SECTION I
EXPERIMENT 1

Histopathology of the roots of *Eclipta alba* infected
with *Meloidogyne incognita*

INTRODUCTION

The following study was carried out to examine the anatomical changes in the roots of *Eclipta alba* resulting from infection with root-knot nematode, *Meloidogyne incognita*. In tomato roots, the second-stage juveniles of *Meloidogyne* spp. penetrated and established in cortex (Christie, 1936) and the giant cells arose from undifferentiated vessel elements or from xylem parenchyma (Christie, 1936; Hodges and Taylor, 1966); or from provascular strand (Krusberg and Nielsen, 1958; Littrell, 1966); or from protophloem elements (Byrne *et al*., 1977). The feeding site of the nematode was mainly confined to stelar region of the root. Cells in the feeding site showed hypertrophy, hyperplasia, thickening of cell walls, granular cytoplasm with enlarged nuclei and nucleoli (Pasha *et al*., 1987). *Meloidogyne* spp. infection accompanies cortical and stelar proliferation (Davis and Jenkins, 1960), hypertrophy and hyperplasia in cortex, pericycle and stele of soybean roots (Ibrahim and Massoud, 1974).

The present work was carried out (i) to trace mode of entry of second-stage juveniles, (ii) to find out mode of migration of juveniles in inner plant tissues, (iii) to investigate the tissue where juveniles settles after movement is stopped, (iv) to trace the origin of giant cells,(v) to find out histological changes during giant cell formation, (vi) to examine development of giant cell (vii) to study abnormalities in xylem and phloem; and (viii) to find out any
relationship between the giant cells and the vascular elements in the roots of _E. alba_ infected with _Meloidogyne incognita_.

**MATERIALS AND METHODS**

**Preparation and Sterilization of Soil Mixture:**

For performing experiments the soil was prepared in the ratio of 7:3:1 comprising of clay, sand and farmyard manure, respectively. The pots were filled with soil at the rate of one kg of soil per pot. The soil was moistened with water prior to transferring the pots into the autoclave. The soil was sterilized in an autoclave at 20 lb pressure for 20 minutes. Sterilized pots were allowed to cool at room temperature before use for experiments.

**Raising and Maintenance of Test Plants:**

The seeds of _Eclipta alba_ were surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2 minutes and washed thrice in sterilized distilled water and were allowed to germinate on moistened filter paper in sterilized petri-dishes. Two leaf stage seedlings were transferred to clay pots of 15 cm diameters having steam sterilized soil.

**Preparation of Inoculum:**

_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-plants (_Solanum melongena_ L.) roots in a glass house by using single egg mass. The egg mass from the galled roots of egg mass were picked with the help of sterilized forceps and washed thrice with distilled water. The eggs in the egg mass were allowed to hatch at 28±2°C under aspecitic conditions in 6 cm diameter sieve lined with tissue
paper and kept in a petri-dish containing sufficient amount of sterilized distilled water. The second-stage juveniles were collected in distilled water and counted with the help of counting dish.

**Inoculation:**

The seedlings of *E. alba* were inoculated by making holes of 5-7 cm depth around the plant within the radius of two centimeters. Through these holes the suspension of second-stage juveniles (2,000 J2 / pot) was introduced with the help of sterilized pipette. The holes were then plugged with sterilized soil soon after inoculation. To maintain soil moisture in the pot, regular watering was done. Each treatment was replicated five times and pots were arranged in complete randomized block design. Uninoculated pots served as control.

**Harvesting:**

Five seedlings (one seedling from each pot) were harvested 24h, 48h, 72h, 6 days, 12 days, 18 days, 24 days and 30 days after inoculation. The roots were washed thoroughly and gently to remove soil particles. The galled roots were then cut into one cm long pieces and processed for histopathological studies.

**Processing for Histopathological Studies:**

(i) **Fixation:** The pieces of galled roots and healthy roots were immersed in a fixative of formalin aceto-alcohol, prepared by mixing 90 ml of 50% ethanol, 5 ml of glacial acetic acid and 5 ml of 37% formaldehyde (Johansen, 1940). Depending upon the thickness, the
galled roots were kept in the fixative for a period of 24h to several days.

(ii) **Dehydration**: The galled and the healthy root tissues were dehydrated through tertiary-butyl alcohol (T.B.A.) schedule as given by Johansen (1940) (Table -1).

(iii) **Infiltration**: After dehydration, paraffin was introduced into the root tissue. The tissue was transferred to a mixture of 100% paraffin oil and tertiary-butyl alcohol (T.B.A.) in the ratio of 1:1. It was kept at least for one hour. Another container was filled 3/4th with melted wax and allowed to solidify. The tissue was placed on wax and filled with T.B.A. paraffin oil mixture. It was kept in an incubator at about 65°C for three hours. After three hours the mixture was poured off and replaced with pure melted wax. The container was kept at the same temperature for three hours. This step was repeated twice.

(iv) **Embedding**: For embedding, paper molds in the form of small shallow trays were prepared. The inner surfaces of the molds were first coated with thin layer of glycerine and afterwards melted paraffin was poured onto the bottom of the molds. The root pieces were then kept gently on the solidifying surface with the help of forceps. More amount of melted wax was added into the molds until the tissue was completely immersed in the wax. The molds were immediately transferred to a container filled with chilled water for solidifying the wax. After hardening, the whole block was cut into smaller pieces according to the position of the root tissue.
(v) **Sectioning:** The small blocks of wax having root tissue were trimmed to remove extra wax. The wax blocks were mounted on the wooden blocks, then fixed in rotary microtome and 10 μm thick transverse and longitudinal sections were obtained in the form of a ribbon.

(vi) **Ribbon mounting:** The ribbon was cut into smaller pieces corresponding to the length of the slides. Surface of the slide was coated with synthetic adhesive. Ribbon was then placed on the slide and flooded with freshly prepared 3% formalin solution. These slides were then kept in an incubator at 40°C for eight hours and then stored in slide boxes.

(vii) **Staining:** The sections were stained with safranin and fast green after removing paraffin wax by the method described by Sass (1951) (Table-2). Staining, Canada balsam was applied on the slide and cover slip was lowered down gradually over the slide. The finished slides were left at room temperature for 24 hours and then kept in an incubator at 60°C. The slides were examined under light microscope, and necessary photographs were taken.

**OBSERVATIONS**

**A. GIANT CELLS**

In young and growing roots of *Eclipta alba* the second-stage juveniles of *Meloidogyne incognita* penetrated near the root tip and entered the roots within 24h of inoculation. The juveniles migrated vertically or parallel to the axis of the root. The juveniles after penetration migrated towards the
differentiating zone of the root apex. Epidermis was found damaged at the entry site of the juveniles. At some places enlargement of the cell and the nucleus was observed near the head of the juveniles. One or more binucleate cells were noticed adjacent to the nematode body. In some enlarged nuclei two or more nucleoli were seen (P.1and 2).

After 48h of inoculation, the longitudinal sections revealed that some of the cells were quite large in size but fewer in number per unit area, and some cells were smaller in size and greater in number. The cells direct in contact with nematode head were very large. These hypertrophied cells, may be designated as incipient giant cells ranged from one to five and enclosed comparatively dense and granular cytoplasm (P.2). The average size of the giant cell measured 156 X 26 μm enclosing up to 8 nuclei. The largest incipient giant cells were almost ten times longer than ordinary cells. The nuclei and nucleoli both were hypertrophied. The nuclei and the nucleoli had variable shapes.

The giant cell became conspicuous after 72h after inoculation and measured 172 X 39 μm. The denseness of the giant cell cytoplasm increased and the vacuoles diminished or disappeared. In the giant cell, the size of the nucleus increased enormously. The number of nuclei increased. The nuclei nearer to nematode head were larger than others. The nucleus enclosed one nucleolus but in some nucleoli there were two or more nucleoli which had different shapes (P.4).

The giant cell dimension increased after 6 days of inoculation. The largest giant cell measured 208 X 72 μm. In all the giant cells, the cytoplasm was very dense and highly granular. The cell wall of the giant cell became very
thick. The thickness of the wall was non-uniform. The cell wall stained only green. The size of the nuclei increased. The nuclei enclosed large nucleoli. In most of the nuclei fragmentation of nucleoli was observed. The dimensions of the larger nuclei were of the order of 13 μm and of nucleoli 6.5 μm. The shapes of the nuclei were non-uniform. Various shapes like spherical, amoeboid, oval were observed. The empty spaces become prominent in some of the nuclei (P. 6, 7, 8, 9 and 10).

The giant cell cytoplasm became more dense after 12 days of inoculation. The nuclei became inconspicuous in the dense and granular cytoplasm. The nuclei were arranged in the form of groups and were not scattered. The nuclei became very large (20 μm) and enclosed large densely stained nucleoli. Average size of the giant cell was 260 x 130 μm. The size of the nucleolus varied from a very small to very large with an average of 10 μm diameter. The shapes of nuclei were variable like globose, ovoid, elongate, triangular and even amoeboid. The number of nuclei per giant cell was upto twenty (P.11, 12 and 13).

The size of the giant cells did not increase, after 18 days of inoculation. The cell wall of giant cell became very thick. The denseness of the cytoplasm decreased. Small to large vacuoles appeared in the giant cells. The number of nuclei varied from one to eight in the giant cells. The nuclei were amoeboid and the nucleoli were globular, triangular or amoeboid in shape (P.9). The size of the giant cell did not increase further. The amount of giant cell cytoplasm decreased to a greater extent. Some of the giant cells lost their entire cytoplasm and comprised of only thick cell walls (P. 14, 15 and 16).
There was no change in the dimension of the giant cells after 24 days of inoculation. The giant cells lost enormous amount of cytoplasm. A large number of abnormal vessel elements were found around the giant cells (P.17 and 18). After 30 days of inoculation, egg masses were found associated with the mature females. Few giant cells developed secondary cell wall (P.11). Some second-stage juveniles of secondary inoculum were observed in parenchyma (P.19, 20, 21, 22, 23 and 24).

**DISCUSSION**

The serial longitudinal and transverse sections revealed that second-stage juveniles of *Meloidogyne incognita* penetrated the roots of *Eclipta alba* at or just behind the root cap. Migration of second stage juveniles into inner tissues was intercellular and no cellular injury was observed throughout their passage. As a result of intercellular migration a prominent passage was formed. The root-knot nematode larvae have been reported to enter the roots of host tissues at or near the root tips in tomato (Christie, 1936); any where from the root tip back to the region of root hair formation in sweet potato (Krusberg and Nielsen, 1958); and in the region of cell differentiation and elongation in wheat (Siddiqui and Taylor, 1970). None of the workers reported intracellular migration and cellular injury. The proponents of inter and intracellular migration of larvae was Nemec (1910) and this concept was supported by Christie (1936), Krusberg and Nielsen (1958) and Siddiqui and Taylor (1970). Later on from the studies performed by several workers it was confirmed that juveniles of root-knot nematodes migrated intercellularly by separating the cell walls along the middle lamella (Endo and Wergin, 1973; Jones and Payne, 1978; Hisamuddin, 1992). The studies on *E. alba* revealed only intercellular
migration, migration through the cells or intracellular migration was not noticed.

After reaching the meristematic zone or differentiating zone of juveniles, the immediate response of the cells was hypertrophy of the cells. Enlargement of the cells near the nematode head and along the length of the nematode body was most probably due to secretion of hormones causing expansion of the cells. This effect was not confined to cytoplasmic components but this also spread to nucleus and nucleolus. The cell was triggered to make proteins in large amount for which enlargement of the nucleus and nucleolus was essential. Increase in size of nucleus and nucleolus indicated hyperactivity of the cell. The active cells usually contain large nucleus and nucleolus (Johnson and Johnson, 1986).

The sections also revealed that in *E. alba* the cells were not induced for enlargement but also for nuclear divisions, after 48h of inoculation. Increase in number of nuclei per cell indicated endomitotic divisions. Formation of incipient giant cells in the vicinity of the nematode is the indication of successful host parasite relationship between *E. alba* and root-knot nematode. In most of the host plants giant cells are formed soon after infection. Jones and Payne (1978) also observed giant cell formation in *Impatiens balsamina* by *M. incognita*, after 48h of inoculation. Siddiqui and Taylor (1970) noticed giant cells caused by *M. naasi* in wheat roots four days after inoculation. In *E. alba* giant cell formation was seen 48h after inoculation but hypertrophy of some cells was observed 24h after inoculation. Multinucleate condition of giant cell arising from repeated mitosis without cytokinesis was proposed by Huang and Maggenti (1969). The nucleus of an incipient giant cell undergoes rapid, synchronous divisions without subsequent cytokinesis and upto eight nuclei
can be seen within 48h of nematode infection (Jones and Payne, 1978). Synchronous nuclear divisions within the same giant cell have been reported in several plants (Bird, 1961; Krusberg and Nielsen, 1958; Smith and Mai, 1965; Owens and Specht, 1964; and Pasha et al; 1987).

In *E. alba*, after 72h and 6 days of inoculation, the cytoplasm of the developing giant cells became very dense and granular as compared to other neighbouring cells. The number of nuclei continued to increase and all these phenomena signified tremendous increase in the metabolism of the incipient giant cells. During mitotic division, it was noticed that the nuclei assumed various shapes indicating transient phase of the newly formed nuclei. The nuclei otherwise were spherical. Surface area of the nuclei is increased by their amoeboid shapes and by the presence of irregular lobes (Huang and Maggenti, 1969). In developing giant cells, cell organelles become abundant (Jones and Northcote, 1972; Jones and Dropkin, 1976; Jones and Gunning 1976). Dense cytoplasm, several nuclei in the cell, more than one nucleolus per nucleus indicated higher rate of metabolism in the giant cell of *E. alba*. Occurrence of less dense cytoplasm taking less stain and presence of vacuolar regions in the cytoplasm, in some of the giant cells, was probably due to feeding by the developing nematode. In *E. alba*, depletion of cytoplasm in the giant cells was noticed after 12 days of inoculation. And the rate of depletion was increased afterwards. Increase in vacuolation, emptying of small giant cells and decrease in number of nuclei of all the giant cells, 18 days after inoculation, further supported that increased rate of nematode feeding caused such intracellular changes. Cytoplasmic deterioration possibly occurred due to consumption of nuclear materials by the nematodes. According to Bird and Loveys (1975) and McClure (1977), *Meloidogyne* functions as metabolic sink in infected plants.
and the photosynthates are mobilized from shoots to roots particularly toward the giant cells. Through Meon et al., (1978) reported that mobilization and accumulation rates of these substances reached to highest level when the mature females started egg laying. Highest rate of depletion of cytoplasm occurred 24 days after inoculation. This supported that the demand rate by the nematode at maturity was highest.

Depletion of cytoplasm from the giant cell, secondary wall deposition in the cell walls and transformation of the giant cell into vessel like elements were the changes observed in the fourth week of inoculation. Formation of abnormal xylem from parenchyma cells, and from cytoplasm depleted giant cells led to the idea that galled region would not collapse. The giant cells are collapsed when the nematode dies and stops feeding (Krusberg and Nielson, 1958).

Thus from the experimental findings it could be concluded that the juveniles entered the roots through meristematic zones; migrated to inner tissues; settled in vascular element differentiating zone or in protophloem region; induced differentiating cells to enlarge (hypertrophy); the enlarged cells developed into giant cells; the number of nuclei first increased and then decreased; the size of nuclei increased; the shapes of nuclei changed; the giant cell cytoplasm in the beginning was very dense but became less dense and vacuolated afterwards; and some giant cells lost their cytoplasm and changed into vessel like elements.

B. GALL FORMATION

*Meloidogyne* spp. elicits an array of responses during its host parasite interactions at the tissue and cellular levels as has been mentioned by many workers in different plant species (Christie, 1936; Davis and Jenkins, 1960).
Root-knot nematode which penetrates the roots induces a conspicuous gall. Gall formation in tomato roots was observed even when the juveniles were outside the tomato roots, (Schuster and Sullivan, 1960). Gall formation by hypertrophy and hyperplasia of pericycle and cortex as early as first day after inoculation was seen by Dropkin and Bonne (1966). Hypertrophy and hyperplasia in cortex, pericycle and stele led to gall formation in soybean roots (Ibrahim and Massoud, 1974). The present work was carried out to investigate the histological changes leading to gall formation in *Eclipta alba* roots infected with root-knot nematode *Meloidogyne incognita*.

**OBSERVATION**

Penetration of second-stage juveniles of *M. incognita* caused enlargement of cells. Increase in size of the cells was noticed in the meristematic; in the zone of cell enlargement and elongation; and in the zone of cell differentiation. Cell enlargement was not restricted only to parenchyma but was also noticed in differentiating vascular elements. The thickness of the root after 48h of inoculation at the infected site increased from 1.0 mm to 1.2 mm in infected root. The size of the giant cells increased; size of other hypertrophic parenchyma. The largest giant cell measured about 140 X 28 μm. Some parenchyma cells adjacent to the giant cells became large (P. 3 and 4). The average size of the gall increased to 1.5 mm, after 72h of inoculation. The width of the normal roots was 1.2 mm. The size of the giant cells increased; the size of parenchyma cells adjacent to giant cells increase; the number of some parenchyma cells increased; the diameter of vessel elements increased; the diameter of sieve tube elements increased and the diameter of cortical and pith cells increased when compared with the cells in normal regions. Average width of the vessel elements near the giant cell was 47.0 μm, but in normal root it was
The width of endodermal cell was 40.0 µm in the galled roots and 22.0 µm in normal roots (P.5).

The size of the gall after 6 days of inoculation reached to 2.3 mm as compared to normal roots which were 1.7 mm in width. The giant cell reached to a maximum size of 178 X 42 µm with an average of 161 X 33 µm. The average diameters of xylem and cortical parenchyma cells were 28 and 17 µm, respectively. The diameter of parenchyma cell in normal root was about 15 µm. In normal root it measured about 31 µm. Endodermal cells did not show proliferation. Number of xylem and phloem parenchyma cells increased in affected than in unaffected part (P. 6, 7, 8, 9 and 10).

After 12 days of inoculation, the galls had become conspicuously large and measured 6.0 mm in width, while normal root measured about 3.0 mm. The size of the largest giant cell was about 208 X 72 µm with an average of about 197 X 63 µm. The xylem and cortical parenchyma cells were having average diameters of 39 and 24 µm, respectively. Average diameter of xylem and cortical parenchyma cells of normal root was 20.0 µm. (P.11, 12 and 13).

The size of the gall after 18 days of inoculation was about 9.0 mm in width. The width of the nematode increased and length decreased. No remarkable change in size of vascular elements, cortical cells, pith cells, pericycle cells was noticed (P.14, 15 and 16). The size of the gall increased to about 12 mm and 14 mm after 24 and 30 days of inoculation, respectively. The size of the giant cells, hypertrophic and hyperplastic tissue, vascular elements, cortical, pericycle and pith parenchyma increased to lesser extent. The egg masses were frequently observed after 30 days of inoculation. In some sections,
second-stage juveniles, hatching out of the egg masses were noticed among the parenchyma cells (P. 17, 18, 19, 20, 21, 22, 23 and 24).

**DISCUSSION**

In *Eclipta alba* galling, the earliest host response of root-knot nematode infection, resulted from hyperplastic and hypertrophic reaction. In tomato galling was resulted when juveniles of *Meloidogyne incognita* entered the roots (Christie, 1936), but galling without entry of the juveniles was also noticed (Schuster and Sullivan, 1960). As a result of secretion of certain substances by the juveniles that stimulated the host tissue to form galls. In *E. alba* the galling was induced when the juveniles reached the region of vascular differentiation. After 24h of inoculation and soon after of penetration of juveniles, hyperplastic and hypertrophic reactions appeared to be responsible for the gall formation in infected roots. Presence of small cells near the giant cells indicated a hyperplastic reaction together with hypertrophic reaction. In addition to giant cell formation and hyperplasia of neighboring parenchyma cells, hyperplasia of phloem parenchyma was also found contributing in root galling, within 48h of inoculation. Increase in diameter of vessel element, near the giant cells, was also a hypertrophic response of root-knot nematode infection. Jones and Payne (1978) observed giant cells and hyperplastic parenchymatous tissue within 48h of inoculation. After 72h of inoculation, xylem and phloem parenchyma cells divided actively. The main contributing factor of the gall formation was either hypertrophy or hyperplasia or both, in all types of cells including vessel elements, xylem and cortical parenchyma, and also endodermal cells.

The size of giant cells was found increasing until 18th day of inoculation. The amount of xylem and phloem parenchyma increased upto 12
days of inoculation. Thus, hyperplastic and hypertrophic reactions of different types of cells, in the affected region, led to the formation of galls until 12th day of inoculation. Increase in size of the nematode was slow upto 18 days after inoculation, but rapid afterwards. Increase in gall size, after 18 days of inoculation, was due to nematode development into adult female. The adult female attained its maximum size after 24 days of inoculation. Production of egg masses by the adult females further contributed in increasing the gall size.

After 30 days of inoculation, secondary infection was noticed in the cortical region. The mature females laid the egg masses and some eggs, hatched into second-stage juveniles that entered the cortex and induced giant cell formation. Due to repeated hypertrophic and hyperplastic reaction, the size of the gall increased rapidly. At some places newly hatched second stage juveniles were found feeding on previously formed giant cells.

Formation of giant cells, hyperplasia in pericycle and xylem parenchyma, hypertrophy of cortex, pericycle, xylem parenchyma, and metaxylem, and enlargement of the nematode body contribute in gall formation (Christie, 1936; Dropkin, 1954; Krusberg and Nielsen, 1958; Davis and Jenkins, 1960; Dropkin and Nelson, 1960; Bird, 1961; 1962; Owens and Specht, 1964; Hodges and Taylor, 1966; Siddiqui and Taylor, 1970; Siddiqui, 1971a; Orr and Morey, 1978; Jones and Payne, 1978; Hisamuddin, 1992; Bhatt, 1999; Yasmeen, 2002; and Parveen, 2006). In E. alba the galls were formed due to hyperplasia in the vicinity of nematode head and around the giant cells; formation of giant cells; hypertrophy in all kinds of parenchyma near the giant cells and hypertrophy of vascular elements. Secondary infection also contributed in enlargement of the gall.
C. VASCULAR ELEMENTS

Infections caused by *Meloidogyne* spp. result in the structural changes in vascular elements and particularly xylem seems to be highly affected. At the infection sites Christie (1936) observed that in tomato roots xylem elements failed to develop initially but at later stage abnormal vessel elements were formed. Abnormal vessel elements of irregular shapes and sizes were derived from xylem parenchyma (Davis and Jenkins, 1960; Siddiqui and Taylor, 1970). In young roots of *Basella alba* Swamy and Krishnamurthy, (1971) observed initial infection in primary phloem resulting in destruction of primary phloem, secondary phloem did not differentiate. Siddiqui and Ghouse (1975) recorded differentiation of secondary phloem but with certain deformities. Primary phloem or adjacent parenchyma were the initial targets of *M. incognita* juveniles in *Glycine max* reported by Byrne *et al.*, (1977). The experiment was aimed at (i) to find out the stages at which abnormalities appear in xylem elements (ii) to examine the affected tissue in relation with the formation of abnormal vessel elements and deformation of normal vessels, (iii) to observed structural abnormalities in phloem elements, (iv) to ascertain relationship between giant cells, xylem elements and phloem elements.

OBSERVATION

(i) Xylem

In most of the cases differentiation of xylem elements was not observed near the migrating juveniles. In some sections, differentiating protoxylem elements were however noticed. In differentiating protoxylem elements the secondary wall thickenings were spiral and annular. The xylem strands near the
developing giant cells exhibited curvature. The treacheary elements adjacent to the giant cells had wider diameter than away from the giant cells.

After 72h of inoculation, orientations and dimension of the vascular elements changed to a greater extent. The vascular strands were curved in the infected zone and straight in non-infected zone. The length of vessel elements decreased and diameter increased. There was an apparent change in the structure of some parenchyma cells near the giant cells. Transformation of parenchyma cells, near the giant cells, into vessel like elements was noticed. The cytoplasm of these cells degenerated and secondary wall deposition took place. The deposition was lignified and stained red with safranine (P. 5).

Longitudinal section revealed that the vascular strands, after six days of inoculation, became more distorted. The orientation of xylem strands changed enormously in the infected zone. The arrangement of vascular strands altered so much that the continuity appeared broken. The vessel elements near the giant cells became very broad which were normally narrow away from the giant cells. Abnormal vessel element formation was more prominent. Transformation of abnormal vessel elements was near the giant cells. There was no effect away from the giant cells. In all the abnormal vessel elements the secondary wall depositions were reticulate (P. 6, 7, 8, 9 and 10).

After 12 days of inoculation, the number of abnormal vessel elements increased. The parenchyma cells around the giant cell complex transformed into vessel like elements which appeared to covered the giant cell cluster completely. The parenchyma lying in between the normal xylem strand and abnormal vessel elements around the giant cells also changed into vessel like elements (P. 11, 12 and 13).
There was not change in size of vessel elements after 18 days of inoculation. The number of vessel elements arising from parenchyma cells, around the giant cell complex, increased (P. 14, 15 and 16). Abnormal vessel elements arising from parenchyma cells was still in progress after 24 days of inoculation (P. 17 and 18). After 30 days of inoculation small and medium size giant cells transformed into vessel like elements (P. 8, 21 and 22).

**DISCUSSION**

In *Eclipta alba*, abnormalities in vascular strand were observed 48h after inoculation. The protoxylem elements were bent near the giant cells due to hyperplasia and hypertrophy in adjacent parenchyma cells. Six days after inoculation metaxylem strand were found arranged in a zig-zag order. There was a marked increase in the diameter of vessel elements. Irregularly scattered vessel elements comparing of protoxylem and metaxylem elements were seen after 12 days of inoculation. In all these changes continuity of the vascular strands was not found broken. The vessels were continuous throughout the galled portion. Christie (1936) observed that vascular strands in galled region of tomato were pushed outward near the giant cells. Interruption in continuity of vascular strands was a subject of discussion for a long time. Some believed that continuity was broken while others thought reverse of it (Davis and Jenkins, 1960; Odihirin and Jenkins, 1965).

Structural abnormalities in vessel elements of *E. alba* were noticed 48h after inoculation in *E. alba* in which the diameter of metaxylem elements in affected part was increased than in unaffected part.

After 72h of inoculation, decrease in vessel length near the giant cells increase in diameter were the consequences of hypertrophic and hyperplastic
reaction. Abnormalities in change in shape might be due to direct effect of auxin on the differentiating vessel elements. Pressure exerted by the hypertrophic and hyperplastic tissue, and the effects of growth regulators contributed in change in shape of the vessel elements. Hypertrophy in vessel elements has also been reported by Siddiqui and Taylor (1970).

Vessel elements are formed produced by the cambium in a definite order and differentiation of vessel elements is subjected to time and space. In E. alba, vessel like elements originating from differentiated parenchyma was noticed in the first of week of infection. Such vessel elements resembled with the shapes of neighbouring parenchyma cells. Abnormal vessel elements from parenchyma in Meloidogyne spp. induced galls have been reported in other host plants (Christie 1936; Krusberg and Nielsen, 1958; Davis and Jenkins, 1960; Odihirin and Jenkins, 1965; Eversmeyer and Dickerson, 1966; Siddiqui and Taylor, 1970; Siddiqui, 1971a, 1971b; Swamy and Krishnamurthy, 1971; Farooq, 1973; Siddiqui et al., 1974; Ngundo and Taylor, 1975; Ediz and Dickerson 1976; Jones and Dropkin 1976; Byrne et al., 1977; Finley, 1981; Jones, 1981; Pasha et al., 1987; Hisamuddin 1992; Hisamuddin and Siddiqui, 1992).

Large to very large abnormal vessel elements originating, from giant cells from which cytoplasm was removed, were seen in the third and fourth week after inoculation. After root-knot nematode infection, lateral roots were frequently developed on primary and secondary roots. It seems that after infection demand for water is increased and to meet this demand very thin, lateral root formation is stimulated (Christie, 1936; Krusberg and Nielsen, 1958; Davis and Jenkins, 1960). Upward translocation is affected due to structural changes in galled portion (Oteifa and Elgindi, 1962; Hanowanik and
Osborne, 1975). Probably to increase the rate of translocation of water and minerals more amount of xylem is produced. From these findings it might be suggested that the plant attempted to increase the rate of absorption of water and consequently the rate of upward translocation. In a mixed response, therefore, diameter of the normal vessel elements was increased and more amount of vessel elements from parenchyma cells were produced. Another phenomenon appeared to be more appealing that newly formed abnormal xylem played a significant role in connection giant cells with normal xylem strands. Giant cells, produced in protophloem, are highly metabolically active cells and function as sinks for metabolites. They might need higher amount of water for their unceasing metabolic activities. Since water can not flow rapidly through parenchyma, therefore, the parenchyma cells in between the giant cells and the xylem strands were transformed into vessel like elements. In addition, it was also speculated that in galled portion the abnormal xylem appeared to protect and provide support to the giant specifically and to entire gall generally. The giant cells which are highly specialized cells are therefore supposed to remain covered by abnormal vessel elements transformed from neighbouring parenchyma cells. Thus the giant cells were provided with extra support to keep them functional for longer time. According to Krusberg and Nielsen, (1958) if mature nematode stops feeding or dies, the giant cell cytoplasm is degenerated and disappears, and finally the giant cells are collapsed. In E. alba it has been found that giant cells are not collapsed but are transformed into mechanical cells, the vessel elements.

From this study it might be concluded that orientation of vascular strands was disorganized 48h after inoculation. Structural abnormality in vessel elements was noticed after 48h of inoculation where lumen of vessel elements
was increased near the giant cells. Shape and size of metaxylem elements became irregular as a result of pressure exerted by the tissue resulting from hypertrophic and hyperplastic reactions. Vessel elements originating from small parenchyma cells were observed 72h after inoculation. After 12 days of inoculation larger parenchyma cells, after 18 days, small giant cells and after 30 days of inoculation larger giant cells transformed into vessel like elements.

(ii) Phloem

The juveniles of *M. incognita* were found in the zone of differentiation of the growing root. The juveniles after penetration reached differentiation zone were found aligned in protophloem. After 48h of inoculation some cells, near the nematode head, changed into discrete giant cells. These giant cells enclosed dense cytoplasm and several nuclei. The size of giant cell increased after 72h inoculation. The parenchyma cells near giant cells increased in number (P. 3, 4 and 5).

The effect of infection was more severe on phloem strands then xylem strands. After six days of inoculation, changes in structure and orientation of phloem strands were noticed. The phloem strands near the giant cells comprised of sieve tube elements of shorter length and wider diameter. Near the giant cells, sieve tube elements resembled with ordinary parenchyma cells and their identification adjacent to the giant cells, became difficult. Near the giant cells and sieve tube elements hyperplastic parenchyma was observed. Although the shape and the size of these sieve tube elements corresponded to the surrounding cells but they could only be identified on the basis of the presence of slime plugs. The sieve tube elements of the phloem away from the giant cells did not show any abnormality. The sieve tube elements near the
giant cells were small and had varying shapes. Their shapes resembled with the neighbouring parenchyma cells. Abnormal sieve tube elements were noticed adjacent to the giant cells (P. 9 and 10).

Normal sieve tube were found to be broken in the zone of infection, after 12 days of inoculation (P. 11). The orientation of sieve tubes became non-uniform near the giant cells after 18 days of inoculation. The sieve tube elements in the infected zone were short as compared to uninfected zone and lacked a definite pattern. Abrupt changes in phloem elements and consequently in their dimensions were observed. In transverse sections, the sieve tube elements were seen alternately arranged in tangential and radial patches near the giant cells (P. 14 and 15).

Abnormal phloem was frequently observed near the giant cells (P. 18) after 24h of inoculation. After 30 days of inoculation, no further changes were observed and the main feature was the occurrence of newly hatched second stage–juveniles which either induced formation of fresh giant cells or fed on old ones. In transverse and longitudinal sections, it was found that abnormal sieve tube elements occurred adjacent to the giant cells. These sieve elements were connected with the giant cell on one side and with normal sieve tubes on the other side (P. 24).

**DISCUSSION**

Cell enlargement was noticed 24h after inoculation and formation of giant cells after 48h of inoculation as a result of hypertrophy induced by the nematode. From the serial sections it was revealed that in *Eclipta alba* the juveniles preferred the meristematic tissue for their feeding sites that would develop into phloem. The heads of juveniles were seen in the differentiating
phloem zone. Ediz and Dickerson (1976) reported most of the giant cells in phloem region. Primary phloem or adjacent parenchyma were selected as feeding sites by nearly all the root-knot nematodes (Byrne et al., 1977; Finley, 1981). Since the giant cells, induced by root-knot nematodes, are the highly metabolically active cells, therefore, in our opinion they must be linked with the phloem. Such association of giant cells with the phloem is essential for a continuous supply of assimilates towards the giant cells. This view was strengthened when sieve tube elements were observed abruptly ending at the giant cells, after 6 days of inoculation. Moreover, formation of sieve tube like elements from the hyperplastic parenchyma of the phloem further supported that giant cells had a strong link with the phloem.

After 12 days of inoculation, phloem appeared to be the highly affected and at this juveniles abnormalities in orientation, structure and origin of sieve tube elements were observed. Orientation of sieve tube elements was probably changed due to giant cell formation and nematode development. These two factors led the phloem strands to change their normal path. The sieve tube like elements were found randomly arranged around the giant cells. In any orientation, continuity of the phloem was not found disturbed. These finding supported the view that nematode did not destroy the galled tissue but instead it manipulated the affected region in favour of giant cell security. Jacobs and Marrow (1958) reported discontinuity in phloem soon after infection. Sieve tube elements of irregular shapes and sizes, were formed probably either because of hypertrophic reaction, or because of transformation of sieve tube elements form phloem parenchyma cells.

The juveniles that penetrated later on, probably, caused giant cell formation in pericycle. The cells of pericycle were induced to give rise to
lateral but fibrous roots which were attacked by other incoming juveniles. These giant cells and their related tissues (hyperplastic and hypertrophic) appeared as if there were in the cortex. Cortical giant cells have been reported by Krusberg and Nielsen, (1958); Ediz and Dickerson,(1976).

The studies on *E. alba*, on the other hand, led to an idea that the giant cells were not derived from cortical parenchyma but from undifferentiated meristematic cells of newly formed lateral roots. Cortical giant cell, therefore were not independent entities but were connected with phloem.

Orientation, shape and size of sieve tube elements were highly affected after 18 days of inoculation. Orientation and shapes of sieve tube elements were changed due to enlargement of nematode. Although there are reports that giant cells remained covered with xylem but did not mention about phloem. The giant cells were formed in the phloem tissue of roots, stolons, and tubers of potato as a result of *Meloidogyne* chitwoodi infection (Finley 1981). Completely suppressed phloem was reported by Swamy and Krishnamurthy .(1971) in *Meloidogyne incognita* infected *Basella* roots. Abnormal sieve tube elements with unusual orientations occurred in *Lagenaria* as a result of *M. javanica* infection (Siddiqui and Ghouse, 1975). Thus from our study it might be concluded that, the giant cells that appeared completely enclosed by abnormal xylem elements, were not actually completely covered. The giant cells had always connected with the phloem. The sieve tube elements appeared diverted towards the giant cells. The metabolites were continuously supplied to the giant cells.
EXPERIMENT 2

Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Eclipta alba*, reproduction of nematode and internal structure of root

INTRODUCTION

*Meloidogyne* species attack more than 3,000 species of plants comprising of almost all the cultivated plants. The characteristic underground symptom of the disease caused by *Meloidogyne* is the formation of knots or galls on the roots. The extent of galling in the roots varies depending on the species of both the host and the nematode involved in the host-parasite relationship (Balasubramanian and Rangaswami, 1964). Dropkin (1954) inoculated tomato and cucumber seedlings with *Meloidogyne incognita acrita* to discern infectivity and the size of the resulted gall. He hypothesized that each individual nematode produced a finite response on the root tissue. The number of nematodes in the gall might be predicted by measuring the gall size. In heavily infested roots it was difficult to predict the nematode number and to follow the hypothesis. Low or high population levels of the nematode produce different effects on plant growth and yield. At low inoculum levels, the plant growth and yield are sometimes enhanced, but most of the times suppressed. Wallace (1971) observed increase in growth at lower population levels and decrease at higher levels. The literature on host parasite relationship between *M. incognita* and *Eclipta alba* is scanty. Furthermore, there are no reports on histopathological studies. The efforts have been made to study the effects of different inoculum levels on: (i) plant growth (ii) number of capitula per plant, number of seeds per capitulum and seed weight (iii) number and size of the
galls (iv) number of egg masses per root system (v) number of eggs per egg mass in roots and (vi) number of mature females per gram root of *Eclipta alba*.

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) nature of the giant cell cytoplasm, nuclei and nucleoli, and (iv) abnormalities in xylem and phloem were studied.

**MATERIALS AND METHODS**

**Preparation and Sterilization of Soil Mixture:**

For performing the experiments, soil was prepared in the ratio of 7:3:1 comprising of clay, sand and farmyard manure, respectively. The pots were filled with soil at the rate of one kg of soil per pot. The soil was moistened with water prior to transferring the pots into an autoclave. The soil was sterilized at 20 lb pressure for 20 minutes. Sterilized pots were allowed to cool at room temperature before use for experiments.

**Raising and Maintenance of Test Plants:**

The seeds of *Eclipta alba* were surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2 minutes and washed thrice in sterilized distilled water. The seeds were allowed to germinate on moistened filter paper in sterilized petri-dishes. The seedlings at the two leaf stage were then transplanted into the pots. Initially there were five seedlings per pot which were thinned to three per pot.
Preparation of Inoculum:

*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-plants (*Solanum melongena* L.) roots in a glass house by using single egg mass. The egg mass from the galled roots of egg mass were picked with the help of sterilized forceps and the egg mass was washed thrice with distilled water. The eggs in the egg mass were allowed to hatch out at 28±2°C under aseptic conditions in a sieve lined with tissue paper and kept in a petri-dish containing sufficient amount of sterilized distilled water. 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 20, 200, 2,000, 20,000 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 complete randomized block design. Uninoculated set of plants served as control. There were five sets of pots as given below:

- **C**: Control
- **T₁**: 20 J₂/pot
- **T₂**: 200 J₂/pot
- **T₃**: 2,000 J₂/pot
- **T₄**: 20,000 J₂/pot

The plants were watered regularly whenever required. The plants were harvested after 60 days of inoculation.
PARAMETERS

Plant Growth:

The roots were washed thoroughly in slow running tap water. Utmost care was taken to avoid loss and injury of root system during the entire operation. The number of leaves per plant were counted visually. For measuring length, fresh and dry weights, the plants were cut with a sharp razor just above the base of root emergence. Lengths of the shoots and the roots were recorded in centimeters from the cut end to the tip of first leaf and the longest root, respectively. The shoots and the roots of the plants of each treatment, when fresh, were weighed separately. These were kept in envelopes separately for drying in an oven running at 80°C for 48 hours and the weight was recorded in grams.

Leaf Area:

Five mature leaves of medium size were randomly selected from each treatment. Outline of the shape of each leaf was drawn on butter paper and the area occupied was measured with the help of planimeter.

Yield:

The yield parameter in terms of number of capitula per plant, seeds per capitulum and weight of 100 seeds, randomly selected from each treatment were considered.

Number and Size of Galls: Total number of galls produced on the root system of each plant was recorded separately. The size of the gall was measured by taking maximum length and width (in mm²) with the meter scale.
Number of Egg Masses:

Total number of egg masses on infected roots was counted by staining egg masses with phloxin B. An aqueous solution of phloxin B 0.15g per liter of water was prepared. The galled roots were placed in this solution for 15-20 minutes. The roots were rinsed in tap water. The egg masses became prominent after staining, which were counted.

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 eggs released from gelatinous matrix of egg masses were stained with acid fuchsin (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 treatment 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 Cobbs sieving and decanting method, and root population was estimated by Blender Baerman tray method (Hooper, 1985).
Reproduction factor (Rf) was calculated by the formula:

\[ Rf = \frac{P_f}{P_i} \]

Where \( P_f \) is the final population and \( P_i \) is the initial population. Rate of population increase (RPI) was calculated by the formula:

\[ RPI = \left( \frac{P_f - P_i}{P_i} \right) \]

Statistical Analysis:

The data obtained were analysed statistically and significance was calculated at \( P = 0.05 \) and \( P = 0.01 \) levels of probability.

HISTOPATHOLOGICAL STUDIES

From the infected roots, few galled portions were selected from each treatment for performing histopathological studies. The galled portions were cut and fixed in formalin aceto-alcohol (F.A.A) and then dehydrated through tertiary butyl alcohol (TBA) 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 10 \( \mu \)m 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 24 hours (Johansen, 1940) (Table –1). The sections were stained with safranin and fast green as described by Sass (1951) (Table–2). The sections were mounted in Canada balsam. For anatomical details, the sections were observed under light microscope and necessary photographs were taken.
RESULTS

Root and Shoot Length:

The length of the root and the shoot of *Eclipta alba*, inoculated with different inoculum levels of second-stage juveniles of *M. incognita* decreased, when compared with uninoculated (control) plants (P. 48). The reduction in plant length of T₁ plant was non-significant at the initial inoculum level of Pi = 20 J₂ per pot. Significant reductions (P ≤ 0.05) in T₂ and (P ≤ 0.01) in T₃ and T₄ plants were observed in comparison to control (C). The reduction was maximum in T₄ plants grown at Pi = 20,000 J₂ per pot (Table-3). There was higher reduction in the length of the roots than of the shoots.

Root and Shoot Weight:

Fresh and dry weight of the roots and the shoots of *M. incognita* inoculated plants exhibited reduction over the control (C). The weight of the roots and shoots reduced non-significantly in T₁ plants and significantly (P ≤ 0.05) in T₂ plants, inoculated with Pi = 200J₂. A significant (P ≤ 0.01) reduction in fresh and the dry weight of the roots and the shoots, in comparison to control, was noticed at Pi = 2,000 J₂ 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 treatment T₄ grown at the initial inoculum level of Pi = 20,000J₂, in comparison to control (Table –3).

Number of Leaves:

The number of leaves showed variation in all the treatments. There was no significant difference in the number of leaves of T₁ plants, when compared with control. A significant reduction was noticed in T₂ (P ≤ 0.05) and T₃ (P ≤
0.01) plants. Reduction in the number of leaves per plants was highest and significant (P<0.01) in T₄ plants, inoculated with Pi= 20,000 J₂ per pot, when compared with the control (Table-3).

**Leaf Area:**

The leaf area of the plants inoculated with *M. incognita*, when compared with control, decreased non-significantly at the lowest initial inoculum level of Pi = 20J₂. The reduction in leaf area was significant (P<0.05) at the next higher initial inoculum level of Pi = 200 J₂ (in T₂ plants), when compared with control. Higher and significant (P<0.01) reductions in leaf area were noticed in T₃ and T₄ plants, inoculated with Pi = 2,000J₂ and Pi = 20,000 J₂, over the control (Table-3).

**Yield:**

The yield of the plant, in terms of the number of capitula per plant and seeds per capitulum, decreased slightly and non-significantly in T₁ and significantly (P ≤ 0.05) in T₂ plants, which were inoculated with Pi = 20J₂ and Pi = 200J₂, respectively. A significant (P≤0.01) reduction in yield was observed in T₃ plants. The number of capitula per plant and seeds per capitulum attained maximum and significant (P ≤ 0.01) reduction at the highest inoculum of Pi = 20,000J₂ (in T₄ plants) (Table-3).

**Seed Weight:**

After maturation and ripening of the capitula, 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 plant. The seed weight of T₁ plants was slightly and non-significantly lower than the control
(C). Significant ($P \leq 0.05$) reductions, in comparison to control, in $T_2$ and ($P \leq 0.01$) $T_3$ plants, that were grown at $Pi = 200 J_2$ and $Pi = 2,000 J_2$ per pot was noticed. Maximum and significant ($P \leq 0.01$) reductions, in comparison to control, were found in the weights of the seeds at the initial inoculum level of $Pi = 20,000 J_2$ per pot, in $T_4$ plants (Table-3).

Number of Galls:

The number of galls per root system increased with the increase in initial inoculum level. The galls were smaller and unnoticeable on the roots of $T_1$ plants that were grown at the lowest initial inoculum level of $Pi = 20 J_2$. The number of galls on the roots of $T_2$ plants at $200 J_2$ was higher and significant ($P \leq 0.05$), 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 ($Pi = 2,000 J_2$), in comparison to lowest inoculum level ($Pi = 20 J_2$) was recorded. The number of galls also increased greatly and significantly ($P \leq 0.01$) at the next higher inoculum level of $Pi = 20,000 J_2$ in comparison to the lowest inoculum level (Table-4).

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 level. A significant ($P \leq 0.05$) increase in gall size was noticed on $T_2$ plants at $Pi = 200 J_2$, when compared with lowest inoculum level ($T_1$ plants). A significant ($P \leq 0.01$) increase in size of the gall was noticed at $Pi = 2,000 J_2$, when comparisons were made with the galls of $T_1$ plants. The galls attained maximum size at the highest inoculum level of $Pi = 20,000 J_2$ which were significantly ($P \leq 0.01$) larger when compared with the size of the galls on $T_1$ plant. (Table-4).
Number of Egg Masses:

The number of egg masses per roots system increased as the number of juveniles introduced per plant increased. At the lowest inoculum level of $Pi = 20 J_2$, the average number of egg masses recorded was 6.2 per root system. The number of egg masses per root system was significantly ($P \leq 0.05$) increased on $T_2$ plants $Pi = 200 J_2$, when compared with the $T_1$ plants. A significant increase ($P \leq 0.01$) was observed at $Pi = 2,000 J_2$ in comparison to the lowest inoculum level $Pi = 20J_2$. The increase in the number of egg masses per roots system was significantly ($P \leq 0.01$) higher as observed at $Pi = 20,000 J_2$, in comparison to $T_1$, $T_2$, and $T_3$ plants (Table- 4).

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 ($P \leq 0.01$) at all the inoculum levels ($Pi = 200, 2,000$ and $20,000 J_2$), as compared with $T_1$ plants (Table –4).

Number of Mature Females:

The number of mature females recovered from the inoculated roots increased with an increase in the initial inoculum level. The number of mature females per gram root was low (4.2) at the initial inoculum level of $Pi = 20J_2$ on $T_1$ plants. A significant ($P \leq 0.05$) increase in the number of the mature females per gram root was observed at the initial inoculum level $Pi = 200 J_2$ ($T_2$ plants) compared with $T_1$ plants. The number of mature females per gram root significantly ($P \leq 0.01$) increased at the initial inoculum level of $Pi = 2,000 J_2$ than $T_1$ plants. Maximum number of mature females was collected from the
roots of the plants at the highest inoculum level ($Pi = 20,000 J_2$) followed by $Pi = 2,000 J_2$, $Pi = 200 J_2$ and $Pi = 20 J_2$ (Table-4).

**Reproduction Factors and Rate of Population Increase:**

Reproduction factor (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 levels (Table-4).

**Histopathology of the infected roots:**

Infestation of root-knot nematode in the roots of *Eclipta alba* was revealed when transverse and longitudinal section of infected roots were examined. The second-stage juveniles after entering the young roots traversed the cortex, or meristematic zone and reached the zone of vascular differentiation. The meristematic cells and some parenchyma cells along the length of second-stage juveniles became enlarged (P.25). The juveniles after reaching the meristematic tissue, or vascular differentiation zone established a successful host parasite relationship by inducing giant cells. They induced 6-8 multinucleate giant cells in vascular tissues sometimes, two or more second-stage juveniles induced a common feeding site that resulted in the formation of more than ten giant cells (P.26). In heavily infested roots, it is a usual phenomenon that two or more nematodes share the same feeding site. The second-stage juveniles finally mature into females that start egg laying soon after maturation.

The giant cells are formed near the nematode head, and in the vicinity of the giant cells hypertrophic and hyperplastic reactions lead to the formation of knot like structure called “gall”. The giant cells had thick cell wall with dense and granular cytoplasm. In each giant cell there were several, enlarged,
scattered or aggregated nuclei (P. 27, 28 and 29). There were differences in the anatomy of galled roots at lower and higher inoculum levels. The number of mature females and the number of giant cell complexes in the galled roots, as revealed by transverse sections, were less at lower (Pi = 20J₂ and 200J₂) inoculum levels than at higher inoculum levels. In most of the transverse sections it was found that one mature female associated with one giant cell complex occupied only one parenchyma ray of the root at lower (Pi = 20J₂ and 200J₂) inoculum levels. At lower inoculum levels, the giant cells were larger and their cytoplasm was dense and granular. There were several nuclei per giant cell. The nuclei were larger having dense nucleoplasm (P.30).

At higher inoculum levels, all the four parenchymatous rays were found occupied by the nematode and the giant cells (P.31, 32). At inoculum levels of Pi = 200, 2000 and 20,000J₂ the giant cell cytoplasm was less dense and vacuolated (P.32). There were several instance where it was found that two or more nematodes contributing in the formation in the giant cell complex. At such location the giant cells were highly vacuolated and nuclei were lesser in number. The shapes of nuclei at lower inoculum levels (Pi = 20 J₂) were amoeboid, circular and oval but at higher inoculum levels the nuclei were mostly amoeboid. The number of nucleoli varied from 4-10 in a single nucleus, but 4-6 were frequently observed (P. 28). Abnormalities in the arrangement of xylem and phloem strands were seen at all the inoculum levels but amount of abnormal xylem was higher at higher inoculum levels than at lower inoculum levels (P. 32). Amount of abnormal phloem near the giant cell complexes was higher at higher inoculum levels than at lower inoculum levels (P.33 and P.34).
DISCUSSION

Plant parasitic nematodes are capable of producing recognizable disease symptoms on many economically important medicinal plants. Root-knot nematode, *Meloidogyne incognita* imparted disease symptoms on the roots of *Eclipta alba* at all the inoculum levels. The most commonly observed above ground symptoms of the root-knot disease were dwarfing and stunting as is evident from Table- 3 and P. 48. The lowest inoculum level caused reduction in the length of the plant through the reduction was non-significant. An increase in the number of juveniles per pot further decreased the length of the plant. Greater decrease in plant length at higher inoculum levels indicated that the severity of the disease was increased. Although there were reports that plant length increased at low inoculum level due to stimulation of the infected root tissue (Wallace, 1971; Yasmeen, 2002), but in *E. alba* reduction in length showed that root tissues were not stimulated for the benefit of the plant.

Change in amount of inoculum level not only caused reduction in plant length but also in fresh as well as dry weights of the roots and the shoots. With an increase in initial inoculum level there was decrease in plant length, correspondingly there was decrease in fresh weight and dry weight of the root and the shoot. From this finding it might be inferred that *E. alba* served as a good host for the root–knot nematode, *M. incognita*.

The deleterious effects on the growth of different plants with an increase in primary inoculum level of *Meloidogyne* spp. have been reported by several workers like Christie (1936) on tomato; Krusberg and Nielson (1958) on sugar beet; Wallace (1974) on tomato; Ferris (1974) on tobacco; Appel and Lewis (1984) on soybean; Ibrahim and Lewis (1985) on soybean; Hisamuddin (1992)

The data revealed that with an increase in initial inoculum levels there was decrease in number of leaves per plant and in leaf area. This indicated that severity of the root-knot disease was expressed as reduction in leaf number and leaf area. Reduction in leaf area and leaf number might be attributed to availability of metabolites at lower rate probably because mineral elements from the roots to the leaves were not transported properly, or the transport of mineral elements towards leaves was checked in the affected tissue of the root, or the minerals transported towards the leaves were not properly metabolized in the leaves, caused production of photosynthates in small amount. It appeared that less availability of photosynthates in growing regions resulted in lesser number of leaves, smaller leaves, shorter height of plant and decrease in weight that ultimately reduced plant growth. Bird (1962, 1968) reported decrease in rate of photosynthesis in tomato with an increase in the initial inoculum levels.

From these findings, it might be inferred that *E. alba* the number of capitula per plant, the number of seeds per capitulum and the average weight of seeds decreased with increase in inoculum levels that resulted in yield
reduction over control plants. There might be probable reasons for yield reduction. First, the rate of metabolism decreased in infected plant and consequently metabolic products were not synthesized in sufficient amount. Second, the metabolic products were unidirectionally transported towards unnatural sinks in the affected parts of the plants, the giant cells. The sedentary endoparasitic nematode removed the metabolites directly from the giant cells. All these unusual phenomena resulted in transport of metabolites in low amount towards the growing parts specifically developing reproductive parts. Due to insufficient supply of photosynthates, the number of capitula, and seeds decreased. Moreover seed weight was also decreased which in other words, affected the next generation.

Wallace (1963) and Oostenbrink (1966) opined that increase in nematode population and subsequently reductions in the yield were directly influenced by initial density of the nematode in the soil. With an increase in the initial inoculum level, there was a corresponding reduction in the seed weight of *E. alba*, with greatest reduction at the highest inoculum level. Retarded shoot growth and nutritional deficiency symptoms in the foliage of root-knot nematode infected plants were reported by Oteifa (1952). The poor nutrient uptake and suppressed plant growth with highly reduced root system are generally the results of root-knot nematode infection. The upward nutrient transport was checked upto certain extent that resulted reduction in leaf area (Hunter, 1958 and Jonathan and Rajendran, 2000a).

Root-knot nematode influenced the ascent of sap from root to shoot and translocation of metabolites from leaves to the growing vegetative organs and caused reduction in the production of photosynthetic pigment (Loveys and Bird, 1973; Wallace, 1974; Melakeberhan *et al.*, 1985; Poornima and Vadivelu,
1998; and Tiyagi et al., 2001). The root-knot nematode infections, the photosynthates were diverted towards the giant cells that served as special transfer cells or metabolic sinks. All these malfunctions contributed in the suppression of plant growth and yield (Hussey, 1985). Olthof and Potter (1972) estimated commercial loss up to 46 and 64% for onions and potatoes, respectively, at an inoculum level of 28,000 J2 per plant. Barker (1977) assessed yield losses from 3.7 to 19.9% due to M. arenaria, M. hapla, M. incognita and M. javanica for each tenfold increase in initial density for each species.

The characteristic symptoms of root-knot disease are the galls or the knots which were observed on the infected roots of E. alba. The number of galls occurring on a system is a measure of disease severity. The severity of the disease on E. alba increased with increase in initial inoculum level and was expressed as higher number of galls on the roots. Higher number of nematodes in the vicinity of the root caused development of the galls in greater number as has also been reported by several workers like McClure and Viglierchio, (1966); Arens et al., (1981); Bhat (1999). Ibrahim and Lewis (1985) observed enhanced gall number in soybean with corresponding increase in initial inoculum level of M. incognita. Parveen (2006) reported this trend might be due to the fact that at higher inoculum levels larger number of juveniles entered into the roots and formed new feeding sites, which resulted in increased number of galls on the infected roots.

Number of mature females recovered per gram root was increased with increase in initial inoculum level on E. alba. Number of females per gram root is a criterion of acuteness of the disease. Usually higher is the number of the galls per root system, higher will be the number of females per gram root.
Sometimes it might not be true as in case of some plants underground symptoms are not well pronounced.

In *E. alba* the number of gall per root system and the number of mature females per gram root were reciprocal. At low inoculum level, number of galls was less and size of galls was small and correspondingly fewer number of females were present in the galls. At higher inoculum levels gall number was high, gall size was larger and relatively higher number of females were recovered. This further supported the view that *E. alba* served as a good host. Similar finding in other plants have been reported by Jonathan and Rajendran (2000a); Yasmeen, (2002), and Parveen (2006).

The present study indicated that the number of egg masses per plant increased with increase in inoculum level. It appeared quite reasonable because lower the inoculum level, the lower would be the number of mature females in the galled roots and consequently fewer would be the egg masses; higher the inoculum level then higher would be the number of mature females as well as number of egg masses. The number of eggs per egg mass significantly decreased at higher inoculum levels. Probably the number of the mature females and the number of egg masses per root system might be interrelated. The number of egg masses per plant and the number of eggs per egg mass were influenced by the nematode density in a root system. The limited food and space were probably disadvantageous for the proper development of the nematode that consequently deterred in egg mass production. Insufficient nutrient supply might be the main cause after the lower rate of 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 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. A negative correlation between nematode multiplication and inoculum level was also reported by Rombati and Dhanachand (2000). Jonathan and Rajendran (2000a) attributed that decrease in multiplication rate at high initial inoculum created crowded conditions, which adversely affected the rate of development of the nematode. 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.

In the roots of Eclipta alba several abnormalities were noticed in the structure of galled portions. The earliest response was the hypertrophy in the vicinity of the nematode. The magnitude of hypertrophy near the oral parts was tremendous than at other places. The hypertrophic response at low magnitude was noticed quite away from nematode head. Near the developing giant cell, hyperplastic response was also encountered. The hypertrophic response near the nematode head resulted in the formation of multinucleate giant cells in the vascular tissue of stelar region. In tomato 3-6 giant cells were reported (Christie, 1936); 4-9 giant cells in sweet potato (Krusberg and Nielsen (1958); 2-5 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). The secretions of nematode stimulated the cells to enlarge that synthesized cytoplasm in large amount. These enlarged cells commonly called as giant cells, adjacent to the nematode head, were much larger than normal cells. The giant cells were developed in tomato as a result of continuous stimulus received from the nematode (Bird, 1962). The giant cell nearest to the nematode head was directly stimulated and hence was larger than those which were further, and received stimulus indirectly. This indicated that formation of giant cells is essential for a successful host parasite relationship. In Eclipta alba the giant cells and nematode were seen in all the four parenchyma rays of tetrarch roots. Formation of giant cells, development of the nematode and formation of hyperplastic tissue in the affected portion caused various anatomical abnormalities. Deviation in course of vascular strands, enlargement of normal vessel elements, formation of abnormal vessel and sieve tube elements were detected in affected roots of E. alba. Average size of the giant cell was smaller at higher inoculum levels probably because of limited amount of food and space.

The depletion of giant cells cytoplasm at higher inoculum levels indicated speedy removal of metabolites by the nematode. Bird and Loveys (1975) and McClure (1977) unequivocally asserted that Meloidogyne acts as a metabolic sink in affected plants. The increased metabolic activity of giant cells stimulates mobilization of photosynthates from the shoots towards the roots and particularly to the giant cells from where the metabolites are evacuated and consumed by the nematode. In E. alba formation of dense cytoplasm in the giant cells at lower inoculum levels indicated that the host plant synthesized more cytoplasm that was made available to the nematode. Less dense and less
granular cytoplasm in the giant cells at higher inoculum levels indicated that the nematode evacuated the cytoplasm at a higher rate than the rate of synthesis. At higher inoculum levels the nematode removed nuclei along with the cytoplasm, therefore the rate of production of cytoplasmic material was decreased. At lower inoculum level, higher number of nuclei in the giant cells maintained higher rate of metabolic activity in the giant cells.

Formation of abnormal xylem near the giant cell complex is a unique phenomenon associated with the root-knot disease development. Excessive amount of abnormal xylem has been reported in some host plants which is an adaptive feature of the plants under disease stress (Siddiqui et al., 1974). At higher inoculum levels several nematodes induced formation of giant cells at a particular site therefore, it was supposed that each nematode had its own affect that increased the over all effect. Higher number of nematodes resulted in hypertrophic and hyperplastic reactions at greater of number of sites. Due to induction of these two kinds of reaction more locations were affected. After certain duration of induction of giant cell formation more amount of abnormal xylem was differentiated. Since xylem elements are conspicuous and can be detected easily therefore it appeared that xylem became abnormal. In all the host parasites interactions abnormalities in phloem were also confirmed. The phloem strands were invariably found associated with the giant cells. Due to various metabolic reaction that were induced by the nematode, the shapes and size of phloem elements, specially sieve tube elements were changed.
EXPERIMENT 3

Effect of Meloidogyne incognita infection on plant growth, chlorophyll pigment, protein and oil content of Eclipta alba

INTRODUCTION

Plant parasitic nematode, Meloidogyne incognita alters the metabolic processes of the host, which are manifested in the form of cellular, physiological and biochemical changes occurring in the infected host. Some of the common responses of the plant to nematode infestation are enhanced respiration, reduced photosynthesis, stimulated protein and nucleic acid synthesis, accumulation of metabolites at the site of infestation, enhanced enzyme activity and hyperauxinity. As a result of Meloidogyne infection, amounts of chlorophyll pigment, protein and oil content are also changed. Reduction in chlorophyll content of the infected plants has been reported by Vashishth et al., (1994); Poornima and Vadivelu (1998); Ramakrishnan and Rajendran (1998); and Parveen et al., (2006a). Increase in protein content in the infected roots was reported in tomato (Bird, 1962); Chilli (Trivedi and Tiagi, 1980, 1986; Pandey and Trivedi, 1991); and okra (Sharma, 1992). Reduction in oil content of various plants has been reported by Pandey (1988); Pandey et al., (1992); Haseeb et al., (1998); Tiyagi et al., (2001) and Parveen, (2006). The present work was performed on Eclipta alba to find out (i) plant growth by measuring the length of roots and shoots and weighing the roots and shoots, (ii) to estimate the amount of chlorophyll a and chlorophyll b, (iii) to estimate protein content of roots and shoots and (iv) to estimate oil contents of the seeds. These results were correlated with the disease occurrence.
MATERIALS AND METHODS

Maintenance and Inoculation of Test Plants:

The seeds of *Eclipta alba* were surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2 minutes and washed thrice in sterilized distilled water. The seeds of *E. alba* were allowed to germinate on moistened filter paper in sterilized petri-dishes. Two-leaf stage seedlings, were transplanted into the clay pots of 15 cm diameters having steam sterilized soil (7 clay: 3 sand: 1 farmyard manure). The required number of freshly hatched second-stage juveniles (J₂) were obtained by the method described in Experiment 2 (Section-I). The plants were inoculated with (0, 20, 200, 2000, and 20,000 J₂) with five replicates in each treatment. The pots were kept in greenhouse condition and were watered regularly.

PARAMETERS

Plant Growth:

The plants were harvested after three months and the roots were washed gently with tap water. The lengths of roots and shoots were recorded in centimeters from the cut end to the tip of first leaf and the longest root. After taking fresh weights of roots and shoots these were placed in envelopes and kept in an oven at 80°C for 24 hours and dry weight was recorded in grams.

Leaf Area:

Leaf area was ascertained by the method described in Experiment 2 (Section-I).
Estimation of Chlorophyll Pigments:

Amount of chlorophyll was estimated by following the method of Mackinney (1941). Fresh leaves (100mg) were homogenized in a mortar with sufficient quantity of 80% acetone. The extract was filtered and supernatant was collected in the volumetric flask. The process was repeated thrice and each time supernatant was collected in the same flask. Finally the volume was made up to 100 ml with 80% acetone. 5 mL sample of leaf, extract was transferred to a cuvette and absorbance was read at 645 and 663 nm on spectrophotometer for the estimation of chlorophyll a and b, respectively. Calculation were performed as follows:

\[
\text{Chlorophyll a} = \left[ 12.7 (D_{663}) - 2.69 (D_{645}) \right] \times \frac{V}{1000 \times W} \text{ (mg g}^{-1})
\]

\[
\text{Chlorophyll b} = \left[ 22.9 (D_{645}) - 4.68 (D_{663}) \right] \times \frac{V}{1000 \times W}
\]

where,

\begin{align*}
V & = \text{ total volume of the solution (mL)} \\
W & = \text{ weight of the tissue (g) used for the extraction of the pigments} \\
D & = \text{ Density of samples at 645 and 663 nm wave lengths}
\end{align*}

Estimation of Total Protein Content:

The plant material of each treatment collected at harvest time was dried and ground to fine powder and passed through a 72 mm mesh sieve. Before analysis, these samples were kept at 80°C in an oven overnight.
The method of the Lowry et al. (1951) was followed. 50 mg of oven dried powder was transferred to a mortar. 1 mL of 5% trichloroacetic acid was added to it. The powder was ground well and then transferred to a centrifuge tube with repeated washing and the final volume was made up to 5 mL with trichloroacetic acid. Complete precipitation of the proteins was allowed to take place by leaving the sample for about 1 hour. The samples were then centrifuged at 4,000 rpm for 15 minutes and the supernatant was discarded. 5 mL of 1N sodium hydroxide was added to the residue and mixed well by shaking. It was left for 30 minutes on a water bath running at 60°C so as to dissolve the precipitated proteins completely. After cooling, for 15 minutes and the supernatant containing the protein fraction together with three washings of 1N NaOH was collected in 25 mL volumetric flask. Volume was made up to the mark with 1N NaOH and used for the estimation of proteins. One ml of sodium hydroxide extract was transferred into 10 mL test tube and 5 mL reagent B was added to it. The solution was mixed well and allowed to stand at room temperature for 10 min. To it, 0.5 mL folin phenol reagent was added rapidly with immediate mixing. The content started turning blue. It was left for 30 minutes for maximum colour development. The absorbance of this solution was read at 660 nm. A blank was run simultaneously with each sample. The protein contents were calculated by comparing the optical density of each sample with calibration curve plotted by taking known graded dilutions of standard solution of bovine serum albumin. The protein contents were expressed in terms of percentage on dry weight basis.

**Standard Curve for Total Protein:**

50 mg bovine serum albumin was dissolved in 50 mL DDW, of this solution was again diluted to 50 mL. Thus, 1 mL of this solution contained
200μg protein. Of this 0.2, 0.4, 0.6, 0.8 and 1.0 mL transferred to 5 test tubes separately. The solution in each test tube was diluted to 1 mL with DDW. A blank was also run with each set of determination. To each test tube, in 5 mL reagent B was added. The content were allowed to stand for 10 minutes. To this, 0.5 mL Folin phenol reagent was added, mixed well and incubated at room temperature in the dark for 30 minutes. The optical density of the blue colour solution thus obtained was read at 660 nm.

**Reagent A:** 2% sodium carbonate in 0.1N Sodium Hydroxide

**Reagent B:** 0.5% Copper Sulphate (CuSO₄·5HO₂)

**Reagent C:** Alkaline Copper Solution: Mix 50mL of Reagent A and 1mL of Reagent B.

**Folin’s Phenol Reagent:**

100g sodium tungstate and 25g sodium molybdate dissolved in 100 ml DDW to which 50 mL 85% phosphoric acid and 100 mL concentrated hydrochloric acid was added. The solution was refluxed on heating mantle for 10h. At the end, 150g lithium sulphate, 50 mL DDW and 3-4 drops liquid bromine were added. The reflux condenser was removed and solution was boiled for 15 minutes to remove excess bromine. Cool and diluted upto 1000 mL. The strength of this acidic solution was adjusted to 1N by titrating it with 1N sodium hydroxide solution using phenolphthalein indicator.

**Estimation of Oil Content of Seed:**

This is an important parameter for oilseeds researchers. The simple procedure adopted for assessing the oil content of seeds is described below:
Preparation of Seed Sample for Analysis:

In seeds were passed through the sieve to remove foreign materials. The sieves used had pore size a bit smaller than the size of the seeds. The impurities larger than the seeds were hand picked.

Grinding of Seed Sample:

In order to get the powder of seeds, grinding was done in an electrical grinder until the formation of a fine meal.

Extraction of Oil:

25g of powdered seed meal was transferred to the flask of soxhlet apparatus and sufficient quantity of pure petroleum ether was added. The apparatus was kept on a water bath, running at 60°C, for about six hr. At the end of each extraction process, the petroleum extract of seeds was left in the air to evaporate the petroleum ether. The oil left after the evaporation of petroleum ether was weighed and expressed as percentage of the mass of the seeds. The percentage of oil was calculated by the following formula:

\[
\text{Oil content (\%)} = \frac{m}{m_0} \times 100
\]

where,

\(m\) = mass of oil in gram; \(m_0\) = mass of seed sample in gram
RESULTS

Root and Shoot Length:

The root and shoot lengths of *Eclipta alba* decreased non-significantly at lower initial inoculum level of Pi = 20 J\(_2\) in T\(_1\) plants, when compared with uninoculated control (C). A significant (P<0.05) decrease in the root and shoot lengths, in comparison to control (C), occurred in T\(_2\) plants. In T\(_3\) and T\(_4\) plants, at higher inoculum levels of Pi = 2,000 J\(_2\), Pi = 20,000 J\(_2\), the root and shoot lengths decreased greatly and significantly (P<0.01), over the control. Reduction in length was highest (48.14% in roots and 37.23% in shoots) in T\(_4\) plants, grown at the highest initial inoculum level, and lowest in T\(_1\) plants (Table-5).

Root and Shoot Weights:

In comparison to control (C), fresh and dry weights of the roots and the shoots, decreased with an increase in initial inoculum level. The reductions were non-significant at lower initial inoculum level of Pi = 20 J\(_2\) and significant (P<0.05) at the next higher inoculum level of Pi = 200 J\(_2\) (T\(_2\) plants). Significant (P<0.01) reductions in T\(_3\) and T\(_4\) plants were observed, when compared with control (C). The extent of reductions in fresh weights of the roots and the shoots were 45.04% and 34.56%, respectively. The reductions in dry weights of the roots and the shoots were 49.13% and 41.14%, respectively (Table-5).

Leaf Area:

The leaf area of the plant, in comparison to control, decreased non-significantly at the lowest initial inoculum level of Pi = 20 J\(_2\) and significantly (P<0.05) at the next higher inoculum level of Pi = 200 J\(_2\) (T\(_1\) and T\(_2\) plants,
respectively). Reductions in leaf area were higher and significant (P<0.01) at an initial inoculum levels of Pi = 2,000 J2 and Pi = 20,000 J2, over the control (C). Highest reduction in leaf area (36.32%) was noticed in T4 plants (Table-5).

**Chlorophyll Pigments:**

In comparison to control (C), the amount of chlorophyll in the leaves of *Eclipta alba* decreased slightly but non-significantly at an initial inoculum level of Pi = 20 J2 in T1 plants. There was a significant (P<0.05) decrease in the amount of chlorophyll pigments at the next higher inoculum level of Pi = 200 J2 in T2 plants. The chlorophyll a and chlorophyll b content decreased significantly (P<0.01) at higher inoculum levels i.e. Pi = 2,000 J2 and Pi = 20,000 J2, when compared with control. Maximum reductions in amount of chlorophyll a, and chlorophyll b (10.0%, and 7.37%, respectively) were observed in T4 plants (Table-5).

**Protein Content:**

**Root:** In comparison to control, the protein content of root of *Eclipta alba* increased at all the initial inoculum levels (T1 to T3 plants), but the increase was non-significant. A significant (P<0.05) enhancement in protein content of the roots was observed (3.24%) in T4 plants (Table-5).

**Shoot:** The protein contents of the shoots of *Eclipta alba* exhibited reduction at all the initial inoculum levels, when compared with the protein content of uninoculated control. The reduction was non-significant in T1 plants, grown at the initial inoculum level of Pi = 20 J2 per pot. In comparison to control, there was a significant (P<0.05) decrease in T2 plants, grown at the initial inoculum level of Pi = 200 J2. A significant (P<0.01) reduction in protein content of
shoots in T3 and T4 plants was observed. The reduction was maximum (8.13%) in T4 plants grown at Pi = 20,000 J2 per pot (Table-5).

Seed Oil Content:

The amount of oil in seeds of *Eclipta alba* decreased non-significantly at the initial inoculum levels of Pi = 20 J2 and significant (P<0.05) at Pi = 200 J2 (T1 and T2 plants, respectively). Significant (P<0.01) reductions in oil content were noticed in T3 and T4 plants grown at Pi = 2,000 J2 and Pi = 20,000 J2. The reductions in oil content was maximum (15.04%) in T4 plants, grown at Pi = 20,000 J2 per pot (Table-5).

Statistical Analysis:

The data obtained were analyzed statistically and significance was calculated at P≤0.05 and P≤0.01 levels of probability

**DISCUSSION**

Initial inoculum levels of *Meloidogyne incognita* produced different effects on *Eclipta alba*. The growth of the plant responded negatively towards the lower as well as higher inoculum levels of *M. incognita*. The effects, in terms of plant growth, leaf area, chlorophyll pigments, protein and oil content were pronounced and significant at both the higher inoculum levels (Pi = 2,000 J2 and Pi = 20,000J2), as was revealed from the data (Table-5). There was an inverse relationship between the population of juveniles and plant growth and other characteristic. Reduction in plant growth might have occurred due to removal of plant metabolites by the nematodes. The threshold level of the population of the juveniles of *M. incognita* that caused significant reduction in plant length and weight was 200 J2 as is obvious from Table - 5. 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. Reductions in plant growth, as a result of M. incognita infection, have been reported by Barker and Olthof (1976); Rodriguez-Kabana and Williams (1981); Kinloch (1982); Ibrahim and Lewis (1985); Gupta et al., (1995); Fazal et al., (1996a); Ramakrishnan and Rajendran (1998); Sharma et al., (1999); Singh and Goswami (2000); Tiyagi et al., (2001); Nehra and Trivedi (2002); Khan (2003); Youssef and El-Nagdi (2004); Kumar and Pathak (2004); Khan and Ashraf (2005); Parveen et al., (2006a); and Park et al., (2007) on various plants.

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 materials 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.

Infection caused by *M. incognita* also leads to reduction in leaf area along with the reduction in height and weights as has been found by Ramakrishnan and Rajendran (1998) and, Jonathan and Rajendran (2000a). The leaves *Luffa cylindrica* become dark green when the plants were grown in excess of nitrogen and inoculated with *M. incognita* (Hisamuddin, 1992). In the root-knot nematode (*M. incognita*) infected plants of *E. alba*, reduction in amount of chlorophyll pigments might be due to decreased rate of translocation of minerals from root to shoot, or due to higher rate of translocation of metabolites from shoots to roots. This phenomenon seems to be operating because of change in anatomical structures of galled roots. Higher amount of abnormal xylem, abnormal shapes of vessel elements, abnormal arrangements of vessel elements might not favour a normal upward movement of water. On the other hand normal phloem in shoot part does not hinder movement of metabolites towards the roots. Availability of nutrients in small amount in tomato due to *M. incognita* infection might be responsible in the reduction of leaf chlorophyll (Loveys and Bird, 1973). In *E. alba*, it seems that metabolic pathways were modified in such a way that synthesis of photosynthetic pigments especially chlorophyll was affected. Due to nematode infection amount of chlorophyll a and chlorophyll b decreased at all the initial inoculum levels. Singh *et al.*, (1978) and Khan *et al.*, (1992) reported reduction in chlorophyll content. The decrease in chlorophyll content was attributed to alteration in host physiology and nutrition (Bergeson, 1966). In *M. incognita* infected plants reduction in amount of chlorophyll has also been reported by a number of workers like Vashisht *et al.*, (1994); Poornima and Vadivelu,
In *Eclipta alba*, protein content was found increased in the root of *M. incognita* infected plants while decreased significantly at Pi = 2,000J₂ and Pi = 20,000J₂ in shoots. One reason of decrease in amount of protein in shoot might be enhanced rate of protease activity and increased level of soluble proteins and amino acids. In host parasite relationship host proteins are broken down into easily available forms of amino acids. In root-knot nematode infections, the proteases are secreted by the nematode into the host tissue for such a proteolytic degradation. The released free amino acids are engaged in synthesizing new types of proteins. Pathogens have been reported to produce proteolytic enzymes which are after detected in diseased tissue (Matsubara and Feder, 1970). In the galled roots, increased rate of protein synthesis was due to availability of amino acid in higher amounts (Roy, 1981). In galled tissue nucleic acid synthesis corresponds very close to protein synthesis. Increased amount of nucleic acid in the giant cell is mainly because of endomitosis that results in higher metabolic activity (Owens and Rubinstein, 1966).

Total protein and proline contents of infected plants exceeded that of the healthy plants of brinjal, and the magnitude of increase was more pronounced with the increase in time of infection. Significant enhancement in protein and total amino acid with simultaneous increase in protease activity were observed in nematode infected roots (Howell and Krusberg, 1966 and Epstein and Cohn, 1971). Higher protein content in the infected roots was reported in tomato (Bird, 1962); Brinjal (Singh *et al.*, 1978); Chilli (Trivedi and Tiagi, 1980, 1986; Pandey and Trivedi, 1991), Pea (Sharma, 1985). Giant cells, nematode bodies and hyperplastic parenchyma around the giant cells were the main sites of
protein accumulation (Owens and Novotny, 1960; Knypl and Janas, 1975; Trivedi and Tiyagi, 1980). Owens and Specht (1964) had observed spreading of proteinaceous material throughout the giant cells. The presence of increased protein amount in galls was due to increased protein synthesis (Owens and Novotny, 1960; Bird, 1961; Littrell, 1966; Chylinska et al., 1972).

Reduction in oil content of seeds, 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 seeds. Oil contents in different plants were found decreased by Sivakumar and Seshadri, 1971 on castor; Pandey et al., 1992 on mint; Haseeb et al., 1996 on O. cannum; Shukla and Haseeb, 1998 on Mentha citrata; Prasad and Narayana, 1999 on sunflower; Tiyagi et al., 2001 on rose; and Parveen, 2006 on tulsi.
SECTION II
EXPERIMENT 4

Histology of the interactions of *Paecilomyces lilacinus* with *Meloidogyne incognita* on *Eclipta alba*

INTRODUCTION

Interactions between antagonists fungi and nematodes have been known to occur in agricultural soils for many years (Barron, 1977; Mankau, 1980). Nematode destroying fungi of diverse biology and affinities are ubiquitous in most soils and undoubtedly, in many instances, play an important role in regulating nematode population dynamics. Opportunistic soil fungi are capable of colonizing nematode reproductive structures and deleteriously affecting them. The opportunistic and nematophagous hyphomycete, *Paecilomyces lilacinus* (Thom.) Samson is an effective bio-control agent (Jatala et al., 1979, 1980; Sayre, 1986; Khan and Williams, 1998) that suppresses populations of root-knot and cyst forming nematodes (Bourne et al., 1999; Amin, 2000; Cannayane and Sivakumar, 2001). The fungus shows a wide antimicrobial activity against some other fungi, yeast, and gram positive bacteria due to the production of peptidal antibiotic (Isogai et al., 1980, 1981).

*Paecilomyces lilacinus* parasitizes eggs of *Meloidogyne* spp. and *Globodera pallida* (Dunn et al., 1982; Jatala, 1986; Bhat, 1999; Jonathan and Rajendran, 2000b; Goswami and Mittal, 2002; Niyaz and Hisamuddin, 2007). *Paecilomyces lilacinus* increased the yield of tomato and okra, lowered the population of *M. incognita* juveniles, at the mid and at the beginning of the next season in treated plots than in untreated plots (Noe and Sasser, 1984). Davide and Zorilla (1986) recorded 66.5-77.3% reduction in population of *M. incognita* when okra plants were inoculated with *P. lilacinus*. The growth and
yield of okra increased progressively with the increase in inoculum level of *P. lilacinus* along with nematodes (Saikia and Roy, 1994). The effects of fungus on *M. incognita* parasitizing the roots of *E. alba* has not yet been reported. Therefore, this experiment was designed to study with inoculation of *P. lilacinus* and *M. incognita* infested *E. alba* plants to find out the most effective sequence of inoculation to use it in biocontrol programmes.

**P. lilacinus Inoculation Sequences:**

The find most effective sequence of inoculation of *P. lilacinus* to minimize the nematode population. *P. lilacinus* was applied at the rate of 2g per seedling. The treatments were designed according the following scheme:

- **C**: Uninoculated control plant – (No nematode, no fungus).
- **N**: Inoculated with 2,000 *J*₂ of *M. incognita* (Nematode alone).
- **T₁**: Inoculated with 2,000 *J*₂ of *M. incognita* and treated with fungus one week before inoculation.
- **T₂**: Inoculated with 2,000 *J*₂ of *M. incognita* and treated with fungus simultaneously.
- **T₃**: Inoculated with 2,000 *J*₂ of *M. incognita* and treated with fungus one week after inoculation.
- **T₄**: Inoculated with 2,000 *J*₂ of *M. incognita* and treated with fungus two weeks after inoculation.
- **T₅**: Inoculated with 2,000 *J*₂ of *M. incognita* and treated with fungus three weeks after inoculation.
MATERIALS AND METHODS

Selection of Test Pathogen, Test Fungus, and Test Plant:

The root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 was selected as a test pathogen. The fungus *Paecilomyces lilacinus* was selected as a test biocontrol agent and *Eclipta alba* as a test plant.

Preparation and Sterilization of Soil Mixture:

For performing experiments, the soil was prepared in the ratio of 7:3:1 comprising of clay, sand and farmyard manure, respectively. The pots were filled with soil at the rate of 1 kg of soil per pot. The soil was moistened with water before transferring the pots into the autoclaved and sterilized at 201b pressure for 20 minutes. Sterilized pots were allowed to cool at room temperature before use for experiments.

Raising and Maintenance of Test Plants:

The seeds of *Eclipta alba* were surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2 minutes and washed thrice in sterilized distilled water. The seeds were placed on a moist sterilized filter paper kept in a sterilized petri-dish for germination. The sprouted seeds were transplanted into 15 cm diameter pots each containing 1kg auto-claved clay loam soil.

Preparation and Inoculation of Nematode Inoculum:

The required number of freshly hatched second-stage juveniles (J₂) were obtained from the procedure described in Experiment Number 1 of Section I. When the seedlings became one week old, holes of 5-7 cm depth around the
plant within a radius of 2 cm were made. Through these holes second-stage juveniles (2,000 J2 per pot) were introduced with the help of sterilized pipette.

**Preparation of Fungal Inoculum and its Inoculation:**

The culture of *Paecilomyces lilacinus* used in the experiments was obtained from Indian Agricultural Research Institute, New Delhi. It was maintained on Potato Dextrose Agar (PDA).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>17.00g</td>
</tr>
<tr>
<td>Potato peeled and sliced</td>
<td>200.00g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20.00g</td>
</tr>
<tr>
<td>Double distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

Richard’s medium (Riker and Riker, 1936) was used for the production of *P. lilacinus*. The composition of Richard’s medium is as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium nitrate (KN03)</td>
<td>10.00g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH₂PO₄)</td>
<td>5.00g</td>
</tr>
<tr>
<td>Magnesium sulphate (Mg SO₄)</td>
<td>2.50g</td>
</tr>
<tr>
<td>Ferric chloride (FeCl₂)</td>
<td>0.02g</td>
</tr>
<tr>
<td>Sucrose (C₁₂ H₂₂ O₁₁)</td>
<td>50.00g</td>
</tr>
<tr>
<td>Double distilled water</td>
<td>1000.00ml</td>
</tr>
</tbody>
</table>

The medium was prepared, filtered through muslin cloth and then sterilized in an autoclave at 15lb, for 15 minutes in 250 ml corning flasks. The liquid medium was then inoculated with small amount of fungus maintained on PDA slants with the help of inoculation needle in an aseptic chamber. These
inoculated flasks were kept in an incubator at 25-28°C for 15 days to allow rapid growth of fungus for experimental studies.

After enmassing of *P. lilacinus*, 100g of mycelia were blended in 1,000 ml of distilled water in warring blender such that 10ml of suspension consisted of one gram mycelium. The fungal suspension of *P. lilacinus* was then incorporated into the soil around the roots of *E. alba*, by making holes, 5-7 cm deep within the radius of 2cm. After inoculation of fungus these holes, were then plugged with sterilized soil. The pots were kept in green house in a simple randomized block design. Regular watering was done to maintain soil moisture. Uninoculated plants served as control.

**PARAMETERS**

After 45 days of inoculation the experiment was terminated and following parameters were taken into account for describing the results.

**Plant Growth:**

The plants were uprooted and gently washed with tap water. The lengths of roots and shoots were recorded in centimeters from the cut end to the tip of first leaf and the longest root, respectively. The number of leaves was counted by visual observation. After taking fresh weights of the roots and the shoots these were kept in envelopes and placed in an oven at 80°C for 24 hours and the dry weight was recorded in grams.

**Leaf Area:**

Leaf area was ascertained by the method as described in the Experiment Number 2 of Section-I.
Yield:

The parameter in terms of number of capitula per plants, number of seeds per capitulum and randomly selected 100 seed weight from each treatments were considered.

Estimation of Chlorophyll Pigments:

The chlorophyll pigment of the leaves was assessed according to the method described earlier in Experiment Number-3 of Section-I.

Estimation of Total Protein Content:

Protein content was estimated according to the method of Lowry et al. (1951) mentioned in Experiment Number-3 (Section-I).

Estimation of Oil Content of Seed:

Oil content of seeds was estimated by soxhlets method mentioned in Experiment 3 (Section -I).

Number of Root Galls:

The galling caused by the root-knot nematode was estimated by counting the number of galls per root system.

Number of Egg Masses:

The number of egg masses on infected roots was counted by staining egg masses with phloxin B. An aqueous solution of phloxin B 0.15 g 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 stained red and were counted directly.
Statistical Analysis:

The data obtained were analysed statistically and significance was calculated at $P \leq 0.05$ and $P \leq 0.01$ levels of probability.

**HISTOPATHOLOGICAL STUDIES**

Inoculated seedlings were uprooted carefully after 45 days. The roots were washed gently and thoroughly to remove all soil particles adhering on them. Galled roots were cut into 1 cm long pieces and processed for histopathological studies as described in Experiment Number-1 of Section-I. Anatomical details were observed under light microscope and necessary photographs were taken.

**RESULTS**

**Root and Shoot Length:**

In comparison to control (C) plants, significant reductions ($P \leq 0.01$) were observed, in the lengths of *Eclipta alba* plants inoculated with the root-knot nematode (*Meloidogyne incognita*) and not integrated with *Paecilomyces lilacinus* (P.49). The significant reductions ($P \leq 0.01$) in plants inoculated with only *M. incognita* were 40.70% and 36.45% in the roots and the shoots, respectively (Table-6). Among all these *P. lilacinus* treated plants, highest reductions (26.99% in the roots and 28.09% in the shoots) were recorded in T₅ plants and lowest (12.38% in roots and 7.35% in shoots) in T₂ plants. The roots and shoots of T₁ plants, compared to control plants were slightly but non-significantly longer (Table-6).
In comparison to nematode (N) alone plants, enhancement in lengths of the roots and the shoots was observed in all the plants inoculated with the nematode and treated with *P. lilacinus* at different time intervals. Increase in length was significant (*P* ≤ 0.01) and highest in T1 plants being 70.14% in roots and 59.47% in shoots. Maximum increase in length of the roots and shoots was observed in T1 plants and minimum increase in T5 plants followed by T4, T3 and T2 plants (Table-6 and H-1).

In all the treatments, in which *P. lilacinus* was applied, enhancement was encountered when the data were compared with nematode alone (N) plants. There were no marked differences in lengths of the root and shoot and total length of the control (C) and T1 plants. A gradual decrease in length of the plant was noticed with an increase in time interval of *P. lilacinus* application after the nematode inoculation.

**Root and Shoot Weight:**

In comparison to control (C), significant reductions (*P*≤ 0.01) were observed, in the fresh as well as dry weights of the roots and the shoots of only nematode inoculated (N) plants and not treated with *P. lilacinus*. The reductions were 38.36% and 27.75% in fresh weights of the roots and the shoots, and 41.6% and 33.45% in dry weights of the roots and the shoots in nematode alone (N) plants, respectively (Table- 6). In all the *P. lilacinus* treated plants, highest reductions in fresh weights (36.06% and 25.83%) and dry weights (29.6% and 26.83%) were observed in the roots and the shoots, respectively, of T5 plants in which *P. lilacinus* was applied after three weeks of nematode inoculation. The reductions were lowest (1.96% and 0.95%), in fresh
and (1.6 and 1.83%) in dry weights of both the roots and the shoots, respectively, in T₁ plants (Table - 6).

On comparing with nematode alone (N) plants. The weights (fresh as well as dry) of the roots and the shoots increased in all P. lilacinus treated plants. Significant (P ≤ 0.01) and highest increases (59.04% and 37.08% in fresh and 68.49% and 47.51% in dry weights) were observed in the roots and shoots of T₁ plants. In T₂ plants, there was a significant (P ≤ 0.05) increase in weight (fresh as well as dry) of the roots and the shoots that were integrated with P. lilacinus simultaneously at the time of nematode inoculation. Among P. lilacinus treated treated plants, the gain in weight was maximum in T₁ plants and minimum in T₅ plants (Table-6, H-2 and H-3).

Number of Leaves:

The number of leaves per plant significantly (P ≤ 0.01) decreased (39.71%) in nematode alone (N) plants when compared with control (C). Among P. lilacinus treated plants, highest reduction in number of leaves over the control was noticed (32.48%) in T₅ and lowest (10.63%) in T₁ plants (Table – 6).

Maximum and significant (P ≤ 0.01) increase in leaf number of T₁ (48.23%) and T₂ (39.52%) plants was observed, when compared with only nematode (N) inoculated plants. In T₃, T₄ and T₅ treatments, enhancement was also encountered when the data were compared with nematode alone (N) plants (Table-6 and H-4).
Leaf Area:

The leaf area of nematode (N) inoculated plants, was significantly (P≤ 0.01) smaller (32.59%) than control (C) plants. In all the *P. lilacinus* treated plants, highest reductions in leaf area (28.88%) was noticed in T5 plants in which *P. lilacinus* was integrated after three weeks of nematode inoculation. The reductions were lowest (1.48%) in T1 plants (Table - 6).

On comparing with nematode (N) alone plants, significant (P≤ 0.01) increase was observed in T1 and T2 plants (46.15% and 35.82%, respectively). The leaf size of T3, T4 and T5 plants was found to be increased when compared with nematode alone (N) plants (Table – 6 and H-5).

Yield:

The yield of the plant in terms of number of capitula per plant and the number of seeds per capitulum was significantly (P≤ 0.01) reduced (43.22% and 50.76%) in nematode alone (N) plants, over the control (C). In *P. lilacinus* treated plants, highest reductions in yield (34.74% and 38.07%) were observed in T5 and lowest (3.38% and 5.76%) in T1 plants that were integrated with *P. lilacinus*, before one week of nematode inoculation (Table – 7).

The number of capitula per plant and the number of seeds per capitulum were significantly (P≤ 0.01) higher (70.14% and 91.40%) in T1 plants and in T2 plants (53.73% and 68.35%) then N plants. An increase in yield was also observed in T3, T4 and T5 plants, over the nematode alone (N) plants (Table – 7 and H-6).
Seed Weight:

In comparison with control (C), significant (P ≤ 0.01) reductions were observed (35.93%) in seed weight of the plants inoculated with *M. incognita* and not integrated with *P. lilacinus*. In T5 plants, the reductions in seed weight were highest (32.81%) in other treatments, inoculated with the nematode and integrated with *P. lilacinus*, when compared with control (C). Lowest reduction in seed weight was noticed (7.81%) in T1 plants, over the control (C) (Table - 7).

The seed weight was significantly (P ≤ 0.01) enhanced (43.90%) in T1 plants and (P ≤ 0.05) in T2 plants then only nematode infected (N) plants. In T3 T4 and T5 plants, enhancement in seed weight was recorded over the nematode alone (N) plants (Table- 7 and H-7).

Chlorophyll Pigments:

Maximum and significant (P ≤ 0.01) reduction in chlorophyll pigments were observed (Chl a 7.57% and Chl b 5.67%) in nematode alone (N) plants, when compared with control (C). Among all these treatments, T2 to T5 a gradual reduction were noticed in chlorophyll pigments with an increase in time interval of *P. lilacinus* application after the nematode inoculation. The highest reductions (Chl a 6.64% and Chl b 4.89%) were recorded in T3 plants and lowest (Chl a 2.52% and Chl b 1.76%) in T1 plants (Table -7).

As compared to only nematode inoculated (N) plants, significant (P ≤ 0.01) and highest increase (5.46% and 4.14%) was observed in T1 plants, in which *P. lilacinus* was applied one week before of nematode inoculation. Significant (P ≤ 0.05) increase in chlorophyll pigments were also observed in T2.
plant, over N plants. In other treatments, T5 plants exhibited lowest increase in chlorophyll pigments when compared with nematode alone (N) plants (Table - 7 and H-8).

**Shoot Protein Content:**

In comparison to control plants, highest and significant (P< 0.01) reductions in protein content of shoots was recorded (5.49%) in nematode alone (N) plants. The data showed a gradual decrease in protein content with an increase in time interval of application of *P. lilacinus* treated (T5) plants exhibited maximum reduction (4.48%) and minimum (1.12%) in T1 plants (Table-7).

In T1 plants, significant (P ≤ 0.01) enhancement in protein content was observed (4.62%) when it was compared with nematode alone (N) plants. Significant (P ≤ 0.05) increase was also noticed in T2 plants then N plants. The increase in protein content of T3, T4 and T5 was higher than N plants (Table- 7 and H-9).

**Seed Oil Content:**

In comparison to control (C), maximum and significant (P≤ 0.01) reductions (9.42%) in oil content of seeds were observed in nematode alone (N) plants which were not treated with *P. lilacinus*. In all the *P. lilacinus* treated plants, highest reduction in oil content was observed (8.19%) in T5 plants in which *P. lilacinus* was applied after three weeks of nematode inoculation. The reduction in oil content was minimum (1.55%) in T1 plants (Table-7).
On comparing with nematode alone (N) plants the oil content of seeds increased in all these *P. lilacinus* treated plants. A significant (P < 0.01) and highest increase (8.68%) was recorded in T₁ plants. The increase in oil content was also significant (P < 0.05) in T₂ plants that were integrated with *P. lilacinus* simultaneously at the time of nematode inoculation. Gain in oil content was minimum in T₅ plants in which *P. lilacinus* was applied after three weeks of nematode inoculation (Table-7 and H-10).

Number of Root Galls:

In nematode inoculated (N) plants, severe galling was noticed the number of galls per root system was very high (75.6). The gall number reduced considerably and significantly (P < 0.01) in T₁ and T₂ plants in which *P. lilacinus* was applied one week before and simultaneously with nematode inoculation. The number of galls per root system was also considerably lower on T₃ plants that were integrated with *P. lilacinus*, after one week of nematode inoculation, respectively. The T₅ plants, maximum number of galls was produced followed by T₄, among *M. incognita* inoculated and *P. lilacinus* treated plants (Table-7).

Number of Egg Masses: *Meloidogyne incognita* produced a large number of egg masses (152.4) on *Eclipta alba* roots. The *P. lilacinus* treated plants reduction in egg masses was observed. The number of egg mass per root system was reduced maximum and significantly (P < 0.01) on T₁ and T₂ plants in which *P. lilacinus* was given one week before and simultaneously with nematode inoculation, respectively. The number of egg mass was also reduced in T₃ plants. Highest number of egg masses was found in T₅ plants among *P. lilacinus* treated plants. The data showed a gradual increase in number of egg
masses with an increase in time interval of application of *P. lilacinus* (Table-7).

**Histopathology:**

Transverse and longitudinal sections of the roots of *E. alba* inoculated with the nematode (*M. incognita*) and not treated with fungus exhibited severe infection. Fully mature females were found feeding on giant cells. Adjacent to the giant cells, large amount of abnormal xylem and abnormal phloem was observed. All the mature females were found associated with egg masses (P.32, 33 and 34). Anatomical details of *M. incognita* infected roots of the plants which were treated with one week before nematode inoculation, showed that the fungus entered into the root tissue successfully. The hyphae traversing through the lumen could be seen in the normal vessel elements of the xylem (P.35). The hyphae and conidiophores bearing the chains of conidia were visible in the normal vessel elements of the xylem (P.36 and 37). In these plants, the galls were smaller as compared to the galls produced on only nematode inoculated plants. The giant cells in the roots of *P. lilacinus* treated plants were smaller than on only nematode infected plants. In the vicinity of the giant cells abnormal xylem and abnormal phloem was present. The hyphae and conidiophores bearing chains of conidia were found in and around the females of *M. incognita* (P.37). The fungal hyphae destroyed eggs and egg masses and also entered into the females (P.38). Around the nematode body fungal growth was abundant (P.39). The fungal growth was profuse around the body of the developing nematode (P.40).

Simultaneous application of root-knot nematode and *P. lilacinus*, not only destroyed eggs and egg masses but also entered the internal tissues of the
root, either intercellularly or intracellularly, as is evident from the transverse section of vessel elements (P.41, P.42). The egg masses were destroyed by the fungus (P. 43).

There was no change in the size of giant cells and the amount of abnormal vascular elements, as compared to only nematode inoculated (N) plants (P.44). Application of *P. lilacinus*, one week after nematode inoculation, in the normal tissues, the fungus spread both inter- and intracellularly. The vessel elements were seen having hyphae and conidia in the normal and galled roots. In the plants, in which *P. lilacinus* was given after two and three weeks of nematode inoculation, the fungal hyphae was observed in the abnormal vessel elements (P.45, 46, and 47).

**DISCUSSION**

From the data it is evident that the plant growth of *Eclipta alba* drastically reduced when infected with *Meloidogyne incognita*. Reduction in the root was higher than in the shoots. *Paecilomyces lilacinus*, an endophytic and opportunistic fungus was applied to the soil to control the root-knot disease and was found that the infected plants managed to improve growth characteristics. As the data indicated, the root as well as the shoot lengths of *E. alba* reduced greatly in the presence of *M. incognita* resulting in stunting of the plant. *Meloidogyne* caused stunting, chlorosis and yield loss in various plants. (Oteifa, 1952; Olthof and Potter, 1972; Barker and Olthof, 1976; Rodriguez–Kabana and Williams, 1981; Haseeb et al., 1996; Pathak et al., 2000; Khan, 2003; Hisamuddin et al., 2004; Khan et al., 2004; Niyaz and Hisamuddin 2007).
In \( T_1 \) plants where \( P. \) \( \textit{lilacinus} \) was applied one week before nematode inoculation, significant increase in root and shoot lengths, as compared with only nematode inoculated plants indicated that the fungus helped the plants to grow in a better way even after infection. The total length was almost equal to that of the control plants (P. 49). Cabanillas and Barker (1989) reported three to four times increase in growth of \( M. \) \( \textit{incognita} \) infected tomato where the fungus was applied 10 days before plantation. In \( \textit{Eclipta alba} \), the time interval was one week before \( P. \) \( \textit{lilacinus} \) application and \( M. \) \( \textit{incognita} \) inoculation. \( \textit{Paecilomyces lilacinus} \) is a saprotroph and an endophyte which grows on humus and in root tissues. The fungus remain established in soil (Brown and Smith, 1957). Since it is also an endophyte (Cabanillas \textit{et al.}, 1988) so the hyphae enter into the cortical and vascular tissues in growing primary and secondary roots. An important characteristic, in addition to saprotroph and endophyte, is that it is also parasite of nematodes and therefore it is referred to as opportunistic fungus. It takes the opportunity to grow in the soil, in plant and animal hosts. It destroys mature females of \( M. \) \( \textit{incognita} \) as well as egg masses (Jatala, \textit{et al.}, 1979, Jatala, 1982, 1986; Morgan Jones and Rodriguez – Kabana, 1984; Freire and Bridge, 1985; Zaki and Maqbool, 1991).

The \( T_2 \) plants, in which \( P. \) \( \textit{lilacinus} \) was applied simultaneously at the time of nematode inoculation, exhibited non-significant reduction, in comparison to control. The plant length increased significantly, when compared with the plants inoculated with only nematode. This observation led to the conclusion that before coming in contact of fungus with nematode, the nematode entered into the roots. After establishment of the nematode in the roots the metabolism of the plants got changed and caused slight reduction in root and shoot lengths. Khan and Goswami (2002) evaluated efficacy of \( P. \)
*P. lilacinus* on rice at 8g/ha that resulted in greater plant vigour and increased shoot length.

In the treatments T3, T4 and T5, the fungus was applied one, two and three weeks after inoculation. The nematode entered into the root and induced infection before coming in contact with fungus. As the time of application of fungus increased, the amount of damage in the plants was also increased.

*Meloidogyne incognita* not only caused reduction in plant length but also in fresh and dry weights of *Eclipta alba* as is evident from the Table – 6. Although the number of lateral fibrous roots in nematode infected plants is high but their contribution in increase in weight is negligible. Reduction in root and shoot weights was mainly due to retarded growth rate of the infected plant. *Meloidogyne incognita* after establishing in the roots withdrew plant nutrients, altered nutrient flow patterns and ultimately caused poor growth of infected plants. (Barker and Olthof, 1976; Hussey, 1985). Oteifa (1952) observed retarded shoot growth and nutritional deficiency symptoms in foliage of root-knot nematode infected plants.

A significant increase in fresh and dry weights of roots and shoots of T1 *Eclipta alba* plants, in comparison to only nematode infected plants might be attributed to the efficacy of the biocontrol agent *P. lilacinus*. Application of *P. lilacinus* before planting the seedling minimized the infection by preventing the nematodes to infect the roots. A check in root-knot disease has also reported by Cabanillas and Barker (1989) in tomato, Goswami and Mittal (2002) in tomato, and Dhawan *et al.*, (2004) in okra. Application of *P. lilacinus*, simultaneously with nematode inoculation was not as much effective as when applied one week before nematode inoculation.
Root-knot nematode infection caused reduction in the number of leaves and in the leaf area of E. alba probably because of uptake of minerals and water in lower amount and their transport to the above ground parts at a retarded rate. An increase in leaf number and leaf area per plant after applying P. lilacinus, before transplanting the seedlings or at the time of nematode inoculation, might be because of its effective controlling ability. Application of P. lilacinus after nematode inoculation did not improve plant growth satisfactorily as can be observed from the data (Table -6) as the fungus was not present in the soil to check the nematode infection.

In M. incognita infected plants, yield was drastically affected as was evident from the data. Root-knot nematode infection has been found to cause reduction in yield of soybean (Kinloch, 1980). Suppression in yield of susceptible tobacco in presence of M. incognita and M. javanica has been reported by Arens and Rich (1981). Our results showed that in T1 and T2 plants P. lilacinus not only improved plant growth but also enhanced yield in terms of number of capitula per plant, number of seeds per capitulum and seed weight. Reduction in nematode population and increase in growth of black gram with P. lilacinus alone and in combination with furadan has been observed by Fazal et al., (1996b).

In other treatments where M. incognita infected plants were treated with P. lilacinus two and three weeks after nematode inoculation exhibit any significant increase in yield as compared to nematode infected plants. Integration of P. lilacinus, before and at the time of nematode inoculation controlled the disease to a greater extent. Nematode infection under such condition is not effectively controlled therefore yield remains unaffected as has
been found in other plants. (Zaki 1998; Hazarika et al., 2000; Nagesh et al., 2001).

Biochemical analysis in terms of amount of chlorophyll in the leaves, protein and oil content in the plant indicated that the M. incognita infected plants exhibited significant reduction. This reduction was, however, lower in T1 plants in which the fungus was applied one week before nematode inoculation.

Under ground symptoms of M. incognita infected plants are the occurrence of galls on their roots (Mueller, 1984; Molliard, 1900; and Christie, 1936). Eclipta alba also exhibited galls on the roots after infection of M. incognita. Integration of P. lilacinus before nematode inoculation or simultaneously, caused reduction in the number of galls on infected plants. The fungus when integrated into soil after one week of inoculation did not cause significant change in gall number as was evident from T3, T4 and T5 plants. This indicated that the nematode had already been established in the roots before the establishment of the fungus. The data revealed gradual increase in size of the galls from the treatment T3 to T5. This indicated that the efficacy of the fungus against M. incognita diminished as the time of application increased. Significant reduction in root-knot nematode population in various plants by P. lilacinus application has been reported by Walia et al., (1991) Khan and Williams (1998) and Khanna (2000).

Eclipta alba infected with M. incognita not treated with biocontrol agent (P. lilacinus), had egg mass on the galls. The egg mass number was reduced to greater extent in T1 and T2 plants. Occurrence of egg masses on the roots of P. lilacinus treated plants indicated that the nematode successfully entered into the root, developed into mature female and laid egg masses. Reduced number
of egg masses on $T_1$ and $T_2$ plants than on inoculated control (N) might be attributed to comparatively fewer number of juveniles penetrated into the roots, or due to slowing down of the development of the nematode, or due to suppression of secondary infection, or due to parasitization of mature females by the fungus. Increase in number of egg masses on $T_4$ and $T_5$ plants indicated that $P. lilacinus$ failed to control the nematode at later stage of infection.

The fungus *Paecilomyces lilacinus* shows diverse modes of habits. Basically it is a saprophyte (Domusch *et al.*, 1980) and can easily be grown on artificial culture media. At one time it acts as an epiphyte and grows on the surface of plant roots (Cabanillas *et al.*, 1988). At other times it grows inside the root tissue and behaves as an endophyte and does not cause any damage to the plant. Still at other times it parasites eggs and egg masses of *Meloidogyne* species and destroys them. Because of this lastly stated behaviour, $P. lilacinus$ has been used, by several workers, as a biocontrol agent against root – knot and other nematodes (Jatala *et al.*, 1979 and Morgan Jones and Rodriguez–Kabana, 1984).

*Paecilomyces lilacinus* was frequently noticed around the root of the plant treated with fungus. While observing transverse and longitudinal section of the nematode infected and $P. lilacinus$ treated *E. alba* plants, the fungal hyphae were found in the tissues of infected roots. This confirmed that the fungus is an endophyte. Moreover, the fungal hyphae were also found associated with the mature females of the nematode which confirmed that the fungus is also an animal parasite taking nourishment from the animal proteins. In our opinion, vessels and vessel elements provided sufficient space for its development and also provided an uninterrupted passage to grow inside the plant tissues. Cabanillas *et al.*, (1988) mentioned its presence in the cortex but
did not disclose its mode of penetration and growth inside the tissues. In our opinion, it grows and develops inside the root tissue inter- and intracellularly. Whether, the fungus is beneficial or not to the plant, but in our opinion it is not as much harmful as in the nematode. In all the sections studied, the fungus was not found damaging the plant tissues even when in abundance.

The fungus did not affect the giant cells in which its occurrence was noticed. The eggs and the egg masses on the roots of *E. alba* were found infected with the fungus, *P. lilacinus*. The fungus destroying the nematode has been reported on the other plants (Jatala *et al.*, 1979; Godoy *et al.*, 1983; Jatala, 1985, 1986; Amoncho and Sasser, 1995; Zaki, 1998; Dhawan *et al.*, 2004). The juveniles and the mature females were not attacked by the fungus on certain plants (Jatala, 1986). The eggs, however, appeared to be the most preferred targets, for obtaining the nutrients, by the fungus. Contrary to this Cardona and Leguizamon (1997) reported 94% infection in *Meloidogyne* spp. by *P. lilacinus* strain 9201. Khan and Williams (1998) found that *P. lilacinus* entered into the mature females through natural opening but did not mention about damaging the female it, however, damaged the eggs inside the egg masses.

On the basis of these observations, we conclude that the fungus can not check root-knot nematode infection at primary level when the plants had already been attacked by the juveniles. However, it can invariably check secondary infection because it destroys eggs as and when deposited (Niyaz and Hisamuddin, 2007). As far as time of application of *P. lilacinus* is concerned, it can be suggested that incorporation of fungus one week before is more effective in controlling the root-knot disease, as compared to later intervals of incorporation.
SECTION III
EXPERIMENT 5

Effect of fly ash amended soil on the plant growth, yield, chlorophyll pigment, protein and oil content of *Eclipta alba*

**INTRODUCTION**

Emissions from thermal power plants are the major source of atmospheric pollution. In India, more than 70% of energy needs are met by coal based thermal power plants. Burning of coal releases, oxides of nitrogen and Sulphur and enormous quantity of fly ash, which gets deposited on the plant and the soil. Some workers pointed out that the fly ash might have a great potential for use in agriculture (Martens, 1971). Fly ash addition could improve the nutrient status of soil and neutralize soil acidity to a level suitable for agriculture depending on the initial pH of the soil (Moliner and Street, 1982). The responses of plants towards fly ash have been found variable ranging from beneficial effects due to presence of essential macro-and micronutrients like Ca, Mg, SO₄, Cu, Zn, Fe, Mo, and Mn to adverse effects due to higher concentrations of heavy metal elements like B, Ni, Cr and also due to high salinity (Adriano *et al*., 1980 and Sikka *et al*., 1994).

In the present experiment, Kasimpur Thermal Power Plant, with a capacity of about 1500 MW, was selected as the source of fly ash for performing soil amendment experiments. It is located 16 km north of Aligarh. The experiment was designed to find out the effects of various concentrations of fly ash on the medicinal plant, *Eclipta alba.*
MATERIALS AND METHODS

Physical and Chemical Analysis of Fly Ash:

The physical and chemical properties of the fly ash, obtained from Kasimpur Thermal Power Plant, were analysed by different methods. The texture was determined by hydrometer method (Allen et al., 1974) and electrical conductivity by conductivity meter (Elico Co. Ltd, Hyderabad, India). The pH was measured with the help of pH meter after obtaining an extract from fly ash and water suspension in the ratio of 1:1 (w/v). Total organic carbon, total nitrogen, and total phosphorus were analysed by Degtjareff method (Walkey and Black, 1934), Microkjeldahl method (Nelson and Sommers, 1972) and Molybdenum blue method (Allen et al., 1974), respectively. The total metal elements were determined by mixed acid digestion using conc. HNO₃ and conc. H₂SO₄, and HClO₄ followed by atomic absorption spectrophotometry (Allen et al., 1974).

Preparation of Soil Amended with Fly Ash:

Sandy loam soil was collected from a fallow field of AMU Campus. The fly ash and sterilized soil were mixed together (v/v) in five proportions with 10, 20, 30, 40 and 50% of fly ash. The pots were sterilized in an autoclave at 20lb pressure for 20 minutes. Sterilized pots were allowed to cool at room temperature before use for experiment.

Raising and Maintenance of Test Plants:

The seeds of E. alba were surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2 minutes and washed thrice in sterilized distilled water. The seeds were placed on a moist sterilized filter paper kept in a sterilized petri-dish for
germination. The sprouted seeds were transplanted into 15 cm diameter pots having steam-sterilized soil amended with fly ash and arranged in a simple randomized design. Normal soil without fly ash was treated as control. There were five replicates for each treatment. The watering was done from time to time.

PARAMETERS

Plant Growth:

Plants were uprooted after 60 days of inoculation and roots were washed thoroughly in slow running tap water. Root and shoot length were measured with the help of meter scale. The number of leaves was counted by visual observation. After taking fresh weights, the roots and the shoots were kept in envelopes separately for drying in an oven running at 80°C for 24 hours and the weight was recorded in grams.

Leaf Area:

Leaf area of the leaves was ascertained described in Experiment.2 (Section I).

Yield:

The yield parameters in terms of number of capitula, seeds per capitulum and weight of 100 seeds, randomly selected from each treatments were considered.

Estimation of Chlorophyll Pigments:

The chlorophyll pigment of the leaves was assessed according to the method of Mackinney (1941) described earlier in Experiment 3 (Section I).
Estimation of Total Protein Content:

Protein content was estimated according to the method of Lowry et al., (1951) mentioned in Experiment 3 (Section-I).

Estimation of Oil Content of Seeds:

Oil content of seeds was estimated by soxhlets method mentioned in Experiment 3 (Section –I).

Statistical Analysis:

The data obtained were analysed statistically and significance was calculated at $P<0.05$ and $P<0.01$ levels of probability.

RESULTS

Fly Ash Analysis:

The fly ash emanated from Kasimpur Thermal Power Plant was alkaline in nature with electrical conductivity 9.84 m mhos cm$^{-1}$. Textural analysis indicated that the amount of silt size particles in the fly ash was greatest, followed by sand and clay size particles. The organic carbon and nitrogen of the fly ash were 0.7% and 0.05%, respectively. Several elements like Pb, Ni, Mn, Co, B, Cu, K, Cr, Cd, Zn and Fe were also present in the fly ash. The concentrations of K, Mn and B were higher than other metallic elements (Table-8 and 9).

Root and Shoot Length:

The root and shoot lengths of Eclipta alba showed variable responses towards the soil containing different concentrations of fly ash levels. In
comparison to control (C) plants, grown in unamended soil, enhancement in length of the roots and shoots was observed in T₁, T₂, T₃, and T₄ plants that were grown in soil amended with 10%, 20%, 30% and 40% fly ash concentration (P. 50). Increase in length of the roots and the shoots was non-significant in T₁, T₂ and T₄ plants, when compared with the control. At 30% fly ash level, (T₃), a significant (P<0.05) increase in the roots and the shoots length, over the control (C) was noticed. In T₅ plants (at 50% fly ash level), the length of the roots and the shoots decreased significantly (P<0.01), when compared with the control (C). Increase in length of the roots and the shoots was highest (27.51% and 21.18%, respectively) at 30% fly ash level in T₃ plants and lowest (4.02% and 3.34%, respectively) at 10% fly ash level, in T₁ plants (Table-10 and H-11).

**Root and Shoot Weights:**

There was an increase in fresh weights and dry weights of the roots and the shoots, from T₁ to T₄ plants, when compared with control. Fresh and dry weights of the roots and the shoots increased non-significantly at 10, 20 and 40% fly ash levels (T₁, T₂ and T₄), and significantly (P<0.05) at 30% fly ash level, in T₃ plants, when compared with control (C). At 50% fly ash level (T₅), significant (P<0.01) reduction was observed in fresh and dry weight of the roots and the shoots, when compared with control. Increase in the weight of the roots and the shoots was highest in T₃ plants (29.41 and 25.71% in fresh and 40.35 and 37.5% in dry weight). Increase in roots and shoots weights was lowest (4.70 and 5.09% in fresh and 9.64 and 4.80% in dry weights, respectively) in T₁ plants (Table-10, H-12 and H-13).
Number of Leaves:

The number of leaves per plant increased non-significantly in T₁, T₂ and T₄ plants, when compared with control (C). A significant (P<0.05) increase in the number of leaves per plant was observed at 30% fly ash level in T₃ plants, in comparison to control. Whereas a significant (P<0.01) reduction in the number of leaves per plant was observed at 50% fly ash level in T₅ plants, in comparison to control (C). Increase in the number of leaves was highest (14.78%) in T₃ plants and lowest (4.92%) in T₁ plants (Table -10 and H-14).

Leaf Area:

Increase in leaf area was observed from T₁ to T₄ plants, when compared with control (C). Increase in leaf area was non-significant at 10% and 20% fly ash levels (T₁ and T₂ plants, respectively). The leaf area increased significantly (P<0.05) at 30% fly ash level in T₃ plants, in comparison to control (C). In (T₄) plants, there was a non-significant increase, over the control. A significant (P<0.01) reduction in the leaf area was observed at 50% fly ash level in T₅ plants, compared with control (C). Increase in leaf area was highest (16.06%) in T₃ plants and lowest (2.39%) in T₁ plants (Table-10 and H-15).

Yield:

The yield of the plant, in terms of the number of capitula per plant and the number of seeds per capitulum, increased non-significantly at 10% and 20% fly ash levels (T₁ and T₂ plants, respectively), when compared with control. Highest and significant (P<0.05) enhancement in number of capitula (35.05%) and in number of seeds per capitulum (23.34%) was noticed in T₃ plants, amended with 30% fly ash level, whereas a non-significant
enhancement was encountered in T₄ plants. A significant (P≤0.01) decrease in yield was observed in T₅ plants at 50% fly ash level, when compared with control. Lowest increase in both the parameters (6.18% and 2.81%) was observed in T₁ plants. (Table-11 and H-16).

**Seed Weight:**

The weight of seeds, increased in the treatments from T₁ to T₄, when compared with control (C). The increase was non-significant at 10% and 20% fly ash levels (T₁ and T₂ plants, respectively). Highest and significant (P≤0.05) increase (22.95%) was noticed at 30% fly ash level in T₃ plants, when compared with control (C). At 40% fly ash level (T₄), increase in seed weight was found but it was non-significant. The weight of seeds significantly (P≤0.01) reduced at 50% fly ash in T₅ plants. Lowest increase (3.27%) was observed in T₁ plants (Table-11 and H-17).

**Chlorophyll Pigments:**

The amount of chlorophyll a and chlorophyll b in the leaves was non-significantly increased at 10% and 20% fly ash levels (T₁ and T₂ plants), when compared with control (C). At 30% fly ash level (T₃), a significant (P≤0.05) increase was noticed. A non-significant increase in the chlorophyll content was also observed, in T₄ plants, compared with control. In T₅ plants, amended with 50% fly ash level, significant (P≤0.01) reduction occurred, in comparison to control (C). Increase in chlorophyll pigments was highest in T₃ plants (5.35% in Chl a, and 4.12% in Chl b) and lowest (1.04% in Chl a, and 0.98% in Chl b) in T₁ plants (Table-11 and H-18).
Shoot Protein Content:

There was a non-significant increase in the protein content of shoot of *E. alba* in T₁ and T₂ plants, amended with 10% and 20% fly ash level. A significant (P≤0.05) increase in the protein content occurred, at 30% fly ash level (T₃), in comparison to control (C). In T₄ plants protein content increased non-significantly, over the control. A significant (P≤0.01) decrease was noticed in the protein content of the shoot at 50% fly ash level, in T₅ plants, in comparison to control. Increase in protein content was highest (7.91%), in T₃ plants and lowest (1.78%) in T₁ plants (Table-11 and H-19).

Seed Oil Content:

In comparison to control (C), the oil content of seeds increased non-significantly at 10% and 20% fly ash levels (T₁ and T₂ plants, respectively). The increase in the oil content of seeds was significant (P≤0.05) at 30% fly ash level in T₃ plants. At 40% fly ash level (T₄), a non-significant increase was observed. There was significant (P≤0.01) reduction in oil content at 50% fly ash level in T₅ plants, when comparison was made with control (C). Increase in oil content was highest (10.78%) in T₃ plants and lowest (2.94%) in T₁ plants (Table-11 and H-20).

**DISCUSSION**

Fly ash as particulate air pollutant has shown great potential in enhancing productivity through soil amendments where it acts as a source of trace elements. Application of fly ash into the soil, in different concentrations, produced both beneficial as well as harmful effects on growth, yield, amount of chlorophyll pigments, protein and oil content of *Eclipta alba*. The data (Table-
10 and 11) revealed that with an increase in amount of fly ash in the soil from 10% to 30%, the growth parameters yield, amount in chlorophyll pigments, protein and oil content significantly enhanced only at 30% fly ash level. The enhancements were non-significant at 10% and 20% levels. From this finding it might be inferred that the soil became more suitable for the plant growth due to change in physio-chemical characteristics of the amended soil.

The responses of plants towards different concentrations of the fly ash were not uniform, as is evident from the data. At 10, 20 and 30% fly ash levels increasing trend was noticed in the length of the roots and the shoots, in fresh and dry weights, in yield parameters such as number of capitula, seeds per capitulum and seed weight, in the amount of chlorophyll a and chlorophyll b, protein and oil content. This showed that amendment of soil with fly ash in increasing concentration probably provided more nutrients, changed pH of the soil, altered porosity of the soil, favoured moisture holding capacity. All these alterations led to develop favourable environment for the plant growth found that amendment with fly ash increased the concentrations of B, Mo, Ca, Cr, K, Mg, P, Se and Sr in alfalfa, barley and brome grass in addition to improvement in growth (Adriano et al., 2002). In centripede grass, fly ash amended soil increased the concentration of B, Mo, As, B and Se and decreased the concentration of Mg, Mn, and Zn (Hammermeister et al., 1998). Increased porosity, pH, electrical conductivity, cation exchange capacity and concentration of SO₄, PO₄, Ca, K, P, carbonate and bicarbonate ions resulted in increased uptake of Fe, Pb, Mn and Zn in sunflower (Siddiqui et al., 2004).

Addition of fly ash also enriched the soil with micro-and macro-elements which were being utilized by the plants. Thus, a change in physical and chemical characteristics of soil by the application of fly ash proved
beneficial to *Eclipta alba*. Various investigators have revealed that occurrence of essential mineral elements in the fly ash that improved the growth, yield and leaf pigments of pulse and vegetables crop plants (Rodgers and Andersen, 1995).

Our experiment was performed to analyze the effects of fly ash on wildly growing medicinal plant as wild plants seem, to be more hardly than cultivated plants. Amendment of soil with 30% of fly ash, seems to be the threshold level, at which the values of plant growth, leaf area, number of capitula per plant, seeds per capitulum and seed weight, amount of chlorophyll pigments, protein and oil content were significantly highest. There are different opinions regarding the actual concentration of fly ash that could be most suitable for the plants as have been reported by Mishra and Shukla (1986) on maize and soybean; Khan (1989) on tomato; Pasha *et al.*, (1990) on cucumber; Singh *et al.*, (1994) on soybean; Singh *et al.*, (1994) on *Beta vulgaris*; Pandey *et al.*, (1994) on sunflower; Srivastava *et al.*, (1995) on *Lactuca sativa*; Krejšl and Scanlon (1996) on blackgram; Tripathy and Tripathy (1998) on *Albizia procera* and *Acacia nilotica*; Kalra *et al.*, (1998) on wheat, chickpea, mustard and lentil; Bharti *et al.*, (2000) on green gram; Yasmeen (2002) on *Lagenaria leucantha*; Srivastava *et al.*, (2002) on *Trigonella foenum-graecum*; Pathan *et al.*, (2003) on *Cynodon dactylon* (L.) Pers, Cv Winter Green; Singh *et al.*, (2005) on *Pisum sativum*; Parveen (2006) on *Ocimum sanctum*; Parveen *et al.*, (2006b) on *Mentha citrata*; Hisamuddin and Singh (2007) on *Pisum sativum*. Their findings indicated that concentration of fly ash for better plant growth varied from plant to plant. Amendment of soil beyond 30% concentration did not favour plant growth of *E. alba* as was evident from the data. At 40% fly ash level, growth of the plant, yield in terms of number of capitula and seed per
capitulum, amount of chlorophyll pigments in the leaves, protein and oil content decreased. From these observations it might be inferred that application of fly ash made the soil conditions less favourable for the plant.

At the highest fly ash level (50%) in T5 plants, reduction in plant growth, yield and other characteristics were greatest. The down hill trend after T3, in T4 and T5 plants might be attributed to the altered physio-chemical characteristics to a level that were not suitable for the plant. The composition of soil was changed to a greater extent. Porosity of the soil was increased which resulted in decreased water holding capacity. The ash particles being chemically inert did not make bonds with soil particles. In physical characteristics ash particles resemble with sand particles. As the sand holds less water similarly ash also retains less water than clay and silt.

Fly ash changed the texture of soil, in addition to other physical characteristics, that proved disadvantageous to Eclipta alba, which had been found growing luxuriantly in sandy loam soils. Probably, physiological and biochemical processes of the plants were influenced by the fly ash amendments.

Leaf pigments (Chlorophyll a and b), protein and oil contents of Eclipta alba increased with increase in fly ash level up to 30% level but decreased beyond it. At lower fly ash levels, increase in chlorophyll content in the leaves might be due to proper utilization of mineral elements by the plants. Higher fly ash levels appeared to be injurious to the plant and therefore, caused reduction in amount of chlorophyll pigment, protein and oil content. This might be due to the toxic effects of metallic ions of heavy metals that interfered in the formation of chlorophyll. Higher amount of protein content at lower fly ash
levels might be attributed to increased amount of chlorophyll content which carried out photosynthesis at an enhanced rate. Higher rate of photosynthesis resulted in synthesis of more amount of enzymes and other proteins. Growth of *E. alba* therefore, was enhanced. In other plants similar responses have been observed by Mishra and Shukla (1986) on maize and soybean; Pasha *et al.*, (1990) on cucumber; Singh (1993) on soybean. Yasmeen (2002) also reported on bottle gourd in higher level of fly ash caused significant reduction in protein content of the plant is quite obvious because of less availability of nitrogen. Pasha *et al.*, (1990) and Parveen (2006) found a positive correlation between leaf pigments and plant growth.

Amendment of soil with fly ash beyond 30% concentration was found harmful effects as evident from the data (Table-10 and 11). The presence of metallic elements like Ni, Ar, Cd, Cr, Pb, Se, Zn, Cu etc., was reported by Wong and Wong (1986) which resulted in poor growth and yield of the plant when fly ash was supplied in higher concentrations. Adverse effects of higher concentration of fly ash on growth and yield of various crops were evaluated by different workers Mishra and Shukla, 1986; Singh, 1989; Singh, 1993; Singh *et al.*, 1994; Khan and Khan, 1996; Kalra *et al.*, 1998; Yasmeen, 2002; Srivastava *et al.*, 2005; Singh *et al.*, 2005; Parveen *et al.*, 2006b; Parveen, 2006; Hisamuddin and Singh, 2007.

After performing the experiment, increasing and decreasing patterns of growth were analyzed critically and it might be concluded that amendment of soil with of fly ash up to 30% level was beneficial for growth, yield, chlorophyll pigments, protein and oil content of *Eclipta alba*. On the other hand higher concentrations were not useful for the plants.
EXPERIMENT 6

Effect of fly ash amended soil on the growth of *Eclipta alba* and development of the root-knot disease caused by *Meloidogyne incognita*

INTRODUCTION

The fly ash, a grayish black particulate waste, is produced in huge quantities (about 15-30% of burnt coal depending on the ash content) during coal-burning in power generating plants. Root-knot nematodes, *Meloidogyne* species particularly, *Meloidogyne incognita* and *M. javanica* are widely distributed in northern India. The degree of damage caused by the nematodes depends on the species, population densities, type of hosts and cultivars and environmental factors (Webster, 1969). They have a wide host range and have an ability to interact synergistically with other plant parasites. These nematodes caused 5-43% yield losses to vegetables in tropical climates (Sasser, 1979). Certain elements such as potassium, phosphorus and boron play important roles in the defense mechanism of plants against nematodes (Kirkpatrick *et al*., 1964; Francois, 1984). All these elements are amply present in fly ash (Elseewi *et al*., 1981; Druzina *et al*., 1983; Wong and Wong, 1989 and Khan *et al*., 1997). An experiment was aimed to investigating the feasibility and potential of fly ash using as a nematicide-cum fertilizer for the management of root-knot disease caused by *M. incognita* on *E. alba*.
MATERIALS AND METHODS

Physical and Chemical Analysis of Fly Ash:

Fly ash collected from Kasimpur Thermal Power Plant, Aligarh was chemically analyzed as has been explained in materials and methods of Experiment 5 (Section –III).

Preparation of Soil Amended with Fly Ash:

Sandy loam soil was collected from a fallow field of AMU Campus. The fly ash and sterilized soil were mixed together (v/v) in five combinations with 10, 20, 30, 40 and 50% of fly ash. The pots were sterilized in an autoclave at 20 lb pressure for 20 minutes. Sterilized pots were allowed to cool at room temperature before use for experiment.

Raising and Maintenance of Test Plants:

The seeds of *E. alba* were surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2 minutes and washed thrice in sterilized distilled water. The seeds were placed on a moist sterilized filter paper kept in a sterilized petri-dish for germination. The sprouted seeds were transplanted into 15 cm diameter pots having steam-sterilized soil amended with fly ash. Unamended soil without fly ash was used as control. There were five replicates for each treatment. The watering was done from time to time as and when required.

Preparation of Inoculum of *Meloidogyne incognita*:

Nematode inoculum was prepared by the method described earlier in Experiment I (Section-1). Two-week old seedlings were inoculated with second-stage juveniles of *M. incognita* (2,000 J₂ pot) by making 5-7 cm deep
holes within the radius of 2 cm. The suspension was introduced with the help of sterilized pipette. The holes were then plugged with sterilized soil soon after inoculation. The pots were arranged in a complete randomized block design. Each treatment consisted of five replicates uninoculated plants served as control (C), inoculated plants grown in unamended soil served as inoculated control (IC).

The treatments were as follows:

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<tbody>
<tr>
<td>C</td>
<td>Control (C)</td>
</tr>
<tr>
<td>IC</td>
<td>0% Fly ash + 2,000 J_2/pot</td>
</tr>
<tr>
<td>T_1</td>
<td>10% Fly ash + 2,000 J_2/pot</td>
</tr>
<tr>
<td>T_2</td>
<td>20% Fly ash + 2,000 J_2/pot</td>
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<tr>
<td>T_3</td>
<td>30% Fly ash + 2,000 J_2/pot</td>
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<tr>
<td>T_4</td>
<td>40% Fly ash + 2,000 J_2/pot</td>
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<tr>
<td>T_5</td>
<td>50% Fly ash + 2,000 J_2/pot</td>
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</table>

**PARAMETERS**

**Plant Growth:**

The experiment was terminated after sixty days and roots were washed thoroughly in slow running tap water. Utmost care was taken to avoid loss and injury of root system during the entire operation. Length of roots and shoots were recorded in centimeters from the cut end to the tip of first leaf and the longest root, respectively. The number of leaves was counted by visual observation. After taking fresh weights, the roots and the shoots were kept in
envelopes and placed in an oven at 80°C for 24 hours and the dry weights were recorded in grams.

Leaf Area:

Leaf area was ascertained which has been already described in Experiment 2 (Section-I).

Yield:

The yield of plants, in terms of number of capitula, seeds per capitulum, and 100 seeds weight was observed by visual counting and weight was recorded in grams.

Estimation of Chlorophyll Pigments:

The chlorophyll pigment of the leaves was assessed according to the method of Mackinney (1941) described earlier in Experiment 3 (Section-I).

Estimation of Total Protein Content:

Protein content was estimated according to the method of Lowry et al., (1951) mentioned of Experiment 3 (Section-I).

Estimation of Oil Content of Seeds:

Oil content of seeds was estimated by soxhlets method mentioned in Experiment 3 (Section –I).

Number of Root Galls:

The galling caused by root-knot nematodes was estimated by counting the number of galls per root system.
Number of Egg Masses:

The number of egg masses on infected roots was counted by 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 counted directly.

Morphometry of Nematode:

The infested roots were fixed in 0.1% cotton blue-lactophenol and nematodes were mounted in lactophenol (Southey, 1970). The length and width of female body; its neck, stylet, median bulb was measured using camera lucida.

Statistical Analysis:

The data obtained were analysed statistically and significance was calculated at P≤0.05 and P≤0.01 levels of probability.

RESULTS

Root and Shoot Length:

In comparison to uninoculated control (C) plants, significant reductions (P ≤ 0.01) were observed, in the lengths of the roots and the shoots of inoculated with the root-knot nematode (Meloidogyne incognita) and grown in the soil not amended with fly ash. The reductions in IC plants were 40.86% and 32.52% in the roots and the shoots, respectively, over the control (C) plants. The reductions were also significant (P ≤ 0.01) in the lengths of roots and the shoots of T₁, T₄ and T₅ plants which were inoculated with the nematode and
grown in fly ash amended soil (Table-12). Among the plants inoculated with root-knot nematode and grown in fly ash amended soil, highest reductions (31.73\% in the roots and 24.22\% in the shoots) were observed in T_1 plants and lowest reductions (14.90\% in roots and 8.65\% in shoots) in T_3 plants (Table-12). Highest reductions in plant length was observed in IC plants followed by T_1 plants, and lowest reduction was observed in T_3 plants.

In comparison to inoculated control (IC) plants, enhancement in the length of the roots and the shoots was observed in the plants inoculated with the nematode and grown in fly ash amended soil. A non-significant increase was noticed in T_1 plants. In T_2 plants, the increase in length was significant (P ≤ 0.05) but in T_4 and T_5 plants it was non-significant. Increase in length was significant (P ≤ 0.01) and highest in T_3 plants being 43.90\% in roots and 35.38\% in shoots. Increase in length was lowest and non-significant in roots and shoots of T_1 plants (Table-12, and H-21). Highest increase in total length was observed in T_3 plants and lowest in T_1 plants (P.51).

**Root and Shoot Weight:**

In comparison to uninoculated control (C), significant reductions (P ≤ 0.01) were observed in the fresh as well as the dry weights of the roots and the shoots of IC plants, inoculated with root-knot nematode (*Meloidogyne incognita*) and grown in unamended soil. The reductions were 37.78\% and 24.51\% in fresh weights of the roots and the shoots, and 39.49\% and 32.57\% in dry weights of the roots and the shoots over the control (C) plants, respectively. The reductions in weights (fresh as well as dry) were also significant (P ≤ 0.05) in other treatments (T_1, T_4 and T_5) inoculated with the nematode and grown in fly ash amended soil. Highest reductions in fresh weight and in dry weight of
both roots and shoots were observed in IC plants followed by T₁ plants. The reductions were lowest (8.01 and 3.27%) in fresh weight of the roots and the shoots and (6.72 and 2.71%) in dry weights of the roots and the shoots, respectively in T₃ plants (Table-12).

In comparison to inoculated control (IC) plants, enhancement in the fresh as well as dry weights of both the roots and the shoots was observed in the plants, inoculated with the nematode and grown in fly ash amended soil. A non-significant increase in weight (fresh as well as dry) of the roots and the shoots was noticed in T₁ plants. In T₂, T₄ and T₅ plants, the increase in weight (fresh as well as dry) of the roots and shoots was significant (P ≤ 0.05). Maximum and significant (P ≤ 0.01) increase in weight of the roots and the shoots (fresh as well as dry) was observed in T₃ plants (47.85% and 28.14%, in fresh and 54.16% and 52.34% in dry weights, respectively) (Table-12, H-22 and H-23).

Number of Leaves:

The number of leaves of all the treatments exhibited a significant reduction (P ≤ 0.01) in comparison to uninoculated control (C) plants. The reductions were maximum (36.55%) in root-knot nematode (*M. incognita*) inoculated (IC) plants grown in the soil not amended with fly ash followed by T₁ plants (29.65%). Lowest reductions (11.58%) were observed in T₃ plants which were inoculated with the nematode and grown 30% fly ash amended soil (Table–12).

An enhancement in the number of leaves was observed in the plants inoculated with the nematode and grown in fly ash amended soil, when comparisons were made with unamended and nematode inoculated (IC) plants.
A non-significant increase was noticed in $T_1$ and $T_3$ plants. The $T_1$ and $T_2$ plants exhibited significant ($P \leq 0.01$) increase whereas, significant ($P \leq 0.05$) increase was also noticed in $T_4$ plants. Maximum increase was observed (39.34%) in $T_3$ plants and lowest (10.86%) in $T_1$ plants. (Table-12 and H-24).

**Leaf Area:**

The leaf area of the plants inoculated with root-knot nematode (*M. incognita*) and grown in soil not amended with fly ash, decreased significantly ($P \leq 0.01$), in comparison to control (C) plants. The reductions in leaf area were also significant ($P \leq 0.01$) in $T_1$ and $T_5$ plants which were inoculated with the nematode and grown in fly ash amended soil. Highest reduction in leaf area (32.27%) was observed in IC plants followed by $T_1$ plants showing 27.21% reduction whereas lowest reduction in leaf area (6.32%) was observed in $T_3$ plants (Table – 12).

An enhancement in the leaf area was observed in the plants inoculated with the nematode and grown in fly ash amended soil, in comparison to inoculated control (IC). The increase in leaf area was non-significant in $T_1$, $T_4$ and $T_5$ plants whereas $T_2$ plants showed significant ($P \leq 0.05$) increase. Highest and significant ($P \leq 0.01$) increase in leaf area (38.31%) was observed in $T_3$ plants and lowest (7.59%) in $T_1$ plants (Table-12 and H-25).

**Yield:**

In comparison to uninoculated control (C), the yield of the plant, in terms of the number of capitula and number of seeds per capitulum, significantly ($P \leq 0.01$) reduced (40.35% and 51.46%) in root-knot nematode (*M. incognita*) inoculated plants grown in the soil not amended with fly ash,
followed by T₁ plants (37.71% and 44.68%). Lowest reductions (11.40% and 22.34%) were observed in T₃ plants inoculated with the nematode and grown in fly ash amended soil (Table-13).

An enhancement in the number of capitula and number of seeds per capitulum was observed in the plants inoculated with the nematode and grown in fly ash amended soil, when comparisons were made with unamended and nematode inoculated (IC) plants. A non-significant increase in yield parameters were observed in T₁ and T₃ plants whereas T₂ and T₄ plants exhibited significant (P ≤ 0.05) increase. Significant (P < 0.01) and highest increase (48.52% and 60.0%) in the number of capitula and number of seeds per capitulum were observed in T₃ plants and lowest (4.41% and 13.96%) in T₁ plants (Table-13, and H-26).

**Seed Weight:**

Significant (P≤ 0.01) reductions in the weight of seeds (100 seeds per plant) were observed in IC plants, and in T₁ and T₅, when comparisons were made with uninoculated control (C) plants. The reduction in seed weight was highest (33.33%) in IC plants followed by T₁ plants, showing 28.57% reduction over control. The reduction was lowest (3.17%) in T₃ plants (Table-13).

In comparison to inoculated control (IC) plants, enhancement in the seed weight was observed in all the treatments inoculated with the nematode and grown in fly ash amended soil. A non-significant increase was noticed in T₁ and T₅ plants. In T₂ and T₄ plants exhibited significant (P ≤ 0.05) increase. Seed weight was significant (P ≤ 0.01) and greatest (45.23%) in T₃ plants but lowest (7.14%) in T₁ plants (Table-13 and H-27).
Chlorophyll Pigments:

In comparison to uninoculated control plants (C), significant reductions (P ≤ 0.01) were observed in the amount of chlorophyll a and chlorophyll b which were highest (7.83% and 6.91%, respectively) in nematode inoculated (IC) plants followed by T1 plants showing 6.75% and 5.89% reductions, respectively. The reductions in the amount of chlorophyll a and b were also significant (P ≤ 0.01) in T2, T4 and T5 plants. The lowest reductions was noticed (3.10 %and 2.23%, respectively) in T3 plants. (Table-13)

In comparison to inoculated control (IC), enhancement in the amount of both chlorophyll a and chlorophyll b was observed in the plants inoculated with the nematode and grown in fly ash amended soil. A non-significant increase in amount of chlorophyll a and chlorophyll b was noticed in T1 and T5 plants whereas in T2 and T4 plants, the increase was significant (P ≤ 0.05). Increase in amount of chlorophyll a and chlorophyll b was significant (P ≤ 0.01) and highest in T3 plants (5.13 and 5.02%, respectively) and lowest (1.17 and 1.09%, respectively) in T1 plants. (Table-13 and H-28).

Shoot Protein Content:

In comparison to uninoculated control plants (C), significant reductions (P ≤ 0.01) in protein content were observed in (IC) plants inoculated with root-knot nematode and grown in unamended soil. The reductions, in comparison to control (C), were also significant (P ≤ 0.01) in T1, T4 and T5 plants which were inoculated with the nematode and grown in fly ash amended soil. Highest reduction (6.42%) in the protein content was observed in IC plants followed by
5.98% reduction in T₁ plants. The lowest reduction (2.76%) was recorded in T₃ plants (Table-13).

An enhancement in protein content was observed in the plants inoculated with the nematode and grown in fly ash amended soil, when comparison were made with unamended IC plants. A non-significant increase was observed in T₁ and T₃ plants but in T₂ and T₄ plants exhibited significant (P ≤ 0.05) increase. Significant (P ≤ 0.01) and highest increase (3.90%) in protein content of shoot was observed in T₃ plants and lowest (0.47%) in T₁ plants (Table -13 and H-29).

**Seed Oil Content:**

In comparison to uninoculated control (C) plants, significant reductions (P ≤ 0.01) in oil content of seeds were observed in IC plants and in other treatments (T₁ and T₃). The reduction in oil content was maximum (10.53%) in IC plants followed by T₁ plants showing 7.91% reduction. Minimum reduction in oil content was observed (1.87%) in T₃ plants. (Table-13).

In comparison to inoculated control (IC) plants, enhancement in oil content of seeds was observed in the plants inoculated with the nematode and grown in fly ash amended soil. A non-significant increase in seed oil content was noticed in T₁ and T₃ plants, but in T₂ and T₄ plants, the increase was significant at (P ≤ 0.05). Maximum and significant enhancement (9.67%) was recorded in T₃ plants and lowest (2.91%) in T₁ plants (Table – 13 and H-30).

**Number of Galls:**

The number of galls per root system decreased in all the nematode inoculated plants, grown in fly ash amended soil, when compared with only
nematode inoculated plants and grown in unamended soil (C). Reduction in the number of galls was non-significant at 10 and 20% fly ash levels (T1 and T2, respectively) and significant (P≤0.05) at 30% fly ash level (T3). The reductions in number of galls were also significant (P≤0.01) at 40 and 50% fly ash level (T4 and T5, respectively). Maximum reduction in the number of galls was observed in T3 plants (35.84%) followed by T4 plants showing 26.49% reduction. The reduction in gall number was minimum (7.01%) in T1 plants (Table-13).

**Number of Egg Masses:**

In comparison to inoculated control (IC) plants, reduction in the number of egg masses was observed in the plants inoculated with the nematode and grown in fly ash amended soil. In T1 and T2, grown in 10 and 20% fly ash levels, the reduction in the number of egg masses was non-significant, where as at 30% fly ash level (T3) significant (P≤0.05) reduction was observed in the number of egg masses. In T4 and T5 plants, the reduction in the number of egg masses was also significant (P<0.01) and higher than T3 plants. Maximum reduction (39.87%) in the number of egg masses was observed in T3 plants followed by T4 plants (30.72%). The T1 plants exhibited minimum reduction (11.20%) in the number of egg masses (Table-13).

**Length and Width of Mature Female:**

The length and the width of mature females decreased in all the treatments from T1 to T5, when compared with inoculated control (IC) plants. In T1 and T2, grown in 10 and 20% fly ash levels, the reduction in the length and the width of mature females was non-significant, where as at 30% fly ash level in T3 treatment, it was significant (P≤0.05). The reduction was significant (P≤0.01) and greatest at 50% fly ash level in T5 plants (4.58% and 9.91%,
respectively) followed by T₄ plants (3.57% and 5.44%, respectively) grown in 40% fly ash level. In T₁ plants, lowest reduction (0.55% and 0.97%) was noticed. (Table -14).

Length and Width of Neck:

In comparison to inoculated control (IC) plants, reduction in length and the width of the neck of the females was observed in all the treatment from T₁ to T₅. A non-significant reduction was noticed at 10 and 20% fly ash levels (T₁ and T₂, plants). In T₃ plants the reduction was significant (P ≤ 0.05). At 40 and 50% fly ash levels, significant (P ≤ 0.01) reduction in length and width of the neck of females was observed, being highest in T₅ (13.18% and 27.20%, respectively) followed by T₄ (6.60% and 19.58%, respectively) plants. Smallest (0.70% and 2.62%) reductions in the length and width of neck of females were noticed grown at 10% fly ash level in T₁ plants, (Table- 14).

Stylet Length and Width:

The length and width of the stylet of the females decreased in all the treatments from T₁ to T₅, when compared with inoculated control (IC). The reductions were non-significant in T₁ and T₂ plants, grown at 10 and 20% fly ash levels, respectively and significant (P ≤ 0.05) in T₃ plants, grown at 30% fly ash level. Significantly (P ≤ 0.01) highest reductions in length and width of the stylet were observed at 40 and 50% fly ash levels (T₄ and T₅ plants, respectively), being highest (1.39% and 1.25%, respectively) in T₅ followed by T₄ plants (1.29% and 1.25%, respectively) (Table-14).
Length and Width of Median Bulb:

The length and the width of medium bulb in all the treatments were compared with inoculated control (IC) and it was revealed that non-significant reductions occurred only in T₁ and T₂ plants, grown in 10 and 20% fly ash levels. At 30% fly ash level, the reductions were significant ($P \leq 0.05$). Highest and significant ($P \leq 0.01$) reductions (32.43% and 12.89%, respectively) were observed in T₅ plants followed by T₄ plants (26.59% and 12.50%, respectively). Smallest reductions were (3.46% and 0.80%) noticed at 10% fly ash level in T₁ plants (Table-14).

DISCUSSION

From the data it became evident that infection of root-knot nematode (*Meloidogyne incognita*) caused reduction in the length, fresh and dry weights of the roots and the shoots, in the number of leaves per plant, in the leaf area, in the number of capitula and seeds per capitulum, in the weight of seeds, in chlorophyll pigments of leaves, in protein and oil content of *Eclipta alba* plant, when grown in unamended or fly ash amended soil over the control plants. From these findings it might be inferred that the nematode successfully established host-parasite relationships with the plant and caused significant damage to it.

The root-knot nematode has been found causing stunting, chlorosis, poor growth and low yield in various plants belonging to different families of dicotyledons and monocotyledons (Christie, 1936; Wallace 1969; Olthof and Potter, 1972; Apple and Lewis, 1984; Samanathan and Sethi, 1996). *Eclipta alba* inoculated with *M. incognita* and grown in fly ash amended soils
responded differently towards increasing amount of fly ash. An increase in fly ash concentration in soil from 10 to 30% level of fly ash, in nematode inoculated plants, developed positive effects in terms of plant length, fresh and dry weights of the roots and shoots, number of leaves and leaf area, yield, amount of chlorophyll pigments, protein and oil content. From these findings it appeared that application of fly ash into the soil probably improved the physio-chemical characteristics of the soil that proved favourable for the plant growth and other plant characteristics. Amendment of soil with fly ash has been applied in several different ways. In the present experiment fly ash amended soil was tested for the growth of the plant under the stress of root-knot disease. Such types of experiments have been performed by several workers (Khan, 1989 in tomato, Singh, 1989 in chickpea and lentil; Pasha et al., 1990 in cucumber, Singh, 1993 on soyabean; Khan et al., 1997 in tomato; Yasmeen, 2002 in Legenaria leucantha; Hisamuddin et al., 2003b in Cicer arietinum; Hisamuddin et al., 2005b in Pisum sativum; Parveen, 2006 in tulsi; Khan et al., 2007 in stem crops; Azam et al., 2007 in Coccinea cordifolia). There are the reports that addition of fly ash into the soil results in increase in cation exchange capacity, pH, and also water holding capacity. These in turn change the characteristics of the natural soil. In certain soils, the fly ash lowered down the porosity and increased concentration of carbonates and bicarbonates (Khan and Khan, 1996; Siddiqui et al., 2004). Iram and Khan (2007) reported retarded rate of penetration and development of juveniles in pepper roots at 5, 10, to 50% fly ash levels.

Some toxic compounds viz. dibenzofuran and dibenzo-p-dioxine mixtures (Helder et al., 1982; Sawyer et al., 1983), metallic elements like arsenic, cadmium, chromium, copper, lead, nickel, selenium and zinc (Khan et
al., 1997) in fly ash, might have been thought of playing some role in killing the nematode juveniles directly in the soil.

Loss of soil porosity, probably resulted in slowing down the movement of the nematodes in the soil. The juveniles could not reach the growing roots and failed to enter into the roots that caused a low degree of disease severity. Organic matter content of the soil is an important factor that affects plant growth as higher the availability of nutrients higher will be the plant growth and consequently plants will be escaped from disease incidence.

Presence of utilizable nutrients such as sulphate, P, K, Ca, B and Mg in fly ash and their accumulation in plant, thereby enhancing natural defence against nematodes has been reported by Kirkpatrick et al., 1964; Francois, 1984; and Khan et al., 1997.

Nitrogen is almost absent in fly ash (Adriano et al., 1980). Nitrogen deficiency in soils slowed down the rate of plant growth and the rate of development of *M. javanica* on tomato (Davide and Triantaphyllou, 1967), and caused abnormal development of nematode juveniles (Singh, 1993). All the considered parameters of plant growth, yield, chlorophyll pigment, protein and oil content exhibited an uphill trend when the plants *T*₁ to *T*₄ were compared with inoculated controls. The plant length was significantly increased in *T*₁, *T*₃ and *T*₅ plants.

The pattern of increase in growth and other parameters was such that at lower concentrations (10 to 30%) of fly ash the parameters like plant length, fresh and dry weights, yield, chlorophyll pigments, protein and oil content increased. The increase in these parameters was highest in *T*₃ plants. From these results it might be suggested that addition of fly ash into the soil,
probably improved soil condition that were expressed as increase in plant growth. The fly ash did not manage the root-knot disease but it improved the plant health. The amount of chlorophyll pigment increased non-significantly in \( T_1 \) and \( T_5 \) plants and significantly at other fly ash levels, when the comparisons were made with inoculated control (IC). Alteration in texture, porosity, \( pH \) and other characteristics of fly ash for amended soil probably affected movement of the nematode and consequently decreased rate of penetration. The disease intensity was decreased which favoured plant growth and played a positive role in the synthesis of chlorophyll pigments at normal rate, in inoculated plants grown at different levels of the fly ash. Due to fly ash amendments, physiological and biochemical activities of the plant might have been influenced. At lower levels, plant showed luxuriant growth which led to improvement in leaf pigments. However, higher levels were found toxic to plant growth, so ultimately the leaf pigment decreased. Higher levels of fly ash in soil affect the growth of other plants as was reported by Mishra and Shukla (1986) on maize and soyabean; Pasha et al., (1990) on cucumber, and Singh (1993) on soyabean. Fluckiger et al., (1978) reported that dust originated from different sources interferes the physiology of plant. Deposition of particulate matter increases leaf temperature and transpiration rate and reduces photosynthesis (Darley, 1966; Fluckiger et al., 1978). The leaf pigments of \( E. alba \) might have been affected adversely because of poor nitrogen content of fly ash amended soils at higher levels.

From the data of morphometrical studies, it was found that fly ash produced deleterious effects on the development of the nematode. The results of the experiments revealed reduction in length and width of the mature female. The measurements of median bulb, neck and stylet also showed reduction, in
comparison to those development on the roots of the plants grown in unamended soil. It appeared that presence of heavy metals and toxic substances in fly ash, probably, intervened in the development of the nematode. Wong and Wong (1986) also reported that higher levels of fly ash, due to accumulation of toxic substances, might have become suppressive for microbial activity like root nodule bacteria and root knot nematode. Helder et al., (1982) attributed the inhibitory effect of the fly ash to the presence of toxic substances and heavy metals. Singh (1993) also reported significant reductions in the morphometrical parameters of *M. javanica* as a result of fly ash amendment. The pathogenic effects like galling and egg mass production in the infected regions, development and reproduction of the nematodes were also greatly suppressed. For instance, at 50% fly ash, inhibition in gall formation and egg mass production was 39.79 % and 28.68 %, respectively.

Due to fly ash amendments, physiological and biochemical activities of the plants might be affected, causing baneful influence on the development through altered supply of the nutrients. Reduced nitrogen content of the plants might have contributed towards the abnormal development of the nematodes. Nitrogen deficiency is reported to retard the rate of development of *M. javanica* on tomato (Davide and Triantaphyllon, 1967). Parveen (2006) also reported the higher concentrations of fly ash were more injurious for the development of the nematode and also found that fly ash addition to any concentration in the soil hampered normal development of the nematode. These facts might be ascribed to the heavy metal elements that probably affected metabolic reactions of the nematode.

Thus, from our findings it might be concluded that fly ash amendment decreased the severity of root-knot disease. Since fly ash changed physical
characteristics of the soil and hence affected plant growth and nematode development. Keeping in view the deleterious aspects of fly ash it is necessary to ascertain the appropriate dosage of fly ash for the amendment of soil as overdose might result in phytotoxicity.
Table 1: Tertiary butyl alcohol dehydration schedule (Johansen, 1940)

<table>
<thead>
<tr>
<th>Steps</th>
<th>% Alcohol</th>
<th>Duration</th>
<th>Quantity (ml) needed for solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Distilled water</td>
</tr>
<tr>
<td>1.</td>
<td>50</td>
<td>2 h or more</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>70</td>
<td>Over night</td>
<td>30</td>
</tr>
<tr>
<td>3.</td>
<td>85</td>
<td>1-2h</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>95</td>
<td>1-2h</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>100</td>
<td>1-3h</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>100</td>
<td>1-3h</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>100</td>
<td>1-3h</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td>100</td>
<td>Over night</td>
<td>0</td>
</tr>
</tbody>
</table>

TBA Changes were carried out at 30°C.
Table-2: Safranin and fast-green (Sass, 1951)

<table>
<thead>
<tr>
<th>Steps</th>
<th>Solution</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Xylene</td>
<td>5 min</td>
</tr>
<tr>
<td>2.</td>
<td>Absolute ethanol</td>
<td>5 min</td>
</tr>
<tr>
<td>3.</td>
<td>95% ethanol</td>
<td>5 min</td>
</tr>
<tr>
<td>4.</td>
<td>70% ethanol</td>
<td>5 min</td>
</tr>
<tr>
<td>5.</td>
<td>50% ethanol</td>
<td>5 min</td>
</tr>
<tr>
<td>6.</td>
<td>30% ethanol</td>
<td>5 min</td>
</tr>
<tr>
<td>7.</td>
<td>1% aqueous safranin O</td>
<td>1-12 h</td>
</tr>
<tr>
<td>8.</td>
<td>Rinse in tap water</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>30% ethanol</td>
<td>3 min</td>
</tr>
<tr>
<td>10.</td>
<td>50% ethanol</td>
<td>3 min</td>
</tr>
<tr>
<td>11.</td>
<td>70% ethanol</td>
<td>3 min</td>
</tr>
<tr>
<td>12.</td>
<td>95% ethanol</td>
<td>3 min</td>
</tr>
<tr>
<td>13.</td>
<td>0.1% fast green FCF in 95% ethanol</td>
<td>5-30 sec</td>
</tr>
<tr>
<td>14.</td>
<td>Absolute ethanol</td>
<td>15 sec</td>
</tr>
<tr>
<td>15.</td>
<td>Absolute ethanol</td>
<td>3 min</td>
</tr>
<tr>
<td>16.</td>
<td>Xylene-absolute ethanol (1:1)</td>
<td>3 min</td>
</tr>
<tr>
<td>17.</td>
<td>Xylene</td>
<td>5 min</td>
</tr>
<tr>
<td>18.</td>
<td>Xylene</td>
<td>3 min or longer</td>
</tr>
</tbody>
</table>
Table 3: Effect of different inoculum levels of *Meloidogyne incognita* on the plant growth and yield of *Eclipta alba*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plant length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>No. of leaves plant(^1)</th>
<th>Leaf area (cm(^2))</th>
<th>No. of capitula plant(^1)</th>
<th>No. of Seeds capitulum(^1)</th>
<th>100 seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatments (Inoculum levels)</strong></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>Control (C)</td>
<td>22.1</td>
<td>28.0</td>
<td>2.96</td>
<td>8.15</td>
<td>1.18</td>
<td>2.50</td>
<td>143</td>
<td>6.45</td>
</tr>
<tr>
<td><strong>T(_1)</strong> (20J(_2))</td>
<td>20.9</td>
<td>27.2</td>
<td>2.79</td>
<td>7.88</td>
<td>1.07</td>
<td>2.34</td>
<td>139</td>
<td>6.20</td>
</tr>
<tr>
<td><strong>T(_2)</strong> (200J(_2))</td>
<td>19.0</td>
<td>24.1</td>
<td>2.42</td>
<td>7.23</td>
<td>0.94</td>
<td>2.09</td>
<td>131</td>
<td>5.60</td>
</tr>
<tr>
<td><strong>T(_3)</strong> (2,000J(_2))</td>
<td>14.0</td>
<td>18.9</td>
<td>1.93</td>
<td>6.18</td>
<td>0.74</td>
<td>1.80</td>
<td>90</td>
<td>4.50</td>
</tr>
<tr>
<td><strong>T(_4)</strong> (20,000J(_2))</td>
<td>11.8</td>
<td>17.9</td>
<td>1.70</td>
<td>5.34</td>
<td>0.58</td>
<td>1.52</td>
<td>80</td>
<td>4.00</td>
</tr>
<tr>
<td><strong>LSD</strong> P≤0.01</td>
<td>5.3</td>
<td>6.9</td>
<td>0.88</td>
<td>1.85</td>
<td>0.39</td>
<td>0.62</td>
<td>20.6</td>
<td>1.53</td>
</tr>
<tr>
<td><strong>LSD</strong> P≤0.05</td>
<td>2.4</td>
<td>3.8</td>
<td>0.42</td>
<td>0.80</td>
<td>0.22</td>
<td>0.38</td>
<td>10.4</td>
<td>0.75</td>
</tr>
</tbody>
</table>

\(J_2\) = Second-stage juveniles of *Meloidogyne incognita*;
Each value is a mean of five replicates.
Table 4: Effect of different inoculum levels of *Meloidogyne incognita* on root galling, size of gall, number of egg masses, number of eggs per egg masses and number of mature females in *Eclipta alba.*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of galls root system^-1</th>
<th>Size of gall (mm^2)</th>
<th>No. of egg masses root system^-1</th>
<th>No. of eggs egg mass^-1</th>
<th>No. of mature females g^-1 root</th>
<th>Pf</th>
<th>Rf</th>
<th>RPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T1 (20J2)</td>
<td>6.8</td>
<td>2.85</td>
<td>6.2</td>
<td>338.8</td>
<td>4.2</td>
<td>228.0</td>
<td>11.4</td>
<td>10.4</td>
</tr>
<tr>
<td>T2 (200J2)</td>
<td>21.6</td>
<td>7.08</td>
<td>36.6</td>
<td>302.0</td>
<td>18.2</td>
<td>1442.8</td>
<td>7.21</td>
<td>6.21</td>
</tr>
<tr>
<td>T3 (2,000J2)</td>
<td>72.4</td>
<td>11.45</td>
<td>168.8</td>
<td>282.4</td>
<td>30.4</td>
<td>2281.4</td>
<td>1.14</td>
<td>0.14</td>
</tr>
<tr>
<td>T4 (20,000J2)</td>
<td>116.0</td>
<td>16.90</td>
<td>228.6</td>
<td>210.2</td>
<td>48.8</td>
<td>20558.6</td>
<td>1.02</td>
<td>0.02</td>
</tr>
<tr>
<td>LSD P≤0.01</td>
<td>16.30</td>
<td>7.75</td>
<td>32.20</td>
<td>35.00</td>
<td>18.60</td>
<td>492.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LSD P≤0.05</td>
<td>10.70</td>
<td>4.08</td>
<td>25.40</td>
<td>28.50</td>
<td>12.80</td>
<td>716.20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

J_2 = Second-stage juveniles of *Meloidogyne incognita*;
Each value is a mean of five replicates
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments (inoculum levels)</th>
<th>Control (C)</th>
<th>T1 (200 mg)</th>
<th>T2 (200 mg)</th>
<th>T3 (2,000 mg)</th>
<th>T4 (2,000 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant length (cm)</td>
<td>Root</td>
<td>21.6</td>
<td>20.0</td>
<td>18.1</td>
<td>13.1</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>28.2</td>
<td>27.0</td>
<td>25.0</td>
<td>19.1</td>
<td>17.7</td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td>Root</td>
<td>2.42</td>
<td>2.22</td>
<td>1.95</td>
<td>1.52</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>7.55</td>
<td>7.26</td>
<td>6.43</td>
<td>5.54</td>
<td>4.94</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>Root</td>
<td>1.16</td>
<td>1.05</td>
<td>0.91</td>
<td>0.71</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>2.09</td>
<td>1.98</td>
<td>1.75</td>
<td>1.46</td>
<td>1.22</td>
</tr>
<tr>
<td>Chlorophyll pigment mg/leaf</td>
<td>Root</td>
<td>6.25</td>
<td>6.00</td>
<td>5.44</td>
<td>4.30</td>
<td>3.98</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>0.750</td>
<td>0.741</td>
<td>0.727</td>
<td>0.700</td>
<td>0.675</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>Root</td>
<td>3.08</td>
<td>2.84</td>
<td>2.71</td>
<td>2.30</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>8.97</td>
<td>8.66</td>
<td>8.48</td>
<td>8.12</td>
<td>7.84</td>
</tr>
<tr>
<td>Seed oil content (%)</td>
<td>Root</td>
<td>12.30</td>
<td>12.00</td>
<td>11.62</td>
<td>11.02</td>
<td>10.45</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>11.00</td>
<td>10.86</td>
<td>10.62</td>
<td>10.12</td>
<td>9.64</td>
</tr>
</tbody>
</table>

**Table 5:** Effect of different inoculum levels of *Meloidogyne incognita* on plant growth, chlorophyll pigments, protein and oil content of *Eclipta alba*.

Each value is a mean of five replicates.

J₁ = Second-stage juveniles of *Meloidogyne incognita*.
Table 6: Interactive effect of *Paecilomyces lilacinus* and root-knot nematode, *Meloidogyne incognita* on plant growth of *Eclipta alba*.

<table>
<thead>
<tr>
<th>Parameters, Treatments</th>
<th>Plant length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Number of leaves plant(^1)</th>
<th>Leaf Area (cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>C</td>
<td>22.6</td>
<td>29.9</td>
<td>3.05</td>
<td>8.36</td>
<td>1.25</td>
</tr>
<tr>
<td>N</td>
<td>13.4</td>
<td>19.0</td>
<td>1.88</td>
<td>6.04</td>
<td>0.73</td>
</tr>
<tr>
<td>T(_1)</td>
<td>22.8</td>
<td>30.3</td>
<td>2.99</td>
<td>8.28</td>
<td>1.23</td>
</tr>
<tr>
<td>T(_2)</td>
<td>19.8</td>
<td>27.7</td>
<td>2.73</td>
<td>7.86</td>
<td>1.11</td>
</tr>
<tr>
<td>T(_3)</td>
<td>18.0</td>
<td>24.8</td>
<td>2.15</td>
<td>6.61</td>
<td>1.09</td>
</tr>
<tr>
<td>T(_4)</td>
<td>17.2</td>
<td>22.2</td>
<td>2.02</td>
<td>6.32</td>
<td>0.98</td>
</tr>
<tr>
<td>T(_5)</td>
<td>16.5</td>
<td>21.5</td>
<td>1.95</td>
<td>6.20</td>
<td>0.88</td>
</tr>
<tr>
<td>LSD (P&lt;0.01)</td>
<td>6.0</td>
<td>9.2</td>
<td>0.92</td>
<td>2.00</td>
<td>0.44</td>
</tr>
<tr>
<td>LSD (P&lt;0.05)</td>
<td>3.5</td>
<td>6.6</td>
<td>0.64</td>
<td>1.26</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Each value is a mean of five replicates.

C = Control. N = Nematode alone. T\(_1\) = *Meloidogyne incognita* + *Paecilomyces lilacinus* one week before nematode inoculation. T\(_2\) = *Meloidogyne incognita* + *Paecilomyces lilacinus* simultaneously. T\(_3\) = *Meloidogyne incognita* + *Paecilomyces lilacinus* one week after nematode inoculation. T\(_4\) = *Meloidogyne incognita* + *Paecilomyces lilacinus* two weeks after nematode inoculation. T\(_5\) = *Meloidogyne incognita* + *Paecilomyces lilacinus* three weeks after nematode inoculations.
Table-7: Interactive effects of *Paecilomyces lilacinus* and root-knot nematode, *Meloidogyne incognita* on yield, chlorophyll pigment, protein content, oil content, root galling and egg mass production on *Eclipta alba*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>No. of capitulo plant&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>No. of seeds capitulum&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>100 seed weight</th>
<th>Chlorophyll content mg g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Shoot protein content (%)</th>
<th>Seed oil content (%)</th>
<th>No. of galls root system&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>No. of egg masses root system&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>23.6</td>
<td>104.0</td>
<td>0.064</td>
<td>0.752</td>
<td>8.92</td>
<td>12.20</td>
<td>75.6</td>
<td>152.4</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>13.4</td>
<td>51.2</td>
<td>0.041</td>
<td>0.695</td>
<td>8.43</td>
<td>11.05</td>
<td>34.2</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>22.8</td>
<td>98.0</td>
<td>0.059</td>
<td>0.733</td>
<td>8.82</td>
<td>12.01</td>
<td>52.4</td>
<td>88.2</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>20.6</td>
<td>86.2</td>
<td>0.052</td>
<td>0.720</td>
<td>8.66</td>
<td>11.72</td>
<td>63.2</td>
<td>120.0</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>17.8</td>
<td>71.2</td>
<td>0.048</td>
<td>0.709</td>
<td>8.59</td>
<td>11.48</td>
<td>69.2</td>
<td>122.8</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>16.0</td>
<td>70.6</td>
<td>0.046</td>
<td>0.703</td>
<td>8.55</td>
<td>11.24</td>
<td>70.0</td>
<td>134.4</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>15.4</td>
<td>64.4</td>
<td>0.043</td>
<td>0.702</td>
<td>8.52</td>
<td>11.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LSD</td>
<td>P&lt;0.01</td>
<td>7.06</td>
<td>27.50</td>
<td>0.014</td>
<td>0.032</td>
<td>0.38</td>
<td>0.89</td>
<td>14.24</td>
<td>34.00</td>
</tr>
<tr>
<td>LSD</td>
<td>P&lt;0.05</td>
<td>4.17</td>
<td>20.00</td>
<td>0.008</td>
<td>0.019</td>
<td>0.22</td>
<td>0.44</td>
<td>10.50</td>
<td>25.40</td>
</tr>
</tbody>
</table>

Each value is a mean of five replicates
Table-8: Physico-chemical characteristics of fly ash under study.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical conductivity (mmhos cm)</td>
<td>9.84</td>
</tr>
<tr>
<td>pH</td>
<td>8.90</td>
</tr>
<tr>
<td>Texture (%)</td>
<td></td>
</tr>
<tr>
<td>Sand size particles</td>
<td>42.5</td>
</tr>
<tr>
<td>Silt size particles</td>
<td>49.0</td>
</tr>
<tr>
<td>Clay size particles</td>
<td>8.5</td>
</tr>
<tr>
<td>Total organic carbon (%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Elements</strong></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>27.56 ppm</td>
</tr>
<tr>
<td>Ni</td>
<td>06.90 ppm</td>
</tr>
<tr>
<td>Mn</td>
<td>22.80 ppm</td>
</tr>
<tr>
<td>Co</td>
<td>03.82 ppm</td>
</tr>
<tr>
<td>B</td>
<td>21.71 ppm</td>
</tr>
<tr>
<td>Cu</td>
<td>01.52 ppm</td>
</tr>
<tr>
<td>K</td>
<td>722.20 ppm</td>
</tr>
<tr>
<td>Cr</td>
<td>13.91 ppm</td>
</tr>
<tr>
<td>Cd</td>
<td>00.24 ppm</td>
</tr>
<tr>
<td>Zn</td>
<td>03.04 ppm</td>
</tr>
<tr>
<td>Fe</td>
<td>02.43 ppm</td>
</tr>
</tbody>
</table>
Table-9: Texture of soil, fly ash, and fly ash amended soil (Black, 1968)

<table>
<thead>
<tr>
<th></th>
<th>Soil</th>
<th>Fly ash</th>
<th>10% fly ash</th>
<th>20% fly ash</th>
<th>30% fly ash</th>
<th>40% fly ash</th>
<th>50% fly ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand %</td>
<td>75.5</td>
<td>42.5</td>
<td>72.2</td>
<td>68.9</td>
<td>65.6</td>
<td>62.3</td>
<td>59.0</td>
</tr>
<tr>
<td>Silt %</td>
<td>16.0</td>
<td>49.0</td>
<td>19.3</td>
<td>22.6</td>
<td>25.9</td>
<td>29.2</td>
<td>32.5</td>
</tr>
<tr>
<td>Clay %</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Loamy sand</td>
<td>Silt loam</td>
<td>Loamy sand</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
</tr>
</tbody>
</table>
Table-10: Effect of different concentrations of fly ash on the plant growth of *Eclipta alba*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plant length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>No. of leaves plant⁻¹</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>C (Control)</td>
<td>20.9</td>
<td>26.9</td>
<td>2.55</td>
<td>8.05</td>
<td>1.14</td>
</tr>
<tr>
<td>T₁ (10% FA)</td>
<td>22.2</td>
<td>27.8</td>
<td>2.67</td>
<td>8.46</td>
<td>1.25</td>
</tr>
<tr>
<td>T₂ (20% FA)</td>
<td>23.6</td>
<td>29.9</td>
<td>3.05</td>
<td>9.14</td>
<td>1.45</td>
</tr>
<tr>
<td>T₃ (30% FA)</td>
<td>25.8</td>
<td>32.6</td>
<td>3.30</td>
<td>10.12</td>
<td>1.60</td>
</tr>
<tr>
<td>T₄ (40% FA)</td>
<td>23.0</td>
<td>28.8</td>
<td>2.76</td>
<td>8.55</td>
<td>1.32</td>
</tr>
<tr>
<td>T₅ (50% FA)</td>
<td>14.8</td>
<td>20.0</td>
<td>1.66</td>
<td>5.85</td>
<td>0.64</td>
</tr>
<tr>
<td>LSD (P≤0.01)</td>
<td>5.2</td>
<td>6.6</td>
<td>0.90</td>
<td>2.10</td>
<td>0.48</td>
</tr>
<tr>
<td>LSD (P≤0.05)</td>
<td>2.8</td>
<td>4.0</td>
<td>0.62</td>
<td>1.31</td>
<td>0.35</td>
</tr>
</tbody>
</table>

FA = Fly ash  
Each value is a mean of five replicates
Table-11: Effect of different concentrations of fly ash on yield, chlorophyll pigments, protein and oil content of *Eclipta alba*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>No. of capitula plant$^{-1}$</th>
<th>No. of Seeds capitulum$^{-1}$</th>
<th>100 seed weight (g)</th>
<th>Chlorophyll pigment g$^{-1}$ leaf</th>
<th>Shoot protein content</th>
<th>Seed oil content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (Control)</td>
<td>19.4</td>
<td>99.4</td>
<td>0.061</td>
<td>0.766</td>
<td>0.509</td>
<td>8.97</td>
</tr>
<tr>
<td></td>
<td>T$_1$ (10% FA)</td>
<td>20.6</td>
<td>102.2</td>
<td>0.063</td>
<td>0.774</td>
<td>0.514</td>
<td>9.13</td>
</tr>
<tr>
<td></td>
<td>T$_2$ (20% FA)</td>
<td>23.8</td>
<td>118.0</td>
<td>0.067</td>
<td>0.792</td>
<td>0.520</td>
<td>9.35</td>
</tr>
<tr>
<td></td>
<td>T$_3$ (30% FA)</td>
<td>26.2</td>
<td>122.6</td>
<td>0.075</td>
<td>0.807</td>
<td>0.530</td>
<td>9.68</td>
</tr>
<tr>
<td></td>
<td>T$_4$ (40% FA)</td>
<td>21.8</td>
<td>108.0</td>
<td>0.065</td>
<td>0.780</td>
<td>0.519</td>
<td>9.32</td>
</tr>
<tr>
<td></td>
<td>T$_5$ (50% FA)</td>
<td>12.0</td>
<td>71.8</td>
<td>0.043</td>
<td>0.721</td>
<td>0.485</td>
<td>8.16</td>
</tr>
<tr>
<td></td>
<td>LSD P$&lt;0.01$</td>
<td>7.20</td>
<td>26.00</td>
<td>0.016</td>
<td>0.043</td>
<td>0.022</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>LSD P$&lt;0.05$</td>
<td>5.80</td>
<td>18.80</td>
<td>0.008</td>
<td>0.028</td>
<td>0.012</td>
<td>0.40</td>
</tr>
</tbody>
</table>

FA = Fly ash
Each value is a mean of five replicates
Table 12: Effect of fly ash and root-knot nematode, *Meloidogyne incognita* on plant growth of *Eclipta alba*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plant length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>No. of leaves plant(^1)</th>
<th>Leaf area (cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Treatments</td>
<td>No. of capitula plant$^{-1}$</td>
<td>No. of Seeds capitulum$^{-1}$</td>
<td>100 seed weight</td>
<td>Chlorophyll content mg g$^{-1}$</td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
<td>-----------------------------</td>
<td>-------------------------------</td>
<td>----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td></td>
<td>C (Control)</td>
<td>22.8</td>
<td>109.2</td>
<td>0.063</td>
<td>0.740</td>
</tr>
<tr>
<td></td>
<td>IC (Inoculated Control)</td>
<td>13.6</td>
<td>53.0</td>
<td>0.042</td>
<td>0.682</td>
</tr>
<tr>
<td></td>
<td>$T_1$ (J$_2$ + 10% FA)</td>
<td>14.2</td>
<td>60.4</td>
<td>0.045</td>
<td>0.690</td>
</tr>
<tr>
<td></td>
<td>$T_2$ (J$_2$ + 20% FA)</td>
<td>18.4</td>
<td>74.6</td>
<td>0.055</td>
<td>0.706</td>
</tr>
<tr>
<td></td>
<td>$T_3$ (J$_2$ + 30% FA)</td>
<td>20.2</td>
<td>84.8</td>
<td>0.061</td>
<td>0.717</td>
</tr>
<tr>
<td></td>
<td>$T_4$ (J$_2$ + 40% FA)</td>
<td>17.8</td>
<td>70.6</td>
<td>0.052</td>
<td>0.704</td>
</tr>
<tr>
<td></td>
<td>$T_5$ (J$_2$ + 50% FA)</td>
<td>16.2</td>
<td>67.0</td>
<td>0.047</td>
<td>0.695</td>
</tr>
<tr>
<td></td>
<td>LSD P$\leq$0.01</td>
<td>6.00</td>
<td>26.50</td>
<td>0.016</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>LSD P$\leq$0.05</td>
<td>4.10</td>
<td>16.80</td>
<td>0.009</td>
<td>0.018</td>
</tr>
</tbody>
</table>

$J_2$ = 2,000 Second-stage juveniles of Meloidogyne incognita per pot; FA = Fly ash; Each value is a mean of five replicates
Table 14: Effect of fly ash on the Morphometrics of *Meloidogyne incognita*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Body length (Female) (μm)</th>
<th>Body width (female) (μm)</th>
<th>Neck length (μm)</th>
<th>Neck width (μm)</th>
<th>Stylet length (μm)</th>
<th>Stylet width (μm)</th>
<th>Median bulb length (μm)</th>
<th>Median bulb width (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (Control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IC (Inoculated Control)</td>
<td>788.40</td>
<td>423.41</td>
<td>252.70</td>
<td>82.70</td>
<td>17.15</td>
<td>3.20</td>
<td>43.01</td>
<td>35.91</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; (J&lt;sub&gt;2&lt;/sub&gt; + 10% FA)</td>
<td>784.02</td>
<td>419.30</td>
<td>250.92</td>
<td>80.53</td>
<td>17.14</td>
<td>3.19</td>
<td>41.52</td>
<td>35.62</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; (J&lt;sub&gt;2&lt;/sub&gt; + 20% FA)</td>
<td>771.20</td>
<td>413.50</td>
<td>248.75</td>
<td>77.10</td>
<td>17.12</td>
<td>3.18</td>
<td>38.23</td>
<td>35.08</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (J&lt;sub&gt;2&lt;/sub&gt; + 30% FA)</td>
<td>765.27</td>
<td>410.25</td>
<td>242.32</td>
<td>73.90</td>
<td>17.00</td>
<td>3.17</td>
<td>36.45</td>
<td>32.92</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (J&lt;sub&gt;2&lt;/sub&gt; + 40% FA)</td>
<td>760.22</td>
<td>400.37</td>
<td>236.0</td>
<td>66.50</td>
<td>16.94</td>
<td>3.16</td>
<td>31.57</td>
<td>31.42</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; (J&lt;sub&gt;2&lt;/sub&gt; + 50% FA)</td>
<td>752.26</td>
<td>398.44</td>
<td>219.39</td>
<td>60.20</td>
<td>16.91</td>
<td>3.16</td>
<td>29.06</td>
<td>31.28</td>
</tr>
<tr>
<td>LSD P&lt;0.01</td>
<td>24.39</td>
<td>16.34</td>
<td>13.22</td>
<td>9.75</td>
<td>0.20</td>
<td>0.04</td>
<td>7.21</td>
<td>3.06</td>
</tr>
<tr>
<td>LSD P&lt;0.05</td>
<td>17.94</td>
<td>11.98</td>
<td>9.68</td>
<td>6.26</td>
<td>0.13</td>
<td>0.03</td>
<td>5.36</td>
<td>1.62</td>
</tr>
</tbody>
</table>

J<sub>2</sub> = 2,000 Second-stage juveniles of *Meloidogyne incognita* per pot; FA = Fly ash
Each value is a mean of five replicates
P-1:  ES : Entry site of J$_2$; Separated epidermis.

P-2:  J  : Second-stage juvenile in meristamatic zone.

P-3:  J  : Second-stage juvenile causing hypertrophy in differentiating phloem

Ph  : Normal phloem.

P-4:  J  : A portion of second-stage juveniles in phloem zone.

VE  : Normal vessel element in Metaxylem.
P-25: J : Second-stage juveniles in abnormal phloem formed in
the vicinity of the giant cell complex.

P-26: N : A mature female feeding on the giant cell cytoplasm
(GCC). A large giant cell became vacuolated HT:
Hypertrophic parenchyma.

P-27: N : A mature female feeding on giant cell cytoplasm from
the giant cell complex (GCC); n: large nuclei; nu: large
nucleoli in the nuclei; HP: Hyperplastic parenchyma.

P-28: N : A mature female; GC: Giant cells containing large, lobed
nuclei with nucleoli; AP: Abnormal phloem near the
giant cells.
P-36: FH : Hyphae of *P. lilacinus* growing on the root surface and in the root tissue.

P-37: C : Conidial chain of *P. lilacinus*; FH: Fungal hyphae; Egg: Hyphae parasitizing the egg.

P-38: EM : Egg mass of *M. incognita*; FH: Fungal hyphae; Egg : Degenerating egg due to fungal infection.

P-39: Egg : Egg of *M. incognita* parasitized by fungal hyphae (FH) of *P. lilacinus*. 
P-33: GC : A giant cell in a typical giant cell complex with the nematode head (N), the giant cell complex appeared to be surrounded by abnormal xylem; AX: Abnormal xylem; AVE: Scattered abnormal vessel elements.

P-34: N : A mature nematode; E.M: Egg mass.

P-35: VE : A vessel elements; FH: Hyphae of the fungus *Paecilomyces lilacinus*. 
P-40: N A degenerating mature female of *M. incognita* due to fungal hyphae (FH) of *P. lilacinus*.

P-41: VE A transverse section of normal vessel elements without fungal hyphae.

P-42: FH Fungal hyphae growing in vessel elements and in parenchyma.

P-43: EM An egg was degenerated by fungal hyphae.
Healthy Plant

M. incognita infected plant

P-48
P-44: FH : Fungal hyphae growing in all the tissues, giant cells and abnormal vessel elements.

P-45: C : Chain of conidia in vessel elements (VE).

P-46: C : Chain of conidia and fungal hyphae on the surface of *M. incognita* infected roots.

P-47: C : Conidia in vessel elements and abnormal vessel elements.