*Original Research Article*

# Pathogenic level of Reniform nematode(*Rotylenchulusreniformis*Linford& Oliveira, 1940) on tomato(*Solanumlycopersicum*L.)


## Abstract:

Pathogenic level of Reniform nematode(*Rotylenchulusreniformis*),was investigated on tomato var.Pusa Ruby by inoculating10, 100, 500, 1000, 5000 and 10000 nematodes per kg autoclavedsoil in root zone of 10 days-old plants. Observations on growth parameters of plants andnematode numbers were recorded at 60 days following inoculation. There was a progressivedecrease 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/kgsoil.Therewasagradualincreaseinthenumberoffemales,eggmassesperrootsystem,and nematodepopulation of*R. reniformis*as theinoculumlevel increased. Therate ofmultiplication decreased with increase in the level of inoculation. The pathogenic level of *R.reniformis*was found to be1000 nematodes per kgsoil.



***Key words:*** Pathogenic level, Reniform nematode (*Rotylenchulusreniformis*),Tomato,Growth parameters,Initial inoculums level, Final population


## INTRODUCTION

The pulpy fruit tomato (*Solanumlycopersicum*L.) alsoknown as 'poor man's orange' areused as vegetable. It is having high nutritive valueas minerals, vitamin A, vitamin C and anti-oxidant are present. (Giovannucci, 1999; Rao andAgarwal,2000).Tomato is grownall over the world in temperate, subtropical and tropicalareas(Blanca*etal*.,2012).In Assam, tomato production is 430.83 thousand tonnes with anaverage yield 20-25 t/ha as reported by National Horticultural Board (NHB) 2021-22. Therequirement for tomato in India and world market is rising day by day. But the production isvulnerablebyseveralbioticandabioticfactors.Amongbioticfactorsplant-parasiticnematodes are considered as one of the most important plant pathogens. The reniform nematode (*Rotylenchulusreniformis*Linford& Oliveira, 1940), is one of the mostimportant plant-parasitic nematodesin the world (Robinson *et al*.,1997;Gaur and Perry, 1991;Jones *et al*.,2013).InIndia, the reniform nematode was first recorded byDas (1960) in Andhra Pradesh and now it is known to present in almost all states of India. Raoand Ganguly, (1996);Gaur *et al*., (2001);Khan, (2005) reported that *R.reniformis*attacks over150 plant species from 50 families.

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Threshold level should be calculated using the pathogenesity level.

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As no work has been conducted on reniform nematodes in Assam therefore the research works on pathogenicity wasdoneon 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)inthenethouseofDepartmentofNematology,AssamAgriculturalUniversity,Jorhat.Thellaboratoryinvestigationwasconductedin thePostGraduateLaboratoryof Departmentof Nematology,AssamAgriculturalUniversity,Jorhat.The isolates of reniform nematode were collected from castor plants from back of nethouse of Department of Nematology, AAU, Jorhat. Permanent slides weremade following Nguyen *et al*., (2019). Morphological identification was done based onRobinson *et al*., (1997) and Dasgupta *et al*., (2011) and it was confirmed morphologicallyas *Rotylenchulusreniformis*. The isolates were used to establish single egg mass cultures.A series of castor plants were grown in sterilizedsoil and then inoculated with egg masses obtained from the previously inoculated plant.Theseinoculatedplantsweremaintainedinpotsandusedasasourceofinoculumsubsequently.From the infested roots, egg masses were collected with thehelp of forceps and needles and placed in a cavity block filled with filter water. Thesuspensionofeggmasseswerettransferredtoaglassbeakerandkeptovernightforhatching. Subsequent collections were made continuously with the same procedure .Thepopulation of *R.reniformis*consisted of mixed vermiform stages obtained a day beforeinoculation. At the time of inoculation, all the suspensions were mixed together and thenumber of nematodes was counted taking 1 ml from the homogenous nematode suspensionon Hawkshley Counting Dish using the Magnüsmicroscope.Seeds of tomato (Var. Pusa Ruby) were surface sterilized with rectified spirit beforesowing.Ineachpot,2seedsweresownatthedepthof5-6cmwhichcontainssterilizedsoilandcoveredwithathinlayerofsoiloverit.Thepotsweresprinkledwith waterwithoutdisturbingthethinsoil cover.Seedlingswerethinnedout afteroneweekofgermination keeping only one healthy seedling in each pot. The pots were arranged in acompletelyrandomized block design(CRD).Sevendaysaftergermination,seedlingswerethinnedtooneplantperpot.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 potalong with control over the

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**Comment [A7]:** what is the age of the seedling? add

surface roots, by carefully exposing the root system up to adepth of 1 cm and then covered with fresh sterilized soil. All the treatments were replicatedfivetimes.Regular watering of plants was done till harvesting of the crop.Harvesting of theplantswas done60 daysafter inoculation.The entire root systemwas taken out from the pot and kept in a plastic bucket half filled with water for half anhour. Then the root system was washed with tap water very carefully to avoid root damageand loss of egg masses from the roots. Observations were made on root length, fresh anddry weight of shoots and roots in all treatments. For recording the dry weight of shoot androots the fresh shoot and root materials were packed in paper bags labeled according to thetreatmentandkeptinanovenrunningcontinuouslyat30-35°C.Thematerialswereweightedafterevery24hourstillaconstantweightwasobserved.Thenematode population density was estimated by extracting 250 cc of soil from each pot. Females ofreniform nematode were stained and numbers present on the whole root system wereenumerated.Root samples were agitated in 0.6% NaOCl for 10 min to dislodge eggs fromeggmasses. Estimation of reproductiveratewas calculated by:

$$\text{Reproductiverate} = \frac{\text{Finalnematodepopulation}}{\text{Initialpopulation}}$$

Theexperimentaldataobtainedwereanalysedbyfollowingthe Fisher'smethodofAnalysis of Variance (Snedecor and Cochran, 1967).

**Comment [A8]:** what about other parameters of plant like flowers, fruits etc.

**Comment [A9]:** add shoot length

**Comment [A10]:** check the reference

## RESULTS AND DISCUSSION

The results indicated that the growth of plants was negatively correlated to the level ofinoculation of reniform nematode (*R. reniformis*) (Table.1).The mean data on shoot lengthshowed that with increase in inoculum levels the shoot length gradually decreases.The treatments with no nematode (uninoculated), 10, 100, and500 nematodes per pot did not show any significant difference in shoot length, however thesetreatments were considerably different from the treatments with 1000, 5000, and 10,000nematodes per pot. Shoot length was significantly reduced at inoculum levels of 1000(60.56cm)andabove.The mean data on root lengthshowed that the root length reduced progressively as the inoculums levelsof 10 and 100 nematodes per pot, there were no significant variations in root length ascomparedtocontrol,thoughthesetreatmentsdifferedsignificantlyfromthetreatmentswith500,1000, 50000and10,000nematodesperpot.Atinoculumslevelsof500(16.00cm)andabovenematodesperpot,

asignificantdecreaseinrootlengthwasobserved.Themeandataonthefreshanddryweightofshootshowed that when the inoculums 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 inoculums 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 inoculums level there was a significantdecrease in dry weight of shoot. However, there were no significant variations in shoot dryweightatinoculumslevelsof10,100,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,thefreshweightofrootdecreasedsignificantly.Therewasnosignificantvariations in fresh root weight between inoculums 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 inoculums 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 andhigherinoculumslevels,therewasasignificant decreaseinrootdryweight(3.03g).

The mean data on the number of females and egg masses per root systemshowed that the number of femalesandeggmassesperrootsystemincreasedprogressivelyasinoculumslevelsincreasedfrom 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

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system.The mean data on the larval populationshowed that increasing the inoculums level from 10 to 10,000 nematodesperpotresultedinasteady increase inlarvalpopulation.The minimum(24.00)andmaximum (4360.00) number of larval populations was at inoculum level 10 and 10,000respectively. In addition, treatments with 10, 100, 500, 1000, 5000 and 10,000 nematodesperkgof soil differed significantlyfrom one another.The mean data on the number of total nematode population per potshowed that the total nematode populationincreasedgradually as the inoculums level increasedfrom 10 to 10,000per pot. Theminimum (118.80) and the maximum (21269.40) number of the total population wererecorded in the lowest and highest inoculum level with 10 and 10,000 respectively. Inaddition, treatments with 10, 100, 500, 1000, 5000, 10,000 nematodes per pot differedsignificantlyfrom oneanother.The mean data on the reproductive rate of nematodes in different levels ofinoculumrevealedthatitdecreased significantly as the inoculum level increased from 10 to 10,000 nematodes perpot. Inoculum levels of 10 and 10,000 nematodes per pot produced maximum (11.42) andminimum (2.18) reproductive rates, respectively. But there was no significant differencebetween the treatments with inoculums level of 10 and 100 nematodes per plant. There wasalsono significant differencein treatments with5000 and 10,000inoculum levels.The decreaseinrateofreproductionwithincreaseininoculumlevelsmaypossiblybeduetocompetition among nematode for space, food etc.

Similar observations relating to thepathogenic 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, Vatsand Dalal (1998) on pea, Sahoo and Padhi (1986) on tomato. Ahmad and Alam (1997)observed32.68% reductionof plantgrowthparametersof tomatovar.Pusa Ruby aftersixty days of inoculation with *R.reniformis*at inoculums level of 5000 nematodes/plant/kgof soil with reproduction rate 3.9. Nguyen*et al.,* (2020) also reported the significantcorrelations between *R.reniformis*and the degree of plant damage (yellowing leaves anddry, rotten rhizomes) in turmeric in the Central Highlands of Vietnam and found density upto480 nematodes/100 ml of soil.

**Comment [A13]:** space

**Comment [A14]:** give supporting article of tomato and related crops of same family

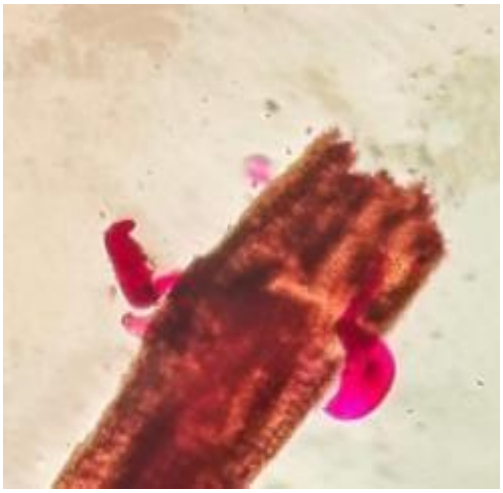

Fig.1. Reniform nematode on tomato root



## REFERENCES

Comment [A15]: give recent reference (2000-2024)


Ahmad, A. and Alam, M.M. (1998). Effect of organic amendments alone and in combinationwith *Paecilomyceslilacinus*on *Rotylenchulusreniformis*attacking some economicallyimportant 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). Variationrevealedby Single nucleotide polymorphisms(SNP), genotypingandmorphologyprovidesinsight into theoriginof the tomato.*PloSone*, **7(**10), e48198.

DasVM.(1960).StudiesonthenematodeparasitesofplantsinHyderabad(AndhraPradesh,India).*Z.Parasitenk*.**19**:553-605.

Dasgupta,D.R.;Raski,D.J.andSher,S.A.(1968).Arevisionofthegenus*Rotylenchulus*LinfordandOliveira,1940(Nematoda:Tylenchiclae).*ProceedingsoftheHelminthologicalSocietyofWashington*35(2): 169–192.

Gaur,H.S.andPerry,R.N.(1991).The biologyandcontroloftheplantparasiticnematode*Rotylenchulusreniformis*.*AgriculturalZoologyReviews*, **4**:177-212.


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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 ApproachforTheir Control.IARI,New Delhi,India.

Giovannucci,E.(1999).Tomatoes, tomato-based products, lycopene, and cancer: review oftheepidemiologicliterature. *Journal oftheNational CancerInstitute*.**91**(4):317-31.

Jones, J.T.; Haegeman, A.; Danchin, E.G.; Gaur, H.S.; Helder, J. and Jones, M.G. (2013).Top10plant-parasiticnematodesinmolecularplantpathology.*MolecularPlantPathology*,**14**: 946–61.

Karmakar, S. G.; Roy, K.; Mukhopadhyay, A. K., and Dasgupta, B. (2004). Damage thresholdlevel of Rotylenchulusreniformis in betelvine (Piper betle Linn.). *Indian Journal ofNematology*,**34**(2): 210-211.

Khan,M.R.(2005).Hostsandnon-hostsofreniformnematode,*Rotylenchulusreniformis*Linford&Oliveira,1940–Acriticalreview.*EnvironmentandEcology*,**23**:124-140.

Misra, R. P. and Padhi, N. N. (1985). Pathogenicity of *Rotylenchulusreniformis*on frenthbean.*Indian Journal ofNematology*, **15**(2): 289-289.

Nguyen,H.T.;Trinh,Q.P.;Couvreur,M.;Singh,P.R.;Decraemer,W.andBert,W.(2019).Molecular and morphological characterisation of anewroot lesion nematode,*Pratylenchushorti*n. sp. (Tylenchomorpha: Pratylenchidae) from Ghent UniversityBotanical Garden.*Nematology*, **21**: 739–752.

Nguyen, H.T.; Trinh, Q.P.; Nguyen, T.D. and Bert, W. (2020). First report of RotylenchulusreniformisinfectingturmericinVietnamandconsequentdamage.*JournalofNematology*, **52**:53.

Patel,R.R.;Patel,B.A.,andThakar,N.A.(2004).Pathogenicityofreniformnematode,*Rotylenchulusrenijiomis*oncotton.*Indian JournalofNematology*,**34**(1), 106-107.

Rao,A.andAgarwal,S.(2000).Roleofantioxidantlycopeneincancerandheartdisease.*JournalofAmericanCollegeNutrition*,**19**:563-569.

Rao,G.M.V.P.andGanguly,S.(1996).Hostpreferenceofsixgeographicalisolatesofreniform nematode, *Rotylenchulusreniformis.Indian Journal of Nematology*, **20**: 19-22.

Robinson, A. F.; Inserra, R. N.; Caswell-Chen, E.; P.;Vovlas, N. and Troccoli, A. (1997).*Rotylenchulus*species:Identification,distribution,hostrangesandcropplantresistance. *Nematropica*,**27**:127-180.

Sahoo,H.andPadhi,N.N.(1986)Susceptibilityofplantstothereniformnematode,*Rotylenchulusreniformis*.*IndianJournalofNematology*.**16**(1):97-98.

Schneider,R.C.;Zhang,J.;Anders,M.M.;Bartholomew,D.P.andCaswell-Chen,E.P.(1992). Nematicide Efficacy, Root Growth, and Fruit Yield in Drip-irrigated PineappleParasitizedby*Rotylenchulusreniformis*. *Journal ofNematology*, **24**(4):540-547.

Vats, R. and Dadal, M.R. (1998). Pathogenicity of the reniform nematode, *Rotylenchulusreniformis*onpea(*Pisumsativum*L.)*Indian Journalof Nematology*,**28**(1):93-95.

**Comment [A17]:** not in text

**Table1.Effectofdifferentinoculumlevelsof*Rotylenchulusreniformis*onplantgrowthparameters oftomato(Meanof5replications)**

Comment [A18]: mention the day after sowing

Comment [A19]:

| Inoculumlevel | Shootlength(cm) | Fresh weight ofshoot(g) | Dry weight ofshoot(g) | Rootlength(cm) | Fresh weight ofroot(cm) | Dry weigh 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) arestatistically*atpar*.



**Table2.Effectofdifferentinoculumlevelof*Rotylenchulusreniformis*onnumberoffemales,eggmassesandnematodepopulationontom**

**ato(Mean of5 replications)**

Comment [A20]: mention the days of observation recorded

| Inoculumlevel | No.offemales / rootsystem | No.ofeggmasses/rootsystem | 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)[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 |