 at 1000, 2000, 4000 and 6000 J<sub>2</sub>/plant respectively. However, highest egg masses/ plant (28.71) was recorded in (T<sub>7</sub>) 8,000 level of inoculum. It showed that as the inoculum level increased from 0 to 8,000, a corresponding increase in the number of egg masses/ plant was also recorded.

### **Nematode population/5g of roots**

The nematode population per 5g of root was minimum (0.00) in (T<sub>1</sub>) uninoculated treatment followed by 332.85, 728.57, 1300.00, 2528.57 and 3485.71 at 500, 1000, 2000, 4000 and 6000 J<sub>2</sub>/plant respectively and maximum (3971.42) at (T<sub>7</sub>) 8000 J<sub>2</sub> (Table 3 and Figure.2). It showed that as the inoculum level increased from 0 to 8000, it was observed that there was a corresponding increase in the root population.

### **Nematode population/200 cc of soil**

The nematode population per 200cc of soil was minimum (985.71) at (T<sub>2</sub>) 500 J<sub>2</sub> level and maximum (5028.57) at (T<sub>7</sub>) 8000 J<sub>2</sub> level, when inoculated initially (Table 3 and Figure. 2). It showed that as the inoculum level increased from 0 to 8000, a corresponding increase in the soil population was also recorded.

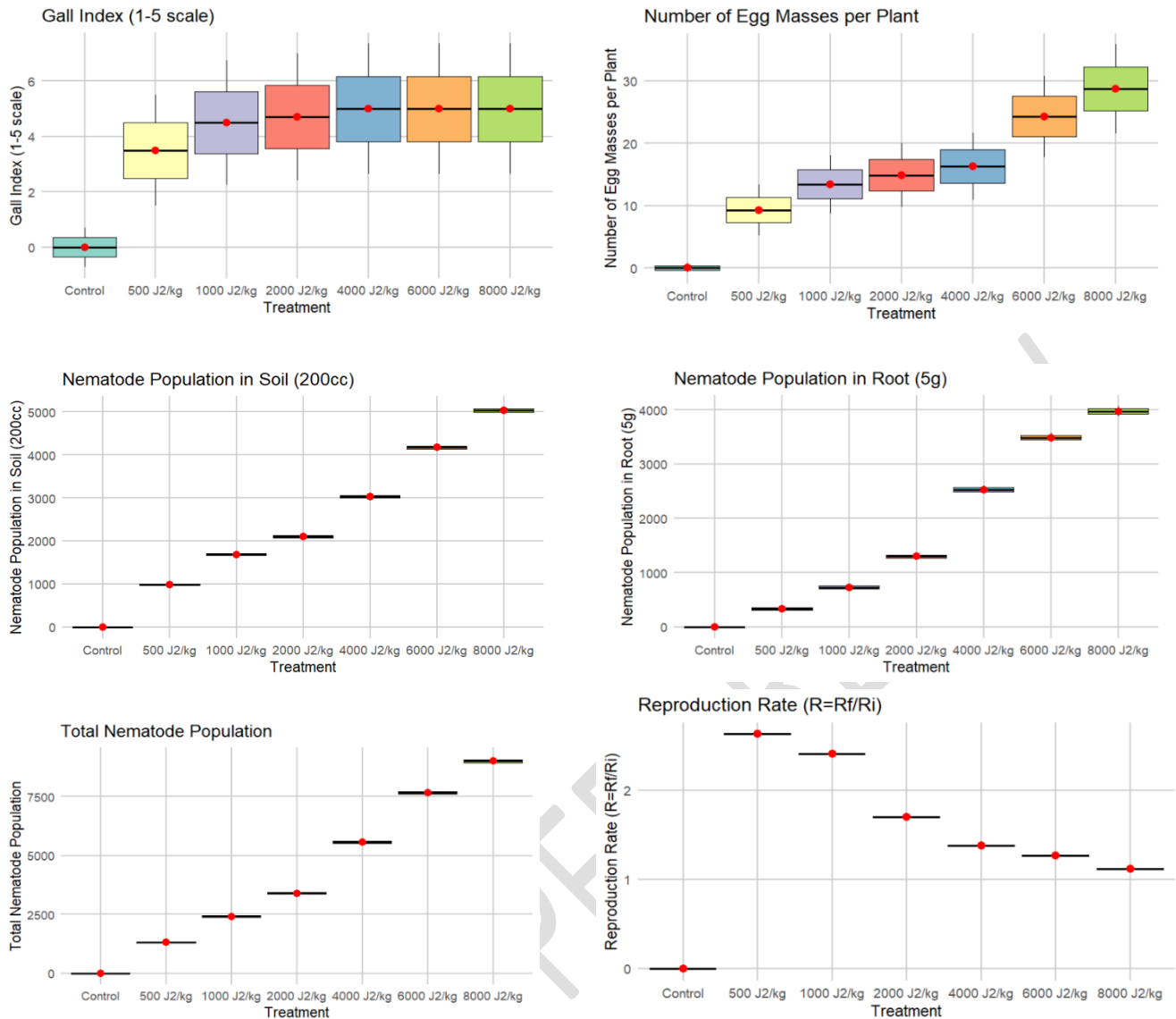
### **Reproduction rate**

Reproduction rate value significantly differed with different inoculum levels (Table 3; Figure.2). The reproduction rate of *M. incognita* was inversely related to the inoculum levels. The maximum Rr value (2.63) was recorded in the inoculum level of (T<sub>2</sub>) 500 J<sub>2</sub>/plant and minimum value (1.12) was recorded in (T<sub>7</sub>) 8000 J<sub>2</sub>/pot. It indicated that as the inoculum level increased from 500 J<sub>2</sub> to 8000 J<sub>2</sub> corresponding Rr values decreased.

**Table 3: Effect of inoculum level of *M. incognita* on gall formation and egg masses per plant in tomato.**

Treatment	Gall index (1-5 scale)	Number of egg masses per plant	Nematode population		Total nematode population	Reproduction rate R=Rf/Ri
			Soil (200cc)	Root (5g)		
<b>T<sub>1</sub>= uninoculated (control)</b>	0.0 (0.71) <sup>c</sup>	0.0 (0.71) <sup>a</sup>	0.0 (0.71) <sup>g</sup>	0.0 (0.71) <sup>g</sup>	0.0 (0.71) <sup>g</sup>	0
<b>T<sub>2</sub>= 500 J<sub>2</sub>/kg soil</b>	3.5 (2.00) <sup>b</sup>	9.28 (4.06) <sup>a</sup>	985.71 (24.14) <sup>f</sup>	332.85 (41.47) <sup>f</sup>	1318.56 (48.00) <sup>f</sup>	2.63
<b>T<sub>3</sub>= 1000 J<sub>2</sub>/kg soil</b>	4.5 (2.23) <sup>a</sup>	13.42 (4.67) <sup>b</sup>	1685.71 (35.61) <sup>e</sup>	728.57 (54.31) <sup>e</sup>	2414.28 (64.99) <sup>e</sup>	2.41
<b>T<sub>4</sub>= 2000 J<sub>2</sub>/kg soil</b>	4.7 (2.29) <sup>a</sup>	14.85 (5.12) <sup>b</sup>	2100.00 (47.52) <sup>d</sup>	1300.00 (60.56) <sup>d</sup>	3400.00 (77.06) <sup>d</sup>	1.7
<b>T<sub>5</sub>= 4000 J<sub>2</sub>/kg soil</b>	5.0 (2.35) <sup>a</sup>	16.28 (5.34) <sup>b</sup>	3028.57 (66.47) <sup>c</sup>	2528.57 (72.75) <sup>c</sup>	5557.14 (98.56) <sup>c</sup>	1.38
<b>T<sub>6</sub>= 6000 J<sub>2</sub>/kg soil</b>	5.0 (2.35) <sup>a</sup>	24.28 (6.55) <sup>b</sup>	4171.429 (78.09) <sup>b</sup>	3485.71 (85.41) <sup>b</sup>	7657.13 (115.75) <sup>b</sup>	1.27
<b>T<sub>7</sub>= 8000 J<sub>2</sub>/kg soil</b>	5.0 (2.35) <sup>a</sup>	28.71 (7.11) <sup>c</sup>	5028.57 (83.36) <sup>a</sup>	3971.42 (93.80) <sup>a</sup>	8999.99 (125.49) <sup>a</sup>	1.12
<b>SEm (±)</b>	<b>0.16</b> <b>(0.04)</b>	<b>3.79</b> <b>(0.35)</b>	<b>1.17</b>	<b>0.97</b>	<b>1.05</b>	-
<b>CD at 5%</b>	<b>0.43</b> <b>(0.12)</b>	<b>9.81</b> <b>(1.04)</b>	<b>3.44</b>	<b>2.86</b>	<b>3.08</b>	-

Data shown correspond to the mean of three replicates. Means with the same alphabet letters on each column are not significantly ( $P < 0.05$ ) different CD-critical difference, CV-coefficient of variation, SE - standard error.



**Figure.2** Box plots representing the effect of inoculum level of RKN on RKN on nematode population

## Discussion:

### 1. Effect of different inoculum level of *M. incognita* on plant growth parameters in tomato

#### Plant height, root length, shoot fresh and dry weight

At 60 DAS, significant damage to plant growth parameters was evident even at the lowest inoculum level of 500 J2 per pot, with maximum damage observed at the highest inoculum density of 8000 J2 per pot. This indicates that a pathogenic threshold for significant damage in tomato begins at 500 J2 under polyhouse conditions. The uninoculated treatment produced the tallest plants (80.00 cm), while the shortest plants (44.5 cm) were found at the highest inoculum level (8000 J2/plant), with a reduction in plant height ranging from 16.96% (500 J2/plant) to 44.37% (8000 J2/plant) compared to the control. Similarly, maximum fresh shoot weight (49.10 g) and dry shoot weight

(28.02 g) were observed in uninoculated plants, while minimum fresh shoot weight (15.12 g) and dry shoot weight (11.62 g) were recorded at the highest inoculum density. The decrease in plant height, fresh, and dry shoot weight with increasing inoculum levels aligns with previous findings by Khan et al. (2004), Sivaprakash et al. (2008), and Khan et al. (2012), who observed similar reductions in plant growth with increasing nematode inoculum densities. Infected plants exhibited stunted growth, yellowing, and basal leaf shedding. Nematode infection reduced shoot length by disrupting nutrient and water uptake, as noted by Karssen and Moens (2006). The shortest root length (9.14 cm) and lowest fresh root weight (11.62 g) were recorded at the highest inoculum level, while maximum root length (21.42 cm) and fresh root weight (48.02 g) were observed in the uninoculated control. The reduction in root length ranged from 15.33% to 57.33%, which is consistent with the findings of Duggal et al. (2017), Ansari et al. (2012), and Di Vito et al. (1986), who demonstrated an inverse relationship between nematode inoculum levels and plant growth parameters.

## **2. Effect of inoculum level of RKN on nematode population parameters in tomato.**

### **1. Gall index/plant, number of egg masses/plant, soil and root population and reproductive rate.**

In the present study, increasing the inoculum level from 500 to 8000 J2 resulted in a significant, progressive increase in root infection by the root knot nematode RKN, as indicated by the root gall index (on a 1-5 scale), the number of egg masses, and nematode population. The highest root gall index (5.00) and egg mass count (28.71) were observed at the 8000 J2 inoculum level, while the lowest (3.5 and 9.28, respectively) were recorded at 500 J2. A higher number of nematodes penetrating the roots at higher inoculum levels led to increased galling and egg mass production, confirming that gall and egg mass formation are inoculum-density dependent. Similar results were found by Ghasolia and Shivpuri (2003) on marigold, Haider et al. (2003) on pulses, and Duggal et al. (2017), who recorded an increase in root galls with increasing inoculum density. The nematode population in the soil also increased with higher inoculum levels, consistent with findings by Ansari and Azam (2005) on green gram and Hussain and Bora (1995) on *Phaseolus vulgaris*. Kankam and Adomako (2014) also reported similar effects of inoculum density on tomato growth. The reproduction rate of nematodes was highest (2.63) at the lowest inoculum level (500 J2/plant) and lowest (1.12) at the highest inoculum level (8000 J2/plant). This decline in reproduction rate with increasing inoculum levels is likely due to reduced competition for host penetration, food, and space at lower densities. Haider et al. (2003) and Jiskani et al. (2008) found similar trends, where reproduction factors decreased with increasing inoculum levels. Prasad and Chawala (1992) attributed the decrease in reproduction rate to overcrowding and competition for resources, as parasitism by nematodes damaged the root system, limiting food and nutrition and reducing the ability of juveniles to find new infection sites.

## Discussion:

## Conclusion:

The study demonstrated a clear relationship between increasing RKN inoculum levels and the severity of plant damage in tomato under polyhouse conditions. Plant growth parameters, including height, shoot and root weights, progressively declined as inoculum levels increased from 500 to 8000 J2, with significant reductions in fresh and dry weights. Root infection, as indicated by the root gall index and egg mass production, also escalated with higher inoculum densities, confirming that nematode-induced damage is directly proportional to the inoculum load. The nematode population in the soil increased with inoculum levels, but the reproduction rate decreased, likely due to competition for resources and space. These findings align with previous research, reinforcing the pathogenicity of RKN at higher inoculum densities, leading to impaired plant growth and reduced reproductive efficiency of the nematodes. This study underscores the importance of managing nematode populations to minimize crop losses and suggests that even low inoculum levels (500 J2) can cause significant damage, highlighting the need for effective control strategies.

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