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Pathogenic Level of Reniform Nematode (*Rotylenchulus reniformis* Linford & Oliveira, 1940) on Tomato (*Solanum lycopersicum* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Author PB performed the experiment and statistical analysis, author GD wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Tomato is an important vegetable crop. Various plant parasitic nematode cause extensive damage during growth stage of tomato. Pathogenic level of Reniform nematode (*Rotylenchulus reniformis*), was investigated on tomato var. Pusa Ruby by inoculating 10, 100, 500, 1000, 5000 and 10000 nematodes per kg autoclaved soil. Observations on plant growth parameters and nematode numbers were recorded at 60 days following inoculation. The inoculated seedlings showed a progressive decrease in the plant growth parameters with increase in inoculum level of *R. reniformis*. Significant reduction in plant growth parameters were recorded at 1000 and above nematodes/kg soil. There was a gradual increase in the number of females, egg masses per root system, and nematode population of *R. reniformis* with increase in inoculum level. The rate of multiplication decreased with increase in the level of inoculation. The pathogenic level of *R. reniformis* was found to be 1000 nematodes per kg soil.

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Keywords: Pathogenic level; reniform nematode (Rotylenchulus reniformis); tomato; growth parameters; inoculums level.

1. INTRODUCTION

The pulpy fruit tomato (*Solanum lycopersicum* L.) also known as 'poor man's orange' are used as vegetable. It is having high nutritive value as minerals, vitamin A, vitamin C and anti-oxidant are present [1,2]. Tomato is grown all over the world in temperate, subtropical and tropical areas [3]. In Assam, tomato production is 430.83 thousand tonnes with an average yield 20-25 t/ha as reported by National Horticultural Board (NHB) 2021-22. The requirement for tomato in India and world market is rising day by day. But the production is vulnerable by several biotic and abiotic factors. Among biotic factors plant-parasitic nematodes are considered as one of the most important plant pathogens. The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira, 1940) is one of the most important plant-parasitic nematodes in the world [4-6]. In India, the reniform nematode was first recorded by Das [7] in Andhra Pradesh and now it is known to present in almost all states of India. Rao and Ganguly [8], Gaur et al. [9], Khan, [10] reported that *R.reniformis* attacks over 150 plant species from 50 families. As no work has been conducted on reniform nematodes in Assam, therefore the present work was undertaken to establish the damaging threshold level on tomato.

2. MATERIALS AND METHODS

The pot experiment on pathogenicity of *R.reniformis* on tomato (Var. Pusa Ruby) was carried out during the rabi season (November, 2022- March, 2023) in the net house of Department of Nematology, Assam Agricultural University, Jorhat. The laboratory investigation was conducted in the Post Graduate Laboratory of Department of Nematology, Assam Agricultural University, Jorhat. The isolates of reniform nematode were collected from castor plants from back of net house of Department of Nematology, AAU, Jorhat. Permanent slides were made following Nguyen et al. [11]. Morphological identification was done based on Robinson et al. [12] and Dasgupta et al. [13] and it was confirmed morphologically as *Rotylenchulus reniformis*. Required inoculums of *R.reniformis* were obtained after processing infested soil collected from culture pots having castor plants. The larvae so collected were kept in water for a week so that all the larvae may reach to preadult stage. The preadults (male female ratio 1: 1) were counted taking 1 ml from the homogenous nematode suspension on Hawkshley Counting Dish using the Magnüs microscope. Seeds of tomato (Var. Pusa Ruby) were surface sterilized with rectified spirit before sowing. In each pot, 2 seeds were sown at the depth of 5-6 cm which contains sterilized soil and covered with a thin layer of soil over it. The pots were sprinkled with water without disturbing the thin soil cover. Seedlings were thinned out after one week of germination keeping only one healthy seedling in each pot. Seven days after germination, seedlings were thinned to one plant per pot. Inoculation was done by adding the counted number of mixers of juveniles, males and pre- mature females of *R. reniformis* in a series 10, 100, 500, 1000, 5000 and 10,000 per pot along with control over the surface roots, by carefully exposing the root system up to a depth of 1 cm and then covered with fresh sterilized soil. The pots were arranged in a completely randomized block design (CRD) with five replications. Regular watering of plants was done till harvesting of the crop. Harvesting of the plants was done 60 days after inoculation. The entire root system was taken out from the pot and kept in a plastic bucket half filled with water for half an hour. Then the root system was washed with tap water very carefully to avoid root damage and loss of egg masses from the roots. Observations were made on root length, fresh and dry weight of shoots and roots in all treatments. For recording the dry weight of shoot and roots the fresh shoot and root materials were packed in paper bags labeled according to the treatment and kept in an oven running continuously at 30-35°C. The materials were weighted after every 24 hours till a constant weight was observed. The nematode population density was estimated by extracting 250 cc of soil from each pot. Females of reniform nematode were stained and numbers present on the whole root system were enumerated. Root samples were agitated in 0.6% NaOCl for 10 min to dislodge eggs from egg masses. Estimation of reproductive rate was calculated by:

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

The experimental data obtained were analysed by following the Fisher's method of Analysis of Variance [14].

3. RESULTS AND DISCUSSION

The results indicated that the growth of plants was negatively correlated to the level of inoculation of reniform nematode (*R. reniformis*) (Table.1). The mean data on shoot length showed that with increase in inoculum levels the shoot length gradually decreases. The treatments with no nematode (uninoculated), 10, 100, and 500 nematodes per pot did not show any significant difference in shoot length, however these treatments were considerably different from the treatments with 1000, 5000, and 10,000 nematodes per pot. Shoot length was significantly reduced at inoculum levels of 1000 (60.56cm) and above. The mean data on root length showed that the root length reduced progressively as the inoculums levels of 10 and 100 nematodes per pot, there were no significant variations in root length as compared to control, though these treatments differed significantly from the treatments with 500, 1000, 50000 and 10,000 nematodes per pot. At inoculums levels of 500 (16.00cm) and above nematodes per pot, a significant decrease in root length was observed. The mean data on the fresh and dry weight of shoot showed that when the inoculums level increased, the fresh weight of the shoot decrease significantly. There was no significant difference in fresh weight of shoot between the uninoculated treatments and the treatments with 10, 100 and 500 nematodes per pot, but these treatments differed significantly from the treatments with 1000, 5000, and 10,000 nematodes per pot. At inoculums levels of 1000 and above nematodes per pot, there was a significant decreased in the fresh weight of the shoot (22.70g) as compared to control. With increase in inoculums level there was a significant decrease in dry weight of shoot. However, there were no significant variations in shoot dry weight at inoculums levels of 10, 100, and 500 nematodes per pot. There was no significant difference in dry weight of shoot between the uninoculated treatments and the treatments with 10, 100, and 500 nematodes per pot, but these treatments differed significantly from the treatments with 1000, 5000, and 10,000 nematodes per pot. At 1000 (8.26g) and higher inoculum levels, there was a significant decreased in the dry weight of the shoot. The mean data on fresh weight of root and dry weight of root showed that when the inoculum level increased, the fresh weight of root decreased significantly. There was no significant variations in fresh root weight between inoculums levels of 10 and 100 nematodes per pot as compared to control but these treatments differed significantly from the treatments with 500, 1000, 5000 and 10,000 nematodes per pot. At 500 and higher inoculum levels, there was a significant decreased in fresh weight of root (9.82g). In the case of the dry weight of the root, increasing the inoculums level resulted in a significant decrease. There was no significant difference in dry weight of root between the uninoculated treatments and treatments with 10 and 100 nematodes per pot, but these treatments differed significantly from the treatments with 500, 1000, 5000, and 10,000 nematodes per pot. At 500 and higher inoculums levels, there was a significant decrease in root dry weight (3.03g). Ahmad and Alam [15] observed 32.68% reduction of plant growth parameters of tomato var. Pusa Ruby after sixty days of inoculation with *R.reniformis* at inoculums level of 5000 nematodes/plant/kg of soil with reproduction rate 3.9. Nguyen et al. [16] also reported the significant correlations between *R.reniformis* and the degree of plant damage (yellowing leaves and dry, rotten rhizomes) in turmeric in the Central Highlands of Vietnam and found density up to 480 nematodes/100 ml of soil.

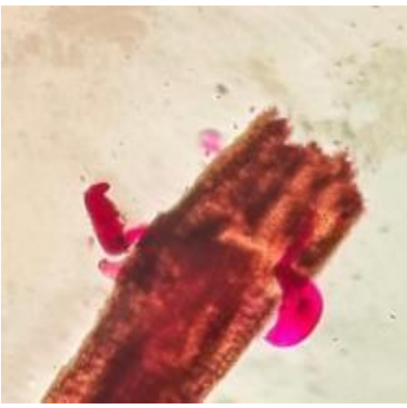

Fig. 1. Reniform nematode on tomato root

Table 1. Effect of different inoculum levels of *Rotylenchulus reniformis* on plant growth parameters of tomato (Mean of 5 replications)

Inoculum level	Shoot length(cm)	Fresh weight of shoot(g)	Dry weight of shoot(g)	Root length (cm)	Fresh weight of root (cm)	Dry weight of root (g)
T1:10	65.80[a]	25.20	9.92[a]	21.86[a]	12.20[a]	4.50[a]
T2:100	65.08[a]	24.60[a]	9.76[a]	21.04[a]	11.92[a]	4.36[a]
T3:500	64.28[a]	24.56[a]	9.54[a]	16.00[b]	9.82[b]	3.03[b]
T4:1000	60.56[b]	22.70[b]	8.26[b]	14.12[b]	9.26[b]	2.71[b]
T5:5000	56.00[c]	19.30[c]	7.24[c]	11.69[c]	7.22[c]	1.56[c]
T6:10000	51.24[d]	17.16[d]	6.34[d]	9.32[d]	5.14[d]	1.04[d]
T7:Control	65.92[a]	25.80[a]	10.00[a]	22.24[a]	12.93[a]	4.45[a]
S.Ed. (±)	0.98	0.81	0.32	0.97	0.96	0.55
C.D(P=0.05)	1.98	1.67	0.66	2.00	1.97	1.13

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

Table 2. Effect of different inoculum level of *Rotylenchulus reniformis* on number of females, egg masses and nematode population on tomato (Mean of 5 replications)

Inoculum level	No. of females / root system	No. of egg masses/ root system	Larval population (200cc of soil)	Total population / pot	Reproductive rate%
T1:10	7.40 (2.75)[e]	6.80 (2.66)[e]	24.00 (4.46)[f]	118.80 (10.91)[f]	11.18[ab]
T2:100	9.60 (3.15)[e]	8.8 (3.00)[e]	266.00 (16.80)[e]	1142.80 (36.80)[e]	11.42[a]
T3:500	26.60 (5.19)[d]	25.6 (5.09)[d]	1138.00 (34.52)[d]	5459.20 (72.82)[d]	10.91[b]
T4:1000	37.40 (6.14)[c]	36.00 (5.86)[c]	1571.8.00 (40.54)[c]	7310.40 (85.52)[c]	7.31[c]
T5:5000	45.20 (6.74)[b]	42.0 (6.51)[b]	3796.00 (62.41)[b]	13446.80 (115.96)[b]	2.68[d]
T6:10000	55.60 (7.48)[a]	51.00 (7.17)[a]	4360.00 (71.98)[a]	21269.40 (145.80)[a]	2.18[d]
T7 :Control	0.00	0.00	0.00	0.00	0.00
S.Ed.(±)	0.14	0.20	0.74	1.35	1.37
C.D(P=0.05)	0.44	0.47	1.52	2.85	2.82

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

The mean data on the number of females and egg masses per root system showed that the number of females and egg masses per root system increased progressively as inoculums levels increased from 10 to 10,000 inoculum levels per plant (Fig.1; Table 2.). The minimum number of females (7.40) and egg masses (6.80) were recorded in the treatment with 10 nematodes per pot which was at par with treatment with 100 nematodes per pot. Treatment with 100, 500, 1000, 5000, and 10,000 nematodes per pot differed significantly from one another. The treatment with 10,000 nematodes per pot produced the maximum number of females (55.60) and egg masses (51.00) per root system. The mean data on the larval population showed that increasing the inoculums level from 10 to 10,000 nematodes per pot resulted in a steady increase in larval population. The minimum (24.00) and maximum (4360.00) number of larval populations was at inoculum level 10 and 10,000 respectively. In addition, treatments with 10, 100, 500, 1000, 5000 and 10,000 nematodes per kg of soil differed significantly from one another. The mean data on the number of total nematode population per pot showed that the total nematode population increased gradually as the inoculums level increased from 10 to 10,000 per pot. The minimum (118.80) and the maximum (21269.40) number of the total population were recorded in the lowest and highest inoculum level with 10 and 10,000 respectively. In addition, treatments with 10, 100, 500, 1000, 5000, 10,000 nematodes per pot differed significantly from one another. The mean data on the reproductive rate of nematodes in different levels of inoculum revealed that it decreased significantly as the inoculum level increased from 10 to 10,000 nematodes per pot. Inoculum levels of 10 and 10,000 nematodes per pot produced maximum (11.42) and minimum (2.18) reproductive rates, respectively. But there was no significant difference between the treatments with inoculums level of 10 and 100 as well as treatments with 5000 and 10,000 nematodes per plant. The decrease in rate of reproduction with increase in inoculum levels may possibly be due to competition among nematode for space, food etc. Similar observations relating to the pathogenic level of *R. reniformis* on other crops were earlier reported by Karmakar et al., [17] on betelvine, Patel et al. [18] on cotton, Misra and Padhi [19] on french bean, Vats and Dalal [20] on pea, Sahoo and Padhi [21] on tomato.

4. CONCLUSION

The study indicates that reniform nematode (*R. reniformis*) can cause significant growth reduction at an inoculum level 1000 nematodes per kg of soil. Root and soil population of *R.reniformis* and number of egg masses per root increased with increase in initial inoculums level. However, further work on *R.reniformis* on different crops under field condition will give better insight to threshold level of this species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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