

Original Research Article

Pathogenic level of Reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira, 1940) on tomato (*Solanum lycopersicum* L.)

Abstract:

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 in root zone of 10 days-old plants. Observations on growth parameters of plants and nematode numbers were recorded at 60 days following inoculation. There was a progressive decrease in the plant growth parameters as the inoculum level of *R. reniformis* increased. Significant reduction in 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* as the inoculum level increased. 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.

Key words: Pathogenic level, Reniform nematode (*Rotylenchulus reniformis*), Tomato, Growth parameters, Initial inoculum level, Final population

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INTRODUCTION

The pulpy fruit tomato (*Solanum lycopersicum* L.) also known as 'poor man's orange' are used as vegetable. It is having high nutritive values as minerals, vitamin A, vitamin C and anti-oxidant are present. (Giovannucci, 1999; Rao and Agarwal, 2000). Tomato is grown all over the world in temperate, subtropical and tropical areas (Blanca *et al.*, 2012). 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 (Robinson *et al.*, 1997; Gaur and Perry, 1991; Jones *et al.*, 2013). In India, the reniform nematode was first recorded by Das (1960) in Andhra Pradesh and now it is known to present in almost all states of India. Rao and Ganguly, (1996); Gaur *et al.*, (2001); Khan, (2005) 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 research works on pathogenicity was done on tomato.

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.*, (2019). Morphological identification was done based on Robinson *et al.*, (1997) and Dasgupta *et al.*, (2011) and it was confirmed morphologically as *Rotylenchulus reniformis*. The isolates were used to establish single egg mass cultures. A series of castor plants were grown in sterilized soil and then inoculated with egg masses obtained from the previously inoculated plant. These inoculated plants were maintained in pots and used as a source of inoculum subsequently. From the infested roots, egg masses were collected with the help of forceps and needles and placed in a cavity block filled with filter water. The suspension of egg masses were transferred to a glass beaker and kept overnight for hatching. Subsequent collections were made continuously with the same procedure. The population of *R. reniformis* consisted of mixed vermiform stages obtained a day before inoculation. At the time of inoculation, all the suspensions were mixed together and the number of nematodes was 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. The pots were arranged in a completely randomized block design (CRD). 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. All the treatments were replicated five times. 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 weighed after every 24 hours till a constant weight was observed. Then 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:

Final nematode population

Reproductive rate = -----

Initial population

The experimental data obtained were analysed by following the Fisher's method of Analysis of Variance (Snedecor and Cochran, 1967).

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 (60.56 cm) and above. The mean data on root length showed that the root length reduced progressively as the inoculum 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, 5000 and 10,000 nematodes per pot. At inoculum levels of 500 (16.00 cm) and above nematodes per pot,

asignificantdecreaseinrootlengthwasobserved.Themeandataonthefreshanddryweightofshootshow ed that when the inoculum level increased, thefreshweightof theshootdecrease significantly.There wasnosignificantdifference infresh weight of shoot between the uninoculated treatments and the treatments with 10, 100and 500 nematodes per pot, but these treatments differed significantly from the treatmentswith 1000, 5000, and 10,000 nematodes per pot. At inoculum levels of 1000 and abovenematodes per pot, there was a significant decreased in the fresh weight of the shoot(22.70g) as compared to control. With increase in inoculum level there was a significantdecrease in dry weight of shoot. However, there were no significant variations in shoot dryweightatinoculumlevelsof10,100,and500nematodesperpot. Therewasnosignificant difference in dry weight of shoot between the uninoculated treatments and thetreatmentswith10,100,and500nematodesperpot,butthesetreatmentsdifferedsignificantly 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 ofthe shoot.The meandata onfreshweightof rootanddry weightofrootshowedthatwhentheinoculumlevelincreased,thefreshweightofrootdecreasedsignifica ntly.Therewasnosignificantvariations in fresh root weight between inoculum levels of 10 and 100 nematodes per potas compared to control but these treatments differed significantly from the treatments with500, 1000, 5000 and 10,000 nematodes per pot. At 500 and higher inoculum levels, therewas a significant decreased in fresh weight of root (9.82g). In the case of the dry weight ofthe root, increasing the inoculum level resulted in a significant decrease. There was nosignificantdifferenceindryweightofrootbetweentheuninoculatedtreatmentsandtreatments with 10 and 100 nematodes per pot, but these treatments differed significantlyfrom the treatments with 500, 1000, 5000, and 10,000 nematodes perpot. At 500 andhigherinoculumlevels,therewasasignificant decreaseinrootdryweight(3.03g).

The mean data on the number of females and egg masses per root systemshowed that the number of femalesandeggmassesperrootssystemincreasedprogressivelyasinoculumlevelsincreasedfrom 10 to 10,000 inoculum levels per plant(Fig.1; Table2.). The minimum number of females (7.40) andegg masses (6.80) were recorded in the treatment with 10 nematodes per pot which was atpar with treatment with 100 nematodes per pot. Treatment with 100, 500, 1000, 5000, and10,000 nematodes per pot differed significantly from one another. The treatment with10,000 nematodes per pot produced the maximum number of females (55.60) and eggmasses(51.00)per root

system. The mean data on the larval populations showed that increasing the inoculum 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 inoculum 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 inoculum level of 10 and 100 nematodes per plant. There was also no significant difference in treatments with 5000 and 10,000 inoculum levels. 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.*, (2004) on betelvine, Patel *et al.*, (2004) on cotton, Misra and Padhi (1985) on french bean, Vats and Dalal (1998) on pea, Sahoo and Padhi (1986) on tomato. Ahmad and Alam (1997) observed 32.68% reduction of plant growth parameters of tomato var. Pusa Ruby after sixty days of inoculation with *R. reniformis* at inoculum level of 5000 nematodes/plant/kg of soil with reproduction rate 3.9. Nguyen *et al.*, (2020) 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.

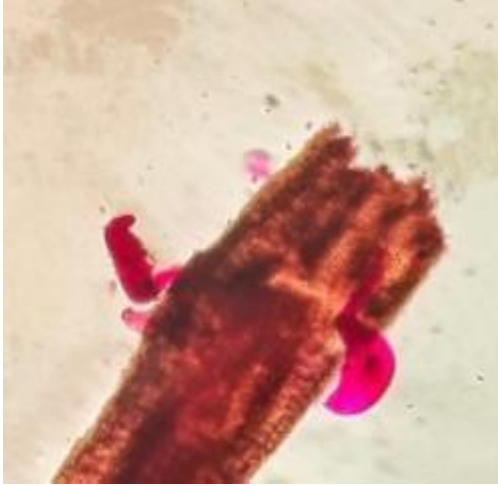


Fig.1. Reniform nematode on tomato root

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REFERENCES

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- Ahmad, A. and Alam, M.M. (1998). Effect of organic amendments alone and in combination with *Paecilomyces lilacinus* on *Rotylenchulus reniformis* attacking some economically important crop plants. *Archives of Phytopathology and Plant Protection*, **31**(5): 439-448.
- Blanca, J.; Cañizares, J.; Cordero, L.; Pascual, L.; Diez, M. J. and Nuez, F. (2012). Variation revealed by Single nucleotide polymorphisms (SNP), genotyping and morphology provides insight into the origin of the tomato. *PloSone*, **7**(10), e48198.
- Das VM. (1960). Studies on the nematode parasites of plants in Hyderabad (Andhra Pradesh, India). *Z. Parasitenk.* **19**:553-605.
- Dasgupta, D.R.; Raski, D.J. and Sher, S.A. (1968). A revision of the genus *Rotylenchulus* Linford and Oliveira, 1940 (Nematoda: Tylenchidae). *Proceedings of the Helminthological Society of Washington* **35**(2): 169-192.
- Gaur, H.S. and Perry, R.N. (1991). The biology and control of the plant parasitic nematode *Rotylenchulus reniformis*. *Agricultural Zoology Reviews*, **4**:177-212.

- Gaur, H.S.; Singh, R.V.; Kumar, S.; Kumar, V. and Singh, J.V. (2001) Search for nematoderesistance in crops. In: AICRP on Plant Parasitic Nematodes with Integrated Approach for Their Control. IARI, New Delhi, India.
- Giovanucci, E. (1999). Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiological literature. *Journal of the National Cancer Institute*, **91**(4):317-31.
- Jones, J.T.; Haegeman, A.; Danchin, E.G.; Gaur, H.S.; Helder, J. and Jones, M.G. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, **14**: 946–61.
- Karmakar, S. G.; Roy, K.; Mukhopadhyay, A. K., and Dasgupta, B. (2004). Damage threshold level of *Rotylenchulus reniformis* in betelvine (*Piper betle* Linn.). *Indian Journal of Nematology*, **34**(2): 210-211.
- Khan, M.R. (2005). Hosts and non-hosts of reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, 1940 – A critical review. *Environment and Ecology*, **23**:124-140.
- Misra, R. P. and Padhi, N. N. (1985). Pathogenicity of *Rotylenchulus reniformis* on French bean. *Indian Journal of Nematology*, **15**(2): 289-289.
- Nguyen, H.T.; Trinh, Q.P.; Couvreur, M.; Singh, P.R.; Decraemer, W. and Bert, W. (2019). Molecular and morphological characterisation of a new root lesion nematode, *Pratylenchus horti* n. sp. (Tylenchomorpha: Pratylenchidae) from Ghent University Botanical Garden. *Nematology*, **21**: 739–752.
- Nguyen, H.T.; Trinh, Q.P.; Nguyen, T.D. and Bert, W. (2020). First report of *Rotylenchulus reniformis* infecting turmeric in Vietnam and consequent damage. *Journal of Nematology*, **52**:53.
- Patel, R.R.; Patel, B.A., and Thakar, N.A. (2004). Pathogenicity of reniform nematode, *Rotylenchulus reniformis* on cotton. *Indian Journal of Nematology*, **34**(1), 106-107.
- Rao, A. and Agarwal, S. (2000). Role of antioxidant lycopene in cancer and heart disease. *Journal of American College Nutrition*, **19**:563-569.
- Rao, G.M.V.P. and Ganguly, S. (1996). Host preference of six geographical isolates of reniform nematode, *Rotylenchulus reniformis*. *Indian Journal of Nematology*, **20**: 19-22.
- Robinson, A. F.; Inerra, R. N.; Caswell-Chen, E.; P.; Vovlas, N. and Troccoli, A. (1997). *Rotylenchulus* species: Identification, distribution, host ranges and crop/plant resistance. *Nematropica*, **27**:127-180.

Sahoo, H. and Padhi, N.N. (1986) Susceptibility of plants to the reniform nematode, *Rotylenchulus reniformis*. *Indian Journal of Nematology*, **16**(1):97-98.

Schneider, R.C.; Zhang, J.; Anders, M.M.; Bartholomew, D.P. and Caswell-Chen, E.P. (1992).

Nematicide Efficacy, Root Growth, and Fruit Yield in Drip-irrigated Pineapple Parasitized by *Rotylenchulus reniformis*. *Journal of Nematology*, **24**(4):540-547.

Vats, R. and Dadal, M.R. (1998). Pathogenicity of the reniform nematode, *Rotylenchulus reniformis* on pea (*Pisum sativum* L.). *Indian Journal of Nematology*, **28**(1):93-95.

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Table1.Effectofdifferentinoculumlevelsof*Rotylenchulusreniformis*onplantgrowthparameters oftomato(Meanof5replications)

| Inoculumlevel | Shootlength(cm) | Fresh weight ofshoot(g) | Dry weight ofshoot(g) | Rootlength(cm) | Fresh weight ofroot(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 |

*Meanfollowed bythe same letter inthesuperscript (s) arestatisticallyatpar.

Table2.Effectofdifferentinoculumlevelof*Rotylenchulusreniformis*onnumberoffemales,eggmassesandnematodepopulationontom

ato(Mean of5 replications)

| Inoculumlevel | No.offemales / rootsy stem | No.ofeggmasses/r ootsystem | Larval population(200 ccofsoil) | Total population /pot | Reproductiverate% |
|---------------|-------------------------------------|-------------------------------|---------------------------------------|-----------------------------------|---------------------|
| 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) ^c | 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 |

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