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Pathogenicity of Root-knot Nematode, *Meloidogyne incognita* on Tuberose (*Polianthes tuberosa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A pot experiment was carried out in the net house of Department of Nematology, Assam Agricultural University, Jorhat, to study the pathogenicity of root-knot nematode, *Meloidogyne incognita,* on tuberose. The study found that as the inoculum level of *M. incognita* increased from 10 to 10,000 second-stage juveniles (J_2) per pot (containing 1 kg of sterilized soil), all plant growth parameters of tuberose decreased progressively. At the highest inoculum level, the plants became severely stunted, chlorotic, and produced very few bulblets. It was noted that at the highest inoculum level, the root systems were significantly reduced, with larger and mostly coalescent galls. The treatments with no nematode (check and associated check) were free from galls and eggmasses. An initial inoculum level of 100 J_2 of *M.incognita* per kg of soil caused significant reduction in all growth parameters and proved to be pathogenic to the tuberose plants. The number of galls and eggmasses increased gradually in the plants with an initial inoculum level of 10 to 1000 J_2 of *M.incognita* per kg of soil, but it declined at highest inoculum level of 10,000 J_2 per kg of soil.

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The experiment also recorded the variation in nematode population in the soil as well as their reproductive rates. These findings can be used to develop effective, targeted methods for minimizing the damage caused by root-knot nematode in tuberose crops.

Keywords: Tuberose; pathogenicity; root-knot nematode; Meloidogyne incognita; inoculum.

1. INTRODUCTION

"Tuberose (*Polianthes tuberosa* L.) is a well-known ornamental crop that grows from bulbs and is perennial in nature, belonging to the Amaryllidaceae family. Originating from Mexico, the Polianthes genus comprises 15 species, with 12 found in Mexico and Central America. Among these, 9 species have white flowers, one is white tinged with red, and two are red. Except for *P. tuberosa* L., all other species are found growing wild. In India, tuberose is popularly known as Rajanigandha or Nishigandha. Tuberose is commercially used both as loose and cut flowers. The spikes of tuberose remain fresh for longer periods, which gives them a distinct place in the flower market. The flowers of tuberose are also used for making artistic garlands, bouquets, floral ornaments, buttonholes, gajras, and for the extraction of essential oil. Tuberose is commercially cultivated from bulbs. There are three types of tuberose in cultivation *viz*., 'single', 'semi double' and double" [1]. "Single flowers bear pure white flowers with one row of corolla segments. These are mostly used as loose flowers. Semi-double flowers consist of two to three rows of corolla segments, and double flowers consist of more than three rows of corolla segments on straight spikes. Single flowers possess more fragrance than double ones. The crop is infested by a number of insects, fungi, as well as plant parasitic nematodes, which leads to reduction in the quality of flowers and can cause complete destruction of the crop. Among the plant-parasitic nematodes that affect tuberose, three main species are known to be harmful: the root-knot nematode (*Meloidogyne* spp.), the reniform nematode (*Rotylenchulus reniformis*), and the foliar nematode" (*Aphelenchoides besseyi*) [2].

For the first time, Melis [3] reported *Meloidogyne* spp. as an important pathogen of tuberose. Jayaraman et al. [4] reported the presence of M. javanica and M. incognita in the tuberose growing fields of Tamil Nadu. Khan and Reddy [5] found that *M. incognita* alone was responsible for reductions in plant weight, number of flowers, spike length, spike weight, and number of bulblets, respectively. Ravichandra [6] found that *M. incognita* causes yield loss up to 13-14% in tuberose. Gall formation in the roots is the diagnostic symptom of root-knot nematode infestation. Besides direct damage, it also forms a disease complex with other pathogens, enhancing the severity of the disease. Affected tuberose plants exhibit stunting, yellowing and drying up of leaves, dwarf tips, rotting of bulbs, and withering with delayed emergence of spikes. Typically, *M. incognita* produces egg masses containing 250-500 eggs on galls. The reproduction of *M. incognita* is affected by various factors, including the host plant's susceptibility, nematode population density, and environmental conditions. The present experiment aims to determine the optimal inoculum density of *M. incognita* to reduce its population to a level that does not cause significant economic damage to tuberose crops. To achieve this, we conducted an experiment to study the pathogenicity of *M. incognita* on tuberose (*Polianthes tuberosa* L.).

2. MATERIALS AND METHODS

The experiment was carried out in the net house of the Department of Nematology during the summer season of 2019 to study the pathogenicity of *M. incognita* on tuberose. Soil from an upland situation was collected and sieved to remove stones and debris. This soil was mixed homogeneously with finely dried cow dung and river sand in the ratio of 2:1:1. The soil mixture was then filled in a gunny bag and sterilized in an autoclave at 121^{o}C at 15lb pressure per square inch for 30 minutes. A single egg mass of *Meloidogyne incognita* was collected from the tomato plants maintained as a pure culture in the net house of the Department of Nematology, AAU, Jorhat. After 24 hours, the hatched second-stage juveniles of *M. incognita* were inoculated into tomato seedlings grown in sterilized soil in pots. These inoculated tomato plants were maintained as a source of inoculum for subsequent inoculations.

Earthen pots were collected, cleaned, and sun-dried, then filled with 1kg of sterilized soil and a few broken bricks. The pots were labeled according to the treatment and replication, and tuberose bulbs (Var. Vaibhav) were planted in each pot. The pots were arranged in a

completely randomized design (CRD) with 5 replications per treatment. Finally, second-stage juvenile of *M. incognita* (10, 100, 1000, or 10,000 per pot) were inoculated into the soil in a logarithmic series. Two sets of checks were conducted, including one that focused on detecting associated micro-organisms. This was achieved by isolating a portion of the original suspension, removing all nematodes, and then using it for inoculation.

The plants received regular watering, and observations were made 45 days after inoculation. Plant height was measured before carefully uprooting the plants with tap water to preserve the roots and egg masses. The number of galls and egg masses per root system were counted, and fresh shoot and root weights were recorded. To determine dry weight, plants and their root systems were placed in labeled paper bags and dried in an oven at 60°C until constant weights were achieved. Additionally, nematodes were extracted from soil samples using the modified Cobb's sieving and decanting technique [7].

$$\text{Reproductive rate} = \frac{\text{Final nematode population}}{\text{Initial nematode population}}$$

The experimental data obtained were analysed by following the Fisher's method of Analysis of Variance [8]. The significance and non-significance of given variance was determined by calculating the respective values of 'F' and comparing the calculated 'F' values with corresponding tabulated 'F' values of 5 percent level of probability. The standard error of deviation (S.Ed) between mean of the treatment of combination was calculated as:

$$\text{S.Ed } (\pm) = \sqrt{\frac{2\text{xError mean square}}{\text{Number of replication}}}$$

When 'F' values were found to be significant, critical difference (C.D) were calculated by multiplying the standard error of difference with corresponding 't' values both at 5 percent level probability.

The mean differences among treatments were tested by arranging the treatment means in descending order. Each of these differences, which are greater than C.D values at 5 per cent level of probability, was declared as significantly different.

3. RESULTS AND DISCUSSION

The experimental results are summarized in Tables 1 and 2, accompanied by their respective CD values. Furthermore, the results are graphically represented in Figs. 1-6 and Plates 1 and 2, providing a visual illustration of the findings, which show the mean data for various plant growth parameters, including plant height, fresh and dry weight of shoot and root, number of galls, egg masses, final nematode population in soil, and reproductive rate. The data reveals a progressive decline in all plant growth parameters of tuberose as the inoculum level of *Meloidogyne incognita* increases from 10 to 10,000 second-stage juveniles per pot. At the highest inoculum level, plants were severely stunted, chlorotic, and produced very few bulblets. Additionally, the root system was significantly reduced, with large, coalescent galls forming on the roots. In contrast, the control treatments (check and associated check) showed no galls or egg masses, indicating the absence of nematodes.

Plate 1. Growth of tuberose plant under different inoculum levels of *Meloidogyne incognita*

Table 1. Effect of different inoculum levels of *Meloidogyne incognita* on plant growth parameters of tuberose (Mean of 5 replications)

Inoculum level (J_2/kg soil)	Plant height (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
T_1: Check	62.54[a]	39.30[a]	8.27[a]	4.69[a]	1.80[a]
T_2: Associated check	60.46[a]	38.17[a]	8.01[a]	4.38[a]	1.77[a]
T_3: 10	59.84[a]	36.18[a]	7.58[a]	4.30[a]	1.72[a]
T_4: 100	46.74[c]	29.98[b]	6.01[b]	3.76[b]	1.56[b]
T_5: 1000	17.68[d]	25.77[c]	4.61[c]	2.55[c]	0.53[c]
T_6: 10,000	13.22[e]	20.15[d]	3.42[d]	1.64[d]	0.44[d]
S.Ed (±)	1.54	1.58	0.34	0.25	0.04
$CD_{0.05}$	3.20	3.29	0.71	0.52	0.08

**Mean followed by the same letter in the superscript(s) are statistically at par.*

Table 2. Effect of different inoculum levels of *Meloidogyne incognita* on number of galls, egg masses and nematode population on tuberose (Mean of 5 replications)

Inoculum level (J_2/kg soil)	No. of galls/root system	No. of egg masses/root system	Final nematode population (200 cc of soil)	Reproductive rate (%)
T_1: Check	0.00 (0.70)[d]	0.00 (0.70)[d]	0.00 (0.70)[d]	0.00
T_2: Associated check	0.00 (0.70)[d]	0.00 (0.70)[d]	0.00 (0.70)[d]	0.00
T_3: 10	10.6 (3.29)[c]	7.80 (2.83)[c]	305.6 (17.47)[c]	30.56
T_4: 100	25.2 (5.05)[b]	16.60 (4.10)[b]	1166.4 (34.04)[b]	11.64
T_5: 1000	49.8 (7.06)[a]	40.80 (6.07)[a]	2377.4 (48.75)[a]	2.37
T_6: 10,000	8.8 (2.99)[c]	5.60 (2.14)[c]	2497.2 (49.94)[a]	0.24
S.Ed (±)	0.31	0.31	1.03	
$CD_{0.05}$	0.65	0.64	2.14	

Values of number of galls, egg masses and final nematode population within in parentheses are square root ($\sqrt{x+0.5}$) transformed data. Mean followed by the same letter in the superscript(s) are statistically at par.

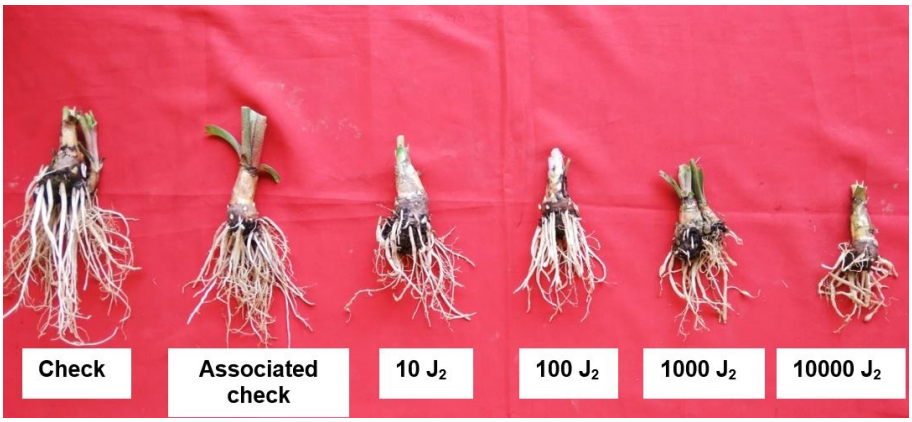

Plate 2. Effect of different inoculum levels on root growth

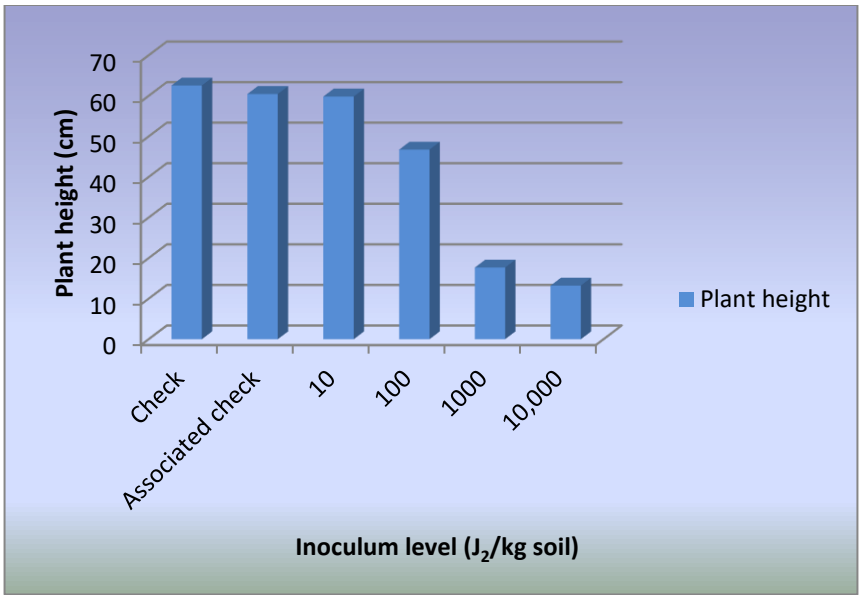

Fig. 1. Effect of different inoculum level on plant height of tuberose

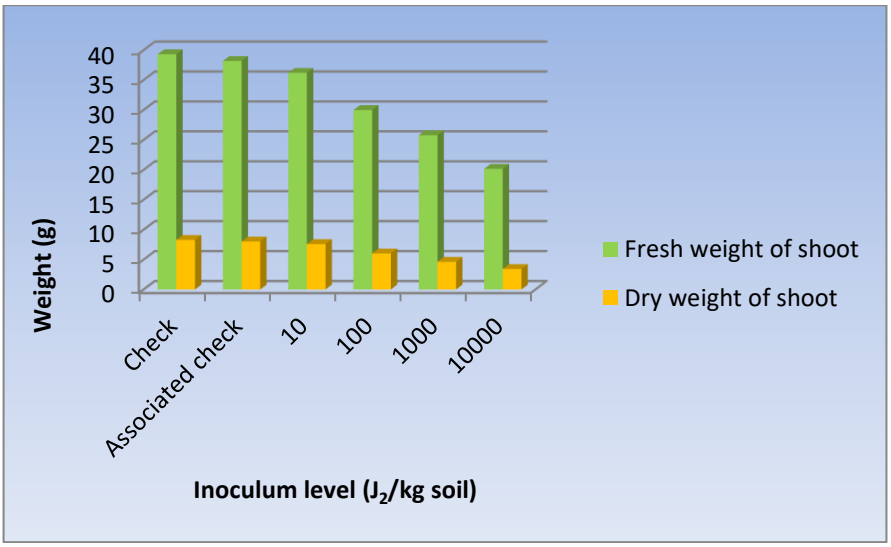

Fig. 2. Effect of different inoculum level on fresh and dry weight of shoot of tuberose

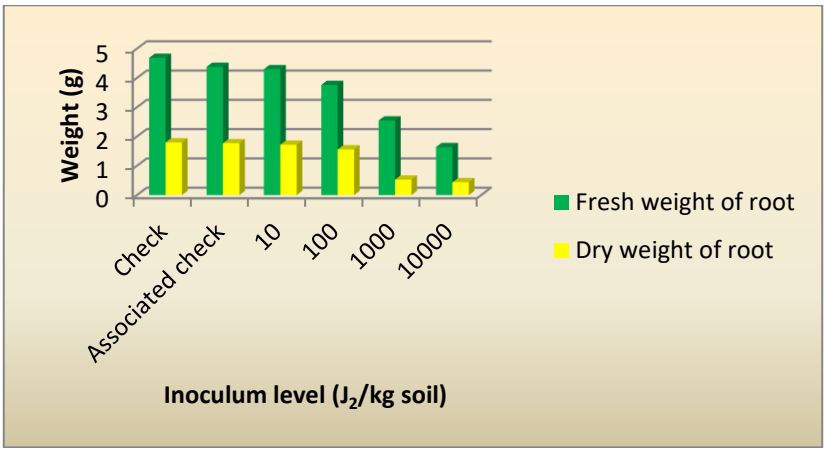

Fig. 3. Effect of different inoculum level on fresh and dry weight of root of tuberose

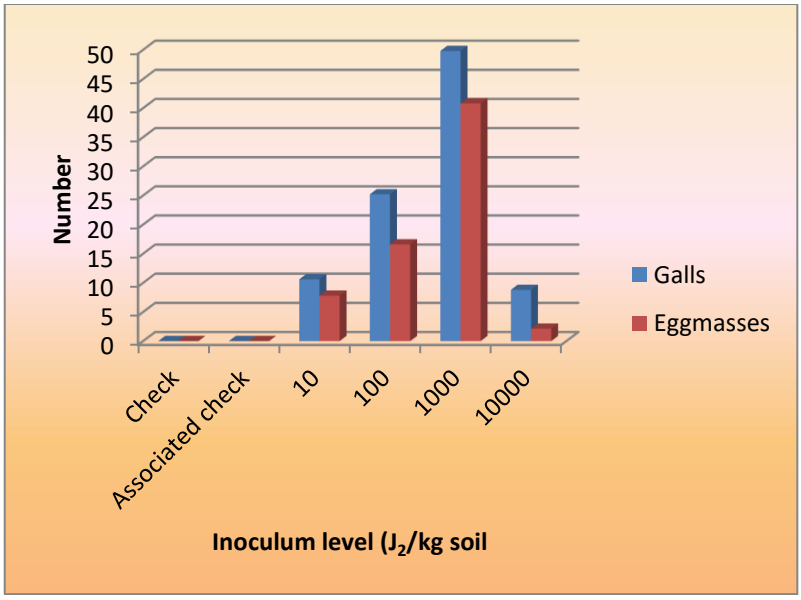

Fig. 4. Effect of different inoculum level on galls and eggmass formation per root system root of tuberose

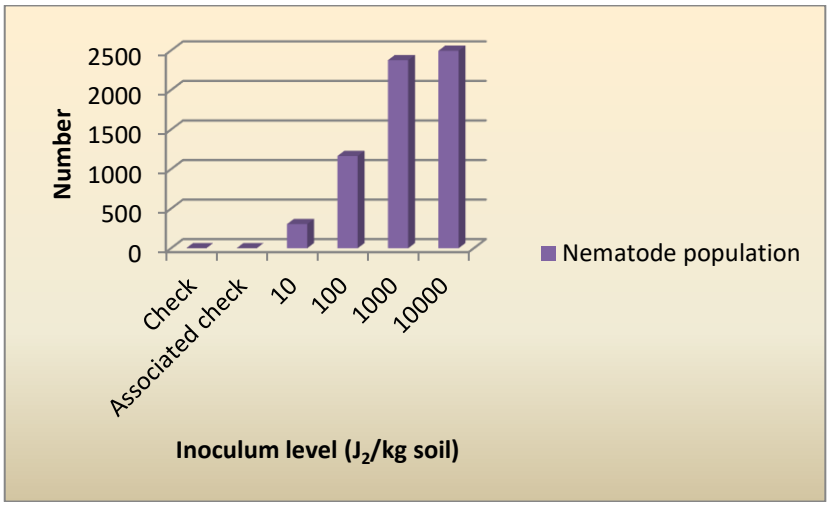

Fig. 5. Nematode population in soil

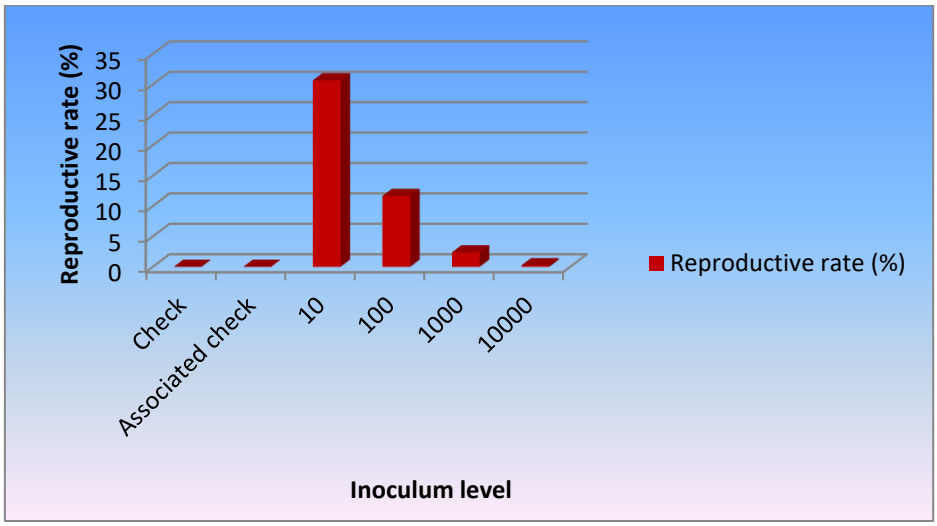

Fig. 6. Reproductive rate of nematode (%) in soil

"Data revealed that plant height decreased progressively in the treatments with an initial inoculum level of 100 to 10,000 nematodes per pot. The highest reduction in plant height was observed in the treatment with 10,000 nematodes per pot. It was observed that there was no significant difference in plant height among the treatments with no nematode (Check and associated check) and the treatment with 10 nematodes per pot but all these treatments differed significantly from the treatments with 100, 1000 and 10,000 nematodes per pot. Further, it was observed that treatments with 100, 1000, and 10,000 nematodes per pot differed significantly from each other. There was no significant difference in the fresh weight of shoots among the treatments with no nematode (Check and associated check) and the treatment with 10 nematodes per pot, but these treatments differed significantly from the treatments with 100, 1000, and 10,000 nematodes per pot" [6]. Further, "treatments with 100, 1000, and 10,000 nematodes per pot differed significantly from each other. No significant difference in the dry weight of shoots was observed among the treatments with check and associated check and the treatment with 10 nematodes per pot. However, these treatments differed significantly from the treatments with 100, 1000, and 10,000 nematodes per pot. Further, treatments with 100, 1000, and 10,000 nematodes per pot differed significantly from each other. Data revealed that there was no significant difference in the fresh weight of roots among the treatments with no nematode (Check and associated check) and 10 nematodes per pot, but these treatments differed significantly from the treatments with 10, 100, and 10,000 nematodes per pot. Further, treatments with 100, 1000 and 10,000 nematodes per pot differed significantly from each other. No significant difference in dry weight of roots was observed among the treatments with no nematode (Check and associated check) and the treatment with 10 nematodes per pot. But the treatments having 100, 1000, and 10,000 nematodes per pot differed significantly from each other. The number of galls and egg masses per root system increased gradually with the increase in inoculum level from 10 to 1000 but it declined at 10,000 inoculum level per pot" [9] The maximum number of galls and egg masses were recorded in the treatment with 1000 nematodes per pot. The minimum number of galls and egg masses were recorded in the treatment with 10,000 and 10 nematodes per pot respectively. However, there was no significant difference in galls and egg masses between the treatments with 10 and 10,000 nematodes per pot, but these treatments differed significantly from the treatments with 100 and 1000 nematodes per pot. There was a progressive increase in nematode population with the increase in level of inoculum from10 to 10,000 nematodes per pot. Further, it was observed that there was no significant difference between the treatments having inoculum levels of 1000 and 10,000 nematodes per pot. But these treatments differed significantly from 10 and 100 nematodes per pot. The reproductive rate of nematodes decreased significantly with an increase in inoculum level from 10 to 10,000 nematodes per pot. The highest (30.56) and lowest (0.24) reproductive rates were observed in the inoculum level of 10 and 10,000 juveniles per pot,

respectively. Further, it was observed that there was no significant difference between the treatments having inoculum levels of 1000 and 10,000 nematodes per pot. But these treatments differed significantly from 10 and 100 nematodes per pot.

The results obtained in the study of pathogenicity of *M. incognita* on tuberose indicated that with the increase in inoculum level of *M. incognita* there was a corresponding decrease in plant height. This finding is in agreement with the findings obtained by Sundarababu and Vadivelu [10] on tuberose. Furthermore, it was observed that there was a significant reduction in plant height of tuberose plants at and above 100 nematodes per pot. Similar results were obtained by Mohanty and Das [11] on tuberose. Additionally, it was observed that at the highest inoculum level (10,000 J_2 per pot), the plants became completely stunted. Similar symptoms were also observed by Dutta [12] and Dungdung [13] on gladiolus.

There was a corresponding reduction in fresh and dry weight of the shoot and root of the plants with increase in level of inoculum from 10 to 10,000 nematodes per pot. Similar results were obtained by Johnson et al. [14] on gladiolus. Ravishankar and Singh [15] also recorded similar results on gladiolus. Furthermore, it was observed that there was a significant reduction in shoot weight and root weight (fresh and dry) of the plants inoculated with 100 or above nematodes per pot. Similar findings had been reported by Duggal et al. [16] on gladiolus. Morever, it was observed that at the highest inoculum level (10,000 J_2 per pot), the plants had very much reduced root system with fewer bulblets.

“The experiment showed that the number of galls and egg masses increased steadily as the inoculum level of *M. incognita* rose from 10 to 1000 per pot, but surprisingly decreased at the highest inoculum level of 10,000 per pot. In contrast, the nematode population in the soil consistently increased as the inoculum level rose from 10 to 1000 nematodes per pot, with no decline observed” [17]. The maximum number of galls and egg masses were recorded in plants inoculated with 1000 nematodes per pot. Similar results were recorded by Manju and Subramanian [18] on gerbera.

As the nematode population increased gradually, the reproductive rate of *M. incognita* decreased correspondingly. The reproductive rate was highest (30.56) at the lowest inoculum level (10 J_2 per pot) and lowest (0.24) at the highest inoculum level (10,000 J_2 per pot). The study revealed an inverse relationship between population growth rate and inoculum level, with the highest growth rate occurring at the lowest inoculum level (10 J_2 per pot) and the lowest growth rate at the highest inoculum level (10,000 J_2 per pot). This trend may be explained by increased competition among nematodes for limited resources, including host penetration, nutrition, and spatial accommodation. These findings are consistent with earlier studies Senthamarai et al. [9] on coleus and Kalaiarasan et al. 2006 on groundnut [19].

4. CONCLUSION

This study conclusively shows that an inoculum level of 100 J_2 of M. incognita per kg of soil is harmful to tuberose plants, causing a consistent decline in plant growth parameters. This finding highlights the intricate dynamics between root-knot nematode inoculum levels and plant responses. With this knowledge, farmers can develop effective nematode management strategies for tuberose cultivation, enabling them to secure better prices in the global flower market and enhance their competitiveness.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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