Pathogenic effect of *Meloidogyne incognita* (a root-knot nematode) on the morphology and root biochemistry of a leguminous crop

REVATHI, M.

(Reg. No. 11 PZO 05)

A THESIS SUBMITTED TO

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

MASTER'S DEGREE IN ZOOLOGY

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Signature of the

Head of the Department

Signature of the Supervisor 7/5/13

1. INTRODUCTION

Nematodes (*Phylum: Aschelminthes*) are the most highly developed of the pseudocoelomates with smooth and unsegmented body covered by colourless cuticles. These nematodes occur in large numbers in various habits like soil, fresh water and marine water. Their occurrence was also reported from the deepest ocean floor to the highest mountains (Maggenti, 1989). Most of them are free living saprophytes but several hundred species are known to attack various plant parts such as roots, bulbs, leaves and flowers and cause immense economic loss to human requirements.

Earlier in 1989 Sasser, conducted a world-wide survey and listed out ten most important genera of plant parasitic nematodes that have caused an estimated overall annual loss of more than 12 per cent yield on large number of crops. These plant parasitic nematodes are prevalent in menacing proportions in most parts of the world, especially in subtemperate, subtropical regions.

Among the best known phytonematodes, root-knot nematodes (*Meloidogyne spp.*) stand out as the most dominant group which have an extensive host range of more than 2000 plant species and were reported to cause approximately 5% of global crop loss (Trivedi, 2001). Over 50 species have been reported (Sasser and Carter, 1985) in the genus *Meloidogyne* with a number of races. They exist in soil in areas with hot climate or short winters.

Being sedentary endoparasites, these root-knot nematodes are nature's most successful parasites. These nematodes infect thousands of different herbaceous and woody monocotyledonous and dicotyledonous plants and thus cause serious losses to numerous agricultural crops worldwide (Castillo, 2008).

In India, 12 species of nematodes were reported (Dasgupta, 1998). *Meloidogyne incognita, M. javanica* and *M. arenaria* are the most predominant species. Krishnappa (1985) has reported that in India, *M. incognita* has the widest range (more than 250 genera) of host plants. Hence, in the recent years *M. incognita* has received continuous attention from the researchers in nematology and agricultural science.

Root-knot nematodes cause morphological, histological and biochemical changes in the host roots by interacting with their tissues. Root-knot nematodes infection results in the formation of galls of root-knots and exhibit the above ground symptoms like dwarfing, chlorosis, yellowing and withering off leaves and finally root decay which results in loss of vigour, poor yield and poor quality of products (Daniel and Boothryod, 1987). Root-knot nematode larvae infect plant roots, causing the development of root-knot galls that drain the plants may be lethal, while infection of mature plants causes decreased yield (Olabiyi, 2004).

The penetration of root-knot nematodes and their further development inside the root tissues cause disruption in the physiological equilibrium by altering the biochemical components of the host plants. The plants in turn, react in a number of ways to offset their disturbances and the overall plant response determines to a large extent, the success or failure of the interactions. Earlier investigations have documented that infection of plants by nematodes trigger mechanism resulting in the alteration of metabolic activities of hosts (Onyeke and Akueshi, 2012).

On infection, it was reported that the proteins of the hosts are likely to change quantitatively resulting in the formation of new polypeptides (Bell, 1981., Dasgupta, 1988 and Nayak, 2010). There are also reports on the increase in the concentration of existing proteins. Such change in proteins following root-knot nematode infection has also been reported by Ganguly and Dasgupta (1981). Alteration in carbohydrate contents were also reported due to the entry of root-knot nematodes into the host plants (Loveys and Bird, 1973 and Wallace, 1987). Phenols, which play a major role in the defence mechanism gets altered based on the susceptibility or resistance of the host plant to the root-knot attack (Meena *et al*., 2011).

Parasitism by root-knot nematodes is characterized by the establishment of permanent feeding sites comprised of giant cells in the cortex, endodermis, pericycle and vascular parenchyma of the host root tissues. These feeding sites are sinks for the plant photosynthesis, and they thus impair plant growth and development. Depending on their feeding mode, plant parasitic nematodes break down root cell structure, remove cell content, disrupt physiological processes, and modify the genetic expression of the host. In addition, deformation and blockage of vascular tissues at the feeding sites limit translocation of water and nutrients, further suppressing plant growth and crop yield (Olabiyi, 2008).

Pulses are leguminous crops which include cowpeas, soybeans, pigeon peas, beans, mucuna, tephrosia and many wild plants. Cowpea (*Vigna unguiculata* L. Walp; family: *Fabiaceae*) are grown throughout the world as annual crops. They tolerate heat and dry condition and grow best in a soil pH of 5.5 to 6.5. This inexpensive crop, packed with nutrition is a widely cultivated crop especially in the developing countries. The different varieties of cowpeas are available in a range of sizes and colours. These seeds which contain a rich source of protein are cooked and prepared as vegetable in many parts of the world. Apart from culinary purpose, cowpeas also serve as rich animal feed and play a major role in the environment by promoting nitrogen fixation (Quin, 1997 and Olowe, 2004).

Several species of nematodes are known to cause losses to cowpea throughout the world. Caveness and Ogunfowora (1985) listed 55 species of plant parasitic nematodes associated with cowpea production. The root-knot nematodes *Meloidogyne incognita* and *M. javanica* are documented to cause major losses.

It was reported that *Meloidogyne incognita* is a serious pest of cowpea, *Vigna unguiculata*, in most parts of the world. These nematodes constitute a major constraint to cowpea production. Symptoms of damage induced by root-knot nematodes include patches of stunted and yellowed plants, presence of root galls, excessive branching of roots and reduced root systems. Poor germination and death of seedlings may be observed in cases of heavy infestations (Sikora *et al*., 2005). An estimated 59% loss of cowpea grain yield caused by *M. incognita* was reported (Singh *et al*., 2003).

Enormous amount of researches were carried out by various agricultural scientists on the pathogenic effect of *Meloidogyne incognita* on different leguminous crops. The present study has been undertaken with a view of assessing the pathogenicity and related biochemical changes in cowpea plants (cv. giant black eye) with the following objectives:

i) To evaluate the pathogenic effect of different inoculum levels of *Meloidogyne incognita* on the growth parameters (shoot length, root length, shoot weight, root weight and number of leaves) of cowpea through pot culture experiment.

ii) To assess the reproductive potentiality of *M. incognita* through galls and egg mass production in the host plant (cowpea).

iii) To estimate the amount of total protein, total amino acids, proline, total carbohydrates and total phenols in the *M. incognita* infected roots of the host plant (cowpea).

2. REVIEW OF LITERATURE

Meloidogyne spp. the root-knot nematodes are the most predominant plant parasitic nematodes which attack almost every type of crop in India. The damage caused by them with regard to yield and the quality of produce is greater. Much attention is given by the nematologists and the agrofarmers to study their pathogenic effect and management strategies in the cultivated areas. A vast number of literature on pathogenicity of these nematodes and their effect on the root biochemistry of various crops are reviewed in this chapter to prove their importance as crop pests.

Earlier in 1855 Berkeley, reported the damage caused by root-knot nematodes in the vegetable crop, namely cucumber. In India, Ayyar (1934) studied the effect of root-knot nematodes in infested vegetable and other crops.

2.1 Pathogenicity of *Meloidogyne spp.* on various crops

Pathogenicity of different species of *Meloidogyne* on number of crops have been studied by several agricultural scientists under different conditions.

Bhagawathi and Phukan (1991) reported a progressive decrease in all plant growth characters with increasing inoculum levels of *Meloidogyne incognita* alone with the leguminous crop like pea. The results obtained by Tripathy and Pandhi (1992) also showed a progressive decrease in plant growth with increasing inoculum levels of *M. incognita* in rice, bean and cowpea respectively.

A progressive decrease in plant growth was recorded with a corresponding increase in the inoculum level of *M. incognita* race three in chickpea (*Cicer arietinum*). Significant reduction in dry shoot weight was observed with an inoculum of 2000 second stage juveniles (Siddiqui and Mahmood, 1992).

Khan *et al.*, (1992) studied the influence of race 2 of *M. incognita* on bottle gourd. An increase in the inoculum levels of nematodes reduction in growth characters was observed by them. However, they reported an increase in gall index and egg mass index with respect to increased inoculum levels.

Dwivedi and Pandey (1993) reported that there was reduction in plant growth of tomato (*Lycopersicon esculentum*) infected with *M. incognita* as the inoculum level increased. The highest number of galls was found at the inoculum level of 1000 nematodes.

Survey conducted by Krishna Rao and Krishnappa (1995) in different chickpea growing areas of Karnataka revealed that an increase in the inoculum level of *M. incognita* juveniles had proportionately decreased the plant growth and increased the root-knot disease on chickpea.

Pathogenicity tests conducted with *M. incognita* on two cultivars of mungbean, namely ps-16 and Pusa Baisakhi revealed that plant growth characters were adversely affected as the level of inoculum was increased (Samathanam and Sethi, 1996).

Experiments proved that as the inoculum level of *Meloidogyne incognita* increased, a progressive decrease in growth of soybean plant was noted. A significant reduction in shoot growth and root length was observed at an initial level of 2000 which was considered as the damaging threshold level (Shalini and Trivedi, 1999).

Mahapatra *et al.*, (1999) conducted a pot culture experiment on the pathogenicity of root-knot nematode *M. incognita* in pointed gourd (*Trichosanthes diocia*). A significant reduction in shoot and root weight of *T. diocia* was observed at an inoculum density of 1000 J_2 of *M. incognita.* This was considered as the damaging threshold level. They reported that the rate of nematode reproduction was density dependent, the maximum being at the highest inoculum density, and the minimum was at the lowest inoculum density.

The effect of different inoculum levels of *M. incognita* on cowpea cv. Pusa komal seedlings were studied by Satyendra and Goswami (2000) under pot culture experiment. They reported that the plant growth was significantly reduced at an initial population of 1000 nematodes per 500 g of soil.

Haider *et al*., (2003) studied the comparative pathogenicity of root-knot nematodes, *Meloidogyne incognita* on six (mung bean, urd bean, lentil, lathyrus, rajma bean and French bean) leguminous crops. The result showed that an initial inoculum level of 100 juveniles of *Meloidogyne incognita* per plant caused significant reduction in growth characters of leguminous crops and proved to be pathogenic. Reduction in growth characters varied from 1.13 to 69.96 per cent in different inoculum levels. Maximum decrease and the multiplication rate of nematode were maximum in lathyrus and French bean.

The experiment on the effect of inoculum levels of *Meloidogyne javanica* on the growth and galling incidence of soybean was conducted in the laboratory by Khalequzzaman (2003). Mixed inoculum of *M. javanica* in five different treatments including control was tested on the growth, galling incidence and development of the nematode on soybean. Maximum length of shoot and root and weight of shoot and root were observed in control treatment. Progressively higher galling incidence, higher number of adult females and juvenile population of *M. javanica* with lower plant growth and plant weight of soybean were observed.

Effect of different initial inoculum levels (10, 100, 1000, 10000) of freshly hatched juveniles of *Meloidogyne incognita* was studied on brinjal (cv. CK) under pot culture experiment (Yadav and Mathur, 2005). Results showed that plant growth characters were inhibited even at lowest inoculum levels when compared to control.

Studies were conducted by Perveen *et al*., (2006) to determine the effect of initial inoculum levels (Pi) of *Meloidogyne incognita* on *Mentha arvensis* cv. Gomti. They found a positive relationship between the initial inoculum level of *M. incognita* and reduction in shoot, root fresh and dry weights. The maximum reduction was recorded at the highest inoculum level (25000 J_2/5 kg soil) as compared to uninoculated control.

Cetintas *et al*., (2007) conducted a study on pathogenicity and reproductive potential of *M. mayaguensis* and *M. foridensis*, two new species recently reported in Florida agriculture were compared to those of *M. arenaria* race 1, *M. incognita* race 4 and *M. javanica* race 1on tomato (*Lycopersion esculentum*). Two levels of each nematode as one egg or second-stage juvenile and three eggs were used with nine replications each. Root-galling, eggs, shoot weight and plant height were recorded and observed the differences between the different nematode species inocula. The study concluded that *M. foridensis* has produced less galling and lower reproductive potential on the tomato plant where as *M. mayaguensis* has produced higher galling and highest reproductive potential on the tomato plant when compared to other nematode species.

Experiment conducted by Olabiyi (2008) on the tomato seedlings, cv. DT 69/257, grown in steam-sterilized soil were inoculated with graded inoculum of

5,000; 10,000; 15,000; 20,000 and 25,000 eggs of *Meloidogyne incognita*. At inoculum levels of 15,000; 20,000 and 25,000 eggs of *M. incognita*, number of leaf per plant, plant height and plant weight were reduced and root galls increased significantly.

Bowen and Hagan (2008) in their study found that *Meloidogyne incognita* caused damage to both cotton and corn. Losses recorded were found to be 3.8 to 11.4 per cent respectively when compared with previous years of their experiment.

An experiment on the plant parasitic nematodes associated with cowpea (*Vigna unguiculata*) was carried out by Sawadogo *et al*., (2009). They identified twelve plant parasitic nematode genera of which six appeared to have significant parasitic potential on cowpea based on their frequency and abundance. They are *Helicotylenchus, Meloidogyne, Pratylenchus, Scutellonema, Telotylenchus* and *Tylenchorhynchus*. These nematodes were found to be suspected to cause pathogenicity to cowpea plants. They caused and reduced the height, weight and number of leaves of the plants.

Chandar *et al*., (2010) conducted a pot culture experiment to study the pathogenic potential of *Meloidogyne incognita* in four species of Cucurbits (*Lageneria ciceraria, Cucumis sativa, Momordica charantia* and *Cucurbita pepo*). 1000 and 10,000 second stage juveniles were inoculated to the seedlings. The results showed that all the four cucurbits were found to be highly susceptible to infection with *M. incognita* and showed reduced growth parameters when compared to control.

Ugwuoke and Eze (2010) conducted an experiment to determine the individual effect of root-knot nematodes, *Meloidogyne incognita* on the growth and development of cowpea plants. When nematodes interact with the roots they increased the galling and as a result the nodulation of the cowpea roots gets lowered. Highest galling cowpea roots produced lesser nodules and also caused pathogenicity on the reduction of height and weight of the plant.

Effects of the root-knot nematode, *M. incognita* on lentil were studied by Hisamuddhi and Azam (2010). Plants were inoculated with 250, 500, 1000, 2000 and 4000 J_2 per plants. The reduction in plant growth was significant at 500 J_2 and at higher inoculum levels over control. An increase in the inoculum level resulted in the production of more galls and egg masses and high nematode population. Therefore it was reported that the reproduction and rate of population was increased with increasing inoculum levels.

Safiuddin *et al*., (2011) conducted a pathogenitic experiment against root-knot nematode, *M. incognita* on okra. The results showed that with an increase in the inoculum of *M. incognita* i.e., from 250-8000 J_2/per plant a considerable reduction in plant growth parameter viz., length, fresh weight and dry weight was noted. The highest reduction in plant growth parameters was recorded in the plants inoculated with the 8000 J_2/kg of *M. incognita* whereas, the leaf reduction was reported from the initial inoculum of 250 J_2/ plant of nematode. However, a significant reduction in plant growth parameter was recorded in the plant inoculated with 2000 and above 2000 J_2/ plant.

An experiment was conducted by Adegbite (2011) on physiological disruption caused by the root-knot nematodes to the cowpea plants. The experiments resulted in the reduction of physiological characters of the cowpea plant that which are infestated by *M. incognita.* They are caused the growth parameters, yield loss and quality of cowpea.

Experiments were carried out to determine the host status of 12 African yam bean accessions to *M. incognita* infection (Onyeke and Akueshi, 2012). They inoculated host plants with 4,000 eggs of *M. incognita* and a control without eggs was maintained for comparison. Results showed that the growth parameters were significantly reduced in inoculated accessions when compared with the controls.

Robab *et al.*, (2012) performed an experiment to study the effects of root-knot disease caused by *Meloidogyne incognita* on *Solanum nigrum*. There were five sets of plants, each with five replicates. The T_1 plants were inoculated with 1000 J_2 per plant, $T_2$with 2000 and T_3 with 3000 J_2. Uninoculated plants served as control (C). The data revealed that plant length, plant weight and number of leaves decreased significantly in the inoculated plants when compared with uninoculated control.

Experiment on infectivity and reproduction of *M. incognita* on African yam bean was conducted by Onyeke and Akueshi (2012). They inoculated different levels of nematode eggs from 1000 eggs to 4000 eggs. Uninoculated control was maintained for comparison. The effect showed that the growth of the plants was decreased on the basis of increased level of nematode reproduction.

2.2 Changes in the biochemical components due to infestation of *Meloidogyne spp.* on various crops

Many investigators have documented the physiological disturbances in various crops that were infected with *Meloidogyne spp.* (root-knot nematodes).

Alam *et al.*, (1976) reported that total protein and amino acids increased in the roots of tomato seedlings infected with *Meloidogyne incognita.*

Earlier in 1977, Bajaj and Mahajan reported an increased level of phenolic compounds in the leaves of root-knot resistant variety NMR-1. An increase in total phenolic content was also reported (Narayana and Reddy, 1980) in the shoots and roots of resistant (NTDR-1) tomato when compared with that of susceptible (Pusa ruby) tomato

Biochemical changes (total proteins and total carbohydrates) in the roots of *Hibiscus esculentus* due to infection of *M. incognita* was analysed by Sinha and Sukal (1983). They reported an increased amount of total proteins and a decreased amount of total carbohydrates content in the inoculated plants when compared with uninoculated plants.

Upadhyay and Banerjee (1986) noted an increase in the amount of proteins in the stem and roots of chick pea infested with the root-knot nematode *M. javanica*.

Nandhini *et al*., (1988) conducted an experiment on the biochemical changes in the infected wheat roots by *M. incognita*. It was reported that the infected wheat roots contained a decreased amount of carbohydrates when compared to uninfected roots of wheat.

Changes were observed both quantitatively and qualitatively in cowpea (cv. Pusa do flash) infected with *M. incognita* (Ganguly *et al*., 1991). They recorded considerable increase in total soluble proteins during post inflectional intervals.

Healthy and *M. incognita* infected roots of fenugreek were collected after 30, 60 and 90 days after inoculation. They showed an decrease in the amount of total sugars in infected roots when compared with the healthy roots (Sharma and Trivedi, 1992).

Experiment was conducted by Anwar (1995) to find out the carbohydrate content of roots and shoots of uninoculated and *M. incognita* inoculated tomato plants. The result showed that the carbohydrate contents of the leaves and roots of uninoculated plants were higher when compared with the infected plants.

Patel *et al.*, (1996) reported that infection of *M. incognita* and *M. javanica* in the resistance plants at 5000 and 10000 second stage juveniles (J_2)/plant. The resistant cowpea plants showed an increased level of total phenol contents i.e., 140.26 per cent and 92.93 per cent respectively over the control i.e., 98.23 and 73.89 per cent respectively.

Mohanty *et al.*, (1997) reported that *M. incognita* infection on green gram interfered the root biochemistry in addition to its pathogenicity. Higher concentration of total sugar was observed in uninoculated roots when compared with the infected roots.

Phenolic accumulation in the pepper *Capsicum annuum* in resistant to *Meloidogyne spp. (M. incognita*, *M. arenaria* and *M. javanica*) was analysed (Pegard *et al.*, 2005). Increased amount of phenolic accumulation in response to root-knot nematodes were showed by the resistant plants.

An increase in root proteins in the *M. incognita* infected grapes was observed by Alsayed *et al.*, (2007). Nematode infection decreased total soluble sugars and total carbohydrates in the roots. The nematode infection also resulted an increase in root protein over those the healthy seedlings. All treatment showed the increase in the root protein when compared with uninfected plants.

Biochemical changes in brinjal cv. Pusa purple long inoculated with root-knot nematode, *Meloidogyne incognita* were observed by Nayak and Mohanty (2010). Biochemical estimation was made on amino acids, total sugar content and crude protein. Results obtained by them showed an increased amount of amino acid, crude protein and decreased content of total sugar in the diseased tissues.

Meena *et al*., (2011) conducted an experiment on systemic resistance in tomato against *Meloidogyne incognita.* The result indicated that the tomato developed resistance against *M. incognita* by increasing the accumulation of phenol by defense mechanism.

Pot culture experiment was conducted by Khan *et al*., (2011) on biochemical and morphological response on selected germplasm of tobacco with inoculation to *Meloidogyne incognita*. Five tobacco cultivars responded to the nematode inoculation level of 2000 J_2/plant. Greater decrease in plant growth and total phenol were recorded in the susceptible tobacco infected with *Meloidogyne incognita* when compared with the resistant tobacco cultivars.

Singha *et al*., (2012) conducted an experiment to study the biochemical responses of tomato roots infected with root-knot nematode *Meloidogyne incognita*. The roots were tested after 10, 20 and 30 days after inoculation. After 30 days there was a very low accumulation of total phenolic compounds in the roots of tomato as a result of the root-knot disease caused by *Meloidogyne incognita.*

Korayem *et al*., (2012) conducted an experiment to study the physiological and biochemical changes in different sugar beet genotypes infected with root-knot nematode *Meloidogyne incognita.* All the tested genotypes were susceptible to nematode infection according to the number of root galls and gall indices. The result showed that the both shoot and root of infected genotypes indicated the decreased level of total phenolic compounds when compared to uninfected genotypes and total proteins were increased across the uninfected genotypes.

A study was conducted by Oliveiraa *et al*., (2012) to evaluate the resistance and susceptibility of ten cowpea cultivars to *Meloidogyne incognita* to analyzing the total proteins and total carbohydrates. The cultivars were highly resistant, moderately resistant and slightly resistant. The results showed an increase of protein in the slightly resistant cowpea cultivars when compared with highly resistant ones. But the carbohydrates were found to be decreased in the slightly resistant cowpea cultivars when compared with the highly resistant cowpea cultivars.

Biochemical analysis was also reported in other plant parasitic nematodes in addition to root-knot nematodes (*Meloidogyne spp.*).

Earlier in 1971, Epstein and Cohn analysed the amino acid content in the terminal root galls of bur marigold (*Bidents tripartita* L.) and grapevine (*Vitis vinifera* L.) infected with *Longidorus africanus*. Among the free amino acids of the galled tissues a large increase in proline was recorded by them.

Mohanty *et al*., (1999) recorded an increase in the amount of protein in cowpea cv. Pusa komal inoculated with reniform nematode, R*otylenchulus reniformis*.

Devarajan and Rajandran (2002) conducted a pot culture experiment with eight banana clones to study the biochemical alterations induced by the burrowing nematodes *Radopholus similis*. The amount of total proteins and amino acids were more in the susceptible clones than the others. Amount of amino acids and total proteins were increased and the amount of total carbohydrates was decreased in the infested roots.

3. MATERIALS AND METHODS

The root-knot nematode, *Meloidogyne incognita* used in the present study entitled **"Pathogenic effect of *Meloidogyne incognita* (a root-knot nematode) on the morphology and root biochemistry of a leguminous crop"** is a phytoparasitic nematode which attacks the root system of various agricultural crops. These nematodes are cultured in a pure form in earthern pots containing sterilized soil. The stock culture was maintained in coleus an ornamental plant.

The host plant selected for the present investigation is cowpea (*Vigna unguiculata*, cv. giant black eye) which belongs to the family *Fabiaceae*. Cowpea is an economically important pulse crop grown throughout the world as annual crop. The seeds of cowpea plants were obtained from the Central Seed Depot, Coimbatore.

3.1 Soil mix

The soil mix used for the present experiment is prepared by mixing one part of fine sand and one part of red soil. This mixture was sieved in a mesh and steam sterilized in order to be free from other soil nematodes. This soil mix is stored in polythene bags for further use to maintain the stock culture, and also for the pathogenic and root biochemical studies.

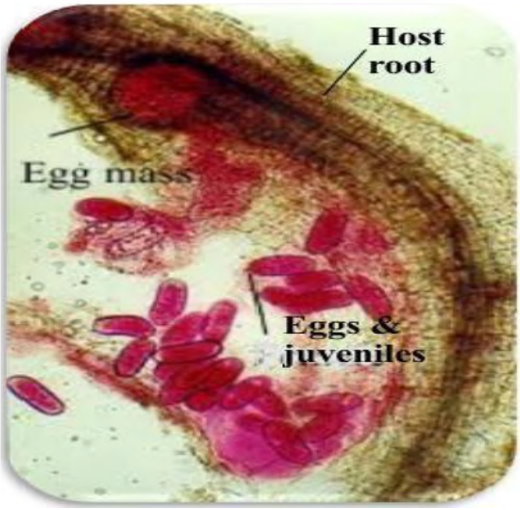
3.2 Maintenance of stock culture of *Meloidogyne incognita*

Pure culture of *M. incognita* obtained from a single egg mass progeny (Plate-I a) was maintained in coleus, an ornamental plant (Plate-I c) in an earthern pot (18 x 20 cm) containing 5 kg of steam sterilized soil. Freshly hatched second stage juveniles (J_2) from the egg masses were collected and poured into the holes made in the soil around the root system of coleus plants and the holes were closed with soil. When the coleus plants mature, the aerial parts were cut off at soil level leaving the roots undisturbed in the soil. The infected roots of coleus plants were carefully pulled out from the soil. The root galls along with egg masses were placed in petri-dishes containing improvised netted chambers covered with tissue paper and were immersed in water for hatching. Second stage juveniles (J_2) hatched out from the egg masses were collected in glass vials and stored in the refrigerator for further studies. New coleus plants were planted again in the soil to maintain the stock culture.

3.3 Raising of cowpea seedlings (study plant)

The seeds of cowpea (cv. giant black eye) were sown in large sized earthern pots (30 x 20 cm) containing steam sterilized soil. The seedlings were thinned out and only healthy plants were allowed to grow for a period of 30 days and were used for various experiments (Plate-I b).

PLATE - I



a. *Meloidogyne incognita*- the parasite

b. Cowpea plants - the host plant

c. Maintenance of stock culture of *Meloidogyne incognita* in coleus plant

3.4 Pathogenic effect of *Meloidogyne incognita* on the growth of cowpea (cv. giant black eye)

Pathogenic effect of root-knot nematode on the host plant cowpea (*Vigna unguiculata*) was studied by pot culture experiment under laboratory conditions at a temperature of 32^{o} C – 34^{o} C.

Four weeks old, three healthy seedlings were picked up and planted in each earthern pot containing 750 g steam sterilized soil. Five days after planting, second stage juveniles (J_2) collected from the egg masses were inoculated to the host plants at three different inoculum levels i.e. 500, 1000 and 2000 per pot around the root system. The roots were covered with sterilized soil followed by light irrigation. A control pot with plants without inoculation of nematodes was also maintained for comparison purpose. Each treatment was replicated thrice. The experiment was terminated 45 days after inoculation. The plants were uprooted from the pots and the roots were washed gently to remove the adhering soil and dried with blotting paper. Data on plant growth parameters (shoot length, root length, shoot weight, root weight and number of leaves), number of galls and egg masses produced per root system were recorded and the data was analyzed statistically (ANOVA).

PLATE – II

Pot culture experiment on pathogenicity of *Meloidogvne incognita* on cowpea plants

3.5 Biochemical studies in the roots of cowpea *(Vigna unguiculata)* plants inoculated with different densities of second stage juveniles (J_2) of *Meloidogyne incognita*

The roots of the inoculated and uninoculated host plant (cowpea) were removed from the soil, washed in water, dried in blotting paper and subjected for biochemical analysis to estimate total proteins, total amino acids, proline, total carbohydrates and total phenols.

Individual chemical components were estimated by adopting the standard methods described by Sadasivam and Manickam (1992) by using Spectrophotometer Spectronic 2000.

3.5.i. Estimation of total proteins

Principle

The blue colour developed by the reduction of the phosphomolybdic-phosphotungstic components in the folin-ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry's method.

Reagents used

Reagent A

2 per cent sodium carbonate was dissolved in 0.1N sodium hydroxide.

Reagent B

0.5 per cent copper sulphate (CuSO45H2O) was added to one per cent potassium sodium tartarate.

Reagent C

Alkaline copper solution

Mixture of 50 ml of A and 1 ml of B was prepared prior to use.

Reagent D

Folin-ciocalteau reagent.

Stock solution

50 mg of bovine serum albumin was dissolved in 50 ml with distilled water in standard flask.

Working standard solution

10 ml stock solution was diluted with 50 ml of distilled water in standard flask.

Extraction of protein from sample

100 mg of root sample from both inoculated and uninoculated cowpea plants were weighed and separated in mortar and pestle using 10 ml of the phosphate buffer. The extracts were centrifuged separately and the supernatants were used for protein estimation.

Estimation of total protein

0.2, 0.4, 0.6, 0.8 and 1 ml of working standard solution were pipetted out in a series of test tubes. 0.1 ml of extracts from four root samples was taken separately in four other test tubes. The volume was made upto 1 ml in each test tube by adding distilled water. A tube with 1 ml water served as the blank. 5 ml of reagent C was added to each tube including the blank and was allowed to stand for 10 minutes. To this mixture 0.5 ml of reagent D was added and incubated at room temperature in dark for 30 minutes. The blue colour was developed.

Readings were taken spectro-photometrically at 660 nm. A standard graph was drawn and the amount of protein in the samples were calculated and expressed as mg/g.

3.5.ii. Estimation of total amino acids

Principle

Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-amino acids and yields an intensely coloured bluish purple product which is colorimetrically measured at 570nm.

Reagents used

- Ninhydrin.
- Diluents solvents (equated volumes of water and n-propanol).

Preparation of root tissue extract

100 mg roots of inoculated and uninoculated host plants was cut into small pieces and homogenized in 80 per cent hot ethanol in a mortar and pestle. The homogenized tissue extract was filtered through Whatman filter paper No.41 and filtrate was collected. Extraction procedure was repeated twice and all three filtrates collected were mixed together and final volume was made upto 20 ml with 80 per cent ethanol. From the basic alcohol extracts, total amino acids were estimated.

Estimation of total amino acids

To 0.1 ml alcohol extracts 1 ml of ninhydrin solution was added and volume was made upto 2 ml with distilled water. After heating the mixture in a hot water bath (100° C) for 20 minutes, 5 ml of diluents solution was added and kept for 15 minutes. The intensity of test solution read against a reagent blank was prepared by taking 0.1 ml ethanol, 1 ml of ninhydrin, 0.9 ml distilled water and 5 ml of diluents and the colour was read at 570 nm in spectronic 2000. The concentration of total amino acid present in the sample was calculated from the standard curve prepared with leucine and expressed as mg/g.

3.5.iii. Estimation of proline

Principle

During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein-sulphosalicylic acid complex.

The extracted proline is made to react with ninhydrin in acidic condition (pH 1.0) to form the chromophore (red colour) and read at 520nm.

Reagents used

- Acid ninhydrin (1.25 g of ninhydrin was warmed in 30 ml acetic acid and 20ml phosphoric acid, was stored at 4°C and used).
- Toluene.

Standard proline

100 mg of proline was dissolved in 100 ml distilled water.

Working Standard

10 ml of standard proline was diluted in 100 ml distilled water.

Preparation of tissue extract

100 mg of root samples from inoculated and uninoculated host plants were weighed and separated. Sample was extracted by homogenizing it in 10 ml of 3 per cent aqueous sulphosalicylic acid.

Estimation of proline

0.2, 0.4, 0.6, 0.8 and 1 ml of working standard solution were pipetted out in a series of test tubes. 0.1 ml of extracts from four root samples was taken separately in four other test tubes. The volume was made upto 1 ml in each test tube by adding distilled water. A tube with 1 ml water served as the blank. 2 ml of acid ninhydrin was added and heated in boiling water bath for 1 hour. The reaction was terminated by placing the tubes in ice bath. 4 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. The red colour was developed.

Reading was taken spectro-photometrically at 520 nm. A standard graph was drawn and the amount of proline in the samples were calculated and expressed as mg/g.

3.5.iv. Estimation of total carbohydrates by Anthrone method

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

Reagents used

- 2.5 N – HCl
- Anthrone reagent (200mg of anthrone was dissolved in 100 ml of ice cold 95 per cent H_2SO_4.Thiswas prepared as fresh before use.)

Standard glucose

100 mg of glucose was dissolved in 100 ml distilled water.

Working standard

This was prepared by diluting 10 ml of stock in 100 ml distilled water. To this few drops of toluene was added and stored in refrigerator.

Extraction of root tissue sample

100 mg of root samples from inoculated and uninoculated cowpea plants were taken in four separate test tubes and hydrolysed by keeping them in water bath for 3 hours each with 5 ml of 2.5 N HCl and were cooled to room

temperature. The sample extracts were then neutralized with sodium carbonate until the effervescence ceased. The volume in each tube was made upto 100 ml by adding distilled water and centrifuged. The supernatant aliquots were used for analysis.

Estimation of total carbohydrates

Working standard solution of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken in series of test tubes. The volume in each tube was made upto 1 ml by adding distilled water. 1 ml of water in a test tube served as blank. 0.1 ml of sample from both inoculated and uninoculated roots was taken in separate test tubes and made upto 1 ml by adding distilled water. To these test tubes, 4 ml of anthrone reagent was added and were heated for 8 minutes in boiling water bath and cooled to room temperature. A dark green colour was developed. This was read at 630 nm in a spectrophotometer. A standard graph was drawn by plotting concentration of the standard on the X-axis and absorbance on Y-axis. The amount of carbohydrates present in the sample was calculated and expressed as mg/g.

3.5.v. Estimation of total phenols

Principle

Phenols react with phosphomolybdic acid in Folin-ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue).

Reagents used

- 80 per cent Ethanol
- Folin-ciocalteau reagent
- $Na_2\ CO_3$, 20 per cent

Stock standard

100 mg catechol was dissolved in 100 ml distilled water.

Working standard

Stock was diluted to 10 times to get working standard.

Extraction of root tissue sample for estimation of total phenols

100 mg of the root samples from both inoculated and uninoculated host plant of the present study were ground in a mortar and pestle with ten times volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatants were collected by reextracting the residues with 80 per cent ethanol and centrifuged. The pooled supernatants were evaporated to dryness. The residues were dissolved in 5 ml distilled water. The aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were pipetted out in test tubes. 0.1 ml of sample was taken in four different test tubes. The test tube was made upto 3 ml with distilled water. 0.5 ml of Folin-ciocalteau reagent was added to each test tube. After 3 minutes, 2 ml of 20 per cent Na_2CO_3solution was added to each

test tube and was mixed well. Tubes were placed in boiling water bath for one minute.

The test tubes were cooled and absorbance was measured at 650 nm with spectrophotometer. Concentration of total phenol were calculated and expressed in mg/g.

Data on the biochemical analysis were recorded and the data was analyzed statistically (ANOVA).

4. RESULTS AND DISCUSSION

The results of the present investigation on the pathogenicity of *Meloidogyne incognita* on cowpea conducted under pot culture experiment and estimation of selected biochemical components in the infected roots of host plant are discussed in this chapter.

The results obtained on the growth parameters (shoot length, root length, shoot weight, root weight and number of leaves) of cowpea using three inoculum levels (500, 1000 and 2000) of second stage juveniles of *Meloidogyne incognita* is presented in the form of Table I, Figures 1a, 1b, 1c, 1d and 1e and Plate III a. Table II, Figures 2a and 2b and Plate III b represents the number of galls and egg masses produced in the cowpea infected with *M. incognita.* Results of estimation of selected biochemical components (total proteins, total amino acids, proline, total carbohydrates and total phenols) in the *M. incognita* inoculated and uninoculated roots of cowpea are presented in Tables III, IV and Figures 3a, 3b, 4a and 4b.

4.1 Pathogenic effect of *Meloidogyne incognita* on the growth parameters in cowpea plants (cv. giant black eye)

4.1.a Shoot length

The values of shoot length of both *Meloidogyne incognita* inoculated and uninoculated (control) cowpea plants are recorded in Table I. The shoot length of the uninoculated control plants recorded was a maximum of 76 cm and that recorded for the plants inoculated with 500, 1000 and 2000 nematodes were 54, 42 and 33cm respectively (Figure 1a and Plate III a).

The shoot length recorded for all the three inoculated plants varied significantly when compared with that of the uninoculated control plant. Between the inoculated plants a significant difference was found.

Percentage reduction in shoot length was a maximum of 56.6 in the plants inoculated with 2000 nematodes/pot, whereas it was 44.7 and 28.9 in the plants inoculated with 1000 and 500 nematodes/pot respectively over the control plants. However, all the inoculated plants showed a reduction in shoot length when compared with uninoculated plants.

4.1.b Root length

Table I, Figure 1b and Plate III a show the values obtained for the root length of inoculated and uninoculated host plants. The root length recorded for all the three inoculated host plants showed a reduction over control plants. A maximum of 6 cm was recorded in uninoculated plants. The values recorded for the inoculated plants were 4.5 cm in 500 nematodes inoculated plants and that of 3.2 cm in 1000 nematodes inoculated plants. Minimum of 2.7 cm root length was observed in 2000 nematodes inoculated plants.

Regarding root length, significant difference was found between the inoculated and uninoculated plants. A significant variation was also found between 500 and 1000 nematodes inoculated plants, whereas the values recorded for 1000 and 2000 nematodes were not statistically significant.

Percentage reduction in the root length of the host plants inoculated with 2000 nematodes was a maximum of 55 and that of 1000 nematodes inoculated plants was 46. Reduction in root length was a minimum of 25 per cent in the host plants inoculated with 500 nematodes when compared with control. However, all the three inoculated plants showed a reduction in their root length when compared with uninoculated plants.

4.1.c Shoot weight

The results obtained for the shoot weight was recorded in Table I and presented in Figure 1c. A maximum of 6.5 g shoot weight was recorded in uninoculated plant (control) which was followed by 3.96 and 3.53 g respectively in the plants inoculated with 500 and 1000 nematodes. A minimum shoot weight of 2.8 g was recorded in the host plants inoculated with 2000 nematodes/pot. Shoot weight showed a significant variation in all the three inoculated plants when compared with the uninoculated plant, whereas the shoot weight between the different inoculum levels was on par with each other and statistically no difference was observed.

Table-I

Pathogenic effect of *Meloidogyne incognita* on the growth parameters of cowpea (cv. giant black eye)

Inoculum levels	Shoot length (cm)	Percentage decrease over control	Root length (cm)	Percentage decrease over control	Shoot weight (g)	Percentage decrease over control	Root weight (g)	Percentage decrease over control	Number of leaves	Percentage decrease over control
Control (Uninoculated plant)	76	-	6	-	6.5	-	0.35	-	27	-
500 nematodes/pot	54	28.9	4.5	25	3.96	39	0.28	20	22	18.5
1000 nematodes/pot	42	44.7	3.2	46	3.53	45.7	0.2	42.9	17.67	37
2000 nematodes/pot	33	56.6	2.7	55	2.8	56.9	0.17	51	11	59
S Ed	1.3123		0.3082		0.3572		0.0151		0.8498	
CD(0.05)	3.2113		0.7542		0.8741		0.0369		2.0796	

*Mean of three replication

Figure 1a. Pathogenic effect of *Meloidogyne incognita* on shoot length of cowpea (cv. giant black eye)

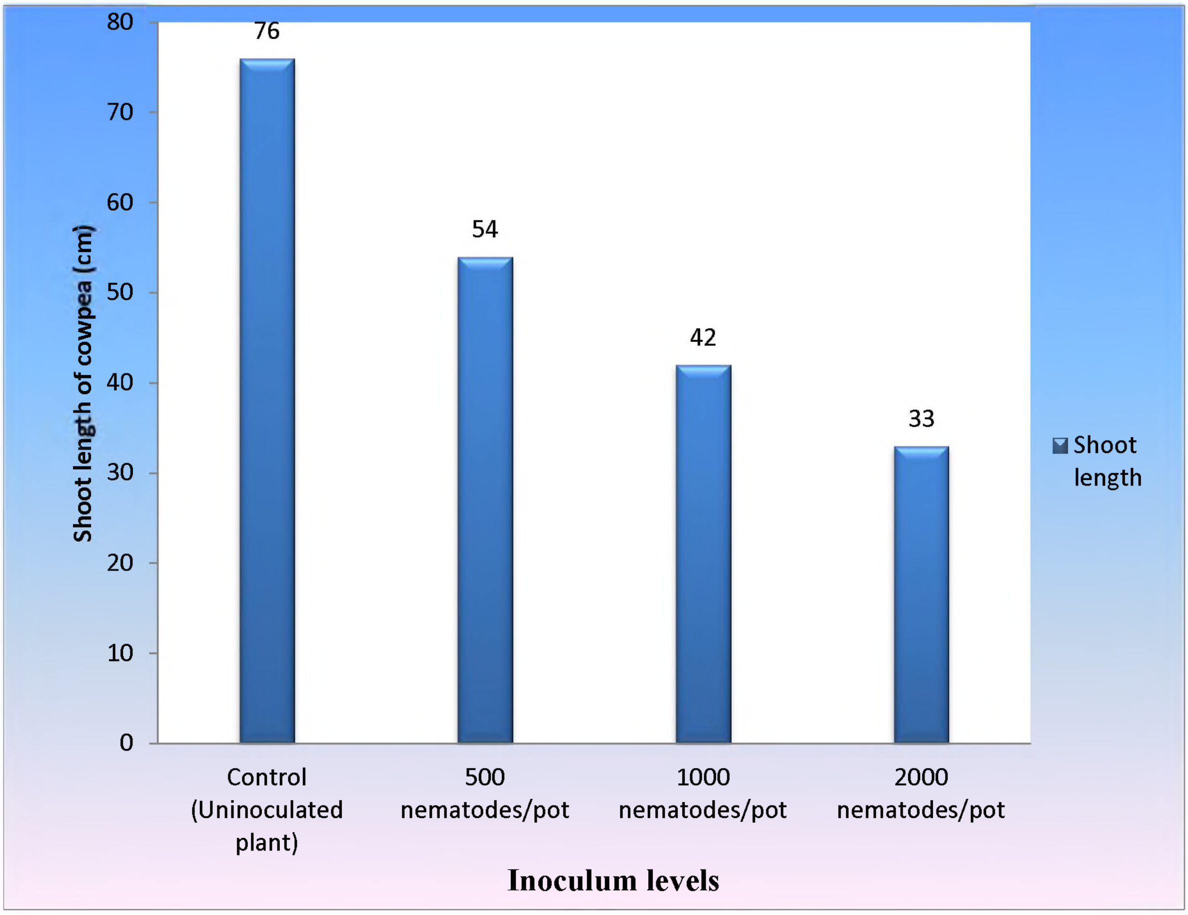
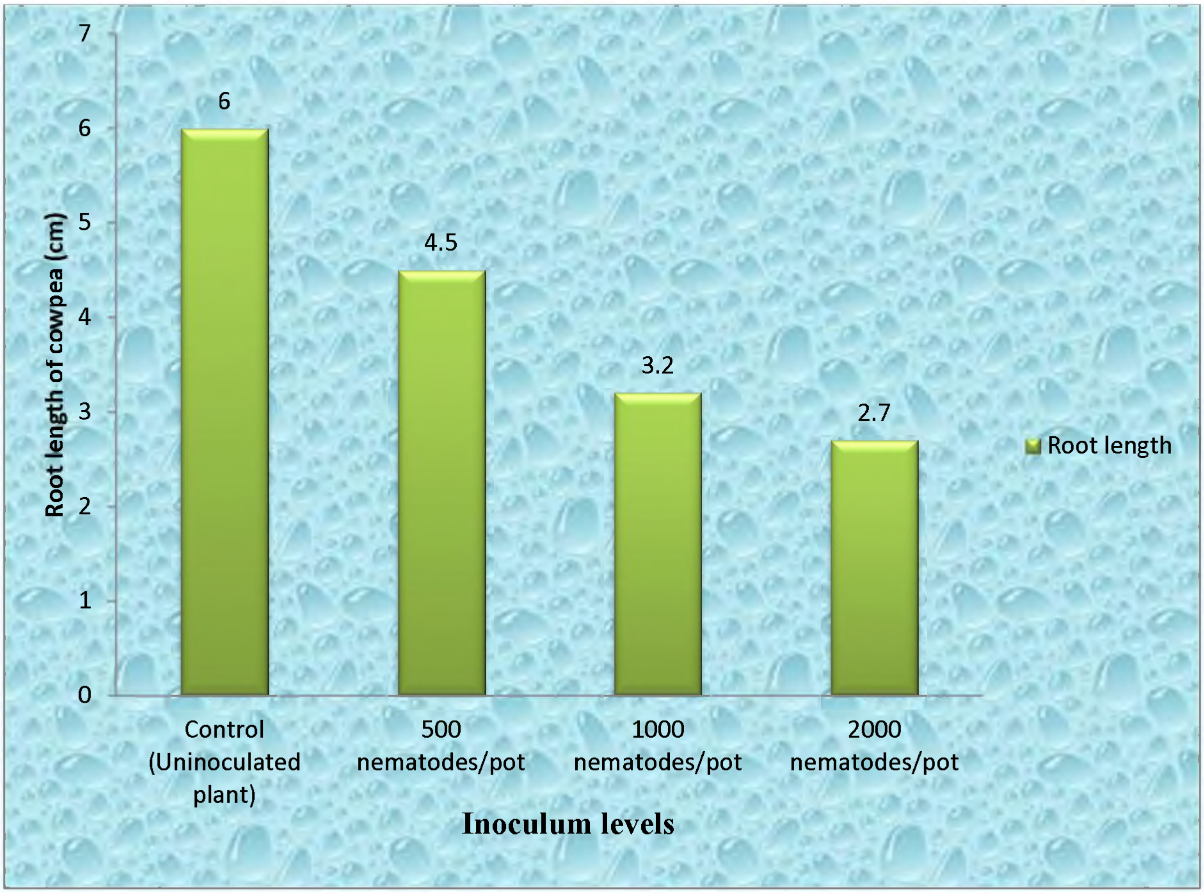


Figure 1b. Pathogenic effect of *Meloidogyne incognita* on root length of cowpea (cv. giant black eye)



The percentage reduction recorded were 39, 45.7 and 56.9 respectively in 500, 1000 and 2000 nematodes inoculated plants

Highest reduction in shoot weight was recorded in the higher density (2000 nematodes/pot) inoculum level.

4.1.d Root weight

In the present study the root weight also showed declining trend as that of the shoot weight (Table I and Figure 1d). As inoculum level increased, weight loss was observed in all three inoculated plants over the uninoculated control. The maximum root weight was recorded in the uninoculated control plant which was 0.35 g. The root weight of the host plants inoculated with 500, 1000 and 2000 nematodes/pot was 0.28, 0.2 and 0.17 g respectively. Significant difference in root weight was observed between 500 nematodes inoculated plants and control. Statistically there was no significant difference found between the 1000 and 2000 nematodes inoculated plants.

The reduction in the root weight was a maximum of 51 per cent in 2000 nematodes inoculated host plants followed by 42.9 per cent in 1000 nematodes inoculated plants. A decrease of 20 per cent in root weight was recorded in the plants inoculated with lowest density level (500 nematodes/pot).

Figure 1c. Pathogenic effect of *Meloidogyne incognita* on shoot weight of cowpea (cv. giant black eye)

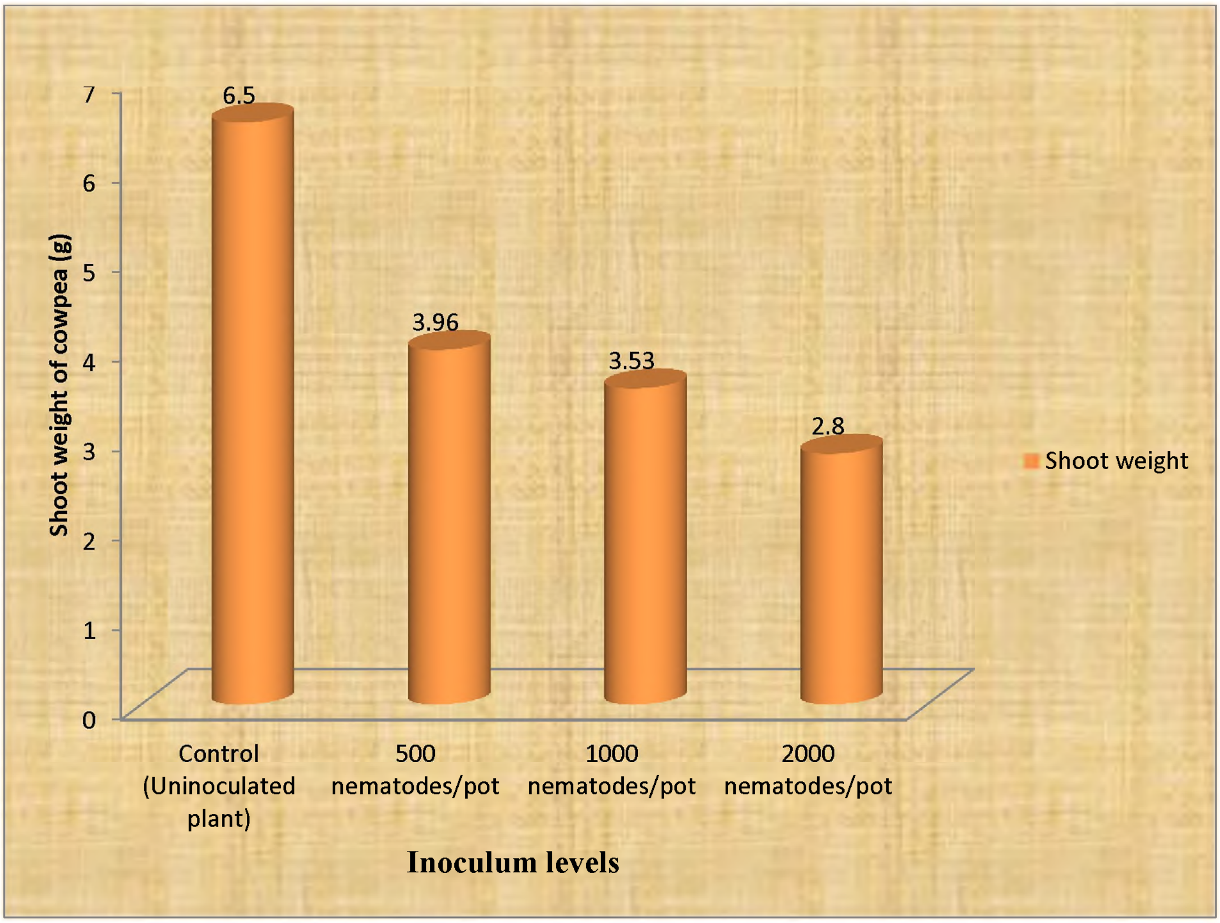


Figure 1d. Pathogenic effect of *Meloidogyne incognita* on root weight of cowpea (cv. giant black eye)

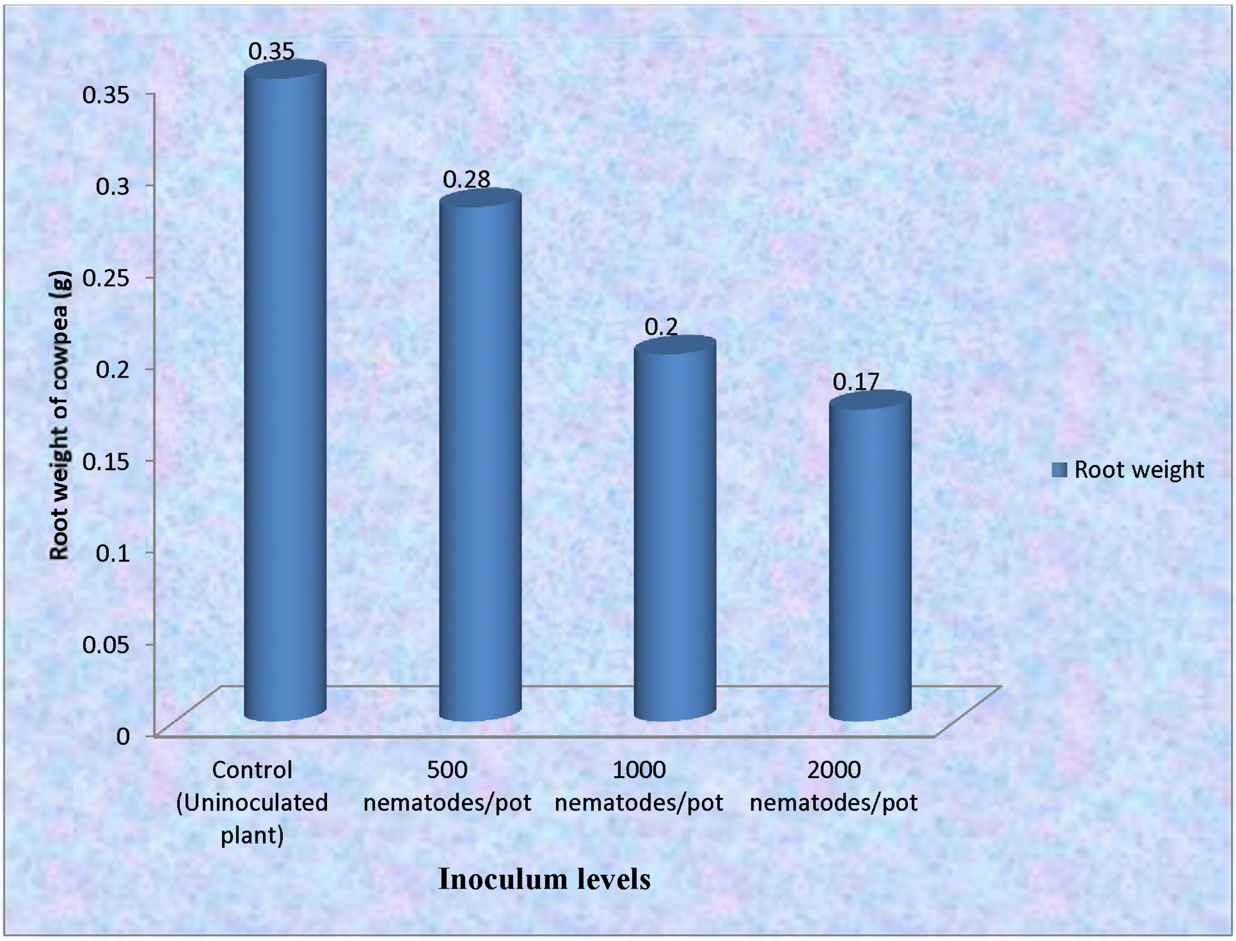
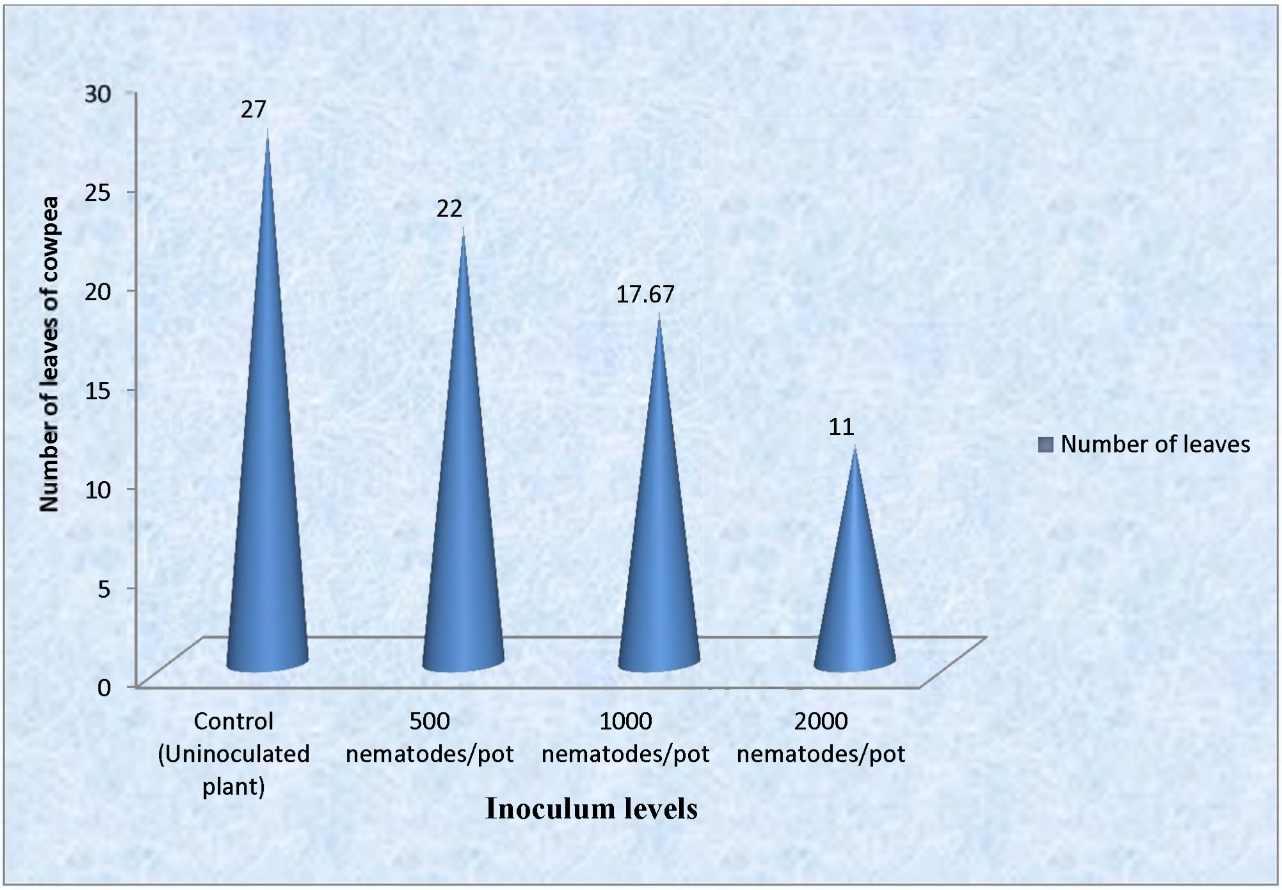


Figure 1e. Pathogenic effect of *Meloidogyne incognita* on the number of leaves of cowpea (cv. giant black eye)



4.1.e Number of leaves

A maximum number of 27 leaves was recorded (Table I and Figure 1e) in the uninoculated plants. The number of leaves progressively decreased as the nematode densities increased. The number of leaves recorded were 22, 17.67 and 11 in the plants inoculated with 500, 1000 and 2000 nematodes/pot respectively. From the statistical analysis it was observed that there was a significant variation between the inoculated and uninoculated plants. Variation was also significant between the plants inoculated with different densities of nematodes.

The percentage reduction in the number of leaves showed a gradual increase with the increase in the inoculum levels of the nematodes. A maximum reduction of 59 per cent was recorded in the number of leaves in 2000 nematodes inoculated plants followed by 37 per cent in 1000 nematodes inoculated plants. Percentage reduction was a minimum of 18.5 per cent in 500 nematodes inoculated plants when compared with uninoculated host plant.

Results obtained from the present investigation on pathogenicity of *M. incognita* on the host plant, cowpea (cv. giant black eye) reveals the fact that there is progressive reduction in all the growth characters (shoot length, root length, shoot weight, root weight and number of leaves) of the study plants as the inoculum level increased.

Reduction in shoot and root length was above 50 per cent in the host plants inoculated with higher density of 2000 nematodes/pot. Similar trend was also observed in the shoot weight, root weight and number of leaves at the highest inoculum level.

Higher the inoculum level of nematodes lower is the growth of the host plant. The decrease in plant growth with increasing density of second stage juveniles of *M. incognita* in the present study has also been supported in earlier years by various authors in cowpea (Khan and Khan, 1996; Satyendra and Goswami, 2000; Ogaraku and Akueshi, 2005 and Sawadogo *et al*., 2006). Such reduction in growth in other crops was also reported by various researchers. Plant growth suppression was reported in cauliflower (Pathak *et al*., 2000) in tomato (Khan and Akram, 2000; Abolusoro *et al*., 2004; Siddiqui, 2004 and Olabiyi, 2008) in *Solanum nigrum* (Robab *et al*., 2012) in cucurbits (Chandra *et al*., 2010) and African yam bean (Onyeke and Akueshi, 2012).

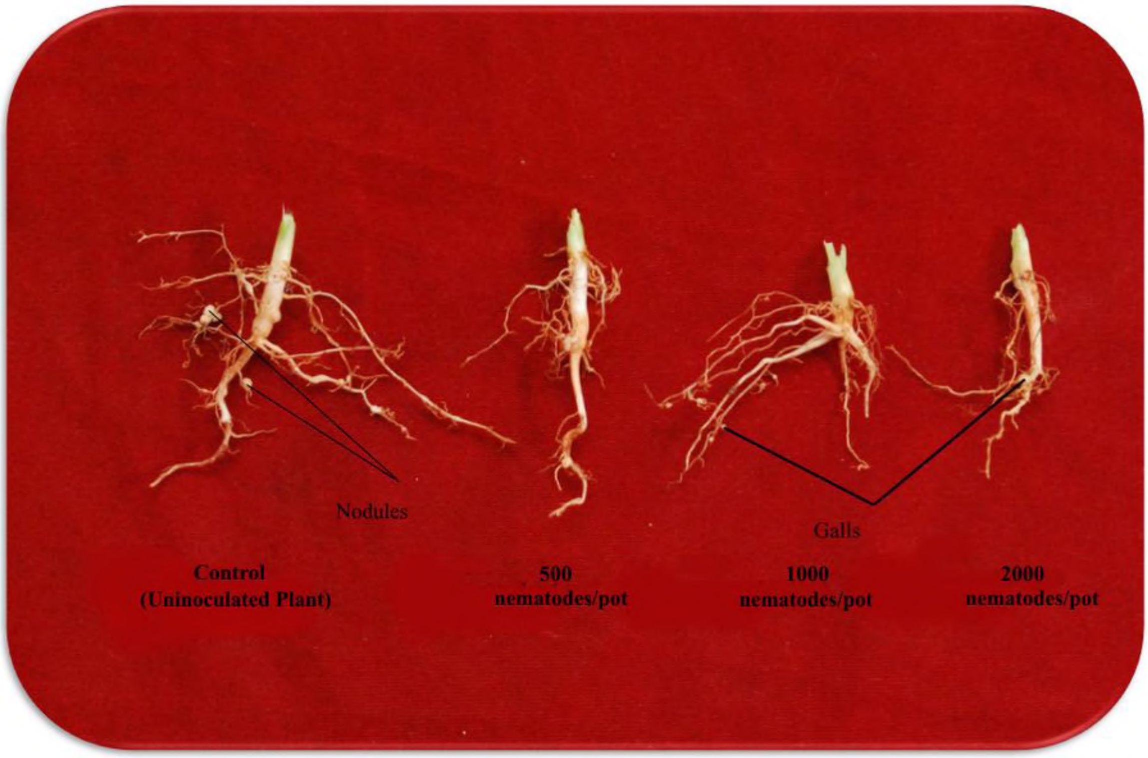
4.2 Pathogenic effect of *Meloidogyne incognita* on the gall and egg mass production in cowpea plants (cv. giant black eye)

4.2.a Number of galls

Table II and Figure 2a showed an increase in the number of galls in all the host plants inoculated with the second stage juveniles of *M. incognita*. Number of galls formed in the plants inoculated with 500 nematodes/pot was 4

PLATE - III

a. Pathogenic effect of *Meloidogvne incognita* on the growth of cowpea plants



b. Gall and egg mass production in the roots of cowpea plants infected with *Meloidogyne incognita*

and that formed in plants inoculated with 1000 nematodes/pot was 8. The plants inoculated with 2000 nematodes/pot recorded a maximum number of 14 galls. Number of galls between the inoculum levels of 500 and 1000 nematodes, 1000 and 2000 nematodes are statistically different from each other.

Progressive increase in the number of galls was observed in the host plant (cowpea) as the inoculum level of *Meloidogyne incognita* increased. Number of gall formation is directly proportional to the initial inoculum levels.

4.2.b Number of egg masses

Egg masses produced in the host plant due to *Meloidogyne incognita* infection is recorded in Table II and Figure 2b. Number of egg masses produced in the plants inoculated with 500 nematodes/pot is 8 and that produced in the host plant inoculated with 1000 nematodes/pot is 16. Highest number of 27.33 egg masses is produced in the host plant inoculated with 2000 nematodes/pot. The egg mass produced in 500, 1000 and 2000 nematodes inoculated host plants are highly significant.

However, there is progressive increase in the number of egg masses produced as the inoculum levels increased.

From the present investigation, it is clearly evident that the number of galls and egg masses produced in the host plant infected by *M. incognita* increased with increasing inoculum levels. Maximum number of galls were

Table – II

Pathogenic effect of *Meloidogyne incognita* on the gall formation and egg mass production in cowpea (cv. giant black eye)

Inoculum levels	Number of galls/plant	Number of egg masses/plant
Control (Uninoculated plants)	-	-
500 nematodes/pot	4	8
1000 nematodes/pot	8	16
2000 nematodes/pot	14	27.33
S Ed	1.3540	2.4495
CD (0.05)	3.3133	5.9940

Figure 2a. Gall formation in *Meloidogyne incognita* infected cowpea (cv. giant black eye)

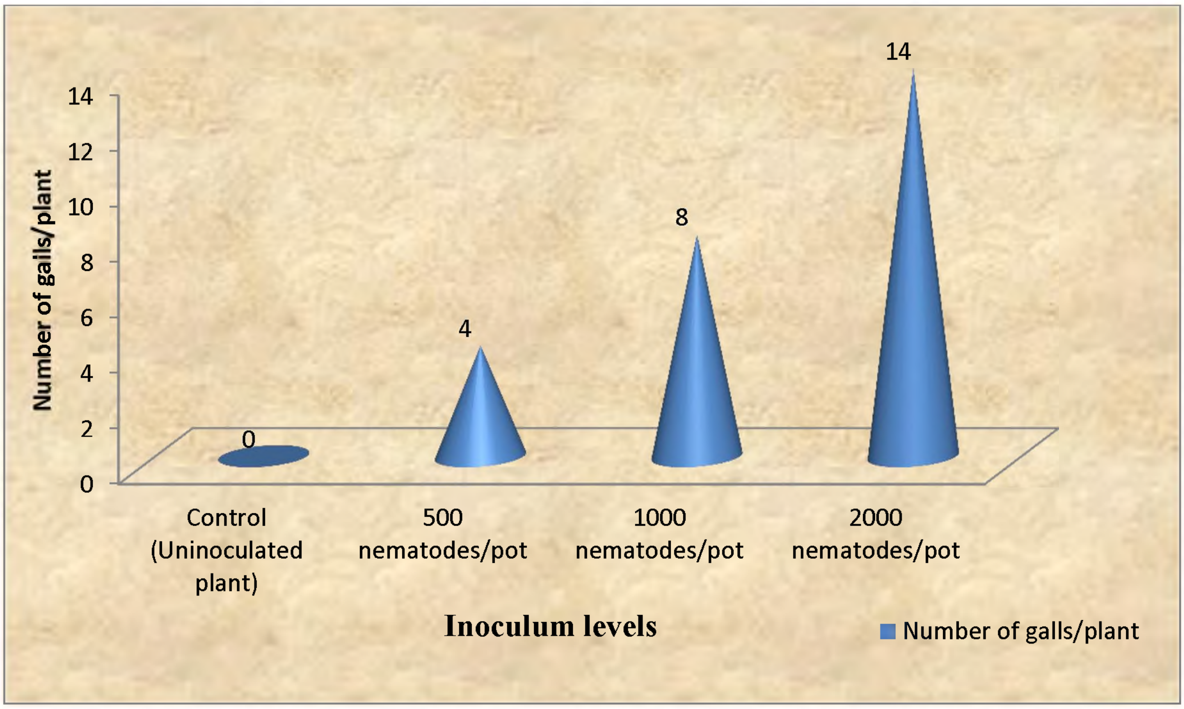
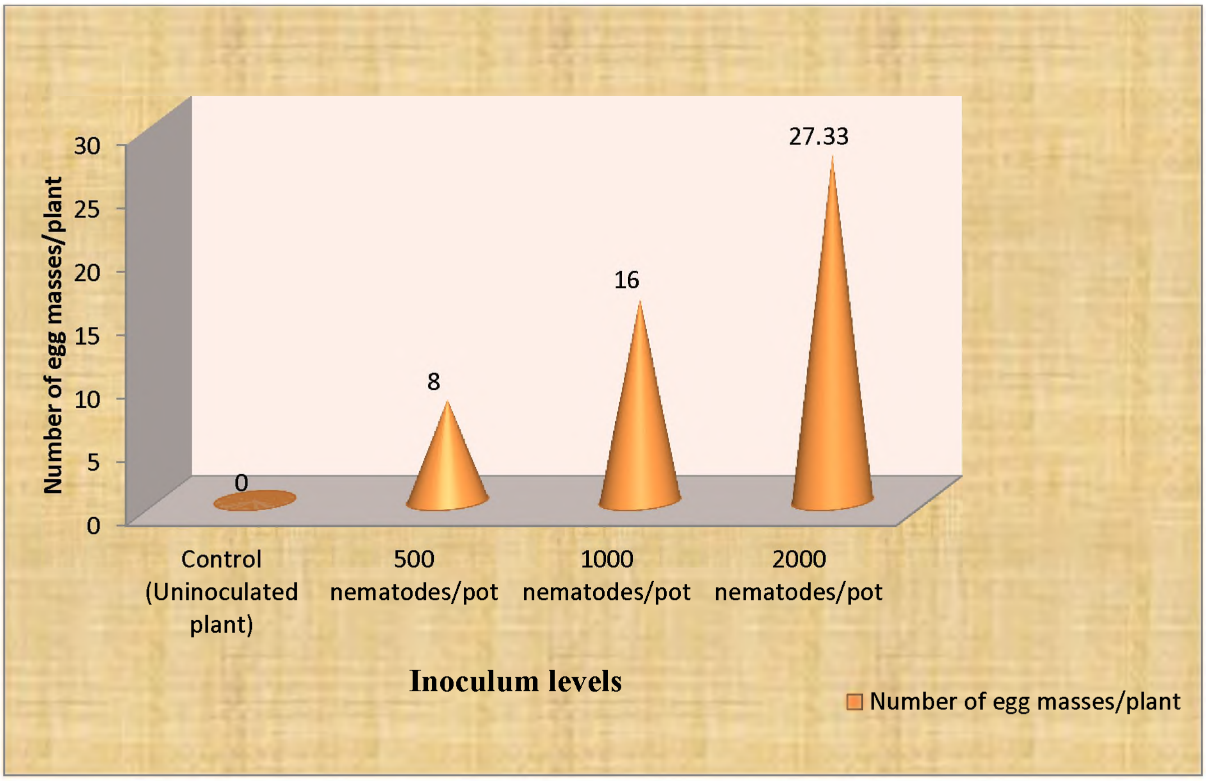


Figure 2b. Egg mass production in *Meloidogyne incognita* infected cowpea (cv. giant black eye)



produced at two higher inoculum levels (1000 and 2000 nematodes/pot) in cowpea infected with *M. incognita*. Egg mass production was progressively increased in all the inoculum levels and it reached a maximum at 2000 nematodes/pot.

Increase in the inoculum density of juveniles of *M. incognita* results in the increase in the number of galls and egg masses in the host plant. As the inoculum level increases the galls and egg masses were also increased. Same was observed by various authors in cowpea (Khan and Khan, 1996; Ugwuoke and Eze, 2010 and Adegbite, 2011). Such increase in the number of galls and egg masses in other crops was also reported by various researchers in tomato (Ogunfowora, 1977 and Cetintas *et al*., 2007) in cucurbits (Wallance, 1973 and Khan *et al*., 2004) in egg plant (Divito *et al*, 1986) in papaya (Khan and Hussain, 1990) in cock's comb (Olabiyi and Ndana, 2003) and in sugar beets (Korayem *et al*., 2012).

4.3 Biochemical analysis of total protein, total amino acids, proline, total carbohydrates and total phenols in *Meloidogyne incognita* infected and uninfected roots of cowpea plants (cv. giant black eye)

The biochemical components estimated in the present study are presented in Tables III and IV and Figures 3a, 3b, 4a and 4b. From the biochemical analysis conducted, it is clearly evident that the host plants inoculated with

M. incognita showed a change in all the components analysed when compared with the uninoculated plants. A significant increase in the amount of total proteins, total amino acids and proline and a significant decrease in the amount of total carbohydrates and total phenols were observed in the roots of *M. incognita* infected host plants when compared with that of uninoculated control plant.

4.3.a Total proteins

The biochemical analysis of total proteins estimated in the inoculated plants showed an increase in the amount when compared with that of uninoculated host plants (Table III and Figure 3a).

Amount of total proteins recorded in the roots of host plants inoculated with 500, 1000 and 2000 nematodes was 3.25, 3.53 and 5 mg/g respectively. The value recoded for control plant was 3 mg/g. The amount of total proteins recorded for all the inoculated plants showed variation when compared with control. A significant difference in total proteins was also noted between the inoculated plants.

Percentage increase in total proteins was 8.33 in 500 nematodes inoculated plants followed by 17.67 in 1000 nematodes inoculated plants. Amount of total proteins increased upto 55.56 per cent in the plants inoculated with highest inoculum density (2000 nematodes) than the uninoculated control plants.

4.3.b Total amino acids

The estimated amount of total amino acids in the roots of uninoculated and *M. incognita* inoculated host plants is presented in Table III and Figure 3a.

Amount of total amino acids estimated from the control roots was 2 mg/g. High amount of total amino acid recorded was 9 mg/g in the roots inoculated with 2000 nematodes whereas it was 4 and 5.5 mg/g respectively in the roots of 500 and 1000 nematodes inoculated host plants.

Amount of total amino acids significantly varied between uninoculated and inoculated host plants and also between different inoculum levels.

Variations were noted between the *Meloidogyne incognita* inoculated plants. More than threefold increase was noted in the amount of total amino acids in the roots of 2000 nematodes inoculated plants when compared with uninoculated plants.

Increase in total amino acids was 100 and 175 per cent in the roots of host plants inoculated with 500 and 1000 nematodes respectively.

All the inoculated plants showed an increase in total amino acids as the inoculum levels increased.

Table - III

Total proteins, total amino acids and proline content in the *Meloidogyne incognita* infected roots of cowpea (cv. giant black eye)

Inoculum levels	Total proteins (mg/g)	Percentage increase over control	Total amino acids (mg/g)	Percentage increase over control	Proline (mg/g)	Percentage increase over control
Control (Uninoculated plant)	3	-	2	-	1	-
500 nematodes/pot	3.25	8.33	4	100	1.2	20
1000 nematodes/pot	3.53	17.67	5.5	175	1.48	48
2000 nematodes/pot	5	55.56	9	350	1.8	80
S Ed	0.0632		0.4684		0.1342	
CD(0.5)	0.1756		1.1463		0.3725	

*Mean of three replications.

Figure 3a. Total proteins and total amino acids in the *Meloidogyne incognita* infected roots of cowpea (cv. giant black eye)

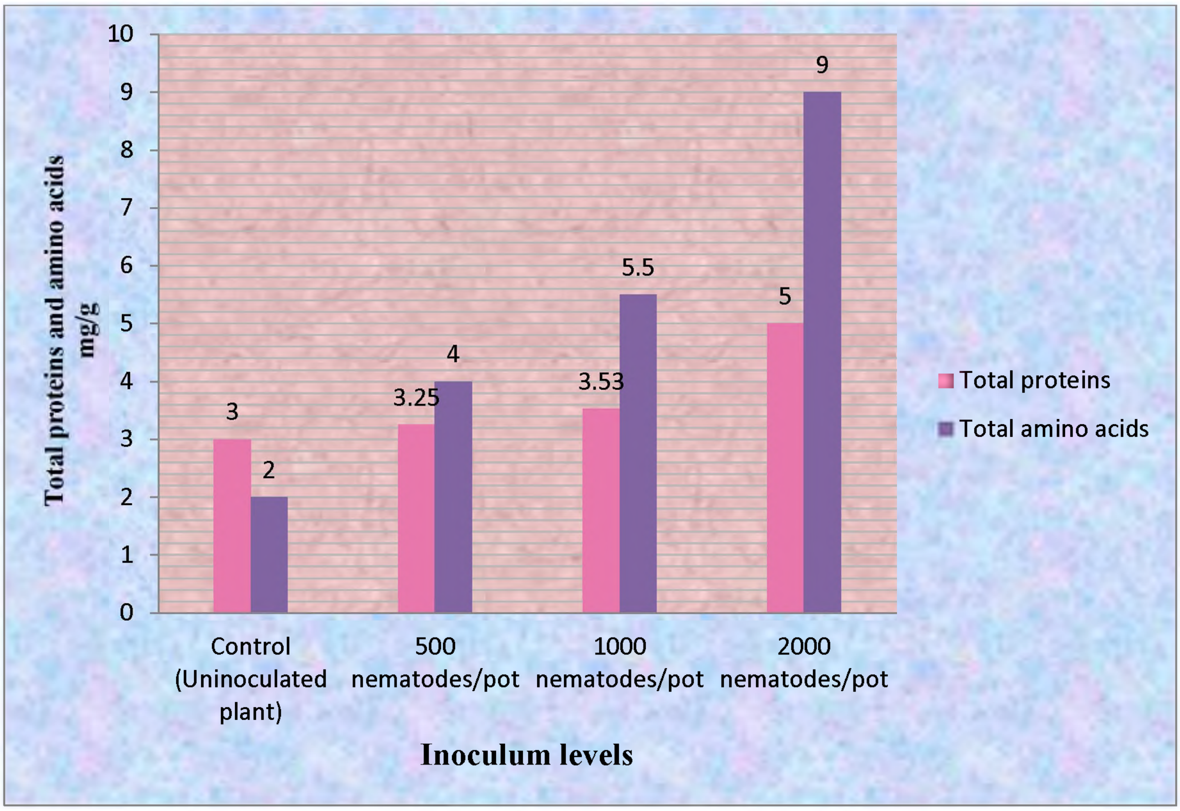
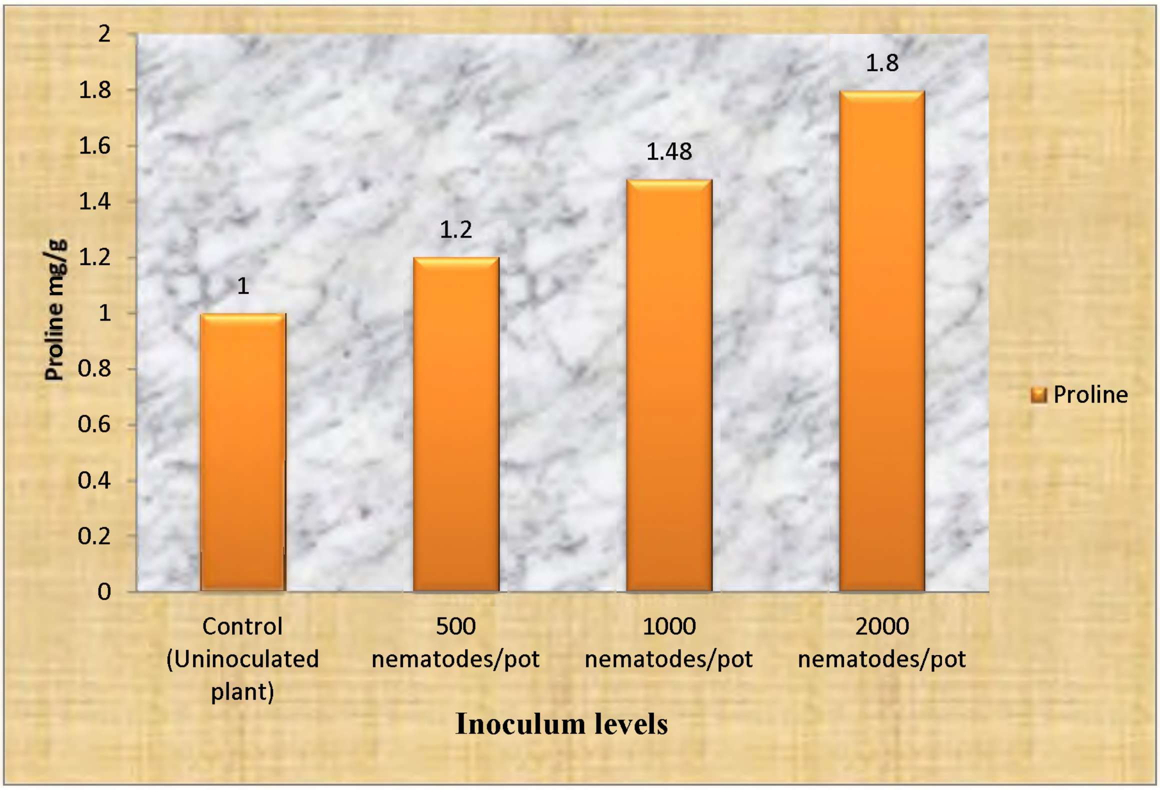


Figure 3b. Proline content in the *Meloidogyne incognita* infected roots of cowpea (cv. giant black eye)



4.3.c Proline

The amount of proline estimated in the roots of uninoculated and *Meloidogyne incognita* inoculated host plants is presented in Table III and Figure 3b.

Amount of proline recorded in the roots of host plants inoculated with 500 nematodes was 1.2 mg/g whereas that of roots inoculated with 1000 nematodes was slightly with higher values of 1.48 mg/g. The amount exceeded in the plants inoculated with higher density of (2000) nematodes which was 1.8 mg/g when compared with the value recorded for control plants which was only 1 mg/g.

All the values recorded for all the inoculated plants showed variations when compared with control.

Increase in the amount of proline was 20 per cent in 500 nematodes inoculated plants followed by 48 per cent in 1000 nematodes inoculated plants. High amount of 80 per cent proline was recorded in the plants inoculated with highest inoculum density (2000) over the uninoculated control plants.

4.3.d Total carbohydrates

The values obtained from the estimation of total carbohydrates for all the three inoculated and uninoculated host plants are presented in the Table IV and Figure 4a. The amount of total carbohydrates estimated in control plant was a maximum of 6 mg/g and that recorded in the plant inoculated with 500, 1000 and 2000 nematodes was 5.4, 4.2 and 3.6 mg/g respectively.

A significant difference in the amount of total carbohydrates was observed between control and plants inoculated with 1000 and 2000 nematodes whereas the values recorded between control and 500 nematodes statistically showed no difference. The amount of total carbohydrates was on par with 1000 and 2000 nematodes inoculated plants.

A decrease of 10, 30 and 40 per cent total carbohydrates was recorded in plants inoculated with 500, 1000 and 2000 nematodes respectively over the control plant.

4.3.e Total phenols

The result obtained on the estimation of total phenols is recorded in the Table IV and Figure 4b. The values showed a decrease in total phenols in the roots of plants infected with *Meloidogyne incognita* with that of control plants. Total phenols recorded in the roots of control plants was a maximum of 7 mg/g followed by 5.5 mg/g in 500 nematodes inoculated plants. Total phenol content recorded in 1000 and 2000 nematodes inoculated host plants was 3.5 and 2 mg/g respectively.

Statistically, there was a significant difference in the content of total phenols between the uninoculated and in all the three nematodes inoculated host

Table – IV

Total carbohydrates and total phenols in the *Meloidogyne incognita* infected roots of cowpea (cv. giant black eye)

Inoculum levels	Total carbohydrates (mg/g)	Percentage decrease over control	Total phenols (mg/g)	Percentage decrease over control
Control (Uninoculated plant)	6	-	7	-
500 nematodes/pot	5.4	10	5.5	21
1000 nematodes/pot	4.2	30	3.5	50
2000 nematodes/pot	3.6	40	2	71
S Ed	0.3317		0.2224	
CD(0.5)	0.9209		0.5441	

*Mean of three replications.

Figure 4a. Total carbohydrates in the *Meloidogyne incognita* infected roots of cowpea (cv. giant black eye)

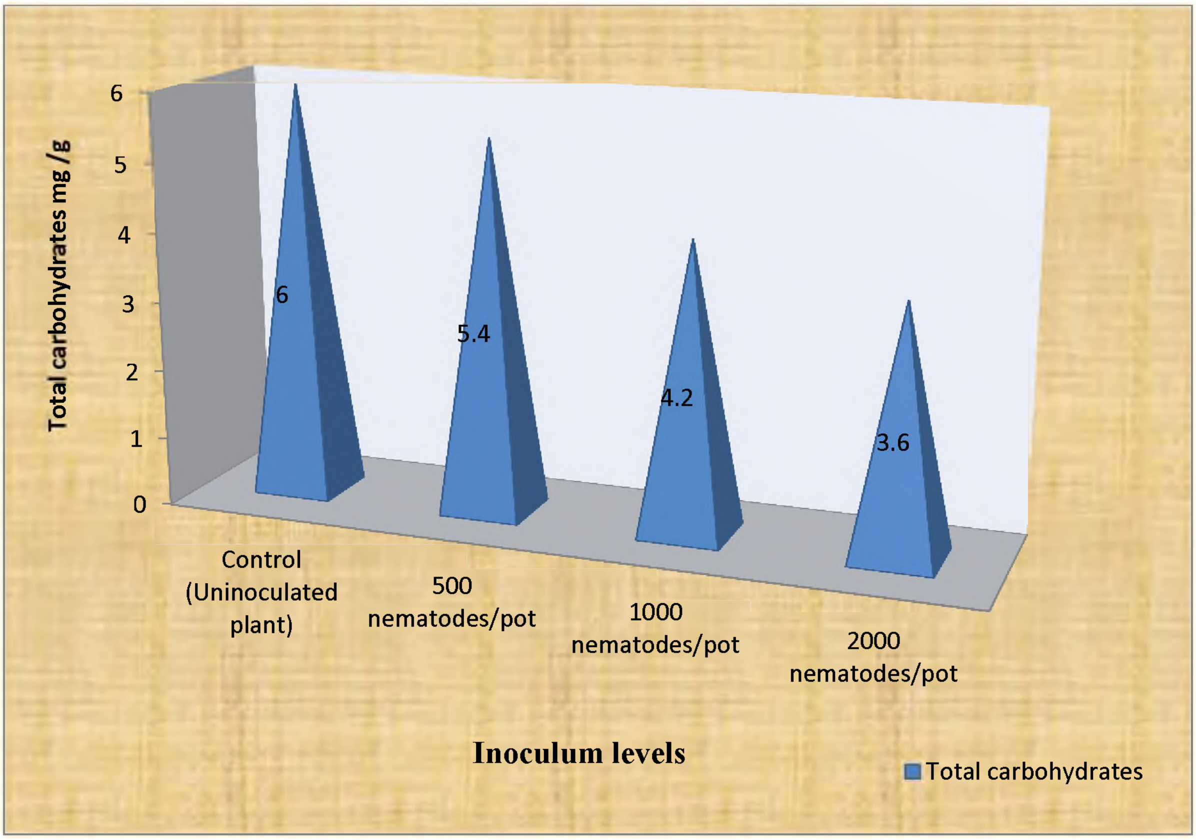
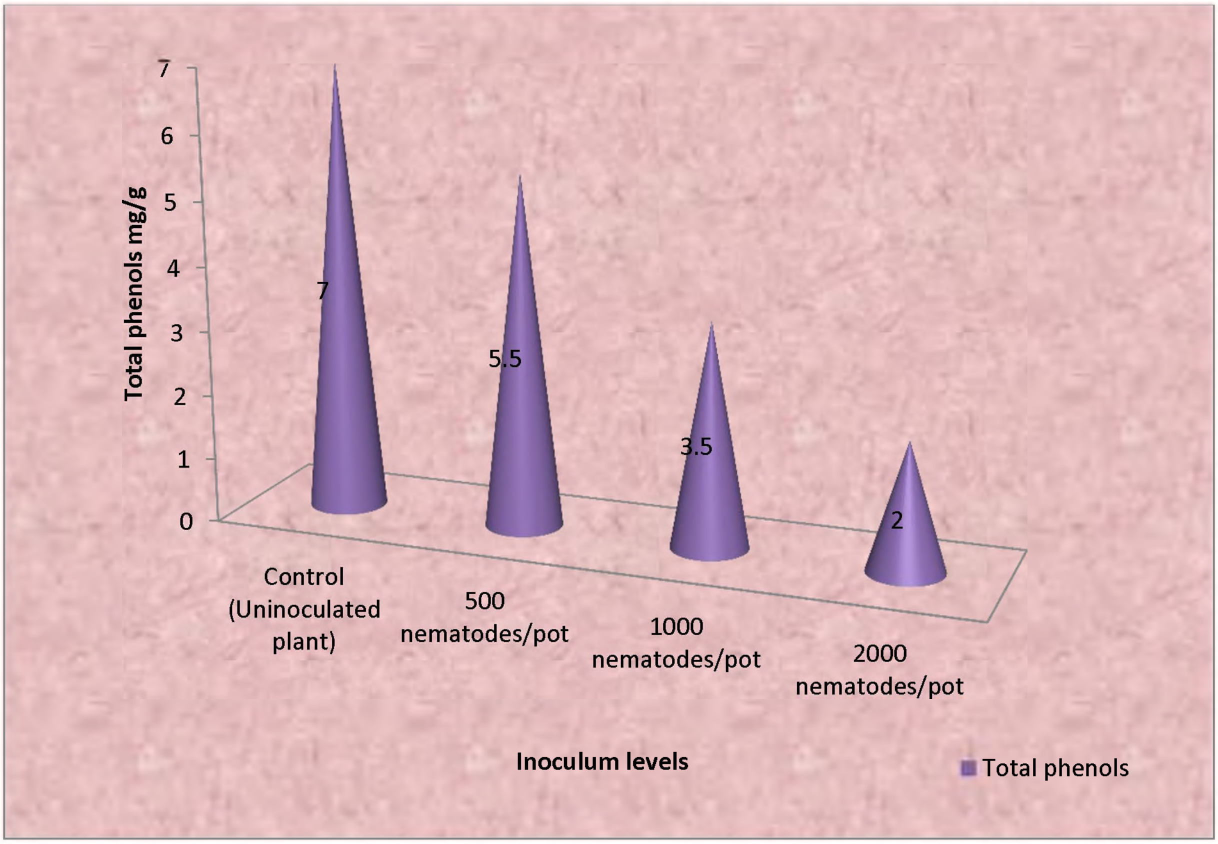


Figure 4b. Total phenols in the *Meloidogyne incognita* infected roots of cowpea (cv. giant black eye)



plants. A decrease in total phenols was observed in all the inoculated plants when compared with control plants.

Percentage decrease in total phenols was 21, 50 and 71 in the plants inoculated with 500, 1000 and 2000 nematodes respectively over the control plants.

From the results obtained on the estimation of biochemical components it is clearly evident that there is tremendous changes in the inoculated cowpea plants due to *Meloidogyne incognita* infection.

An increase in the amount of total proteins, total amino acids and proline was observed in all the inoculated plants over the uninoculated plants, whereas the amount of total carbohydrates and total phenols in the inoculated plants were decreased over the uninoculated plants.

Data presented in Table III indicates the fact that the amount of total proteins, total amino acids and proline present in the host plants inoculated with 1000 and 2000 nematodes/pot was found to be more when compared with that of the uninoculated (control) plants and the magnitude of increase was more pronounced at inoculum density of 2000 nematodes. Increase in total proteins, total amino acids and proline in the host roots might be due to the nematode infection. Such increase of these chemical components due to invasion of pathogenic nematodes was also reported earlier by several authors in different crops (Daney *et al.*, 1971; Singh *et al.*, 1978 and Nayak and Mohanty, 2010).

Increase in total proteins was also reported by various authors in different cultivars of cowpea (Ganguly *et al.*, 1991 and Oliveiraa *et al.*, 2012). Increase in total proteins in the host plants following infection with root-knot nematodes was also reported in tomato and egg plant (Alam *et al.*, 1976) in lady's finger (Chatterjee and Sukul, 1981) and in chickpea (Upadhyay and Banerjee, 1986). Further, it can be explained that the increase in the concentration of total proteins may be due to the synthesis of new enzyme proteins or its contribution from the parasitic nematode itself (Ganguly and Dasgupta, 1981; Simite and Dasgupta, 1987 and Gopinatha *et al.*, 2002).

Significant increase in total amino acids in the roots of infected plants might have resulted due to host parasitic interactions or due to the blockage of protein synthesis (Goodman *et al.*, 1967). Similar increase in total amino acids due to pathogenesis was reported in several other crops by various authors (Narayana and Reddy, 1980; Mohanty and Pradhan, 1990 and Hofmann *et al.*, 2010).

Accumulation of high amount of total amino acids in infected roots of host plants may serve as a readily available source of nutrition for the growing nematodes (Khan and Khan, 2010) and might have helped in the gall formation (Jonathan *et al.*, 2009 and Nayak and Mohanty, 2010).

Proline is a natural compound with a greater solubility than other amino acids. Accumulation of increased amount of proline in plants during the stress condition may be due to pathogenic attack. Its accumulation, therefore helps in increasing the amount of osmotically bound water, which in turn enables the plant to conserve water during drought, stress caused by parasitism of nematodes (Khan and Husain, 1989).

Significant increase in proline content in the roots was observed in the present investigation. Greater accumulation of proline in response to nematode infection of plants has been reported two decades ago by several workers (Sharma *et al.*, 1980 and Khan *et al.*, 2004).

Proline is a readily available storage compound in moisture deficient tissues, the hydrolysis of plant proteins by enzymes secreted by nematodes might have resulted in the greater accumulation of proline in the diseased plants, or the nematodes by themselves might have contributed to the increase in proline content (Khan *et al.*, 2006).

Reduction in total carbohydrates was recorded in the host plant which was infected by second stage juveniles of *Meloidogyne incognita* when compared with uninoculated control plants. Decrease in the content of total carbohydrates was higher at 1000 and 2000 inoculum density of nematodes.

Decrease in total carbohydrates might have resulted due to the action of hydrolyzing enzymes secreted by *M. incognita* or its influence in the production of these enzymes in host which might have helped in the conversion of total carbohydrates (Alsayed *et al*., 2007) or may be due to rapid consumption of these substances by nematodes (Basu and Sukul, 1983; Mohanty *et al*., 1997; Jain *et al*., 2007 and Oliveiraa *et al*., 2012).

Decrease in total carbohydrates may alter host physiology by affecting host tissues (Sharma and Trivedi, 1992). Such reduction in total carbohydrates due to root-knot nematode infection which alter the metabolism of several other host plants was also reported by Bajaj et al., (1983); Upadhyay and Banerjee (1986) and Anwar (1995). However, reduction in total carbohydrates in the host tissues is suggested due to pathogenesis of the root-knot nematodes.

Phenolic compounds are the most influential and widely distributed secondary products in the plants and has a different action in the defence of plants against pathogen attack (Takahama and Oniki, 1992).

Amount of phenol content in the present study shows a decrease in *M. incognita* inoculated plants indicating that the defence mechanism is very poor in the host plant due to its susceptibility. Same was earlier reported by Patel *et al*., (1996) in susceptible cowpea cultivars.

Decrease in the total phenols was found in the plants susceptible to *M. incognita*. This indicates that the phenolic compounds contribute to the defence of plants against nematode attack (Nicholson and Hammerschmidt, 1992; Kosack and Jones, 1996 and Khan *et al*., 2011).

Decrease in the total phenols in different susceptible plants was also reported by several researchers. Earlier it was reported in tomato (Bajaj and Mahajan, 1977 and Singha *et al*., 2012) in *Capsicum annuum* (Pegard *et al*., 2004) in tobacco (Khan *et al*., 2011) and sugar beets (Korayem *et al*., 2012).

5. SUMMARY AND CONCLUSION

A study entitled "**Pathogenic effect of *Meloidogyne incognita* (a root-knot nematode) on the morphology and root biochemistry of a leguminous crop**" was undertaken to assess the pathogenicity of *M. incognita* on cowpea (cv. giant black eye) and biochemical changes that resulted in the host plants at different inoculum levels. A pot culture experiment was carried out to study the severity of damage caused by these nematodes in the host plant of study using different inoculum levels (500, 1000 and 2000 nematodes/pot).

The result of the present study on the pathogenicity with special reference to the growth characters (shoot length, root length, shoot weight, root weight and number of leaves) of the cowpea (cv. giant black eye) infected with *Meloidogyne incognita* are summarized in this chapter and conclusion was drawn from the above study.

A reduction in shoot length was observed in all the inoculated host plants when compared with uninoculated control plant. Shoot length reduction was maximum (56.6 per cent) at highest inoculum density (2000 nematodes/pot).

Similar trend was also observed in root length of all the inoculated plants. Percentage reduction was above 50 per cent in 2000 nematodes inoculated host plants.

Shoot weight reduction recorded was nearly 60 per cent at 2000 nematodes inoculated plants when compared with control plants.

Maximum root weight was recorded in uninoculated control plants, whereas a reduction was noted in all three inoculated plants. Maximum weight loss was observed in 2000 nematodes inoculated plants.

Decrease in the number of leaves was observed in all three inoculated plants. Maximum decrease of 59 per cent was observed in the plants inoculated with 2000 nematodes.

Due to pathogenicity of *M. incognita*, the morphological changes observed in the host plants were stunted growth, yellowing and shedding off leaves in the above ground (shoot) system and formation of root-knots or galls and egg masses in the below ground (root) system. From the above results, it is clearly evident that all the growth characters of the host plants correspondingly decreased with increased inoculum levels.

Production of galls and egg masses in the host plants showed an increase in their number as the inoculum levels increased. Maximum number of galls were observed in the plants inoculated with higher densities of nematodes. Root rotting was also observed at the highest inoculum levels.

An attempt was also made to study the root biochemistry of the host plants. Selected biochemical components such as the amount of total proteins, total amino acids, proline, total carbohydrates and total phenols were estimated in the roots of inoculated and uninoculated host plants to support the present investigation.

Amount of total proteins estimated in all the inoculated plants showed an increase over the uninoculated plants. Maximum amount was recorded in the plants inoculated with higher density of 2000 nematodes.

Similar observation was also made in the amount of total amino acids. More than threefold increase of total amino acids was recorded in 2000 nematodes inoculated plants. All the three inoculated plants showed an increase in total amino acids when compared with uninoculated cowpea plants.

Increase in the amount of proline was observed in all the three inoculated plants. The amount of proline exceeded in the host plants inoculated with higher density of 2000 nematodes.

Amount of total carbohydrates recorded in the present study showed a decrease in all the inoculated plants in an increasing trend. Maximum reduction was observed in 2000 nematodes inoculated plants.

The phenolic content estimated from the roots of inoculated plants showed a decrease over the uninoculated control plants. Percentage reduction was above 50 per cent in 1000 and 2000 nematodes inoculated host plants.

The observations and the data recorded in the present study revealed the fact that the cowpea cultivar (giant black eye) selected for the investigation proved to be susceptible to the attack of root-knot nematodes *Meloidogyne incognita* which was evident through the pathogenic and biochemical studies.

From the present study it was observed that the damage caused by the root-knot nematode, *Meloidogyne incognita* was above 50 per cent in the selected host plant (cowpea cv. giant black eye) which was evident from the morphological observations where the host plants showed stunted growth and reduction in the number of leaves.

The above study has also been supported by the root biochemistry of infected plants by estimating certain biochemical components such as total proteins, total amino acids, proline, total carbohydrates and total phenols also proved the damage caused by the root-knot nematodes in the host plants due to their interaction.

The entry of root-knot nematode larvae might have altered the physiological functioning of the root system which in turn would have reduced the photosynthetic ability of the host plants.

From the above study it can be concluded that the cowpea plants (cv. giant black eye) selected as the host plant is prone to the attack of *Meloidogyne incognita* and hence, is a susceptible host for the nematode.

Damage caused is not much severe at these inoculum levels, though it may be severe at still more higher inoculum levels in the soil. Therefore, these root-knot nematodes must be controlled through various means. The agrofarmers must be alarmed at this point of time and necessary steps are to be taken.

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