Section-II
EXPERIMENT 2

Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Ocimum sanctum*, reproduction of the nematode and internal structure of root.

INTRODUCTION

*Meloidogyne incognita*, a parasitic nematode causes severe damage to a large number of plant species including the members of the family Lamiaceae. Low or high population densities of this nematode produce different effects on plants. At low inoculum levels, sometimes the plant growth is stimulated, but at other times it is suppressed. Wallace (1971) found an increased plant growth at lower population densities and decreased at higher population densities. Dropkin (1954) inoculated tomato roots with a single juvenile of *M. incognita* and measured the size of the resulted gall. He therefore hypothesized that each individual nematode produced a finite response on the root tissue and by measuring the gall size, number of nematodes in the gall might be predicted. In heavily infested roots, it is very difficult to follow the hypothesis.

The following study was carried out to determine the effects of different inoculum levels on: (i) the plant growth, (ii) the plant yield, (iii) the number and size of the galls, (iv) the number of egg masses per plant, (v) the number of eggs per egg mass in roots and (vi) the number of mature females per gram of root, of *Ocimum sanctum*.

Histopathological studies, of the galls produced as a result of different inoculum levels, were also carried out. For this
purpose (i) size of the mature females (ii) size of the giant cells (iii) the number of giant cell, nuclei and nucleoli and abnormalities in xylem and phloem were studied.

MATERIALS AND METHODS

Raising the Test Plant:
The seeds of *Ocimum sanctum* L. (green variety), Krishna were procured from National Seeds Corporation, New Delhi. The seeds were axenized by NaOCl method (Koenning and Barker, 1985). About 200 seeds were placed in a sterilized beaker containing a mixture of 95% ethanol and 5.25% NaOCl in the ratio of 1:1. The mixture was stirred gently and the seeds were allowed to soak for about 10 minutes. The mixture was drained off and the seeds were rinsed thrice with distilled water.

The axenized seeds were sown in 30 cm diameter clay pots containing steam sterilized soil (7 clay: 3 sand: 1 farmyard manure). Initially there were five seedlings per pot, these were thinned to one plant per pot, when the seedlings reached three-leaf stage.

Inoculation with Nematode:

*Meloidogyne incognita* (Kofoid and White) Chitwood was selected as test pathogen. To perform experiments during the period of research, pure culture of *M. incognita* was maintained on egg plant (*Solanum melongena* L.) roots in the glass house by using single egg mass. The egg masses from galled roots of egg plant were picked with the help of sterilized forceps and were allowed to hatch. The second-stage juveniles were collected in distilled
water and counted with the help of counting dish. Three-leaf stage seedlings were inoculated by making holes of 5-7 cm depth around the plant within the radius of two centimeters. The second-stage juveniles at the rate of 5J2, 50J2, 500J2 and 5,000J2 per 10 ml of water were pipetted into the soil through the holes. The holes were then plugged with the sterilized soil soon after inoculation. Each treatment was replicated five times and the pots were arranged in randomized complete block design. Uninoculated set of plants served as control. There were five sets of pots as given below:

- C : control
- T1 : 5J2/pot
- T2 : 50J2/pot
- T3 : 500J2/pot
- T4 : 5,000J2/pot

Watering was done regularly and the plants were uprooted after three months of inoculation. The data for different parameters were collected and statistically analysed.

PARAMETERS

Plant Growth:

Root length, shoot length and internodal length of the plants were measured with the help of meter scale. Number of branches of the shoots was counted by visual observation. The roots and the shoots of the plants of each treatment, when fresh, were weighed separately and afterwards, these were placed in bamboo paper envelopes. These envelopes were kept in an incubator and
left for 48 hours at 80°C. The dried shoots and the roots were weighed again to obtain dry weights.

**Leaf Area:**

Five medium size mature leaves from each treatment were randomly selected for this purpose. Outline of the shape of each leaf was drawn on rice papers and the area occupied was measured with the help of planimeter.

**Yield:**

The weight of 100 seeds, randomly selected from each treatment, was taken as yield.

**Number and Size of Galls:**

The number of galls per root system was counted by visual observation. The size of the gall was obtained by measuring maximum length and width (in mm²) on a meter scale.

**Number of Egg Masses:**

The number of egg masses per root system on infected roots was counted after staining egg masses with phloxin B. An aqueous solution of phloxin B 0.15g per litre of water was prepared. The galled roots were placed in this solution for 15-20 minutes. The roots were gently rinsed in tap water. The egg masses were stained red and were counted directly.

**Number of Eggs per Egg Mass:**

About 10 mature egg masses were selected randomly from root galls of each treatment. The egg masses were treated with 20 ml of NaOCl (2%) solution and stirred vigorously for one minute.
The egg masses were stained with acid fuschin (Byrd et al., 1972) and then counted under stereoscopic microscope.

**Number of Mature Females:**

For counting the number of mature females the root samples taken from each treatments were blended with 200 ml water in a warring blender for 30 seconds at low speed. The resultant suspension was passed through coarse and 100 mesh sieves in order to separate root tissues. The total female population was number of female nematodes in the suspension was divided by the weight of each root system to derive population per gram root.

**REPRODUCTION FACTOR (Rf) AND RATE OF POPULATION INCREASE (RPI):**

For final population (Pf), soil population was estimated by Cobb's sieving and decanting method, and root population was estimated by Blender- Baerman tray method (Hooper, 1985). Reproduction factor (Rf) was calculated by the formula:

\[
Rf = \frac{Pf}{Pi}
\]

where Pf is the final population and Pi is the initial population. Rate of population increase (RPI) was calculated by the formula:

\[
RPI = \frac{(Pf - Pi)}{Pi}
\]

**HISTOPATHOLOGICAL STUDIES:**

From the infected roots, few galled portions were selected from each treatment for performing histopathological studies. The
galled tissues were fixed in formalin aceto-alcohol (F.A.A.) and then dehydrated through tertiary butyl alcohol (T.B.A) schedule (Johansen, 1940) (Table-1). The galls were infiltrated with paraffin oil and then embedded in paraffin wax. The wax embedded galls were trimmed to small blocks and then fixed on wooden blocks. Sections of 10μm thickness were obtained in the form of ribbon with the help of rotary microtome. The ribbons were cut and mounted on the slides which were kept in an incubator at 40°C for 24h (Johansen, 1940) (Table-1). The sections were stained with safranin and fast green as described by Sass (1951) (Table-2). Anatomical details were observed under light microscope and necessary photographs were taken.

RESULTS

Plant Length:

The length of the root and the shoot of Ocimum sanctum, inoculated with different inoculum levels of second-stage juveniles of Meloidogyne incognita decreased, when compared with uninoculated (control) plants (P. 60). The reduction in plant length of T1 and T2 plants was non-significant at the initial inoculum levels of Pi = 5J2 and Pi = 50J2 per pot. Significant reductions (p < 0.05) in T3 and (p < 0.01) T4 plants were observed in comparison to control (C). The reduction was maximum in T4 plants grown at Pi = 5,000J2 per pot (Table 3A, H-1). There was higher reduction in the length of the roots than the shoots.

Root and Shoot Weight:

Fresh weights and the dry weights of the roots and the shoots of root-knot nematode inoculated plants exhibited
reduction over the control (C) (H-1). The weights of the roots and shoots reduced non-significantly at the inoculum levels of Pi = 5J₂ and Pi = 50J₂, in comparison to control. A significant (p ≤ 0.05) reduction in fresh and dry weight of the roots and the shoots, in comparison to control, was noticed at Pi = 500J₂ per pot. Reduction in fresh as well as dry weights of both the roots and the shoots was much higher and significant (p ≤ 0.01) in the treatments T₄ grown at the initial inoculum levels of Pi = 5,000J₂, respectively, in comparison to control (Table 3A).

**Internodal Length:**

The length of internodes of the shoot showed variation in all the treatments (H-2). There was no significant difference in internode length of T₁ and T₂ plants, when compared with control. Significant reductions were noticed in T₃ (p≤0.05) and T₄ (p≤0.01) plants. At the highest inoculum level (Pi = 5,000J₂), in T₄ plants, the reduction in internode length was maximum (Table 3A).

**Number of Shoot Branches:**

In the root-knot nematode inoculated plants, the number of shoot branches per plant decreased with the increase in initial inoculum levels (H-2). The number of shoot branches decreased but non-significantly at the initial inoculum levels of Pi = 5J₂ and 50J₂, when compared with control. A significant (p ≤ 0.05) decrease in the number of branches per plant was observed in T₃ plants inoculated with 500J₂ per pot. Reduction in branch number was higher in T₄ and was also significant (p ≤ 0.01). At an initial inoculum level of Pi = 5,000J₂, reduction in branch number was the greatest (Table 3A).
Leaf Area:

The leaf area of the plants inoculated with the root-knot nematode, when compared with control, decreased non-significantly at lower \((\text{Pi} = 5J_2 \text{ and } \text{Pi} = 50J_2)\) and significantly at higher \((\text{Pi} = 500J_2 \text{ and } \text{Pi} = 5,000J_2)\) inoculum levels (H-2). The reduction in leaf area was significant at \(p \leq 0.05\) at an initial inoculum level of \(\text{Pi} = 500J_2\) (in T3 plants), when compared with control. Higher and significant \((p < 0.01)\) reductions in leaf area were noticed at the initial inoculum levels of \(\text{Pi} = 5,000J_2\) per plant over the control (Table 3A).

Seed Weight:

After maturation and ripening of the fruits, the seeds were gathered, dried and weighed. The weights of the seeds collected from all the treatments were found to be lower than the weights of the seeds of control plants (H-2). The seed weights of T1 and T2 plants were slightly and non-significantly lower than the controls. Significant \((p \leq 0.05)\) decrease in weight of 100 seeds was observed at an initial inoculum level of 500J2, in comparison to control. There were significant \((p \leq 0.01)\) reductions, in comparison to control, in the weights of the seeds at the initial inoculum level of \(\text{Pi} = 5,000J_2\) per pot, in T4 plants (Table 3A).

Number of Galls:

The galls were smaller and almost unnoticeable on the roots of T1 plants that were grown at the lowest initial inoculum level of \(\text{Pi} = 5J_2\). The number of galls per plant increased with the increase in initial inoculum level. The number of galls on the roots of T2 plants at \(\text{Pi} = 50J_2\) was higher but non-significant, when compared
with the gall number at the lowest inoculum level. A significant ($p \leq 0.01$) increase in gall number on T$_3$ plants ($Pi=500$ J$_2$, per pot), in comparison to lowest inoculum level ($Pi = 05$J$_2$), was observed. The number of galls also increased greatly and significantly ($p \leq 0.01$) at the next higher inoculum level of $Pi = 5,000$J$_2$ in comparison to the lowest inoculum level (Table 3B).

**Size of Galls:**

The size of the galls was found to be increased at higher inoculum levels when compared with the galls at the lowest inoculum levels (Table 3B). A non-significant increase in gall size was noticed on T$_2$ plants at $Pi = 50$J$_2$ in comparison to the gall size at $Pi = 5$J$_2$. A significant ($p \leq 0.01$) increase in the gall size at the inoculum level of $Pi = 500$J$_2$ was observed when the comparisons were made with the galls of T$_1$ plants. A significant ($p \leq 0.01$) increase was also seen in the size of the galls at $Pi = 5,000$J$_2$ and the galls attained their largest size at this inoculum level when compared with the size of the galls on T$_1$ plants (Table 3B).

**Number of Egg Masses:**

An increase in the number of juveniles introduced per plant resulted in the increase in the number of egg masses per plant. The average number of egg masses recovered from the lowest inoculum level of $Pi = 50$J$_2$ was very low (2.1 per plant). A non-significant increase, in comparison to T$_1$, in the number of egg masses per plant was observed at the initial inoculum level of $Pi = 50$J$_2$. The number of egg masses per plant significantly ($p \leq 0.01$) increased at the initial inoculum level of $Pi = 500$J$_2$ when compared with T$_1$ and T$_2$. The increase in the number of egg
masses per plant was significantly (p ≤ 0.01) higher as observed at Pi = 5,000J2, in comparison to T1, T2 and T3 plants (Table 3B)

**Number of Eggs Per Egg Mass:**

The number of eggs per egg mass decreased with an increase in initial inoculum level. The differences were significant (p ≤ 0.01) at all the inoculum levels (Pi = 50, 500 and 5,000 J2), when compared with T1, at the lowest inoculum level of Pi = 5J2 (Table 3B).

**Number of Mature Females:**

The number of mature females recovered from the inoculated roots increased with the increase in the initial inoculum level. The number of mature females per gram root was very low (2.2) at the initial inoculum level of Pi = 5J2 on T1 plants. A non-significant increase in the number of mature females per gram root was noticed at Pi = 50J2 (T2) as compared with (T1) plants, at the lowest initial inoculum level (Pi = 5J2). The number of mature females per gram root increased significantly (p ≤ 0.01) at the initial inoculum level of Pi = 500J2 when compared with T1. Their number was greatest at the highest inoculum level of Pi = 5,000J2 per pot (Table 3B).

**Reproduction factors (Rf) and Rate of Population Increase:**

Reproduction factors (Rf) and rate of population increase (RPI) decreased with an increase in initial inoculum level, maximum being at the lowest and minimum at highest inoculum level (Table 3B).
Histopathological Studies:

Longitudinal and transverse sections of Ocimum sanctum infected with root-knot nematode (Meloidogyne incognita) exhibited severe infection, when compared with the healthy ones. The juveniles that entered the roots established a successful host-parasite relationship by inducing giant cells. After penetration into the roots of O. sanctum, the second-stage juveniles led to the induction of 6-8 multinucleate giant cells. The giant cells were induced in the phloem region. (P. 52) A cluster of more than 10 or more giant cells was observed when two nematodes were found lying side by side and feeding on the same giant cell complex. (P. 53).

There were great variations in the size of the giant cells. At lower inoculum levels, the nematode and the giant cells were found at one or two places (P. 54), when seen in transverse section. At higher inoculum levels, all the four parenchymatous rays were seen occupied by the nematode and the giant cells (P. 55).

The giant cell cytoplasm was dense and more granular (P. 56) at lower inoculum levels (Pi = 5 and 50J2) whereas at higher inoculum levels (Pi = 500 and 5,000J2) it was less dense (P. 57). The multinucleate condition of giant cells varied at different inoculum levels. The number of nuclei was higher at lower initial inoculum level (Fig. 56 and 57) than at higher inoculum levels. The nuclei were amoeboid, circular and oval in shapes at lower inoculum level but at higher inoculum levels the nuclei were mostly amoeboid. The increase or decrease in initial inoculum level had no effect on size and shape of nucleoli. The nucleoli varied from 4-10 in a single nucleus, but 4-6 nucleoli were observed more
frequently (P. 58). Distortion of xylem strands occurred as a result of nematode infection but the amount of abnormal xylem was higher at the higher inoculum levels than at the lower inoculum levels. Similarly, the orientation of phloem strands was also less distorted at lower than at higher inoculum levels (P. 52). Phloem elements were found associated with every giant cell cluster (P. 52 and 59).

**DISCUSSION**

Inoculation of *Ocimum sanctum* with second-stage juveniles of *Meloidogyne incognita* caused reduction in plant length as is evident from H-1 and Table 3A. An increase in the number of juveniles at different inoculum levels decreased the length of the plant. The relationship between the juvenile population and the plant length was inversely proportional, and conversely the relationship between the juvenile population and reduction in plant length was directly proportional. The inoculum level comprising of 500J2 per plant was the lowest level that caused significant reduction in plant length. Increase in inoculum level from 5J2 per pot to 5,000 J2 per pot brought about a gradual decrease in length, fresh weight and dry weight (H-1 and Table 3A). Adverse effects on growth of different plants with an increase in primary inoculum level of *Meloidogyne* spp. have been reported by several workers (Barker and Olthof, 1976; Barker, 1977; Nordacci and Barker, 1979; Kinloch 1980, 1982; Rodriguez Kabana and Williams, 1981; Appel and Lewis, 1984; Ibrahim and Lewis, 1985; Verma and Ali, 1993; Vashisth *et al.*, 1994; Gupta *et al.*, 1995; Haseeb *et al.*, 1996, Fazal *et al.*, 1996; Ramakrishnan and
Rajendran et al., 1998; Sharma et al., 1999; Rombati and Dhanachand, 2000; Singh and Goswami, 2000; Jain et al., 2000; Jonathan and Rajendran, 2000; Kumar, 2000; Pathak et al., 2000; Tiyagi et al., 2001, Gergon et al., 2002, Nehra and Trivedi, 2002; Khan, 2003; Hisamuddin et al., 2003, 2004; Khan et al., 2004; Manoj and Pathak, 2004; Pathan et al., 2004; Youssef 2004; and Hisamuddin et al., 2005).

In *O. sanctum*, the length of the internode was also observed to be affected by the nematode infection. The number of the branches of the shoot also decreased at higher inoculum levels. The leaf area was reduced with increase in inoculum levels. All these findings led to an outcome that low or high population of *M. incognita* was detrimental to the plant as a whole. This was further supported by the fact that yield, in terms of weight of seeds, was decreased in root-knot nematode inoculated plants.

The plant responses towards *M. incognita* could be reflected by the symptoms developed on the roots and the shoots. The infested plants generally show the symptoms of stunting, yellowing and burning of lower leaves, and severe galling on the root system. Dwarfing and stunting however, are the most commonly observed symptoms of root-knot disease. The characteristic symptoms sometimes may or may not develop on the plant. From our studies, it is evident that every increase in the primary inoculum level from 5 to 5,000 J2 caused a corresponding decrease in the plant growth and yield characters.

The damage caused to the plant as a result of *M. incognita* infection involves several mechanisms. The plant growth might be
affected due to removal of nutrients by the nematode. There are several reports narrating that the root-knot nematode influenced the transport mechanism from root to shoot and translocation of metabolites from leaves to other organs, and reduced the amount of photosynthetic pigment (Loveys and Bird, 1973; Wallace, 1974 and Melakeberhan et al., 1985). The nutrient transport from the root towards shoot was curtailed probably due to anatomical abnormalities developed in the galled regions. Moreover, the photosynthates were diverted towards the giant cells that acted as metabolic sinks. All these malfunctions contributed in the suppression of plant growth and yield (Hussey, 1985).

Fazal et al., (1996) suggested a threshold level of 1,000 J$_2$ of *M. incognita* on mungbean whereas further higher inoculum levels, proved detrimental to the plant growth. Singh and Goswami (2000) observed significant reduction in growth of cowpea plants at 1,000 nematodes per 500g of soil. Pathak et al. (2004) reported minimum damaging threshold level of 500 J$_2$/kg soil for spinach and fenugreek. Khan and Ashraf (2005) reported detrimental or pathogenic effect of 2,000 J$_2$ of *M. incognita* and 1,000 J$_2$ of *M. javanica* per plant on lettuce (*Lactuca sativa*).

In the presence of higher number of nematodes the chances of infection are greatly increased. The growing roots as well as newly emerged roots are frequently attacked by the juveniles causing severe infection in roots leading to heavier damages to the plants. The reduction in internodal length of the shoot might have contributed in stunting and dwarfing of the plant that are the characteristic symptoms established by *M. incognita*. 
The number of shoot branches reduced non-significantly at lower inoculum levels. On the other hand, significant reductions in the number of branches occurred at higher inoculum levels. The root-knot nematode infection markedly retards the rate of absorption by the roots and also affects the rate of translocation towards the shoot apices. Oteifa (1952) compared Meloidogyne infected plants with the plants grown in nutrient deficient soils and observed that similar nutritional deficiency symptoms occurred in the foliage of both kinds of plants. Hunter (1958) correlated poor nutrient uptake and suppressed plant growth with highly reduced root system as a result of root-knot nematode infection. The nutrient materials were prevented, up to certain extent to reach the top of the plant which resulted in reduction of total photosynthetic leaf area. Jonathan and Rajendran (2000) also reported significant reduction in leaf area of Musa sp. at 1,000 and 10,000 juveniles per kg of soil.

With an increase in the initial inoculum level, there was a corresponding reduction in the seed weight, with a greatest reduction at highest inoculum level. The disturbances caused by Meloidogyne in plant roots impaired some physiological phenomena of the plant. The translocation patterns from the root to the shoot and vice versa were drastically influenced which might have affected the yield of the plant. Barker (1977) assessed yield losses from 3.7 to 19.9% in tobacco due to M. arenaria, M. hapla, M. incognita and M. javanica for each ten fold increase in initial density for each species. Ramana et al. (1998) reported 29.60 to 33.35% yield loss in ginger at Pi= 0.2 per 100 cc soil. Reduction in yield of Papaver rhoeas and Eclipta alba as a result of
M. incognita infection has been reported by Hisamuddin et al. (2003, 2004).

With an increase in initial inoculum level, the number of mature females recovered per gram root was found to be increased. However, a limited supply of food in a limited space, probably, induced detrimental effects on the normal development of the nematode. Our results are in accordance with the studies carried out by Bhat, (1999), Jonathan and Rajendran, (2000) and Yasmin, (2002). Our data revealed that the number of egg masses per plant increased with the increase in inoculum level and it seems quite reasonable that the lower is the inoculum level, the lower will be the number of mature females in the galled roots and consequently fewer will be the egg masses; higher is the inoculum level, correspondingly higher will be the number of mature females as well as number of egg masses.

The number of eggs per egg mass significantly decreased at all inoculum levels and it seems that size of the mature female and the number of eggs produced by it are interrelated. The number of egg masses per plant and the number of eggs per egg mass is influenced by the nematode density in a root system. The limited food and space are probably disadvantageous for the maximum development of the nematode that consequently interfered in egg mass production. Insufficient supply of nutrients seemed to be the main reason behind the low production of egg masses.

Reproduction factor (Rf) and rate of population increase were found decreased with the increase in initial inoculum level
which was highest at the lowest inoculum level and lowest at the highest inoculum level.

The reductions in the multiplication rate of *M. incognita*, on banana were noticed by Adiko (1989). Jonathan and Rajendran (2000) attributed that decrease in multiplication rate to high initial inocula created crowded conditions, which adversely affected the rate of development of the nematode. The decrease in the rate of nematode multiplication was perhaps due to destruction of the root system with high population of the nematode and due to competition for nutrition among the developing nematodes within a given root system as was also reported by Chitwood (1951) for *M. hapla*, Samathanam and Sethi (1996) and Pathak *et al.* (2000) for *M. incognita*. Khan and Ashraf (2005) also reported a decrease in reproduction factor of *M. incognita* and *M. javanica* with an increase in the inoculum levels from 250 to 8,000 J2.

*Ocimum sanctum* roots, in response of feeding by *M. incognita* juveniles, showed pronounced anatomical changes. The development of elaborate permanent feeding sites, called giant cells, were the main among the other various changes observed. Our studies revealed that *M. incognita* induced 6–8 multinucleate giant cells in the vascular tissues of *O. sanctum*; Christie (1936) reported 3–6 giant cells in tomato, similarly 4–9 giant cells were reported in sweet potato, (Krusberg and Nielsen, 1958), 2–5, 10–12 giant cells in *Gardenia*, (Davis and Jenkins, 1960), 5–9 in soybean (Dropkin and Nelson, 1960); 4–7 in *Hibiscus* (Littrell, 1966), 4–5 in tifdwarf, (Heald 1969); 3–5 in barley (Ediz and Dickerson, 1976); 5–6 in *Impatiens* (Jones and Payne, 1978) 3–5 in banana (Kheir *et al.*, 2004).
As observed in the transverse and longitudinal sections, the shape of the giant cells was circular, ovate or oblong. The giant cells were generally oblong because they arose from elongated cells which in normal development would lead to the formation of vessels (Christie, 1936). In contrast, our conclusion is that giant cells are formed from these cells that under normal conditions would develop into phloem elements as has been proposed by Byrne et al. (1977), Hisamuddin and Siddiqui (1992) and Bhat (1999).

The giant cells adjacent to the nematode head were larger as compared to those which were away from the nematode. The giant cells were produced as a result of continuous stimulus received from the nematode (Bird, 1962). The giant cell nearest to the nematode head gets direct stimulus and hence is larger than that which is away, and receives an indirect stimulus. This indicated that formation of giant cells is an essential phase for a successful host parasite relationship. The tissue preferred for the formation of giant cells, was primary phloem or adjacent parenchyma (Christie, 1936; Krusberg and Nielsen, 1958; Byrne et al., 1977). The site where giant cells were produced was occupied by parenchyma ray during normal development of root. As a result of secondary growth, the protophloem was crushed and pushed towards the periphery. Formation of giant cells and development of the nematode, in the protophloem, exerted considerable pressure on the surrounding tissues causing abnormality in the neighbouring cells and tissue. Average size of the giant cell reduced at higher inoculum levels probably because all the parenchyma rays were occupied by the giant cells and the nematode at higher inoculum densities.
Bird and Loveys (1975) and McClure (1977) unequivocally asserted that *Meloidogyne* acts as a metabolic sink in diseased plants. The increased metabolic activity of giant cells stimulates mobilization of photosynthates from shoots to roots and particularly to the giant cells where they are removed and utilized by the feeding nematode. In our findings, the dense cytoplasm in giant cells at the lower inoculum levels indicated that the host plant had sufficiently supplied metabolites to the giant cells. Depletion of dense cytoplasm and much vacuolation in the giant cells, at higher inoculum levels, indicated that either there was an inadequate supply of the photosynthates or there was more demand by the nematodes as compared with the rate of production. At lower inoculum level, the higher number of nuclei in the giant cells induced higher metabolic activity of giant cells. The depletion of giant cell cytoplasm at higher inoculum levels governed the speedy removal of metabolites by the nematodes.

Formation of abnormal xylem as a result of root-knot nematode infection has been reported in almost all the histopathological studies. Since several nematodes induce giant cells at a particular site, at higher inoculum level, therefore, it is supposed that each nematode produces its own affect. The results are evident in the form of multiple hypertrophic and hyperplastic reactions, and also formation of abnormal xylem at various sites. The combined effects of these reactions stimulated abnormal xylogenesis. Increase in number of phloem elements was probably due to formation of higher number of giant cells that was essential for regular supply of food to the giant cells (Hisamuddin, 1992).
EXPERIMENT 3
Effect of different inoculum levels of Meloidogyne incognita on growth, chlorophyll and oil content of Ocimum sanctum

INTRODUCTION

Meloidogyne incognita infection causes a number of changes in the structure, composition and also metabolic activities of host plant. This results in alteration in amount of chlorophyll content and oil content of the leaves. Reduction in chlorophyll content of the infected plants has been reported by Vashishth et al., (1994); Poornima and Vadivelu (1998); and Rajendran (1998). Reduction in oil content of leaves has been reported by Pandey (1988); Pandey et al., (1992); Haseeb (1998) and Tiyagi et al., (2001). The present study was carried out to estimate chlorophyll and oil contents in infected plants and comparisons were made with the healthy ones.

MATERIALS AND METHODS

The seeds of Ocimum sanctum L. (green variety) were procured from National Seeds Corporation, New Delhi. The seeds were axenized by NaOCl method (Koening and Barker, 1985). About 200 seeds were placed in a sterilized beaker containing a mixture of 95% ethanol and 5.25% NaOCl in the ratio of 1:1. The mixture was stirred gently and the seeds were allowed to soak for about 10 minutes. The mixture was drained off and the seeds were rinsed thrice with distilled water.
The axenized seeds were sown in 30 cm diameter clay pots containing steam sterilized soil (7 clay: 3 sand: 1 farmyard manure). The required number of freshly hatched second-stage juveniles (J2) were obtained from the procedure as described in experiment number 2. The plants were inoculated with (0, 50, 500 and 5000 J2) with five replicates per treatment at sowing time. The pots were kept in glass house and were watered regularly. The plants were harvested after three months. After harvesting, plant length, fresh weight, dry weight and other parameters were recorded and statistically analyzed.

Parameters

Plant growth:

The root and the shoot lengths of the plants were measured with the help of meter scale. The roots and shoots of the plants of each treatment, when fresh, were weighed separately and afterwards were placed in bamboo envelopes. These envelopes were kept in an incubator and left for 48 hours at 80°C. The dried shoots and roots were weighed again to obtain dry weights.

Leaf Area:

The leaf area of the plants was ascertained by the same method as mentioned earlier in Experiment 2.

Estimation of chlorophyll:

The chlorophyll content was estimated by following the method of Hiscox and Israelstam (1979). 100 mg of fresh plant material was weighed and kept in 10 ml dimethyl sulphoxide (DMSO). The test tubes were then kept in an incubator at 55°C for
an hour to facilitate the extraction of chlorophyll into the solution. The optical densities were taken at 645 and 663 nm wavelength on Spectronic-20 spectrophotometer and chlorophyll (chl-a, chl-b and total chlorophyll) content was calculated according to the following formulae given by Arnon (1949).

\[
\text{Chlorophyll a (mg g}^{-1} \text{ fresh tissue)} = \frac{12.7(\text{O.D.663}) - 2.69(\text{O.D.645}) \times V}{1000 \times W}
\]

\[
\text{Chlorophyll b (mg g}^{-1} \text{ fresh tissue)} = \frac{22.9(\text{O.D.645}) - 4.68(\text{O.D.663}) \times V}{1000 \times W}
\]

\[
\text{Total Chlorophyll (mg g}^{-1} \text{ fresh tissue)} = \frac{20.2(\text{O.D.645}) + 8.02(\text{O.D.663}) \times V}{1000 \times W}
\]

where, \(V\) = total volume of chlorophyll extracts in DMSO

\(W\) = fresh weight of plant tissue (g)

\(\text{O.D.}\) = optical density of samples at 645 and 663 nm wavelengths

12.7, 2.69, 22.9, 4.68, 20.2 and 8.02 are the dilution factors.

**Oil content:**

The oil content in fresh leaves was measured according to the method of Clevenger (1928). Fresh leaves (100g per plant) were chopped and were left for sometime for air dry. The chopped material was packed in a distillation flash and water was added to the packed material upto mark of flask. Heating was supplied by heating mantle. Before heating the distillation flask, water was run into the graduated receiver keeping the tap open until the water overflowed. Air bubbles in the tube were carefully removed by pressing the tube. The distillation was continued at a rate which kept the lower end of the condenser cool, by
continuous supply of tap water with the help of rubber tubings. After some time steam was formed in distilling flask. The mixture of water vapour and essential oil passed into the condenser. As distillation proceeded the distillate was collected in the graduated part of the receiver. The oil being lighter than water and insoluble in it, floated on the top of the receiver. At the end of 90 minutes, heating was discontinued, the apparatus was allowed to cool for 10 minutes. As soon as the entire quality of oil had entered the graduated part of receiver the volume was read directly. The measured amount of oil was taken to be the content of essential oil in the leaves.

**RESULTS**

**Root and Shoot length:**

The root and the shoot length of *Ocimum sanctum* decreased non-significantly at lower inoculum levels (Pi=5 and 50 J2/pot) in T1 and T2 plants, when compared with uninoculated control (C). A significant (p < 0.05) decrease in the root and the shoot length in comparison to control (C), occurred in T3 plants. In T4 plants, at the inoculum levels of Pi=5,000 J2/pot, the root and the shoot length decreased greatly and significantly (p ≤ 0.01), in comparison to control (C). The reduction in length was maximum in the plants grown at the highest inoculum level (P. 61, Table 4 and H-3).

**Root and Shoot weights:**

In comparison to control (C), fresh and dry weights of the roots and the shoots, decreased with the increase in initial inoculum level. The reductions, however, were non-significant at
lower inoculum level (T1). Significant (p ≤ 0.05) reductions in T3 and (p ≤ 0.01) in T4 plants were observed in comparison to control. Highest reduction in the weight of the roots and the shoots was recorded in T4 plants that were grown at the initial inoculum level of Pi = 5,000J2 per pot (Table 4, H-3).

**Lateral Roots:**

The root system of the control plants as well as the plants grown at lowest inoculum level Pi=5J2 (T1) comprised of normal roots having primary, secondary and tertiary branches. There was no sign of thin and fibrous lateral root formation from the normal primary or secondary roots. Small, thin fibrous roots emerged laterally from the galled portions of infected roots. At lower inoculum levels, the number of fibrous roots was low but at higher inoculum level, the number was quite high. The number of these lateral roots was maximum at the highest inoculum level (Pi=5,000J2) in T4 plants followed by T3 and T2 plants.

**Leaf Area:**

The leaf area of the plants, in comparison to control, decreased non-significantly at the lower (Pi=5 and 50J2) and significantly at higher (Pi=500 and 5,000J2) inoculum levels (Table 4, H-4).

**Chlorophyll content:**

The amount of chlorophyll a,b and total chlorophyll content in the leaves of *O. sanctum* decreased non-significantly at the initial inoculum level of Pi=5 and 50J2 (T1 and T2, respectively). In comparison to control (C), there was significant (p ≤ 0.005) reduction in the chlorophyll content of leaves in T3 and (p ≤ 0.01)
T4 plants, when comparisons were made with the control (C). The reduction in chlorophyll content was highest at the highest inoculum level of Pi=5,000J2 per pot (Table 4, H-4).

**Oil Content:**

Total oil contents in the leaves of *O. sanctum* exhibited reduction at all the initial inoculum levels, when compared with the oil content of uninoculated control plants (Table 4 and H-4). The reduction was non-significant in T1 plants at the initial inoculum level of Pi= 5 and 50J2, respectively. Significant reductions (p < 0.05) in leaf oil content of T3 and T4 (p < 0.01) were observed. The reduction in oil content was maximum in T4 plants grown at Pi = 5,000J2 per pot (Table 4).

**DISCUSSION**

The experiment, carried out to investigate the pathogenicity of root-knot nematode on *Ocimum sanctum*, indicated that the plants were quite susceptible towards *Meloidogyne incognita*. The growth of the plants responded negatively towards the lower as well as higher inoculum levels of the nematode. The effects, in terms of plant growth, chlorophyll content and oil content of the leaves were pronounced and significant when the inoculum level was 500J2 per plant or higher. Reduction in the considered parameters indicated susceptibility of the plant towards the root-knot nematode. From the data (Table 4) it was revealed that there was an inverse relationship between the juvenile population and plant length, or fresh and dry weights of plants, or chlorophyll and oil content of the leaves. The threshold level of the population of the juveniles of *Meloidogyne incognita* that caused significant
reduction in growth and other parameters was \( \Pi = 500J_2 \) as is obvious from Table 4. This could be attributed to either immobilization or slow mobilization of nutrients from galled roots to above ground plant parts resulting in retardation in plant growth. The nematode manipulates the root tissue in such a way that the minerals and plant metabolites are diverted towards the affected tissue. It causes scarcity of nutrients in growing regions. Drastic changes in the internal structure of root, caused by the root-knot nematode, might not be able to provide nutritional and other requirements in sufficient amount for the plant growth. Our results regarding the reduction in plant growth, as a result of \( M. incognita \) infection, are in conformity with the observations made by Barker and Olthof (1976); Nordacci and Barker (1979); Kinloch (1980, 1982; Rodriguez Kabana and Williams (1981); Appel and Lewis (1984); Ibrahim and Lewis (1985); Verma and Ali (1993); Gupta et al., (1995); Fazal et al., (1996); Ramakrishnan et al., (1998); Sharma et al., (1999); Singh and Goswami (2000); Tiyagi et al., (2001); Nehra and Trivedi (2002); Khan (2003); Youssef (2004); Kumar and Pathak (2004); and Khan and Ashraf (2005) on various plants. The number of lateral fibrous roots increased with increased in initial inoculum level of nematode. As the xylem and the phloem are the most affected tissues in the galled roots, abnormalities in vascular tissue resulted in irregular transport of water and minerals towards the photosynthetic tissues. Inadequate supply of water and minerals towards leaves caused lowering down in the rate of photosynthesis. Abnormalities in phloem, as a result of root-knot infection, impaired the translocation of photosynthates towards normally growing roots.
Unavailability of food material appeared to be responsible for the reduction of root length and production of abnormally large number of lateral branches. All anatomical malformations and physiological malfunctions contributed in suppressing the plant growth and yield (Hussey, 1985). Formation of lateral hairy roots in paddy plants as a result of *M. incognita* infection has been reported by Kamalwanshi *et al.*, 2002.

It is thought that the root-knot nematode infection caused abnormalities in the phloem tissues of affected roots which prevented the translocation of nutrients to the growing regions of the roots, and on the other hand abnormalities in xylem caused hindrance in ascent of sap. These abnormalities resulted in reduction of leaf size. Reduction in leaf area of plants as a result of *M. incognita* infection has been reported by several workers like Ramakrishnan and Rajendran (1998) and Jonathan and Rajendran (2000). The reduction in chlorophyll, in the leaves of root-knot nematode infected plants might be due to unavailability of micro- or-macronutrients. Availability of nutrients, in small amount might be responsible in the reduction of leaf chlorophyll in tomato due to *M. incognita* (Loveys and Bird, 1973). Reduction in chlorophyll content was attributed to reduced uptake of water and minerals; poor translocation; and accumulation of the nutrients in the nematode infested galled roots. Reduction in chlorophyll content of leaves as a result of *M. incognita* infection has been reported earlier by Vashisht *et al.*, 1994; Poormina and Vadivelu, 1998; Ramakrishnan and Rajendran, 1998 and Yasmeen, 2002.
Reduction in oil content of leaves, might be ascribed to reduced photosynthetic rate due to nematode infection. Being sedentary endoparasite, this nematode influences translocation of nutrient by modifying structure and function of the conducting tissues. Availability of photosynthates in abnormally low concentration seem to be responsible in reducing oil content of leaves. Similar observations have been reported on various plants (Pandey, 1988; Pandey et al., 1992 on Mint; Haseeb, 1996 on O. cannnum; Shukla and Haseeb, 1998 on Mentha citrata; Tiyagi et al., 2001 on rose).