5. SECTION-3

Determination of economic threshold level of root-knot nematode, *Meloidogyne incognita* race 2 on chickpea var. Avrodhi

5.1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) occupies an important place in the pulse cultivation and ranked second in the global farming after beans (*Phaseolus vulgaris* L.) (FAO, 2000). It is grown in 33 countries and is a significant component of cropping systems of subsistence farmers in the Indian subcontinent, West Asia and North Africa (Ansari et al., 2004). The crop suffers from several biotic and abiotic stresses. Among the biotic stresses, nematodes are potential constraints in the successful cultivation of chickpea (Greco, 1987). Many species of plant-parasitic nematodes have been found associated with chickpea in seventeen countries and they cause an estimated 13.7% annual loss (Nene et al., 1989; Sasser, 1998). Two root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) Chitwood and *Meloidogyne javanica* (Treub) Chitwood are the key pests of chickpea in the tropics (Sharma and Mc Donald, 1990). *Meloidogyne* spp. cause more than 10% loss in the world’s total crop production (Topp et al., 1988; Whitehead, 1998). Estimate of crop losses suggest that this nematode causes 22-84% loss in chickpea yield in two states of Northern India (Ali, 1997). Plants infected with *Meloidogyne* spp. show typical symptoms of root galling (Siddiqui and Akhtar, 2008). In addition to the formation of root-galls, the nematode interferes with the process of symbiotic nitrogen-fixation in legume plants and suppress nodule formation (Balasubramanian, 1971).

Less investigations concerning the relationship between chickpea yield and initial population densities of *M. incognita*, were achieved or to estimate the the economic threshold level (i.e., the lowest level of nematode density to cause economic loss and at which the cost of control measures is equal to the damage caused by nematodes). This level is now considered very essential for nematode management programs.
Consequently, the main objective of this section is to relate the growth of chickpea with different population densities of *M. incognita* and to determine the economic threshold level of this nematode on chickpea var. Avrodhi.

5.2 MATERIALS AND METHODS

5.2.1 Seedlings for maintaining nematode culture

Seedlings of tomato (*Lycopersicon esculentum*) cv. Pusa Ruby were raised in clay pots (30cm diam.) from seeds surface sterilized with 0.01% mercuric chloride. The seeds were sown in the pots filled with autoclaved sandy loam soil mixed with washed river sand and farmyard manure in the ratio of 3:1:1 (v/v/v).

5.2.2 Root-knot nematode culture

In this study root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, one of the common species in the area, was used. Field population of *M. incognita* were collected from tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena* L.). Species of root-knot nematode infecting roots of vegetables (tomato or eggplant) in fields were tentatively identified on the basis of the characteristics of perennial pattern of the females. Roots infected with *M. incognita* were chopped and added to the tomato plants (3 weeks old) raised earlier as mentioned above. After 50 days, single eggmass culture of the nematode was established. Now seedlings of the tomato plants were transplanted in clay pots (15cm diam.) containing autoclaved soil. Single eggmass of the nematode, *M. incognita* obtained from the roots of the plants were added in each pot near the roots of the seedlings. The pots were kept in glasshouse with temperature 27±2 °C. Subculturing was done approximately at every 3 months by inoculating new tomato seedlings with at least 15 eggmasses, each obtained from a single eggmass culture. In this way, single eggmass culture of *M. incognita* was maintained on tomato seedlings, which can be used for further experimental studies.

5.2.3 Identification of the root-knot nematode

The root-knot nematodes were identified on the basis of the characteristics of perineal patterns of females and races were identified by conducting North Carolina
Differential Host Test (Eisenback et al., 1981; Taylor and Sasser, 1978; Hartman and Sasser, 1985). Fifteen perineal patterns of females of each single mass population were prepared and their characteristics were examined in order to identify the species (Eisenback et al., 1981).

For North-Carolina differential host test, seedlings of tomato cv. Rutgers, tobacco cv. NC-95, pepper cv. California Wonder, peanut cv. Florunner, watermelon cv. Charleston Grey and cotton cv. Deltapine-61 were grown in clay pots (15cm diam., one seedling/pot) filled with sterilized soil. Five replicates were maintained for each crop. Two additional replicates of tomato were included to determine the time of termination of the test.

After determining the number of second stage juveniles (J2) per ml, plants in each pot were inoculated with 5000 J2. Juveniles were added around the roots of the seedlings. Inoculated plants were kept in glasshouse with temperature ranging from 27-30 °C. Fifty to sixty days after inoculation, roots were harvested and thoroughly washed with tap water and examined for the presence of galls. Roots with infection were stained with Phloxine-B to determine the number of eggmasses. Number of galls and eggmasses were counted and gall index (GI) and egg mass index (EMI) were rated on 0-5 scale (Taylor and Sasser, 1978).

After the rating of root system, results were compared with the differential host test reaction chart (Table 13). This confirmed the identity of the species determined by the perineal pattern method.

5.2.4 Preparation of inoculum and inoculation

Second stage juveniles (J2) of *M. incognita* race 2 were used as nematode inoculum in the study. Second stage juveniles were obtained by incubating eggmasses collected from the roots of tomato plants on which a single eggmass culture of *M. incognita* was maintained. Large number of *M. incognita* eggmasses were handpicked using sterilized forceps, washed in distilled water and then placed in 9cm diameter 15-mesh coarse sieves which were previously mounted with cross-layered tissue paper. The sieves were then placed in Petriplates containing distilled water just enough to contact the egg masses and were incubated at 27 °C for hatching. After 72 h, the hatched juveniles (J2) were collected from the Petri plates in a beaker and the number
Table 13. North Carolina differential host test reaction chart

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Species &amp; Race</td>
<td>Deltapine-61</td>
<td>NC-95</td>
<td>California Wonder</td>
<td>Charleston Grey</td>
<td>Florunner</td>
<td>Rutgers</td>
</tr>
<tr>
<td>M. incognita</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race 1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Race 2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Race 3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Race 4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. javanica</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. arenaria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race 1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Race 2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. hapla</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Susceptible; - = Resistant; Box indicate key differential host plants
of juveniles per ml was standardized by counting the juveniles from ten 1ml samples. Average number of juveniles was then calculated to represent the number of second stage juveniles (J2) per ml of the suspension.

5.2.5 Test plants

Seedlings of chickpea (Cicer arietinum L.) var. Avrodhi were grown as described earlier in Section-2. Seeds were surface sterilized with 0.01% mercuric chloride for 2 min and then washed three times with distilled water. Five sterilized seeds of chickpea were then sown in 15 cm diameter earthen pots containing 1 kg sterilized soil and later thinned to one seedling per pot.

5.2.6 Inoculation of nematode

On germination, plants were inoculated with 0, 500, 1000, 2000, 5000 and 8000 freshly hatched second stage juveniles (J2) of M. incognita plant⁻¹ in the rhizosphere. For inoculation, soil around the roots of the plant was carefully removed without damaging the roots. The suspension containing J2 was taken in micropipette controller and was poured around the roots of the seedlings and the soil was replaced. An equal volume of sterile water was added to the control treatment.

5.2.7 Experimental design

There were six treatments each with five replications, were arranged in a completely randomized block design and maintained on a glasshouse bench with air temperature ranging from 22±3 °C. Uninoculated plants served as control and all the plants were watered regularly.

5.2.8 Observations

The plants were terminated 90 days after nematode inoculation for determining the plant growth, nutrient status of the plants and nematode related parameters. The performance of the crop inoculated with different levels of M. incognita juveniles (J2) were compared with that of control and the population density of juveniles at which a significant damage is firstly observed was selected as the
economic threshold level of *M. incognita* second stage juveniles (*J*₂) for chickpea var. Avrodhi.

### 5.2.9 Parameters studied

After termination of the experiment, the following parameters were determined for each treatment:

- Plant length (cm)
- Plant fresh weight (g)
- Plant dry weight (g)
- Pods plant⁻¹
- Nodules plant⁻¹
- Chlorophyll content (mg g⁻¹ fresh leaves)
- Nutrient contents (mg g⁻¹ fresh leaves)
- Nematode population (both in soil and root)
- Number of galls root system⁻¹
- Number of egg masses root system⁻¹
- Number of eggs eggmass⁻¹
- Root-knot index (0-5)
- Reproduction factor (pf/pi)

### 5.2.10 Plant growth and chemical parameters

Plant growth and chemical parameters were studied by the methods mentioned in Section-2.

### 5.2.11 Nematode population

A 250g sub-sample of well-mixed soil from each treatment was processed by Cobb’s sieving and decanting method, followed by Baerman’s funnel extraction to determine the final nematode population in soil (Southey, 1986). Nematode suspensions were collected after 24 h, and the number of nematodes were counted in five aliquots of 1 ml of suspension from each sample. The means of the five counts
were used to calculate the population of nematodes per kg soil. To estimate the number of juveniles, eggs and females inside the roots, 1g sub-sample of roots were macerated for 30-40 sec in a waring blender and counts were made from the suspension thus obtained. The total number of nematodes present in the roots were calculated by multiplying the number of nematodes present in 1g of root by the total weight of root.

5.2.12 Galls and egg masses

At termination of the experiment, roots of harvested plants were washed under the tap and examined for the presence of galls. Number of galls per root system were counted. For the assessment of egg masses, plant roots were immersed for 15 minutes in 0.015% Phloxine B, which specifically stains the gelatinous matrix of nematode egg masses bright red and the egg masses per root system were counted (Taylor and Sasser, 1978).

5.2.13 Fecundity

The number of eggs per egg mass is known as fecundity. It was measured by shaking vigorously 10 egg masses in 5.25% NaOCl solution. The eggs were separated from egg mass and collected over 500 mesh sieve. From the sieve, eggs were transferred into a beaker and 0.35% acid fuchsin (in 25% lactic acid) was added into 20 to 25ml of suspension with boiling for 1 min for staining the eggs. After cooling, the eggs were counted and the eggs per egg mass were calculated to find out the fecundity.

The eggs were extracted from the root of each treatment separately by Chlorox method of Hussey and Barker (1973). Roots from each treatment were cut into 1-2 cm pieces. One gram of the root pieces were shaken vigorously in 200 ml of 1.0% NaOCl solution for 1 to 4 min. Then NaOCl solution with root pieces was passed through a 200 mesh sieve, rested over a 500 mesh sieve to collect free eggs. After this 500 mesh sieve with eggs was placed under a stream of cold water to remove residual NaOCl (rinsed for several minutes). The rinsed roots pieces were put under water to remove additional eggs and were collected by sieving. The number of eggs were then counted in counting dish under stereoscopic microscope. The total number of eggs was
calculated by multiplying the number with the fresh weight of the root in the treatment.

5.2.14 Root-knot index (0-5)

The root-knot index (RKI) was determined by scoring on a scale ranging from 0-5 scale where 0 = no disease and 5 = maximum disease intensity.

5.2.15 Reproduction factor (pf/pi)

Reproduction factor was calculated by using the formula, \( R = \frac{pf}{pi} \), where pf represents final and pi represents initial population of nematodes.

5.2.16 Statistical analysis

All the data were analyzed statistically by the method of Panse and Sukhatme (1985). Minimum difference required for significance (C.D.) at \( P=0.01 \) and \( P=0.05 \) was calculated by the ANOVA model 3 given in Appendix.

5.3 RESULTS

5.3.1 Plant length (cm)

The effect of different inoculum levels (500, 1000, 2000, 5000 and 8000 \( J_2 \) plant\(^{-1} \)) of *M. incognita* on plant length was examined in terms of shoot, root and total length of chickpea plant (Table 14). There was a progressive decrease in plant length with increase in inoculum levels.

Maximum shoot length was obtained in uninoculated plants followed by plants inoculated with 500 and 1000 \( J_2 \) plant\(^{-1} \) but the difference was not significant. Highest reduction in shoot length (30.18%) was observed when nematode population was 8000 \( J_2 \) plant\(^{-1} \). An inoculum level of 2000 \( J_2 \) plant\(^{-1} \) significantly reduced the shoot length as compared to control.

The root length also decreased with an increase in the inoculum level of nematode. Significant reduction (13.11%) observed at 2000 \( J_2 \) plant\(^{-1} \).
Maximum enhancement in total plant length was observed in uninoculated plants. An inoculum of 2000 J\(_2\) plant\(^{-1}\) significantly reduced the total plant length (13.21%) over control followed by 5000 and 8000 J\(_2\) plant\(^{-1}\) (Table 14 and Fig. 8).

### 5.3.2 Plant fresh weight (g)

With the increase in inoculum levels of *M. incognita*, there was a corresponding increase in plant fresh weight (shoot, root and total) reduction (Table 14).

Highest reduction in shoot fresh weight (42%) was recorded at an inoculum level of 8000 J\(_2\) plant\(^{-1}\) and the lowest (5.33%) was at an inoculum level of 500 J\(_2\) plant\(^{-1}\). Significant reduction was observed at 2000 J\(_2\) plant\(^{-1}\).

Inoculation with 500 and 1000 J\(_2\) plant\(^{-1}\) did not cause a significant reduction in root fresh weight. Plants inoculated 2000 J\(_2\) plant\(^{-1}\) and above densities of *M. incognita* showed a significant reduction in root fresh weight.

Total plant fresh weight significantly reduced (20.05%) at 2000 J\(_2\) plant\(^{-1}\). However, when plants were inoculated with 500 and 1000 J\(_2\) plant\(^{-1}\), there was no significant reduction in total fresh weight of plant over control (Fig. 8).

### 5.3.3 Plant dry weight (g)

Plant dry weight in terms of shoot, root and total plant dry weight decreased with the corresponding increase in the inoculum levels of *M. incognita* (Table 14).

Significant reduction in shoot dry weight with respect to control was found only when 2000 or more second stage juveniles of *M. incognita* plant\(^{-1}\) were inoculated. The largest reduction (43.01%) was observed in plants receiving the highest inoculum i.e. 8000 J\(_2\) plant\(^{-1}\).

Root dry weight also showed the similar trend as that of shoot dry weight. 500 J\(_2\) plant\(^{-1}\) failed to cause a significant reduction in root dry weight. Significant reduction (20.37%) was observed at 2000 J\(_2\) plant\(^{-1}\).

Different inoculum levels of *M. incognita* reduce the total plant dry weight. An inoculum level of 2000 J\(_2\) plant\(^{-1}\) brings significant reduction in total plant dry weight (20.42%) over control (Table 14 and Fig. 8).
Table 14. Effect of different inoculum densities of root-knot nematode, *Meloidogyne incognita* race 2 on the growth parameters of chickpea plant

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant length (cm)</th>
<th>Plant fresh weight (g)</th>
<th>Plant dry weight (g)</th>
<th>Pods plant(^{-1})</th>
<th>Nodules plant(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot Root Total</td>
<td>Shoot Root Total</td>
<td>Shoot Root Total</td>
<td>Shoot Root Total</td>
<td>Shoot Root Total</td>
</tr>
<tr>
<td>Control</td>
<td>43.64±2.18 21.82±1.09 65.46±3.27</td>
<td>43.36±2.17 10.84±0.54 54.20±2.71</td>
<td>6.51±0.33 1.62±0.08 8.13±0.41</td>
<td>31.0±1.55 4.0±0.20</td>
<td></td>
</tr>
<tr>
<td>500 J(_2)</td>
<td>42.36±2.12 20.64±1.03 63.00±3.15</td>
<td>41.05±2.05 10.22±0.51 51.27±2.56</td>
<td>6.22±0.31 1.50±0.08 7.72±0.39</td>
<td>26.0±1.30 2.0±0.10</td>
<td></td>
</tr>
<tr>
<td>1000 J(_2)</td>
<td>41.13±2.06 19.87±0.99 61.00±3.05</td>
<td>38.77±1.94 9.68±0.48 48.45±2.42</td>
<td>5.89±0.29 1.45±0.07 7.34±0.37</td>
<td>23.0±1.15 1.0±0.05</td>
<td></td>
</tr>
<tr>
<td>2000 J(_2)</td>
<td>37.85±1.89 18.96±0.95 56.81±2.84</td>
<td>34.66±1.73 8.67±0.43 43.33±2.17</td>
<td>5.18±0.26 1.29±0.06 6.47±0.32</td>
<td>18.0±0.90 0.0±0.00</td>
<td></td>
</tr>
<tr>
<td>5000 J(_2)</td>
<td>35.00±1.75 17.25±0.86 52.25±2.61</td>
<td>30.44±1.52 7.58±0.38 38.02±1.90</td>
<td>4.66±0.23 1.14±0.06 5.80±0.29</td>
<td>15.0±0.75 0.0±0.00</td>
<td></td>
</tr>
<tr>
<td>8000 J(_2)</td>
<td>30.47±1.52 15.24±0.76 45.71±2.29</td>
<td>25.15±1.26 6.63±0.33 31.78±1.59</td>
<td>3.71±0.19 1.02±0.05 4.73±0.24</td>
<td>11.0±0.55 0.0±0.00</td>
<td></td>
</tr>
<tr>
<td>C.D. ((P=0.05))</td>
<td>3.68 1.81 5.49</td>
<td>3.45 0.86 4.32</td>
<td>0.521 0.129 0.649</td>
<td>2.10 0.181</td>
<td></td>
</tr>
<tr>
<td>C.D. ((P=0.01))</td>
<td>5.23 2.58 7.80</td>
<td>4.91 1.23 6.14</td>
<td>0.740 0.183 0.924</td>
<td>2.98 0.258</td>
<td></td>
</tr>
</tbody>
</table>

Data mean±SD of five replicates
T1 = Control; T2 = 500 J$_2$; T3 = 1000 J$_2$; T4 = 2000 J$_2$; T5 = 5000 J$_2$; T6 = 8000 J$_2$

Fig. 8 Effect of different inoculum densities of root-knot nematode, *Meloidogyne incognita* race 2 on the growth parameters and chlorophyll content of chickpea plant
5.3.4 Pods plant\(^{-1}\)

All the inoculum levels of *M. incognita* significantly decreased the number of pods in the plant. Maximum reduction (64.52\%) being observed at 8000 J\(_2\) plant\(^{-1}\) and lowest reduction (16.13\%) at 500 J\(_2\) plant\(^{-1}\) (Table 14 and Fig. 8).

5.3.5 Nodules plant\(^{-1}\)

Root-nodulation decreased considerably due to parasitism of *M. incognita*. Significant reduction in nodule number was observed when plants were inoculated with inoculum density of 500 and 1000 J\(_2\) plant\(^{-1}\). No nodules were present at higher inoculum levels i.e. 2000, 5000 and 8000 J\(_2\) plant\(^{-1}\) (Table 14 and Fig. 8).

5.3.6 Chlorophyll content (mg g\(^{-1}\) fresh leaves)

The result presented in table 15 clearly revealed that the root-knot nematode, *M. incognita* significantly reduced the chlorophyll content of the plants at different inoculum levels. But this variation was not found significant at minimum inoculum level (500 J\(_2\) plant\(^{-1}\)). Significant reduction (24.02\%) being observed at 2000 J\(_2\) plant\(^{-1}\) (Fig. 8).

5.3.7 Nutrient contents (N, P & K) (mg g\(^{-1}\) fresh leaves)

Nutrient contents in terms of N, P and K also decrease with an increase in the inoculum levels of nematode. Significant reduction in N, P and K content was found at the inoculum level of 2000 J\(_2\) plant\(^{-1}\), with the maximum reductions 35.45\%, 54.16\% and 45.31\% respectively occurring at 8000 J\(_2\) plant\(^{-1}\) (Table 15 and Fig. 9).

5.3.8 Root-knot development

5.3.8a Nematode population

With an increase in the inoculum densities of *M. incognita*, the nematode population in soil and root was also increased. The nematode population both in soil and root was highest in and around the plants inoculated with 8000 J\(_2\) plant\(^{-1}\) while the lowest population was recorded in and around the plants inoculated with 500 J\(_2\) plant\(^{-1}\) (Table 15 and Fig. 9).
5.3.8b Number of galls root system\(^{-1}\)

The number of galls root system\(^{-1}\) were directly proportional with the increasing inoculum levels of *M. incognita*. Significant increase in number of galls were observed in all the inoculum levels of *M. incognita* i.e. 500-8000 J\(_2\) plant\(^{-1}\) (Table 15 and Fig. 9).

5.3.8c Number of eggmasses root system\(^{-1}\)

Similar trend was observed here as in the case of number of galls root system\(^{-1}\). Significant increase in number of eggmasses root system\(^{-1}\) was observed at all the inoculum density, maximum increase (124) being at 8000 J\(_2\) plant\(^{-1}\) (Table 15 and Fig. 9).

5.3.8d Number of eggs eggmass\(^{-1}\)

Significant increase in number of eggs eggmass\(^{-1}\) was observed at all the inoculum density of *M. incognita*. Number of eggs eggmass\(^{-1}\) increased significantly from inoculum levels of 500-8000 J\(_2\) plant\(^{-1}\). This shows that number of eggs eggmass\(^{-1}\) is also directly proportional to the increasing levels of *M. incognita* (Table 15 and Fig. 9).

5.3.8e Root-knot index (0-5)

The root-knot index on 0-5 scale was observed at all the inoculum levels of *M. incognita* (500-8000 J\(_2\) plant\(^{-1}\)). Lowest disease intensity (2) being observed at 500 J\(_2\) plant\(^{-1}\) and highest (5) being observed at 8000 J\(_2\) plant\(^{-1}\) (Table 15 and Fig. 9).

5.3.8f Reproduction factor (pf/pi)

Whilst the nematode population (soil and root), number of galls root system\(^{-1}\), number of eggmasses root system\(^{-1}\), number of eggs eggmass\(^{-1}\) and root-knot index increased with an increase in the inoculum levels of *M. incognita* from 500-8000 J\(_2\) plant\(^{-1}\), the reproduction rate of nematode decreased with an increase in inoculum levels. Maximum nematode multiplication (22.0) occurred at the lowest (500 J\(_2\)) and minimum (4.4) at the highest (8000 J\(_2\)) inoculum levels (Table 15 and Fig. 9).
Table 15. Effect of different inoculum densities of root-knot nematode, *Meloidogyne incognita* race 2 on the chlorophyll content, nutrient status and root-knot development in chickpea plant

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll content (mg g(^{-1}))</th>
<th>Nutrient contents (mg g(^{-1}))</th>
<th>Nematode population</th>
<th>Reproduction factor (pf/pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>Control</td>
<td>2.402±0.120</td>
<td>2.68±0.13</td>
<td>0.24±0.012</td>
<td>1.92±0.096</td>
</tr>
<tr>
<td>500 J(_2)</td>
<td>2.232±0.112</td>
<td>2.60±0.13</td>
<td>0.22±0.011</td>
<td>1.86±0.093</td>
</tr>
<tr>
<td>1000 J(_2)</td>
<td>2.173±0.109</td>
<td>2.51±0.13</td>
<td>0.20±0.010</td>
<td>1.79±0.089</td>
</tr>
<tr>
<td>2000 J(_2)</td>
<td>1.825±0.091</td>
<td>2.30±0.12</td>
<td>0.16±0.008</td>
<td>1.54±0.077</td>
</tr>
<tr>
<td>5000 J(_2)</td>
<td>1.601±0.080</td>
<td>2.07±0.10</td>
<td>0.14±0.007</td>
<td>1.33±0.067</td>
</tr>
<tr>
<td>8000 J(_2)</td>
<td>1.308±0.065</td>
<td>1.73±0.14</td>
<td>0.11±0.006</td>
<td>1.05±0.052</td>
</tr>
<tr>
<td>C.D. ((P=0.05))</td>
<td>0.188</td>
<td>0.236</td>
<td>0.018</td>
<td>0.154</td>
</tr>
<tr>
<td>C.D. ((P=0.01))</td>
<td>0.267</td>
<td>0.336</td>
<td>0.025</td>
<td>0.220</td>
</tr>
</tbody>
</table>

Data mean±SD of five replicates
Fig. 9 Effect of different inoculum densities of root-knot nematode, *Meloidogyne incognita* race 2 on the nutrient status and root-knot development in chickpea plant.
5.4 DISCUSSION

The results revealed that the plant growth of chickpea was adversely affected by the root-knot nematode, *M. incognita*. Though all the inoculum densities caused damage to chickpea plants but no significant reduction was estimated up to 1000 J$_2$ plant$^{-1}$. An inoculum level of 2000 J$_2$ plant$^{-1}$ caused a significant reduction in plant growth parameters, followed by other higher levels. The progressive decrease in plant growth with the increasing inoculum levels of *M. incognita* has also been reported by various workers in different crops (Rakesh et al., 1992; Siddiqui et al., 1995; Gupta et al., 1995; Patel et al., 1996; Pathak et al., 2000; Jonathan and Rajendran, 2000; Khan, 2003b, Pofu et al., 2009; Chandra et al., 2010). Stunted plants with chlorotic foliage at higher population densities are due to the damage caused by penetration and migration of large number of nematode juveniles through root tissues at early stage of the plant growth. Wallace (1971) observed that when the nematode population is high, there is a severe effect on root growth, root hair development and that these changes reduce water and mineral absorption and translocation in the plant. These effects results in lower photosynthesis rate and reduced plant growth.

Pods and nodules numbers were also severily affected by the root-knot nematode, *M. incognita*. Balasubramaniyam (1971) observed that when the nematode population is high, they interfere directly with the establishment of *Rhizobium japonicum* bacterium due to lowered production of root hairs in *M. incognita* infected plants. It caused reduction in nodulation. Reduced number of nodules might also be due to overall reduction of the root system as a result of nematode infection. These results reveal that the infection of *M. incognita* could considerably affect the plant growth of chickpea due to the formation of deformed galled roots, which inhibit the uptake, and absorption of nutrients from soil. The nematode also effects the chickpea nodulation due to the inhibition of development of lateral roots and root hairs. The severity of this effect is directly proportional to the level of nematode infection.

In the study, the root-knot development increase with an increase in the inoculum density of *M. incognita*. The results of host infestation recorded in terms of nematode population (soil and root), number of galls root system$^{-1}$, number of eggmasses root system$^{-1}$ and number of eggs eggmass$^{-1}$ were directly proportional
with the inoculum level. Proportional increase in root-knot index confirm the findings of Ahmed and Husain (1988). Wallace (1973) reported that the increase in nematode population and subsequent reduction in the yield of crops or other manifestations of pathogenic effects are directly influenced by initial density of nematodes in soil. The decrease in nematode multiplication at the highest population level was perhaps due to the destruction of the root system and competition for food and nutrition among developing nematodes within the root system and also due to the inability of the larvae to find out the new infection sites of subsequent generations (Ogunfowora, 1977). Similar observations on the reduction in nematode multiplication were also noted by Khan and Husain (1989), Pathak et al. (2000), Khan (2003a) and Chandra et al. (2010).

The reproduction factor of *M. incognita* significantly reduced with the increase in the inoculum level, i.e. a significant linear relationship was found between the initial and final population. Thus, the reproduction factor decreased with an increase in inoculum level, which revealed that nematode multiplication showed a decline trend, suggesting it to be a density dependent phenomenon (Siddiqui and Mahmood, 1992; Mahapatra et al., 1999; Patel et al., 2001; Khan et al., 2006).

In our study, the threshold level of *M. incognita* on chickpea was 2000 $J_2$ plant$^{-1}$. Our results/findings are in accordance with Mani and Sethi (1984), Krishnarao and Krishnappa (1995), Siddiqui and Mahmood (1992). Tiyagi and Alam (1986) reported an inoculum level of 1000 and 5000 *M. incognita* $J_2$ plant$^{-1}$ to be the threshold level on two cultivars of chickpea i.e. cv. Pusa 209 and cv. K-850 respectively. Differences in threshold level may be due to variation in susceptibility of test cultivars. There was a direct correlation between the inoculum level and reduction in plant growth.

Economic threshold level determined under this type of experiment (greenhouse) may not coincide with those determined in field experiments, since the nematode-crop relationship must be evaluated in the context of agricultural ecosystem (Barker et al., 1985).
5.5 CONCLUSION

In our study, increase in inoculum densities (500, 1000, 2000, 5000 and 8000 J$_2$ plant$^{-1}$) of *M. incognita* resulted in reduction in plant growth and development. However all the levels reduce the plant growth, but the first significant reduction was observed at 2000 J$_2$ plant$^{-1}$. Thus, the economic threshold level of *Meloidogyne incognita* race 2 was found @ 2000 J$_2$ plant$^{-1}$ in chickpea var. avrodhi.

5.6 SUMMARY

1. A study was conducted to determine the economic threshold level of *Meloidogyne incognita* on chickpea (*Cicer arietinum* L.) var. Avrodhi. in the greenhouse conditions.

2. Different inoculum levels (500, 1000, 2000, 5000 and 8000 J$_2$ plant$^{-1}$) of *M. incognita* were taken into consideration.

3. The parasitic activity of the nematode adversely affected nodulation and there was an increase in the root-knot development.

4. An inoculum level of 2000 J$_2$ plant$^{-1}$ was the economic threshold level of *M. incognita* on chickpea plant. Lower inoculum levels (500 and 1000 J$_2$ plant$^{-1}$) failed to cause a significant reduction, highest reduction being observed at 8000 J$_2$ plant$^{-1}$.

5. In general, determination of economic threshold level is considered essential in the integrated nematode management programs. Accordingly, this parameter should be considered before undertaken of any decision of nematode control.