Initial Population Density its Effect on the Pathogenic Potential and Population Growth of Rotylenchulus reniformis on Cowpea (Vigna unguiculata L.)

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Abstract

A glasshouse experiment was conducted to assess the potential of Rotylenchulus reniformis population of Delhi on cowpea (Vigna unguiculata) cultivar Pusa komal. In the present study, different inoculums levels viz., 10, 100, 1000 and 10000 with immature females and equal number of males were inoculated in the rhizosphere of ten day old cowpea plants. Significant reduction in growth parameters were recorded at 1,000 and 10,000 females/plants. However, multiplication rate of population was roughly 84.9 times highest at minimum inoculum level while lowest 2.31 times at maximum inoculum level. In the present investigation threshold level of R. reniformis was found to be 1000 immature females/one kg soil.

Keywords

Cowpea; Inoculum levels; Multiplication rate; Pathogenic potential; R. reniformis

Introduction

Rotylenchulus reniformis is considered economically important plant parasitic nematode next to root knot nematode in agriculture. The reniform nematode, Rotylenchulus reniformis [1] is one of the most important plant parasitic nematode species distributed in subtropical and tropical parts of the world. Linford and Oliveira (1940) described reniform nematode for the first time on cowpea root in pineapple field in Island of Oahu, Hawaii (U.S.A). It is known to be associated with more than 115 plant species belonging to more than 30 plant families mainly Leguminosae, Cucurbitaceae, Solanaceae, Euphorbiaceae and Malvaceae, including some monocot too. Some of the important host crops on which damage has been recorded are cotton, papaya, pineapple, cowpea, castor bean, banana, tea, sweet potato, coffee, pigeon pea, tomato, tobacco, okra, eggplant, beans, soybean, cantaloupe. Its pathogenic capabilities have been established at least on 35 agriculturally important crops. Crop yield losses as high as 60 % have been reported due to infection by reniform nematodes [2]. Cowpea is the most productive legume used agronomically and thrive well in hot moist zones but require high temperature for optimal growth. It makes an excellent nitrogen source and attract

In India, R. reniformis was first recorded by Siddiqi and Basir [3] from coffee roots. Subsequently, its occurrence was recorded by several researchers from different parts of country. Analysis of nematode communities has shown this species to be one of the five top ranking nematodes of India based upon their frequency of occurrence, density and pathogenic capabilities [4,5]. Despite this, not much research work has been carried out on reniform nematodes. This might be attributed to their extremely small sized egg masses which can hardly be seen with unaided eye and also to their non specific type of root symptoms. R. reniformis is known to be occurring in almost all the states of India, except temperate hilly region of north India. Its widespread distribution in varied agro climatic condition shows its highly adaptive capabilities for survival at different geographical locations. Plants infected with reniform nematode showed drying and yellowing of leaf margin and noticed within one month of post inflectional period. The drying of leaves spreads gradually towards lamina followed by premature shedding of leaves. The roots infected with reniform nematode show moderate discoloration with root lets as dark brown and sometimes necrotic. Dasgupta and Seshadri [6] distinguished two host races (Race A and B) of reniform nematode. Race A reproduces well on cowpea, castor and cotton but race B multiples on cowpea only. Meliodogyne incognita and R. reniformis are considered most important nematode pests of cowpea. The other nematode associated with cowpea include Belonolaimus gracilis, Criconemella curvata, C. xenoplax, Criconemoides (Criconemella) Sp, Ditylenchus dipsaci, Helicotylenchus cavenessi, H. dihyusta, H. pseudorobustus, Hemicyclonema arenaria, Heterodera cajani, H. glycines, H. sachachtii, H. vinga, H. pararobustus, H. seinhorstii, Paratylenchus minutus, Pemphigus nigeriensis, Pratylenchus brachyurus, P. coffee, P. goodeyi, P. minyus, P. penetrans, P. pruten, P. scribneri, P. safaensis, P. thornei, P. vulnus, P. zeae, Radopholus similis, Scutellonema cavenessi, S. brady, S. elaecraudatum, Tylenchorhynchus brevidentis, Xiphinema americanum, X. basiri, X. ecaulcom and Zygotylenchus guerava[7].

The avoidable yield loss of cowpea due to R. reniformis to the tune of 10.5 to 14.5% was estimated. Roy K et al. [8] losses are aggravated more when initial nematode population is high and favourable biotic conditions viz. soil type, temperature, soil moisture etc. prevail. Hence, this study was undertaken with an objective to find out the threshold levels of Delhi population of R. reniformis and its effects on the growth parameters of cowpea and multiplication rate of nematodes in respect of different density levels.

Materials and Methods

R. reniformis inoculum was collected from brinjal and tomato roots uprooted from culture pots. The egg masses were carefully removed, picked and finally kept in petridish containing water. The second stage juveniles hatched out of the egg mass were kept in water for seven days to become immature females egg masses kept for hatching contain both males and females . However males do not have any effect on inoculum and do not parasite the roots. Moreover there is no methods to separate males and females used for inoculation in the experiment. Earthen pots of 15 cm diameter were filled with 1 kg of steam sterilized soil. Then, two seeds of cowpea were
sown in each pot and after the germination only one seedling was allowed to grow in each pot. Inoculation was done after 10 days on seeding emergence at different inoculum levels viz. 0, 10, 100, 1000, 10,000 immature females & equal number of males of R. reniformis per pot. The required number of nematodes was placed around the root zone at about 2 cm depth. After inoculation the holes were closed with sterilized soil and then slight watering was done. Besides this an un inoculated control was also maintained. The observations on various plant growth parameters viz. shoot length, root length, fresh shoot and root weight were taken after forty five days of inoculation while dry shoot weight was taken after keeping the shoot in oven at 60 °C for five days. The uprooted plant roots were gently washed in slow running tap water for 1-2 minutes to get rid of adhered soil particles. The roots were then immersed in 1.5% sodium hypochlorite solution for one minute and agitated occasionally. The roots were again kept in water for overnight to remove the bleaching agent. After 24 hrs, roots were transferred for a minute in boiling mixture of 100 ml of water and four ml of stain from stock solution (3.0g of acid fuchsin +250 ml of acetic acid +750 ml distilled water). The stained roots were again rinsed in tap water for 1-2 minutes to remove excess of stain [9]. The sodium hypochlorite acid fuchsin glycerine method was adopted for staining as it prevents the exposure of root materials to the toxic phenols utilized in other staining methods. The roots were cut off from shoots pressed between 2-3 folds of blotting paper to absorb the excess water and finally kept in glass petriplates containing glycerine. Ten egg masses were randomly picked and taken in a small glass vial in which fifteen ml of 0.53 % sodium hypochlorite (NaOCl) was added. Then it was quickly passed through 200 mesh sieve held over 500 mesh sieve after vigorously shaking for about 30 sec. Eggs collected on 500 mesh sieve were rinsed with water to remove NaOCl and then transferred to counting dish. Three aliquots of each five ml solution were counted thrice and then their average number multiplied with total volume of solution prepared.

Observation on the different parameters such as nematode multipication viz. number of females/root system, number of egg mass/root system, eggs/egg mass, total number of eggs/root system, soil and total population/pot were recorded. The soil population was determined by Modified Cobb’s sieving and Baermann’s funnel technique [10]. Each soil sample was thoroughly mixed and 200 cm³ was determined by Modified Cobb’s sieving and Baermann’s funnel soil and total population/pot were recorded. The soil population mass/root system, eggs/egg mass, total number of eggs/root system, multiplication viz. number of females/root system, number of egg mass/root system were valued same for non significant difference between the treatments. If the treatments were found significant the treatment represented different value (Tables 1 and 2).

Regression analysis was calculated out for the above growth parameters and equations which are as follows (Figure 1).

Shoot length  
Y = 21.72 – 2.22 X

Fresh Shoot weight  
Y = 17.48 – 1.33 X

Dry Shoot weight  
Y = 2.51-0.23X

Root length  
Y = 31.86 – 2.01 X

Fresh Root weight  
Y = 3.79 – 0.57 X

No. of nodules  
Y = 28.3-3.15X

Here, Y = crop growth parameter and X = nematode inoculum level (Log transformed)

Results

Data in Table 1 revealed that maximum number of females of R. reniformis (342.50) was observed at inoculum level of 10,000 immature females/one kg soil. The significant differences were observed among 10, 100, 1000 and 10,000 inoculum levels of immature females/one kg soil. The minimum (8.50) egg mass/root system was observed at lowest inoculum level and maximum (293.50) egg mass/root system at highest inoculum level of R. reniformis. However, formation of eggs/egg mass reduced progressively with increasing inoculum level. The lowest eggs/eggmass (56.67) was observed at 10,000 immature female /kg soil of R. reniformis. Maximum number (16,611.15) of eggs/root system was observed at 10,000 inoculum levels and minimum (579.10) eggs/root system was recorded at lowest inoculum level of R. reniformis (Figure 1).

The observation on soil population and total nematode population (root + soil) exhibited similar trend as observed for total no. of eggs/root system .Multiplication rates (pf/pi) at initial inoculum levels 10, 100, 1000 and 10000 were found 84.9, 43.3, 13.4 and 2.31 respectively. The pf/pi was found to be highest at 10 inoculum level followed by 100, 1000 and at 10000 inoculum level. However, the increase was not found to be proportional to inoculum levels but density dependent.

Significant reduction in shoot length was obtained at 1000 immature females/one kg soil of R. reniformis compared to control. (Table 2) Nematode inoculum level of 1000 and 10,000 were found to be at par than other treatments. No significant reduction was observed in fresh shoot weight between 10 and 100 immature female /kg soil of R. reniformis. Statistically significant variation was observed in fresh shoot weight at 100 and 1000 immature female/ one kg soil. Maximum reduction 1.47 g in dry shoot weight was observed at 10,000 immature female/ kg soil of R. reniformis as compared to 2.30 g in control. Root length decreased with increasing initial inoculum levels. All the treatments were found statistically non significant, maximum reduction in root length was observed at 10,000 nematodes inoculum level of R. reniformis. Maximum reduction 1.42 g in root weight
Table 1: Effect of initial inoculum levels on the female number, fecundity and final population of *R. reniformis* on cowpea cv. Pusa komal.

<table>
<thead>
<tr>
<th>Inoculum levels/kg soil</th>
<th>No. of females /root system</th>
<th>No. of eggmass/ root system</th>
<th>Eggs/ eggmass*</th>
<th>Total no. of eggs/root system</th>
<th>Soil population/pot</th>
<th>Total population/pot (Eggs + soil pop.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0.61)^a</td>
<td>0 (0.61)^a</td>
<td>0</td>
<td>0</td>
<td>0 (0.61)^a</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>11.50 (3.41)^b</td>
<td>8.5 (2.94)^b</td>
<td>68.65 (8.30)^a</td>
<td>579.10 (23.45)^b</td>
<td>270.73 (16.04)^b</td>
<td>849.83 (28.64)^b</td>
</tr>
<tr>
<td>100</td>
<td>67.25 (8.17)^c</td>
<td>51.75 (7.11)^c</td>
<td>66.66 (8.17)^c</td>
<td>3520.63 (58.11)^c</td>
<td>801.98 (27.20)^c</td>
<td>4322.61 (64.24)^c</td>
</tr>
<tr>
<td>1000</td>
<td>208.75 (14.43)^d</td>
<td>167.50 (13.10)^d</td>
<td>63.32 (7.95)^d</td>
<td>10435.51 (102.08)^d</td>
<td>2968.75 (54.27)^d</td>
<td>13404.26 (115.69)^d</td>
</tr>
<tr>
<td>10000</td>
<td>342.50 (18.49)^e</td>
<td>293.50 (17.13)^e</td>
<td>56.67 (7.54)^e</td>
<td>16611.15 (128.78)^e</td>
<td>6541.56 (80.81)^e</td>
<td>23152.71 (152.05)^e</td>
</tr>
<tr>
<td></td>
<td>S. Em 0.39</td>
<td>0.46</td>
<td>0.22</td>
<td>3.77</td>
<td>2.72</td>
<td>4.29</td>
</tr>
<tr>
<td></td>
<td>CD (0.05)</td>
<td>1.19</td>
<td>1.39</td>
<td>0.67</td>
<td>11.38</td>
<td>8.20</td>
</tr>
</tbody>
</table>

*Average of 10 eggmass/plant.

Each data represent average of four replications.

Figures in parentheses represent (X+ 0.375)^1/2 transformed values.

Within a column, data followed by the same letter are not significantly different (P ≥0.05).

Table 2: Effect of initial inoculum levels of *R. reniformis* on different plant growth parameters of cowpea cv. Pusa komal.

<table>
<thead>
<tr>
<th>Inoculum levels/kg soil</th>
<th>Shoot length (cm)</th>
<th>Fresh Shoot weight (g)</th>
<th>Dry Shoot weight (g)</th>
<th>Root length (cm)</th>
<th>Fresh Root weight (g)</th>
<th>Root length (cm)</th>
<th>No of nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20.18^a</td>
<td>16.40^a</td>
<td>2.30^a</td>
<td>31.10^a</td>
<td>3.41^a</td>
<td>27.25^a</td>
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</tr>
<tr>
<td>10</td>
<td>20.20^a</td>
<td>16.80^a</td>
<td>2.41^a</td>
<td>30.00^a</td>
<td>3.40^a</td>
<td>24.25^a</td>
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</tr>
<tr>
<td>100</td>
<td>19.90^a</td>
<td>16.22^a</td>
<td>2.29^a</td>
<td>29.00^a</td>
<td>3.38^a</td>
<td>26.25^a</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>14.20^a</td>
<td>13.10^a</td>
<td>1.75^a</td>
<td>26.12^a</td>
<td>1.72^a</td>
<td>17.25^a</td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td>12.00^a</td>
<td>11.61^a</td>
<td>1.47^a</td>
<td>23.00^a</td>
<td>1.42^a</td>
<td>15.00^a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. Em 1.08</td>
<td>0.63</td>
<td>0.20</td>
<td>2.35</td>
<td>0.51</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD (0.05)</td>
<td>3.27</td>
<td>1.92</td>
<td>0.61</td>
<td>NS</td>
<td>1.54</td>
<td>6.42</td>
</tr>
</tbody>
</table>

Each data represent average of four replications.

Within a column, data followed by the same letter are not significantly different (P ≥0.05).

**Figure 1:** Effect of different inoculum levels of *R. reniformis* on plant growth parameters of cowpea cv. Pusa komal.
was observed at 10,000 immature females/kg soil of \( R. \text{reniformis} \) as compared to 3.41g in control. The nodules formation decreased as the inoculum levels increased from 10 to 10,000 and significant reduction was obtained at 1000 and 10,000 immature females/one kg soil. The data given in Tables 1 and 2 revealed that an increase in initial inoculum level of the nematode resulted in a progressive decrease in all plant growth parameters.

**Discussion**

Susceptibility of a host for plant parasitic nematodes is expressed as the ability of the nematode to multiply in the plant and is measured by the ratio of the number of nematodes recovered at the end of the experiment (Pi) to the number of nematode used in the initial inoculum (Pi) \([13-15]\). Maximum number of females of \( R. \text{reniformis} \) (342.50) was observed at inoculum level of 10,000 immature females/kg soil. However, the increase was not found to be proportional to inoculum level but density dependent. The minimum (8.50) egg mass/root system was observed at lowest inoculum level and maximum (293.50) egg mass/root system was noticed at highest inoculum level of \( R. \text{reniformis} \). The formation of eggs/egg mass reduced progressively with increasing inoculum level.

The general trend in reduction of plant growth parameters influenced by the nematode inoculation was in agreement with Khan and Hussain \([16]\). Similar observation on the reduction in nematode multiplication with increased population density was also observed \([17-19]\). Multiplication rates (pf/pi) at initial inoculum levels 10, 100, 1000 and 10000 were found 84.9, 43.3, 13.4 and 2.31 respectively. The pf/pi was found to be highest at 10 inoculum level followed by 100, 1000 and least at 10000 inoculum level as also observed by Parveen et al., Patel et al., Volvas et al. \([20-22]\). Khan and Hussain \([19]\) observed that in one cowpea variety Pusa barsati crowding of nematode at high inoculum densities created competition for root surface among nematodes which resulted in many, not finding a site for penetration, resulting natural death for lack of nutrition and reduced multiplication. The high rate of reproduction factor at low level of inoculum on the other hand could possibly be due to factors like abundance of food, lack of competition and ability of host to support population levels. Results of \( R. \text{reniformis} \) on cowpea cv. Pusa Komal also provided convincing evidence regarding damaging nature of this nematode. It is apparent from the data that there was progressive decrease in length and weight of shoot and root with the increase in the initial nematode inocula. Further, some damage to various plant growth parameters were observed at all levels of initial inocula. However, the minimum threshold level of \( R. \text{reniformis} \) in the present investigation to cause appreciable losses in cowpea cv. Pusa Komal was found 1000 immature females/kg of soil. However, the threshold level 10000 immature females/kg soil in present studies were differed from the result of Gupta and Yadav \([23]\) who reported threshold level 1000 immature female /500 g soil. In the present investigation the reproduction factor of \( R. \text{reniformis} \) showed a declining trend with increasing initial inoculum levels, as root surface area for both the lower and higher inoculum level remained the same. Crowding of nematode at high inoculum densities created competition for root surface among nematodes which resulted in many not finding a site for penetration, resulting natural death for lack of nutrition and reduced multiplication. The high rate of reproduction factor at low level of inocula on the other hand, could possibly be due to positive factors like abundance of food, lack of competition and ability of host to support population levels.

The reduction in fresh weight of the shoot and the root were significant only at the inoculum level of 1000 and 10,000 of each nematodes inoculation /one kg soil. This might be due to the same or nearby infection sites. Reduction in nodulation might be due to suppression of lateral root formation by \( R. \text{reniformis} \) which might cause reduction in the number of sites for nodule initiation.

**References**


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