Experiment No. 1

Effect of fly ash amended soil on the plant growth, yield, chlorophyll pigment and protein content of *Lagenaria leucantha*

The coal based Thermal Power Plants emit fly ash, which is collected in large pits. The fly ash spreads in the surrounding areas. It alters the soil quality affecting physical and chemical characteristics. The amended soil produces harmful affects on the vegetation depending upon the extent of the deposition. The Thermal Power Plant, Kasimpur, was selected as the pollution source for this study. The soil, in windward direction of this Power Plant was found to have heavy depositions of fly ash. In this experiment effects of fly ash, on growth of *Lagenaria leucantha* and development of root-knot nematode (*Meloidogyne incognita*) have been examined. The experiment was performed in glass house. An amended soil was prepared by adding varying but constant concentration of fly ash into the soil.

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 analyzed by different methods. The texture of the fly ash in relation to particle size was determined by hydrometer method (Allen *et al.*, 1974). For electrical conductivity of fly ash, conductivity meter (Elico., Co. Ltd., Hyderabad, India) was used. The pH was measured with pH meter by using an extract prepared
from 1:1 fly ash / water suspension (w/v). Total organic carbon, total nitrogen and total phosphorus were analyzed 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).

**Soil amendment with Fly ash:** Sterilized sandy loam soil (7 clay : 3 sand : 1 farmyard manure) was amended by adding fly ash in different proportions (v/v) i.e. 0% (control), 10%, 20%, 30%, 40% and 50% to carry out experiments in the first year. In the second and the third years same soil was used except that farmyard manure was added proportionately.

**Plant Culture and Treatments:** Seeds of *Lagenaria leucantha* obtained from National Seed Corporation, New Delhi were axenized with 0.5% NaOCl solution. The seeds were then transferred to sterilized petridishes on moist filter paper for germination. The sprouted seed were then transferred to clay pots of 30 cm diameter having steam sterilized soil amended with fly ash. The inoculations were performed when the seedlings were three weeks old. Each treatment consisted of five replicates and the pots were kept in a randomized complete block design in the glass house. Uninoculated plants served as control.

**Plant Growth:** After termination of the experiment, lengths, fresh weights and dry weights of roots and shoots of inoculated and uninoculated plants were determined. Root and shoot length of plant was measured with the help of meter scale. After taking fresh weights of the roots and the shoots these were
kept in bamboo envelopes and placed in an incubator for 48 h at 80° C and weighed to obtain their dry weights.

**Number of Flowers and Fruits** : The number of flowers and fruits per plant of each treatment was counted by visual observation.

**Leaf Area** : Leaf area was ascertained by gravimetric method. The method of leaf area measurement was already described in experiment no. 1 of section II.

**Estimation of Chlorophyll** : The amount of chlorophyll a, b and total was estimated. The method of chlorophyll estimation was described earlier in experiment no.3 of section -I.

**Estimation of Protein** : The amount of soluble and insoluble protein was determined according to the method of Lowry *et al.* (1951). The method protein estimation was described earlier in experiment no. 3 section -I.

**RESULTS**

**Fly ash analysis**: The fly ash emanated from the Thermal Power Plant was alkaline in nature and its electrical conductivity was 9.84mmhos.cm⁻¹. Textural analysis indicated that the amount of silt size particles was greatest followed by sand and clay size particles. The organic carbon and nitrogen of the fly ash were 0.07% 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 concentration of K, Mn and B was higher than other metal elements (Table-6).
Root and shoot lengths: I Year: The roots and shoots of *Lagenaria leucantha* showed variable growth responses towards the soils containing varying fly ash levels. In comparison to control, a non-significant increase was observed at 10% and 20% fly ash levels. At 30% fly ash level, there was a significant ($P \leq 0.05$) increase in the root and shoot length, in comparison to control. A non-significant increase in the root and shoot length was also observed at 40% fly ash level when compared with control. In comparison to control, a significant ($P \leq 0.01$) reduction in the root and the shoot lengths was observed at 50% fly ash level (Table-8).

II Year: In comparison to control, a non-significant increase was observed in the root and shoot length of the plants grown in 10% and 20% fly ash amended soils. A significant ($P \leq 0.05$) increase in the root and shoot length was observed at 30% fly ash level when compared with control. A non-significant decrease, in comparison to control was observed at 40% fly ash level and at 50% fly ash level the reduction was significant ($P \leq 0.01$) (Table-9).

III Year: In the third year, a non-significant decrease was observed in the length of the roots and the shoots at 10%, 20% and 30% fly ash levels, when compared with control. Significant reductions ($P \leq 0.05$) at 40% fly ash level ($P \leq 0.01$) at 50% level were observed in the length of the root and the shoot (Table-10).

Roots and shoot weights: I Year: The root and the shoot weights non-significantly increased at 10% and 20% fly ash levels, in comparison to control. A significant ($P \leq 0.05$) increase at 30% fly ash level was observed in the root and the shoot weights, when compared with control. At 40% fly ash level there
was a non-significant increase in the root and the shoot weights, in comparison to control plants. A significant \( P \leq 0.01 \) reduction in the root and the shoot weight was also observed at 50% fly ash level, when compared with control (Table-8).

**II Year:** In comparison to control, a non-significant increase in the root and shoot weight was observed at 10% and 20% fly ash levels. At 30% fly ash level there was a significant \( P \leq 0.05 \) increase in the root and shoot weight, when compared with control. A non-significant decrease in the root and shoot weights was observed, in comparison to control, at 40% fly ash level. At 50% fly ash level there was a significant \( P \leq 0.01 \) decrease in the root and shoot weight, when compared with control (Table-9).

**III Year:** The root and shoot weights non-significantly decreased at 10%, 20% and 30% fly ash levels. There was a significant \( P \leq 0.05 \) decrease in the root and shoot weight, in comparison to control at 40% fly ash level; a significant \( P \leq 0.01 \) reduction was also observed at 50% fly ash level (Table-10).

**Number of flowers per plant: I Year:** The number of the flowers increased non-significantly at 10% and 20% fly ash levels, when compared with control plant. In comparison to control, a significant \( P \leq 0.05 \) increase in the number of flowers per plant at 30% fly ash level, and a non-significant increase at 40% level was observed. A significant \( P \leq 0.01 \) decrease was observed at 50% fly ash level, when compared with control (Table-8).

**II Year:** In comparison to control, a non-significant increase was observed in the number of flowers of plants grown in soil containing 10% and 20% fly ash.
At 30% fly ash level a significant ($P \leq 0.05$) increase in the number of flowers was observed. A non-significant decrease in the number of flowers was observed at 40% fly ash level, in comparison to control. At 50% fly ash level there was a significant ($P \leq 0.01$) decrease in the number of flowers when compared with control (Table-9).

**III Year:** There was a non-significant decrease in the number of flowers per plant at 10%, 20% and 30% fly ash levels, in comparison to control. A significant ($P \leq 0.05$) reduction in the number of flowers per plant occurred at 40% fly ash level, when compared with control. The reduction was also significant ($P \leq 0.01$) at 50% fly ash level as compared to control (Table-10).

**Number of fruits per plant: I Year:** The number of fruits per plant increased non-significantly at 10% and 20% fly ash levels, when compared with control. A significant ($P \leq 0.05$) increase in the number of fruits per plant was observed at 30% fly ash level in comparison to control. A non-significant increase in comparison to control, was noticed at 40% fly ash level; there was a significant ($P \leq 0.01$) reduction in the number of fruits at 50% fly ash level (Table-8).

**II Year:** There was a non-significant increase in the number of flowers per plant at 10% and 20% fly ash levels, when compared with control. A significant ($P \leq 0.05$) increase, in comparison to control, was noticed at 30% fly ash level. At 40% fly ash level there was a non-significant decrease in the number of fruits per plant, when compared with control. A significant ($P \leq 0.01$) decrease in the number of fruits per plant was noticed at 50% fly ash level (Table-9).
III Year: The number of fruits decreased non-significantly at 10%, 20% and 30% fly ash levels, when compared with control. At 40% fly ash level, a significant (P ≤ 0.05) reduction in the number of fruits, in comparison to control was noticed. A significant (P ≤ 0.01) reduction in the number of fruits was also observed at 50% fly ash level, when compared with control (Table-10).

Leaf area: I Year: The leaf area increased non-significantly at 10% and 20% fly ash levels, in comparison to control. A significant (P ≤ 0.05) increase in the leaf area occurred at 30% fly ash level. At 40% fly ash level a non-significant increase, in comparison to control, was noticed in the leaf area of the plants. A significant (P ≤ 0.01) reduction in the leaf area was observed at 50% fly ash level, when compared with control (Table-8).

II Year: In comparison to control, a non-significant increase in the leaf area was observed at 10% and 20% fly ash levels. A significant (P ≤ 0.05) increase in the leaf area was noticed at 30% fly ash level when compared with control. At 40% fly ash level there was a non-significant decrease in the leaf area, in comparison to control. There was a significant (P ≤ 0.01) decrease in the leaf area at 50% fly ash level, when compared with control (Table-9).

III Year: The leaf area non-significantly reduced at 10%, 20% and 30% fly ash levels, when compared with control. At 40% fly ash level there was a significant (P ≤ 0.05) reduction in the leaf area, in comparison to control. At 50% fly ash level a significant (P ≤ 0.01) decrease in the leaf area was also observed when compared with control (Table-10).
**Chlorophyll content: I Year:** The chlorophyll content of the leaves of the plant non-significantly increased at 10% and 20% fly ash level when compared with control. At 30% fly ash level a significant ($P \leq 0.05$) increase was noticed in the chlorophyll content of the leaves in comparison to control. A non-significant increase in the chlorophyll content was observed, in comparison to control, at 40% fly ash level. A significant ($P \leq 0.01$) decrease in chlorophyll content occurred at 50% fly ash level (Table-8).

**II Year:** There was a non-significant increase in the chlorophyll content of the leaves of the plants grown in 10% and 20% fly ash amended soils. A significant ($P \leq 0.05$) increase in the chlorophyll content occurred, in comparison to control, at 30% fly ash level. At 40% fly ash level a non-significant decrease was observed in the chlorophyll content of leaves, when compared to control. In comparison to control, a significant ($P \leq 0.01$) decrease was noticed in the chlorophyll content of the leaves at 50% fly ash level (Table-9).

**III Year:** In comparison to control, there was a non-significant decrease in the chlorophyll content of the leaves at 10%, 20% and 30% fly ash levels. A significant ($P \leq 0.05$) reduction in the chlorophyll content of leaves occurred at 40% fly ash level when compared with control. At 50% fly ash level there was a significant ($P \leq 0.01$) decrease in the chlorophyll content of the leaves (Table-10).

**Protein content: I Year:** In comparison to control, there was a non-significant increase in total protein at 10% and 20% fly ash levels. A significant ($P \leq 0.05$) increase in protein content occurred at 30% fly ash level when
compared with control. At 40% fly ash level there was a non-significant increase, in comparison to control. A significant ($P \leq 0.01$) reduction, in comparison to control, was also observed at 50% fly ash level (Table-8).

II Year: A non-significant increase was observed in total protein content at 10% and 20% fly ash levels, when compared with control. A significant ($P \leq 0.05$) increase in the protein content was observed at 30% fly ash level, in comparison to control. At 40% fly ash level a non-significant decrease in the protein content in comparison to control was noticed. A significant ($P \leq 0.01$) decrease in the protein content in comparison to control had occurred at 50% fly ash level (Table-9).

III Year: The total protein content of the plant non-significantly reduced at 10%, 20% and 30% fly ash levels, when compared with control. At 40% fly ash level there was a significant ($P \leq 0.05$) reduction in total protein content, when compared with control. A significant ($P \leq 0.01$) reduction, in comparison to control, was observed in the protein content at 50% fly ash level (Table-10).

DISCUSSION

Application of fly ash into the soil, in different concentrations, produced both beneficial and harmful effects on plant growth, yield, leaf area, leaf pigments and protein content of *Lagenaria leucantha* var. Kasturi. The data (Table-9) revealed that an increase in the rate of application of fly ash from 10% to 30% level improved plant growth in the first year. Increase in growth parameters, amount of chlorophyll pigments, and protein content and yield was significant only at 30% fly ash level. It was, however, 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 physico-chemical characteristics of the amended soil. In our study 30% fly ash level proved to be optimally useful for the plant growth. The observed responses of plants are in line with the results of other experiments carried out by Mishra and Shukla (1986) on maize and soybean; Khan and Khan (1989) on tomato; Pasha et al. (1990) on cucumber; Singh (1993) on soybean; Khandkar et al. (1996) on rice, soybean and black gram; Srivastava et al. (1995) on Lactuca sativa; Krejsl et al. (1996) on bean; Tripathy and Sahu (1997) on wheat; Tripathy and Tripathy (1998) on Albizia procera and Acacia nilotica; Kalra et al. (1998) on wheat, chickpea, mustard and lentil; and Bharti et al. (2000) on green gram. Similar results were also reported by Plank and Martens (1973); Plank et al. (1975); Schnappinger et al. (1975); Martens and Beahm (1976); Elseewi et al. (1978a,b; 1980). Seedlings of some trees and shrubs had shown luxuriant growth in fly ash amended soils (Hodgson and Towns, 1973; Scanlon and Duggan, 1979). In the present study, the increase in plant growth, chlorophyll content of the leaves, protein content, flowering and fruiting, in the soil having 10% to 30% fly ash, might be attributed to the presence of utilizable plant nutrients in fly ash. Druzhina et al., 1983 advocated the presence of phytoutilizable nutrients in fly ash. Martens and Beahm (1978) observed increase in growth of plant and yield. The fly ash neutralizes the acidity of soil where the pH of soil is low; it increases ion exchange capacity, water holding capacity and porosity of the amended soil (Jones and Straughan, 1978; Adriano et al., 1980; Elseewi et al., 1981).

The fly ash of Kasimpur Thermal Power Plant was found to contain some utilizable nutrients like zinc, potassium, boron manganese etc., (Pasha,
1990). These nutrients might have increased the plant growth and yield of bottle gourd. Higher level of fly ash (50%) was found to be harmful for the plant growth and yield. High alkalinity and excess of mineral elements in fly ash might have caused toxic effects on plant growth, which led to poor yield, at higher levels. At higher rates of fly ash application adverse effects of fly ash on plant growth, have been attributed to these micro-nutrients particularly boron (B) (Capp and Engle, 1967; Capp and Faber, 1970; Mulford and Martens, 1971; Adriano et al., 1980). The harmful effects of fly ash at higher concentration might also be due to toxic effects of the compounds like dibenzofuran and dibenzo-p-dioxin mixture and heavy metals found in fly ash. (Kamath, 1979; Helder et al., 1982; Mishra and Shukla, 1986; Wong and Wong, 1986). Adverse effects of higher concentration of fly ash on plant growth and yield of several crops were reported by different workers (Mishra and Shukla, 1986; Singh, 1989; Pasha et al., 1990; Singh, 1993; Singh et al., 1994; Khan and Khan, 1996; Kalra et al., 1998). Phytotoxicity due to excessive uptake of boron in fresh bean and rhodes grass was reported by Aitken and Bell (1985). Excessive uptake of the elements and subsequent accumulation in the plant might have caused reduced growth and yield of bottle gourd. Metallic elements like Ni, Ar, Cd, Cr, Pb, Se, Zn, Cu etc., (Wong and Wong, 1986), which are reported to occur in fly ash, might have contributed towards poor growth and yield of bottle gourd at higher levels of fly ash.

Addition of 50% fly ash to the soil changes physical characteristics of the soil; porosity of soil decreases and water holding capacity increases since the normal soil is sandy loam and the texture of the fly ash is loamy. The change in texture and other physical characteristics of the soil might be
disadvantageous to bottle gourd, which grows luxuriantly in sandy loam soil. Probably, physiological and biochemical activities of the plants had been influenced by the fly ash amendments. Leaf pigments (chlorophyll a and b) and protein content of bottle gourd increased with increase in fly ash level upto 40% but decreased at 50% level. At lower fly ash level increase in chlorophyll content in the leaves might be because of increase in leaf surface area. However, higher fly ash levels were probably injurious to the plant and caused reduction in chlorophyll content. Higher amount of protein content at lower fly ash levels might be attributed to increased chlorophyll content. Since the plant growth increased, therefore the plants, probably, synthesized more enzymes and other structural proteins. Similar responses had been observed by Mishra and Shukla (1986) on maize and soybean; Pasha et al. (1990) on cucumber; and Singh (1993) on soybean. Nitrogen was altogether absent in fly ash (Adriano et al., 1980), therefore chlorophyll pigments of bottle gourd might have affected adversely because of insufficient availability of enzymes. Reduction in protein content of the plant is quite obvious because of less availability of nitrogen. Singh (1988) and Pasha (1990) found a positive correlation between leaf pigments and plant growth.

The present study showed that amendment of soil with the concentration of the fly ash upto 30% was beneficial for plant growth, yield, leaf pigment, protein content of bottle gourd and toxic at higher level.

In the second year, significant difference in comparison to control, was not observed at 10% and 20% levels. Even at 30% level, which had shown a significant increase in the first year, exhibited a non-significant increase in the second year. The total yield of the crop increased non-significantly in the
second year at 10%, 20% and significantly at 30% level. At higher levels of fly ash, the yield reduced non-significantly at 40% and significantly at 50% level. Similar trend of increase and decrease was also observed in leaf area, chlorophyll pigment, and protein content of *L. leucantha*. Highest amount of chlorophyll pigment and protein contents at 30% level and lowest at 50% level might be due to the fact that the soil had lost most of its nutritive elements in the first year which were unavailable in the next year. Further it might be considered that excessive amount of heavy metals as well as micro-nutrients which had retained in the soil caused harmful effects on the plants. Plank and Martens (1973); Schnappinger et al. (1975); Elseewi et al. (1978a,b; 1980); Mishra and Shukla (1986); Khan Khan (1989); Singh (1993); Tripathy and Sahu (1997); Kalra et al. (1998) and Bharti et al. (2000) suggested that low levels of fly ash were beneficial for the plant growth and yield. They did not mention for how long the amended soil would remain beneficial or after how long the soil would require the same amount of fly ash to regain its nutritive status. In either the case, the soil texture would be affected (Table-7).

*Lagenaria leucantha* showed retardation in plant growth, yield, chlorophyll pigment and protein content in the third year. A non-significant reduction occurred in plant growth at 10%, 20% and 30% level while a significant reduction was observed at 40% and 50% levels, when compared with control. The yield of the crop was also found to be reduced, non-significantly, at 10%, 20% and 30% levels. A non-significant decrease in the chlorophyll pigment and protein content was noticed at 10%, 20% and 30% levels while a significant reduction was observed at 40% and 50% levels.
Increase in plant growth at lower fly ash levels was upheld by several workers like Neeta et al. (1997) on Hardwickia binata; Singh et al. (1997) on Albizia procera; Kuchnawar and Matte (1997) on groundnut and Malewar et al. (1998) on nilgiri, neem, custard apple and jamun. But their studies were converged only on one year. In the third consecutive year, our findings are in contrary to all the above mentioned work. In comparison to control, reduction in all the parameters at all the fly ash levels might be attributed to (i) change in texture of soil (ii) increase in pH of soil (iii) reduction in porosity of soil (iv) presence of heavy metals (v) presence of toxic substances in fly ash amended soil. Our findings are further strengthened by comparing the results of the first year with the second and the third year. It was found that plants exhibited reduction in the growth and yield almost at all the fly ash levels in the second and the third year, when comparison was made with results of first year. Reduction in all parameter was highest at 50% fly ash level in the third year than the second year. The chlorophyll pigment and protein content were also reduced non-significantly at 10% fly ash level, and significantly from 20% to 50% level, when compared with the first year. From our results, it might be suggested that due to poor availability of nitrogen, the growth of the plant was retarded and also the chlorophyll pigment and protein content were reduced in the third year. Singh (1988) and Pasha (1990) found a positive correlation between plant growth and chlorophyll pigments.

In all the parameters studied in the first year, an increasing trend was observed from 10% to 40% fly ash level, as compared to control. Increase was significant only at 30% fly ash level. However a significant reduction was observed at 50% fly ash level. Our results are in accordance with those of
Mishra and Shukla (1986); Pasha et al. (1990) Khandakar et al. (1996); Neeta et al. (1997) Singh et al. (1997), Kuchnawar and Matte (1997) and Malewar et al. (1998). The increase in all the parameters of the plant from 10% to 30% fly ash level might be attributed to the presence of utilizable nutrients in fly ash (Druzina et al., 1983). The fly ash neutralizes the acidity of the acidic soil and increases ion exchange capacity, water holding capacity, and decreases the porosity of the amended soil which might have caused favourable effect on plant growth yield, chlorophyll pigment and protein content.

In the second year, a similar increasing trend was observed except at 40% fly ash level, which also exhibited reduction. The quantum of increase in growth and other parameters, however, was lower than that of the first year. At 50% fly ash level, an increase in the reduction was observed. Regarding the effect of fly ash on the growth of the plant in the second year no work has been traced so far. Although some workers repeated the experiment in the next year but they did not use the same soil of the previous year. In the second year all the parameters showed retardation as compared to the first year because the soil had lost most of its nutritive elements in the previous year which became unavailable in the next year. The excessive amount of heavy metals and the micro-nutrients that were retained in the soil caused harmful effects on plants in the current year.

In the third year, while comparing with control, reduction in all the growth parameters was observed at all fly ash levels. The extent of reduction was lower at 10% to 30% fly ash levels and higher at 40% and 50% fly ash levels. No report has been traced so far by any other worker regarding the result of the third year. Retardation in all the growth parameters in the third
year was due to increased pH, increased cation exchange capacity, increased water holding capacity and decreased porosity of amended soil. The binding capacity of fly ash particles was lower because of absence of charges on the particles than the soil particles. Therefore fly ash particles were loosely bound to the roots of the plant that is why the growth of the plant was reduced in the third year. Further fly ash contained positively charged ion and the pH of the fly ash was above neutral (pH > 7); the fly ash neutralizes the acidity of the acidic soil. The organic matter present in the fly ash was absorbed by the plant in the previous year so the growth of the plant was retarded in the third year. The metallic elements that were responsible for the growth of the plant were probably present in the fly ash in the weathered form; these elements in the soil are present in ore form. All the minerals occur in the soil in oxidized form but not in fly ash.

The growth pattern in terms of increase or decrease in three consecutive years, was also analyzed. On considering first year as the base year, it was observed that the plants exhibited reduction at all the fly ash levels, without any exception in the second and third year. In comparison to first year, smaller reduction was observed in both the second and the third year, from 10% to 30% fly ash levels. Greater reduction was observed at 50% fly ash level compared to that of first year. This was because of utilizable nutrients present in the fly ash were observed in the previous year and became unavailable in the next year. The heavy metals and micro-nutrients that became toxic to the plants were retained in the soil and had harmful effects on the plants.

In our opinion, if the same amount of soil is added year after year, it would change not only the texture but also the physico-chemical characteristics
of the soil. Therefore, soil amendment with 10% or higher amount of fly ash can not be recommended to increase the fertility of the soil every year. It is, thus, suggested that the addition of fly ash is harmful to the plants in long run, and fly ash can not be used as a fertilizer.
Experiment No. 2

Effect of fly ash amended soil on the development of root-knot nematode (*Meloidogyne incognita*) and growth of bottle gourd (*Lagenaria leucantha*)

Fly ash is a fairly stable particulate pollutant and it alters the quality of the soil. The Thermal Power Plant, Kasimpur was selected as the source of pollution of present study. The experiment was performed in the glass house. Different proportions of fly ash were used to amend the soil. The effects of fly ash amendment on the plant growth, yield chlorophyll pigment and protein content, and the development of root-knot disease caused by *Meloidogyne incognita* were examined.

**Materials and Methods**

**Fly ash Analysis:** Fly ash was analyzed as has been explained in materials and methods of experiment no. 1 of section – III.

**Soil amendment with Fly ash:** Sterilized sandy loam soil (7 clay : 3 sand : 1 farmyard manure) was amended by adding fly ash in different proportions (v/v) i.e. 0% (control), 10%, 20%, 30%, 40% and 50%.

**Plant Culture and Treatments:** Seeds of *Lagenaria leucantha* obtained from National Seed Corporation, New Delhi were axenized with 0.5% NaOCl solution. The seeds were then transferred to sterilized petridishes on moist filter paper for germination. The sprouted seeds were then transferred to clay pots of 30 cm. diameter having steam sterilized soil amended with fly ash. The
inoculations were performed when the seedlings were three weeks old. Each treatment consisted of five replicates and the pots were kept in a randomized complete block design in the glass house. Uninoculated plants served as control. The treatments were as follows in the first year:

\[ T_1 = \text{Control} \]

\[ T_2 = 0\% \text{ fly ash} + 2000 J_2 / \text{pot} \]

\[ T_3 = 10\% \text{ fly ash} + 2000 J_2 / \text{pot} \]

\[ T_4 = 20\% \text{ fly ash} + 2000 J_2 / \text{pot} \]

\[ T_5 = 30\% \text{ fly ash} + 2000 J_2 / \text{pot} \]

\[ T_6 = 40\% \text{ fly ash} + 2000 J_2 / \text{pot} \]

\[ T_7 = 50\% \text{ fly ash} + 2000 J_2 / \text{pot} \]

In the second and third years, same soil and population of the nematode was used except that farmyard manure was added proportionately.

**Preparation and Inoculation of Root-knot nematode**: The egg masses were obtained from the infected roots of egg plants from cropfields. The egg masses were collected and then allowed to hatch in sterilized distilled water at 30°C in sterilized petridishes. The suspension was collected for three days at an interval of 24 h. The juveniles were counted with the help of counting dish. Three week old seedlings were then inoculated with freshly hatched juveniles (2000 J₂ /pot) by making holes, 5–7 cm deep within the radius of 2 cm. The holes were then plugged with steam sterilized soil. The plants were harvested 45 days after inoculation. To maintain moisture in the soil regular watering was
done. The data for different parameters were collected and statistically analyzed.

**Plant Growth**: After termination of the experiment, lengths, fresh weights and dry weights of roots and shoots of inoculated and uninoculated plants were determined. Root and shoot length of plant was measured with the help of a meter scale. After taking fresh weights of the roots and the shoots, these were kept in bamboo envelopes and placed in an incubator for 48 h at 80°C and weighed to obtain their dry weights.

**Number of Flowers and Fruits**: The number of flowers and fruits per plant of each treatment was counted by visual observation.

**Leaf Area**: Leaf area was ascertained by gravimetric method. The method of leaf area measurement was already described in experiment no. 1 of section II.

**Estimation of Chlorophyll**: Chlorophyll a, b and total was estimated. The method of chlorophyll estimation was described earlier in experiment no. 3 of section I.

**Estimation of Protein**: The amount of soluble and insoluble protein was determined according to the method of Lowry *et al.* (1951). The method of protein estimation was described earlier in experiment no. 3 of section I.

**Number of Galls**: The number of galls was counted by visual observation.

**Number of Egg masses**: Number of egg masses in infected roots was counted by staining egg masses with phloxin B.
RESULTS

Root and shoot length: I year: In comparison to control (T₁), a significant (P ≤ 0.01) reduction was observed in the root and the shoot length of Lagenaria leucantha inoculated with the nematode, Meloidogyne incognita (T₂). The reduction in the length of the roots and the shoots was also significant (P ≤ 0.01) in the plants inoculated with M. incognita and grown in fly ash amended soil.

The root and the shoot lengths increased non-significantly at 10% fly ash level inoculated with M. incognita (T₃), when compared with nematode inoculated plant (T₂). A significant (P ≤ 0.05) increase in the root and the shoot length occurred at 20% fly ash level (T₄) in comparison to only nematode inoculated plant (T₂). There was a significant (P ≤ 0.01) increase in the root and the shoot length, in comparison to nematode inoculated plant (T₂), at 30%, 40% and 50% fly ash level inoculated with M. incognita (T₅, T₆ and T₇ respectively). In comparison to control (T₁), maximum reduction was observed in T₂ and minimum in T₇ plants. In comparison to T₂, a gradual increase was noticed from T₃ to T₇ plants (Table- 11).

II year: The root and the shoot length of L. leucantha decreased significantly (P ≤ 0.01) in nematode inoculated plants, when compared with control (T₁). A significant (P ≤ 0.01) decrease in the root and the shoot length was also noticed in the plants treated with different levels of fly ash and inoculated with M. incognita (T₃ to T₇), as compared to control (T₁).

The root and the shoot length of the plant increased non-significantly at 10% fly ash level inoculated with M. incognita (T₃) when compared with
nematode inoculated plant (T2). A significant (P < 0.05) increase in the root and the shoot length of *L. leucantha* was noticed at 20% fly ash level inoculated with *M. incognita* (T3), as compared to nematode inoculated plants (T2). On comparing with nematode inoculated (T2) plants there was a significant (P < 0.01) increase in the root and the shoot length at 30% and 40% fly ash levels inoculated with *M. incognita* T5 and T6 plants. The increase in length of T7 plants was non-significant than the length of T2 plants. In comparison to control (T1), reduction in length was maximum in (T2) plants followed by (T7) plants. When a comparison was made with T2 plants, first the length gradually increased from T3 to T5 and than gradually decreased from T5 to T7. Enhancement was highest in T5 plants and lowest in T7 plants (Table-12).

**III year:** In comparison to control, the root and the shoot length decreased significantly (P < 0.01) in nematode inoculated plants. The reduction in the root and the shoot lengths from T2 to T7 was significant (P < 0.01), when compared with control.

On comparing with nematode inoculated plants (T2) there was a non-significant increase in the root and the shoot length of plant at 10% and 20% fly ash levels, inoculated with *M. incognita* (T3 and T4). A significant (P < 0.05) increase in the root and the shoot length of plant at 30% fly ash level inoculated with *M. incognita* (T5) when compared with only nematode inoculated plants (T2). The root and the shoot length increased non-significantly at 40% fly ash level inoculated with *M. incognita* (T6), and decreased non-significantly at 50% level (T7), as compared to nematode inoculated plants. In comparison to control (T1), maximum reduction was
observed in T7 plants and minimum in T5 plants. In comparison to T2 maximum increase was observed in T5 and minimum in T7 plants (Table- 13).

**Root and shoot weight: I year:** The fresh and dry weight of the roots and the shoots decreased significantly (P ≤ 0.01) in *M. incognita* inoculated plants (T2). A significant (P ≤ 0.01) reduction, in comparison to control (T1), also occurred in plants inoculated with *M. incognita* and grown in fly ash amended soil.

In comparison to *M. incognita* inoculated (T2) plants, the fresh and the dry weight of both the roots and the shoots increased non-significantly at 10% fly ash level (T3). A significant (P ≤ 0.05) increase in the weights of the root and the shoot was observed at 30% fly ash level inoculated with *M. incognita* (T4), when compared with nematode inoculated plants (T2). On comparing with nematode inoculated plants (T2), the fresh and the dry weight of both the root and the shoot increased significantly (P ≤ 0.01) at 30, 40, and 50% fly ash levels, inoculated with *M. incognita*. In comparison to control (T1) highest reduction in weight was observed in T2 plants. Highest weight was observed in T5 plants among the nematode inoculated plants grown in fly ash amended soil (Table- 11).

**II year:** In comparison to control, a significant (P ≤ 0.01) reduction was noticed in the root and the shoot weight of *L. leucantha* inoculated with *M. incognita* (T2). Significant reductions in the fresh and the dry weight of both the root and the shoot also occurred in the plants inoculated with *M. incognita* and treated with fly ash, as compared to control (T1).
The fresh and the dry weight of both the root and the shoot increased non-significantly at 10% fly ash level inoculated with *M. incognita* (T3), when compared with *M. incognita* inoculated plants (T2). A significant (P ≤ 0.05) increased in the fresh and dry weights of the root and the shoot occurred at 20% fly ash level inoculated with *M. incognita* (T4), in comparison to only *M. incognita* inoculated plants. The fresh and the dry weights of the root and the shoots increased significantly (P ≤ 0.01), in comparison to *M. incognita* inoculated plants (T2), at 30% fly ash level inoculated with *M. incognita* (T5). At 40% and 50% fly ash levels (T6 and T7), the fresh and the dry weights of the root and the shoot increased significantly (P ≤ 0.01), when compared with only *M. incognita* inoculated plants (T2). In T2 plants, the reduction in weight was highest as compared to control (T1). In fly ash amended soil, the nematode inoculated plants first exhibited an increase in gain of weight from 10% fly ash level to 40% fly ash level and then decrease at 50% level, when compared with only nematode inoculated plants (T2) (Table -12).

**III Year:** The fresh and the dry weights of both the roots and the shoots decreased significantly (P ≤ 0.01) in *M. incognita* inoculated plants when compared with control (T1). A significant (P ≤ 0.01) reduction in the fresh and the dry weights of both the root and the shoot was also noticed in the plants inoculated with *M. incognita* and treated with fly ash as compared to control (T1).

In comparison to nematode inoculated plant (T2), the fresh and the dry weights of root and shoot increased non-significantly in the plants inoculated with *M. incognita* and treated with 10% and 20% fly ash. A significant (P ≤ 0.05) increase in the weight of the root and the shoot was observed at 30% fly
ash level inoculated with *M. incognita*, when compared with T2 plants. The fresh and the dry weights of both root and shoot were increased non-significantly in comparison to *M. incognita* inoculated plant (T2), at 40% fly ash level (T6). There was a non-significant decrease in the fresh and the dry weights of both the root and the shoot of the plants inoculated with *M. incognita* and treated with 50% fly ash (T7), when compared to nematode inoculated plants (T2).

Significant reductions, in comparison to control (T1) were observed in all the nematode inoculated plants grown in fly ash amended or unamended soil. However, T7 plants exhibited maximum reduction over control. In comparison to T2 plants, a significant increase in plant weight was observed in T5 plants, whereas in all other treatments, the difference was non-significant (Table -13).

**Number of flowers: I Year:** There was a significant (*P* ≤ 0.01) reduction in the number of flowers of *L. leucantha* infected with *M. incognita*, when compared with control (T1). In comparison to control (T1), the number of flowers per plant decreased significantly (*P* ≤ 0.01) in the plants treated with fly ash and inoculated with *M. incognita*.

In comparison to nematode inoculated plants (T2), the number of flowers increased non-significantly in *L. leucantha* treated with 10% fly ash inoculated with *M. incognita*. A significant (*P*≤0.05) increase in the number of flowers was observed at 20% fly ash level inoculated with *M. incognita* (T4), when compared with nematode inoculated plants (T2). From 30% to 50% fly ash levels (T6 and T7, respectively), the number of flowers per plant increased.
significantly. \( P \leq 0.01 \). In comparison to control reduction in number of flowers per plant was observed in the treatment \( T_2 \). In comparison to \( T_2 \) plants the number of flowers per plant increased to maximum in the treatment \( T_7 \) (Table -11).

II year: The number of flowers per plant decreased significantly \( (P \leq 0.01) \) in \( M. \text{incognita} \) inoculated plant (\( T_2 \)), when compared with control (\( T_1 \)). In comparison to control (\( T_1 \)), the number of flowers of \( L. \text{leucantha} \) decreased significantly \( (P \leq 0.01) \) in fly ash treated and \( M. \text{incognita} \) infected plants.

There was a non-significant increase in the number of flowers per plant at 10% fly ash level inoculated with \( M. \text{incognita} (T_3) \), when compared with nematode inoculated plants (\( T_2 \)). A significant \( (P \leq 0.05) \) increase in the number of flowers was observed in the plants treated with 20% fly ash and inoculated with \( M. \text{incognita} (T_4) \), as compared to only nematode inoculated plants (\( T_2 \)). On comparing with nematode inoculated plants, the number of flowers per plant increased significantly \( (P \leq 0.01) \) at 30%, 40% and 50% fly ash levels (\( T_5, T_6 \) and \( T_7 \), respectively). The number of flowers per plant was lowest in the treatment \( T_2 \) as compared to control (\( T_1 \)). On comparing with \( T_2 \), the number of flowers was highest in \( T_3 \) at 30% fly ash level. An increasing trend in the number of flowers was observed from 10% to 30% fly ash levels and a decreasing trend from 40% to 50% fly ash levels (Table -12).

III year: The number of flowers per plant, as compared to control, decreased to minimum value in \( T_7 \), which was lower than \( T_2 \). In comparison to \( T_2 \) plants,
the number of flower increased from 10% to 30% fly ash level and then retarded at 40% and 50% levels.

In comparison to nematode inoculated plant (T2), the number of flowers per plant increased non-significantly at 10% and 20% fly ash level (T3 and T4, respectively). Increase in number of flowers was significant (P ≤ 0.05) in the plants at 30% fly ash level (T5), as compared to only nematode inoculated plant (T2). A non-significant increase in the number of flowers per plant was observed at 40% fly ash level inoculated with *M. incognita* (T6), when compared to *M. incognita* inoculated plant (T2). The number of flowers per plant decreased non-significantly in the plants inoculated with *M. incognita* and treated with 50% fly ash, in comparison to plants inoculated with *M. incognita* only (T2) (Table -13).

**Number of fruits: I year:** In comparison to control (T1), the number of fruits per plant decreased significantly (P ≤ 0.01) on *M. incognita* inoculated plants (T2). The reduction in number of fruits per plant was also significant (P ≤ 0.01) in the plants inoculated with *M. incognita* and treated with fly ash.

The number of fruits per plant increased non-significantly in the plants inoculated with *M. incognita* and treated with 10% fly ash (T3), as compared to *M. incognita* inoculated plants (T2). A significant increase in the number of fruits per plant was observed at 20% fly ash level inoculated with *M. incognita* (T4). On comparing with *M. incognita* inoculated plants (T2), the number of fruits per plant increased significantly (P ≤ 0.01) in the plants inoculated with *M. incognita* and treated with 30%, 40% and 50% fly ash (T5, T6 and T7, respectively). In the first year, in comparison to control, highest number of
fruits was recorded on the plants of T5. The number of fruits per plant was lowest on T2 plants (Table -11).

**II year:** The number of fruits per plant decreased significantly (P ≤ 0.01) on *M. incognita* inoculated plant (T2) as compared to control (T1). A significant (P ≤ 0.01) reduction, in comparison to control, was also observed in the plants treated with fly ash and inoculated with *M. incognita*.

In comparison to nematode inoculated plants (T2) the number of fruits per plant increased non-significantly at 10% fly ash level inoculated with *M. incognita* (T3). A significant (P ≤ 0.05) increase in the number of fruits was noticed in the plants inoculated with *M. incognita* and treated with 20% fly ash (T4), when compared to nematode inoculated plants (T2). The increase in the number of fruits per plant was also significant (P≤0.01) in the plant inoculated with *M. incognita* and treated with 30%, 40% and 50% fly ash (T5, T6 and T7, respectively). In the second year, in comparison to control, maximum reduction was observed in T2 plants. The reduction was maximum but significant in T5 plants (Table -12).

**III year:** A significant (P ≤ 0.01) decrease in the number of fruits per plant was observed in *M. incognita* inoculated (T2) plants, when compared with control (T1). The reduction in the number of fruits per plant was also significant (P ≤ 0.01) in fly ash treated plants inoculated with *M. incognita*, as compared to control (T1).

On comparing with *M. incognita* inoculated plants (T2) the number of fruits per plant increased non-significantly at 10% and 20% fly ash level inoculated with *M. incognita* (T3 and T4, respectively). A significant
(P < 0.01) increase in the number of fruits of *L. leucantha* occurred in the plants inoculated with *M. incognita* and treated with 30% fly ash (T₃), as compared to only nematode inoculated plants (T₂). At 40% fly ash level inoculated with *M. incognita* (T₆) there was a non-significant increase in the number of fruits per plant, when compared with nematode inoculated plants (T₂). The number of fruits per plant decreased non-significantly at 50% fly ash level inoculated with *M. incognita* (T₇). In the third year, in comparison to control reductions in the number of fruits per plant was maximum in T₂ and minimum in T₅ plants. In comparison to T₂ plants a maximum and significant (P < 0.05) increase was observed in T₅ plants (Table -13).

**Leaf area: I year:** The leaf area of the plant decreased significantly (P ≤ 0.01) in the plants inoculated with *M. incognita* (T₂) when compared with control (T₁). A significant (P ≤ 0.01) reduction in the leaf area was also observed in the plant inoculated with *M. incognita* and treated with different levels of fly ash, in comparison to control (T₁).

In comparison to *M. incognita* inoculated plants (T₂), the leaf area non-significantly increased at 10% fly ash level inoculated with *M. incognita* (T₃). A significant (P ≤ 0.05) increase in the leaf area was also noticed in the plants inoculated with *M. incognita* and treated with 20% fly ash (T₄). The increase in the leaf area was also significant (P ≤ 0.01) in the plants inoculated with *M. incognita* and treated with 30%, 40% and 50% fly ash levels, when compared to *M. incognita* inoculated plants (T₂) (Table -11).

**II year:** In comparison to control (T₁), the leaf area of *L. leucantha* decreased significantly (P ≤ 0.01) in nematode inoculated plants (T₂). A significant (P ≤
0.01) reduction in the leaf area was also noticed in the plants inoculated with *M. incognita* and treated with fly ash, when compared to control (T₁).

On comparing with only *M. incognita* inoculated plants, the leaf area increased non-significantly in the plants inoculated with *M. incognita* and treated with 10% fly ash (T₃). A significant (P ≤ 0.05) increase in the leaf area, as compared to *M. incognita* inoculated plants (T₂), was observed at 20% fly ash level inoculated with *M. incognita* (T₄). There was a significant (P ≤ 0.01) increase in the leaf area of the plant at 30%, 40% and 50% fly ash levels inoculated with *M. incognita* (T₅, T₆ and T₇, respectively), when compared with *M. incognita* inoculated plants (T₂) (Table -12).

III year: There was a significant (P ≤ 0.01) reduction in the leaf area of the plants inoculated with *M. incognita* (T₂), when compared with control (T₁). A significant (P ≤ 0.01) decrease in the leaf area also occurred in the plants inoculated with *M. incognita* and treated with fly ash as compared to control (T₁).

The leaf area increased non-significantly in the plants inoculated with *M. incognita* and treated with 10% and 20% fly ash (T₃ and T₄, respectively) as compared to only *M. incognita* inoculated plants (T₂). There was a significant (P ≤ 0.05) increase in the plants inoculated with *M. incognita* and treated with 30% fly ash (T₅), when compared to *M. incognita* inoculated plants (T₂). In comparison to *M. incognita* inoculated plants (T₂), the leaf area increased non-significantly at 40% fly ash level inoculated with *M. incognita* (T₆). At 50% fly ash level and *M. incognita* inoculated plants (T₇) the leaf area non-
significantly decreased when compared with *M. incognita* inoculated plants (T2) (Table -13).

**Chlorophyll content: I year:** In comparison to control (T1), the chlorophyll content of the leaves of *L. leucantha* decreased significantly (*P* ≤ 0.01) in *M. incognita* inoculated plants (T2). The reduction in the chlorophyll content was also significant (*P* ≤ 0.01) in the plants inoculated with *M. incognita* and treated with fly ash, when compared with control.

The chlorophyll content of the leaves increased non-significantly at 10% fly ash level inoculated with *M. incognita* (T3), when compared with plants inoculated with *M. incognita* only (T2). A significant (*P* ≤ 0.05) increase in chlorophyll content occurred in the plants inoculated with *M. incognita* and treated with 20% fly ash (T4), in comparison to *M. incognita* inoculated plants (T2). On comparing with only *M. incognita* inoculated plants (T2) the chlorophyll content of the leaves increased significantly (*P* ≤ 0.01) in the plants inoculated with *M. incognita* and treated with 30%, 40% and 50% of fly ash (T5, T6 and T7, respectively) (Table -11).

**II year:** The chlorophyll content of the leaves of *L. leucantha* decreased significantly (*P* ≤ 0.01) in the plants inoculated with *M. incognita* (T2), in comparison to control (T1). A significant (*P* ≤ 0.01) reduction in the chlorophyll content of the leaves was also observed in plants inoculated with *M. incognita* and treated with fly ash, when compared with control.

In comparison to *M. incognita* inoculated plants (T2), the chlorophyll content of the leaves of *Lagenaria leucantha* increased non-significantly at 10% fly ash inoculated with *M. incognita* (T3). The increase in the chlorophyll
content was also significant (\( P \leq 0.05 \)) in the plants inoculated with \( M. \) incognita and treated with 20% fly ash (T4), when compared with \( M. \) incognita inoculated plants (T2). The chlorophyll content of leaves of \( L. \) leucantha also significantly (\( P \leq 0.01 \)) increased in the plants inoculated with \( M. \) incognita and treated with 30%, 40% and 50% fly ash (T5, T6 and T7, respectively), in comparison to nematode (\( M. \) incognita) inoculated plants (T2) (Table -12).

**III year:** The chlorophyll content of the leaves significantly (\( P \leq 0.01 \)) reduced in the plants inoculated with \( M. \) incognita only, when compared to control (T1). A significant (\( P \leq 0.01 \)) decrease in the chlorophyll content, in comparison to control (T1), occurred in the plants inoculated with \( M. \) incognita and treated with fly ash.

In the third year, the chlorophyll content of leaves non-significantly increased at 10% and 20% fly ash (T3 and T4, respectively) inoculated with \( M. \) incognita, as compared with \( M. \) incognita inoculated plants (T2). At 30% fly ash level (T3) the chlorophyll content increased significantly (\( P \leq 0.05 \)), when compared with \( M. \) incognita inoculated plants (T2). On comparing with \( M. \) incognita inoculated plants (T2), the chlorophyll content of the leaves of \( L. \) leucantha increased non-significantly in the plants inoculated with \( M. \) incognita and treated with 40% and 50% fly ash (T6, T7) respectively (Table -13).

**Protein content: I year:** In comparison to control (T1), the protein content of the plant decreased significantly (\( P \leq 0.01 \)) in \( M. \) incognita inoculated plant (T2). The decrease in the protein content was also significant (\( P \leq 0.01 \)) in the
plant inoculated with *M. incognita* and treated with fly ash, when compared with control.

The protein content of the plant increased non-significantly in the plants inoculated with *M. incognita* and treated with 10% fly ash (T3), in comparison to *M. incognita* inoculated plants (T2). A significant (P ≤ 0.05) increase in the protein content was noticed at 20% fly ash level inoculated with *M. incognita* (T4), when compared with *M. incognita* inoculated plants (T2). The increase in the protein content was also significant (P ≤ 0.01) in comparison to nematode (*M. incognita*) inoculated plants at 30%, 40% and 50% fly ash level inoculated with *M. incognita* (T5, T6 and T7, respectively) (Table -11).

**II year:** The protein content of *L. leucantha* decreased significantly (P ≤ 0.01) in the plants inoculated with *M. incognita* only (T2), as compared to control (T1). The reduction in the protein content was also significant (P ≤ 0.01) in the fly ash treated plants inoculated with *M. incognita*, when compared with control.

On comparing with *M. incognita* inoculated plants (T2), the protein content of the plant was found to be increased non-significantly at 10% fly ash level inoculated with *M. incognita* (T3). A significant (P ≤ 0.05) increase in the protein content also occurred in the plants inoculated with *M. incognita* and treated with 20% fly ash (T4), as compared to plants inoculated with *M. incognita* only. The increase in the protein content was also significant (P ≤ 0.01) in the plants inoculated with *M. incognita* and treated with 30%, 40% and 50% fly ash (T5, T6 and T7, respectively), when compared to *M. incognita* inoculated plants (T2) (Table -12).
III year: In comparison to control (T₁), the protein content of the plant decreased significantly (P ≤ 0.01) in the plants inoculated with *M. incognita* (T₂). A significant (P ≤ 0.01) reduction in the protein content also occurred in fly ash treated plants inoculated with *M. incognita* when compared with control (T₁).

The protein content of the plant increased non-significantly at 10% and 20% fly ash levels inoculated with *M. incognita* (T₃ and T₄), when compared with *M. incognita* inoculated plant (T₂). At 30% fly ash level inoculated with *M. incognita* there was a significant (P ≤ 0.05) increase in the protein content of the plant in comparison to *M. incognita* inoculated plants (T₂). On comparing with *M. incognita* inoculated plants (T₂) the protein content of the plant increased non-significantly at 40% fly ash level inoculated with *M. incognita* (T₅). A non-significant decrease was observed in the protein content of the plant at 50% fly ash level inoculated with *M. incognita* (T₆), when compared to the protein content of *M. incognita* inoculated plants (T₂) (Table - 13).

**Number of galls I year**: In the first year, the number of galls per plant, non-significantly decreased on nematode inoculated plants grown at 10% and 20% fly ash levels, when compared with only *M. incognita* inoculated plants (T₂). The nematode inoculated plants grown at 30% fly ash level, showed a significant (P ≤ 0.05) reduction in the number of galls per plant in comparison to only *M. incognita* inoculated plants (T₂). A significant (P ≤ 0.01) reduction was observed at 40% and 50% fly ash level in plant inoculated with *M. incognita* when compared with only *M. incognita* inoculated plants (T₂).
Maximum number of galls was found in T2 and minimum in T7 treatments (Table -11).

II year: In the second year, the number of galls decreased non- significantly on nematode inoculated plants grown at 10% and 20% fly ash levels (T3 and T4), as compared to only M. incognita inoculated plants (T2). At 30% level, the number of galls per plant decreased significantly (P ≤ 0.05) when compared with T2 plants. Reduction in the number of galls was also significant (P ≤ 0.01) at 40% and 50% level in the treatments T6 and T7, in comparison to untreated nematode inoculated controls (T2) (Table -12).

III year: A non- significant reduction in the number of galls per plant was observed at 10% and 20% fly ash level in T3 and T4 plants, when compared with only M. incognita inoculated plants (T2). At 30% fly ash level the number of galls decreased significantly (P ≤ 0.01) as compared to T2 plants. There was a significant (P ≤ 0.01) reduction in the number of galls per plant at 40% and 50% fly ash level, when compared to only M. incognita inoculated (T2). A gradual decrease in the number of galls per plant was noticed from T2 to T7 (Table -13).

Number of egg masses I year: The number of egg masses per plant decreased non- significantly at 10% and 20% fly ash levels in the treatments T3 and T4, as compared to nematode inoculated T2 plants. In T5, at 30% fly ash level the number of egg masses per plant decreased significantly (P ≤ 0.05) when compared with only nematode inoculated plants. A significant (P ≤ 0.01) decrease had occurred in the number of egg masses in the plants at 40% and
50% fly ash levels and inoculated with nematodes when compared to nematode inoculated plants (T2) (Table -11).

II year: There was a non-significant decrease in the number of egg masses per plant in the treatments T3 and T4, when compared with only nematode inoculated plants (T2). A significant (P ≤ 0.05) reduction had occurred in number of egg masses per plant at 30% fly ash level inoculated with *M. incognita* (T5), when compared with only nematode inoculated plants (T2). The number of egg masses per plant decreased significantly (P < 0.01) at 40% and 50% fly ash levels in the treatments T6 and T7, in comparison to only *M. incognita* inoculated plants (Table -12).

III year: The number of egg masses per plant decreased non-significantly at 10% and 20% fly ash levels T3 and T4 treatments as compared to T2 plants. At 30% fly ash level, the number of egg masses per plant decreased significantly (P ≤ 0.01) when compared with nematode inoculated plants (T2). At 40% and 50% fly ash levels, the number of egg masses per plant decreased significantly (P ≤ 0.01) in comparison to only nematode inoculated T2 plants (Table- 13).

**DISCUSSION**

Addition of fly ash into the soil causes changes in physical and chemical characteristics of the soil. For instance, fly ash results in increase in cation exchange capacity, pH and water holding capacity; decrease in porosity; and increase in concentration of carbonate and bicarbonates. Higher concentration of carbonates and bicarbonates (Khan and Khan, 1996) and a pH above neutral (Khalil and Shah, 1979) caused adverse effects on nematode penetration and subsequently disease development. Lowering in porosity probably caused slowing down of the movements of the nematodes into the soil and also adverse effects on nematode penetration and finally lowering down disease severity. Some toxic compounds, like dibenzofuran and dibenzo-p-dioxime mixtures (Helder et al., 1982; Sawyer et al., 1983) and certain metals like Ar, Cd, Cr, Cu, Pb, Se, Zn etc. have been reported to be toxic that killed nematode juveniles directly in the soil (Khan et al., 1997). Nitrogen is almost absent in fly ash (Adriano et al., 1980). Nitrogen deficiency in soils declined the rate of development of *M. javanica* on tomato (Davide and Triantaphyllou, 1967) and abnormal development of nematode juveniles (Singh, 1993). Excessive uptake of certain elements like B, P and K and their accumulation in plant enhanced natural defence against nematodes (Kirkpatrick et al., 1964; Francois, 1984).

A gradual increase in the fly ash concentration in the soil caused a corresponding decrease in the number of galls per plant and number of egg masses per root system. Increased soil porosity favours movement of juveniles in the soil (Sasser, 1954; O’ Bannon and Reynolds, 1961). Increased soil porosity might have favoured the greater root ingress of juveniles. Greater ingress of juveniles caused more root galling and egg mass production at lower levels. But at higher levels increased concentration of substances that were
toxic to the nematodes adversely affected root galling, suppressed reproduction which was reflected as lower number of eggs per egg mass of the nematode. The significant increase in the body length and width of the mature females of the nematode in plant roots grown at lower levels of fly ash, might be due to greater availability of potassium present in fly ash and moderate range of pH (7.4 - 7.6). Oteifa (1953) found rapid development of the nematode and egg mass deposition of *M. incognita*, as the level of potassium increased in soil. The toxicity of the chemicals present in the amended soils was evident from the fact their water extracts caused death of the newly hatched juveniles. These effects could be correlated with the amount of fly ash added to the soil. Electrical conductivity and pH of the soil were found to be influenced by the amount of fly ash content in the amended soil. The toxic substances present in fly ash inhibited hatching and increased juvenile mortality. The toxic effects of fly ash suppressed the penetration of juveniles of nematodes and may have killed the juveniles thus caused less number of galls and egg masses at higher concentration of fly ash.

Singh (1989) observed similar trend response of galling and egg mass production by *M. incognita* and *M. javanica* to fly ash on chickpea and lentil. Khan (1989) found that fly ash at 10-40% increased the root penetration of juveniles and root-knot disease intensity on tomato whereas from 40% onwards root penetration and reproduction of *M. incognita* race was gradually inhibited and disease intensity was also reduced. Pasha *et al.* (1990) reported decreased soil population of *M. javanica* at 10-100% fly ash. Singh (1993) observed suppression of morphometrics of *M. javanica* females, egg mass production and fecundity gradually decreased with the increasing fly ash levels.
The chlorophyll pigment and protein content of the plant also decreased non-significantly at 10% fly ash level and significantly from 20% to 50% fly ash levels when compared with only nematode inoculated plants. Increase in fly ash levels could cause deficiency of nitrogen in soil as a result, the growth of the plant was checked, at the higher fly ash levels. The chlorophyll pigment and protein content of the plants also decreased when the growth of the plant was reduced. Singh (1988) and Pasha (1990) found a positive correlation between plant growth and chlorophyll pigment of the plant.

In the first year, root-knot nematode, *Meloidogyne incognita* caused reduction in the growth of *Lagenaria leucantha* grown in unamended or fly ash amended soil, when compared with uninoculated control plants grown in unamended soil. The reduction in growth was highest in nematode inoculated plants grown in unamended soil. The growth reduction was lowest at 50% fly ash level. In comparison to nematode inoculated control (T2) plants, an improvement in the plant growth of inoculated plants grown in amended soil. These findings indicated that incorporation of fly ash into the soil produced some beneficial effects on the growth of the plant. Although amendment of soil with fly ash did not control the disease, however, it improved the growth of inoculated plants, in comparison to inoculated control plants. Significant enhancement in plant growth at 50% fly ash level, over inoculated control might be attributed to the occurrence of utilizable mineral elements in higher concentration. These mineral elements, when absorbed by the plants, probably worked as nutrient elements (Misha and Shukla, 1986; Khan and Khan, 1989; Pasha et al., 1990; Singh, 1993; Khandkar et al., 1996; Srivastava et al., 1995; Tripathy and Sahu, 1997; Tripathy and Tripathy, 1998; Kalra et al., 1998;
Bharti et al., 2000). Increase in growth of nematode inoculated plants at various concentrations of fly ash, over inoculated control, showed that the fly ash has some role in overcoming the loss caused by the nematode (Singh, 1989; Khan and Khan, 1989; Pasha et al., 1990; Singh, 1993).

In the first year, the data revealed that fly ash amendment reduced gall number as well as number of egg masses per plant. This finding support that application of fly ash did not favour root-knot nematode infection. As the amount of fly ash increased, the number of galls and number of egg masses decreased. This reciprocal relationship indicated that fly ash changed physical characteristics of the soil. Increase in porosity might have resulted in lowering down the penetration of the juveniles into the roots.

In the second year, in comparison to uninoculated controls, maximum reduction in plant growth was observed in T2 plants and minimum in T5 plants. This showed that amendment of soil with fly ash at 30% level was beneficial for the plants infected with nematodes even after one year. In comparison to inoculated controls (T2), increase in plant growth was observed in all the fly ash amended treatments. An increasing trend in plant growth from T3 to T5 with 10%, 20% and 30% fly ash respectively, might be due to increase in concentrations of mineral elements and also due to favourable physical characteristics of the amended soil. A decreasing trend from T5 to T7 might be attributed to presence of toxic elements in higher fly ash concentrations as well as physical characteristics, like lower porosity of the amended soil. For all the growth parameters, amendment of soil with 30% fly ash seems to be more beneficial than other concentrations. This idea is further strengthened by observing a similar trend in protein and chlorophyll contents. From this study it
might be inferred that fly ash concentration above and below 30% is not advantageous for the plants.

In the second year, the number of galls and number of egg masses exhibited a trend similar to the first year. Concentration of fly ash and number of galls and egg masses per plant were reciprocally proportional. However, it was also discovered that in the second year, the number of galls and number of egg masses per plant at the same fly ash level decreased when the data was compared with the data of the first year. Thus, from this finding, the view that addition of fly ash is not beneficial for the plant, was further supported.

In the third year, in comparison to uninoculated control, reduction in plant growth was observed in all the treatments. The reduction was maximum in T7 plants followed by T2 plants. In comparison to inoculated controls (T2), reduction in plant growth, although non-significant, was observed in T7 plants. This indicated that higher concentration of fly ash caused damage to the plants, probably because of undesirable physico-chemical attribute of the amended soil. A similar trend in the results of biochemical analysis further support this viewpoint. Thus, it can, very safely, be concluded that soil amendment with fly ash in any concentration is not beneficial in long term for the plant growth, because it changes permanently unfavourably the physio-chemical characteristics of the soil.

The data of pathogenicity for the third year was similar to that of first and the second years. In the third year, however, further reduction in gall number and egg mass production was noticed at the same fly ash levels, as compared to the previous year.
In comparison to control, it was found that gall formation and egg mass production reduced at higher levels in the third than first and second years. From this finding it may be concluded that fly ash amendment is not favourable for root-knot nematode infection in long run. It seems that amendment of soil with fly ash permanently changes physical characteristics of the soil, which are harmful to plant (host) as well as the nematode (pathogen).