



## PATHOGENICITY OF DIFFERENT INOCULUM LEVELS OF *MELOIDOGYNE INCOGNITA* INFECTING *VIGNA RADIATA* AND MANAGEMENT BY FLY ASH APPLICATION

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### ABSTRACT

The experiment was conducted in pot, to evaluate the damage potential of *Meloidogyne incognita* against *Vigna radiata* at different inoculum levels, by inoculating with 200, 400, 800 and 1,600 J2. The results showed that the threshold level of inoculum was (800 J2) at which the rate of reproduction of nematode was increased and prominent symptoms were developed. Plant growth parameters including plant length, fresh weight, dry weight, leaf area, nodule number were significantly decreased at 800 inoculum level in addition to decrease in number of pods, seeds and seed weight. Fly ash was added @ 15%, 30%, 45% and 60% into the soil. Fly ash amendment at 15% was found to be most effective which probably acted as stimulant for the growth and yield of *Vigna radiata* and accorded negative impact on the nematode at fly ash concentrations.

**Keywords:** Inoculum level; Fly Ash; *Meloidogyne incognita*; *Vigna radiata*.

### INTRODUCTION

Plant-parasitic nematodes are cosmopolitan in distribution and are a continuous threat to the agricultural crops, and are among the most difficult crop pests to be controlled (Chitwood, 2002). The root-knot nematodes (*Meloidogyne spp.*) are sedentary endoparasites and, attack a wide range of crops including green gram (Sikora and Greco, 1993). The characteristic symptom of the disease caused by *Meloidogyne spp.* is the formation of galls on the root. The minimum number of nematodes used as initial inoculum formed the maximum number of galls and with further increase in the number of nematodes there was a decrease in number of galls/plant as was reported on tomato (Jones and Nirula, 1963), cowpea (Sharma and Sethi, 1976) and pea (Chahal and Singh, 1984). The results showed that the optimum level of inoculum

for maximum reproduction of nematodes was different from that for symptom development. Hence the present study was carried out to ascertain the threshold level of the pathogen that might be helpful in formulating nematode disease management strategies in mung bean.

The use of chemical compounds to manage populations of plant parasitic nematodes has been the preferred method by agriculturalists the world over (Evenson and Gollin, 2003). There is growing awareness regarding the repercussions of indiscriminate use of chemical pesticides which are not only toxic to human life but identified as one of the major cause of environmental pollution and deterioration of agricultural land and ecosystem as a whole. The necessity for alternative nematode management has led soil and plant specialists to

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apply a wide range of by-products as soil amendments (Akhtar and Alam 1993; Stirling *et al.*, 2005; Walker 2007). Fly ash has shown great potential in enhancing productivity through soil amendments where it acts as a source of trace elements which are beneficial to the plants (Hammermeister *et al.*, 1998). Since leguminous plants could grow well on FA amended soils without manifestation of any injury symptoms (Singh *et al.*, 1997), therefore in the present investigation fly ash was used to find out its effect on plant vigour and nematode multiplication.

## 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 of 10 inch diameter were filled with soil at the rate of 1.5 kg of soil per pot. A little water was poured into each pot to just wet the soil surface before sterilization at 20 lb pressure for 20 min. Sterilized pots were allowed to cool at room temperature before use for experiments.

### Raising and Maintenance of Test Plant

The seeds of *Vigna radiata* L. var. PDM 139 were procured from Indian Institute of Pulse Research, Kanpur. The seeds were axenized by NaOCl method (Koenning and Barker, 1985). The seeds were placed on a moist sterilized filter paper kept in a sterilized petri-dish for germination. The sprouted seeds were sown in clay pots. Initially there were five seedlings per pot, these were thinned to one plant per pot, when the seedlings reached three leaf stage.

### Preparation of Inoculum

*Meloidogyne incognita* (Kofoid and White) Chitwood was selected as test pathogen. To perform experiments, pure culture of *M. incognita* was maintained on egg plant (*Solanum melongena* L.) roots in glass house by using single egg mass. The egg masses from galled roots of egg plant were picked with the help of sterilized forceps and were allowed to hatch at  $28 \pm 2^\circ\text{C}$  under aseptic conditions in the sieves, lined with tissue paper and kept in petri-dishes containing sufficient amount of sterilized distilled water.

### Inoculation with Nematode:

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 200J<sub>2</sub>, 400J<sub>2</sub>, 800J<sub>2</sub>, 1,600J<sub>2</sub> 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. To maintain soil moisture in the pot, regular watering was done. Each treatment was replicated five times and the pots were arranged in randomized complete block design. Un-inoculated set of plants served as control. There were five sets of pots as given below:

C: Control

T1: 200 J<sub>2</sub>/pot

T2: 400 J<sub>2</sub>/pot

T3: 800J<sub>2</sub>/pot

T4:1,600 J<sub>2</sub>/pot

### 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 four proportions with 15, 30, 45 and 60% 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 using for experiments.

### Inoculation with Nematode in Fly Ash Amended Soil

The seeds were sown in fly ash amended soil and at three leaves stage seedlings were inoculated with second stage juveniles of *M. incognita* (800 J<sub>2</sub> 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; un-inoculated plants served as control (C); inoculated plants grown in un-amended soil served as inoculated control (IC).

The treatments were as follows:

C: Control (C)

IC: 0% Fly ash + 800 J<sub>2</sub>/pot

T1: 15% Fly ash + 800 J<sub>2</sub>/pot

T2: 30% Fly ash + 800 J<sub>2</sub>/pot

T3: 45% Fly ash + 800 J<sub>2</sub>/pot

T4: 60% Fly ash + 800 J<sub>2</sub>/pot

### Growth and yield parameters

The plants were harvested after 60 days of inoculation. Length of the plants was measured with the help of meter scale. After taking fresh weight, the plants were dried in an oven at 80°C. The dry weight was taken using single pan balance. The number of leaves was counted by visual observation. For leaf area, outline of the shape of each leaf was drawn on butter paper and the area occupied was measured with the help of planimeter. The nodules were counted with the help of hand lens. Yield parameters in terms of pods per plant, number of seeds per pod and randomly selected 100 seed weight from each treatment, were considered.

### Photosynthetic Pigments, Leghaemoglobin and Seed Protein

Chlorophyll content was estimated after extraction in 80% chilled acetone following the method of Mackinney (1941) by using the following formulae

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

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

Where,

V = total volume of solution (mL)

W = weight of the tissue (g) used for the extraction of the pigments

D = Density of samples at 645 and 663 nm wave lengths

For the measurement of leghaemoglobin from the excised root nodules, a method of Appleby and Bergerson's described by Sadasivm and Manickam (1992) was followed. The optical density was recorded at 556 and 539 IUU. The Lb content was calculated using the following formula:

$$\text{Lb concentration (mM)} = \frac{\text{OD 556} - \text{OD 539} \times 2D}{23.4}$$

where, OD 556 and 539 represent absorbance (OD). Values recorded at 556, 539 IUU, respectively and D is the initial dilution

The protein content in the seeds was estimated by Folin-phenol method (Lowry *et al.*, 1951) using bovine serum albumin as a standard.

### Parameters related to nematode infection

The number of galls was counted by visual observation like size of galls was measured by taking maximum length and width (in mm<sup>2</sup>) with the meter scale. Egg masses per root system were counted by staining with Phloxin B. The number of mature females was counted by blending the infected roots with 200 ml water in a waring 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.

For Morphometry of nematodes 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 was measured using camera lucida.

### Data Analysis

The data obtained were analyzed statistically and significance was calculated by R software (R development core team, 2011) at P ≤ 0.05 and P ≤ 0.01 levels of probability.

### RESULTS

In the present study it was disclosed that *M. incognita* at different inoculum levels produced varying effects on the growth of plant. The plant length of *V. radiata* was non-significantly different at lower initial inoculum level of Pi = 200J<sub>2</sub> in T1 plants, when compared with un-inoculated control (C). A significant (P ≤ 0.05) decrease, in comparison to control (C), occurred in T2 plants at the initial inoculum level of Pi = 400J<sub>2</sub> (T2 plants). In T3 and T4 plants, at higher inoculum levels of Pi = 800J<sub>2</sub>, Pi = 1,600J<sub>2</sub>, the plant length was decreased greatly and significantly (P ≤ 0.01), over the control (Table-1)

In comparison to control (C), fresh weight (whole plant) and dry weight (shoot and root), decreased with an increase in initial inoculum level of Pi = 200J<sub>2</sub> (T1 plants) and significant (P ≤ 0.05) at the next higher inoculum level of Pi = 400J<sub>2</sub> (T2 plants). While significant (P ≤ 0.01) reductions in T3 and T4 plants were observed, when compared with the control (C) (Table-1).

From the results it was clear that in comparison to control there was progressive decrease in leaf number with increase in initial inoculum levels (Pi =

200J<sub>2</sub> to 1,600 J<sub>2</sub>). Non significant decrease occurred in T1 plants followed by significant ( $P \leq 0.05$ ) reduction in T2 plants with higher and significant ( $P \leq 0.01$ ) reduction in T3 and T4 plants, over the control. The leaf area of the plants, in comparison to control, decreased non-significantly at lowest inoculum level of Pi= 200J<sub>2</sub> and significantly ( $P \leq 0.05$ ) at the next higher inoculum level of Pi= 400J<sub>2</sub> (T2 plants). Reductions in leaf area were higher and significant ( $P \leq 0.01$ ) at the initial inoculum levels of Pi= 800J<sub>2</sub> and Pi= 1,600J<sub>2</sub>, over the control (C) (Table-1).

On comparing the number of root nodules of uninoculated plants (C) with those of inoculated plants, higher reduction was observed in inoculated plants. The reductions were non-significant at lower

initial inoculum level of Pi= 200 J<sub>2</sub> and significant ( $P \leq 0.05$ ) at the next higher inoculum level of Pi= 400J<sub>2</sub> (T2 plants). At higher inoculum levels (Pi= 800J<sub>2</sub> and 1600J<sub>2</sub>) significantly higher ( $P \leq 0.01$ ) reductions occurred in T3 and T4 plants (Table-1).

The yield of the plant, in terms of the pod number, seeds per pod, was decreased slightly and non-significantly in T1 and significantly ( $P \leq 0.05$ ) in T2 plants, which were inoculated with Pi= 200J<sub>2</sub> and Pi= 400J<sub>2</sub>, respectively. A significant ( $P \leq 0.01$ ) reduction in yield was observed in T3 plants. The number of pods and seeds per pod attained maximum and significant ( $P \leq 0.01$ ) reduction at the highest inoculum level of Pi= 1600 (in T4 plants) (Table-1).

**Table 1: Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Vigna radiata*.**

| Treatments                          | Plant length (cm) | Fresh weight (g) | Dry weight (g) |             | No. of leaves plant <sup>-1</sup> | Leaf area (cm <sup>2</sup> ) | No. of nodules root system <sup>-1</sup> | No. of pods plant <sup>-1</sup> | No. of seeds pod <sup>-1</sup> | 100 seed weight (g) |
|-------------------------------------|-------------------|------------------|----------------|-------------|-----------------------------------|------------------------------|--|---------------------------------|--------------------------------|---------------------|
|                                     |                   |                  | Shoot          | Root        |                                   |                              |  |                                 |                                |                     |
| C (Control)                         | 41.53             | 10.11            | 1.89           | 0.97        | 20.88                             | 67.32                        | 8.90                                     | 12.85                           | 11.43                          | 3.87                |
| T1 (200J <sub>2</sub> )             | 38.51             | 8.95             | 1.75           | 0.92        | 18.57                             | 63.34                        | 7.25                                     | 11.34                           | 11.00                          | 3.30                |
| T2 (400J <sub>2</sub> )             | 37.35             | 8.26             | 1.65           | 0.89        | 17.31                             | 61.73                        | 6.65                                     | 9.98                            | 10.00                          | 3.09                |
| T3 (800J <sub>2</sub> )             | 28.18             | 5.75             | 0.96           | 0.48        | 12.23                             | 42.87                        | 3.42                                     | 5.27                            | 9.15                           | 1.89                |
| T4 (1600J <sub>2</sub> )            | 25.23             | 4.68             | 0.92           | 0.28        | 8.54                              | 33.31                        | 2.37                                     | 2.45                            | 8.52                           | 1.40                |
| <b>LSD <math>P \leq 0.05</math></b> | <b>3.07</b>       | <b>1.77</b>      | <b>0.23</b>    | <b>0.06</b> | <b>2.62</b>                       | <b>4.15</b>                  | <b>1.71</b>                              | <b>2.62</b>                     | <b>1.28</b>                    | <b>0.59</b>         |
| <b>LSD <math>P \leq 0.01</math></b> | <b>4.24</b>       | <b>2.45</b>      | <b>0.31</b>    | <b>0.09</b> | <b>3.63</b>                       | <b>5.69</b>                  | <b>2.34</b>                              | <b>3.55</b>                     | <b>1.75</b>                    | <b>0.81</b>         |

Each value is a mean of five replicates

**J<sub>2</sub>**= Second stage juveniles of *Meloidogyne incognita*

The weights of the seeds collected from inoculated plants found to be lower than the weights of the seeds of control plants. 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 T2 and ( $P \leq 0.01$ ) T3 plants, at Pi= 400J<sub>2</sub> and Pi= 800J<sub>2</sub> per pot were 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= 1,600J<sub>2</sub>, in T4 plants (Table-1).

The length of plants showed variable responses towards the soil containing different concentrations of fly ash levels. In comparison to control (C) plants, grown in un-amended soil, enhancement in length of plants occurred in T1 plants, with significant ( $P \leq 0.05$ ) increase in the soil amended with 15% fly ash concentration. There was non-significant difference in T2 plants, at 30% fly ash concentration, over the control (C). In T3 plants (at 45% fly ash level), the plant length decreased significantly ( $P \leq 0.05$ ) followed by highest and significant ( $P \leq 0.01$ ) decrease at 60 % fly ash level in T4 plants, when compared with the control (Table- 2).

Fresh weight and dry weight of the shoots and roots, were significantly ( $P \leq 0.05$ ) increased in T1 plants, grown in the soil amended with 15% fly ash, followed by minimum decrease at 30% of fly ash, where the difference over the control was non-significant. Subsequent increase in fly ash concentration (45% and 60%), caused significant ( $P \leq 0.05$ ) reduction in T3 plants, and significant ( $P \leq 0.01$ ) and highest reduction in T4 plants, when compared with the control (C) (Table- 2).

The number of leaves per plant increased significantly ( $P \leq 0.05$ ) on T1 plant, grown in the soil amended with 15% fly ash, over the control (C). Progressive decrease in leaf number was recorded on increasing fly ash levels (30%, 45% and 60%). At 30% fly ash level, the number of leaves was reduced non-significantly in T2 plants, whereas significant reductions were observed in T3 and T4 plants at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively, as compared to the control (Table- 2).

**Table 2: Effect of various fly ash amendments on the growth of *Vigna radiata*.**

| Treatments        | Plant length | Fresh weight | Dry weight (g) |      | No. of leaves plant <sup>1</sup> | Leaf area (cm <sup>2</sup> ) | No. of nodules root system <sup>1</sup> |
|-------------------|--------------|--------------|----------------|------|----------------------------------|------------------------------|---|
|                   |              |              | Shoot          | Root |                                  |                              |   |
| C (Control)       | 42.89        | 10.38        | 2.19           | 1.20 | 22.12                            | 68.42                        | 8.96                                    |
| T1 (15% FA)       | 46.79        | 12.12        | 2.43           | 1.27 | 24.65                            | 72.45                        | 9.00                                    |
| T2 (30% FA)       | 41.12        | 9.73         | 2.02           | 1.15 | 20.35                            | 66.66                        | 8.50                                    |
| T3 (45% FA)       | 38.93        | 8.68         | 1.95           | 1.12 | 19.48                            | 63.19                        | 6.43                                    |
| T4 (60% FA)       | 27.00        | 5.81         | 1.28           | 0.54 | 16.26                            | 59.59                        | 4.15                                    |
| LSD $P \leq 0.05$ | 2.89         | 1.29         | 0.18           | 0.06 | 2.10                             | 3.80                         | 1.37                                    |
| LSD $P \leq 0.01$ | 4.00         | 1.79         | 0.25           | 0.09 | 2.87                             | 5.26                         | 1.90                                    |

Each value is a mean of five replicates

FA= Fly ash

**Table 3: Effect of various fly ash amendments on yield, chlorophyll content, seed protein content and leghaemoglobin of *Vigna radiata*.**

| Treatments                          | No. of pods plant <sup>1</sup> | No. of seeds pod <sup>1</sup> | 100 seed weight (g) mg g <sup>-1</sup> leaf | Chlorophyll (a+b) (mg g <sup>-1</sup> ) | Seed protein nodules | Leghaemoglobin mg g <sup>-1</sup> fresh |
|-------------------------------------|--------------------------------|-------------------------------|---|---|----------------------|---|
| C (Control)                         | 13.00                          | 12.65                         | 3.88  | 1.812                                   | 29.25                | 4.70                                    |
| T1 (15% FA)                         | 15.90                          | 12.70                         | 4.00  | 1.964                                   | 30.15                | 4.68                                    |
| T2 (30% FA)                         | 12.93                          | 12.13                         | 3.58  | 1.800                                   | 29.10                | 3.35                                    |
| T3 (45% FA)                         | 10.63                          | 11.86                         | 3.12  | 1.660                                   | 28.24                | 2.16                                    |
| T4 (60% FA)                         | 8.86                           | 10.25                         | 2.35  | 1.600                                   | 26.76                | 1.12                                    |
| <b>LSD <math>P \leq 0.05</math></b> | <b>1.74</b>                    | <b>1.02</b>                   | <b>0.55</b>                                 | <b>0.149</b>                            | <b>2.37</b>          | <b>0.52</b>                             |
| <b>LSD <math>P \leq 0.01</math></b> | <b>2.41</b>                    | <b>1.41</b>                   | <b>0.77</b>                                 | <b>0.206</b>                            | <b>3.27</b>          | <b>0.70</b>                             |

Each value is a mean of five replicates

FA= Fly ash

There was significant ( $P \leq 0.01$ ) enhancement in leaf number occurred in T1 plants followed by non-significant reduction at the increased fly ash level (30% fly ash), over the control. Further increase in fly ash concentration to 45% and 60%, caused significant ( $P \leq 0.05$ ) decrease in T3 plants, while significant ( $P \leq 0.01$ ) and highest decrease in T4 plants, when compared with the control (C) (Table-2).

When the comparison was made between control plants (C) and the plants grown in fly ash amended soil, there was a remarkable decrease in nodule number, in all the treatments viz., T2, T3 and T4 except non-significant improvement was seen in T1 plants, which were grown at lowest concentration of fly ash (15 %). A non-significant decrease had occurred in T2 plants, at 30% fly ash application. At 45 and 60% of fly ash, there was a significant ( $P \leq 0.01$ ) reduction in T3 and T4 plants, when compared with the control (C) (Table- 2).

Number of pods per plant was significantly ( $P \leq 0.01$ ) increased and seeds per pod were increased non-significantly, over control (C) at 15% fly ash concentration. At higher fly ash levels (30%, 45% and 60%), the decrease was non-significant and significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively in T2, T3 and T4 plants, in seed and pod numbers, over the control plants (C), grown in un-amended soil (Table-3).

The weight of seeds increased non-significantly in the treatment T1 (at 15% fly ash), when compared with the control (C). Increase in fly ash level, caused reduction in seed number of T2, T3 and T4 plant. Non-significant reductions were observed in T2 plants (at 30% fly ash) followed by a significant ( $P \leq 0.05$ ) reduction in T3 plants (at 45%), while highest and significant ( $P \leq 0.01$ ) reduction in T4 plants over the control (C) (Table- 3).

The amount of chlorophyll in the leaves was significantly ( $P \leq 0.01$ ) increased at 15% fly ash level, when compared with the control (C). At 30% fly ash level (T2), a non-significant decrease over the control, was noticed. A significant ( $P \leq 0.05$ ) decrease in chlorophyll content was observed, in T3 plants, over the control. In T4 plants, amended with 60% fly ash level, significant ( $P \leq 0.01$ ) and highest reduction was occurred in comparison to the control (C) (Table- 3).

There was a significant enhancement in T1 plants, with 15% fly ash level. Non-significant reduction in protein content occurred, at 30% and 45% fly ash

levels in (T2 and T3), respectively. Highest and significant ( $P \leq 0.05$ ) decrease was observed in T4 plants, in comparison to the control (C) (Table- 3).

In comparison to control (C), the leghaemoglobin content of nodules decreased non-significantly at 10% fly ash level (T1 plants). Further increase in fly ash concentration resulted in decreased level of leghaemoglobin content of the nodules. There was a significant ( $P \leq 0.01$ ) decrease in leghaemoglobin content in all the treatments (T1, T3 and T4) amended with 30%, 45% and 60% fly ash, in comparison to the control (Table- 3).

Reduction in the number of galls was non-significant at 15% fly ash level (T1 plants) and significant ( $P \leq 0.05$ ) at 30% fly ash level (T2). The reductions in the number of galls were higher and significant ( $P \leq 0.01$ ) at 45% and 60% fly ash level (T3 and T4, respectively), when compared with the control (C) (Table- 4).

The size of galls decreased in all the treatments from T1 to T4, when compared with the inoculated controls (IC). Least and non-significant reduction was observed in T1 plants, where amendment was made with 15% fly ash, over the control (C). With further elevation in fly ash levels, significant ( $P \leq 0.05$ ) reduction was observed in T2 (at 30%), followed by T3 (at 45%) with significant ( $P \leq 0.01$ ) decrease, and maximum and significant ( $P \leq 0.01$ ) decrease in T4 (at 60%), when compared with the nematode inoculated plants without fly ash (Table-4).

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. Considerable decrease in egg masses was observed from T2 to T4 plants except in T1, which showed non-significant decrease as compared to inoculated control (IC). In T2 plants, grown in 30% fly ash concentration, exhibited significant ( $P \leq 0.05$ ) reduction. Whereas in T3 and T4 plants, higher and significant ( $P \leq 0.01$ ) decreases were observed in the egg masses at 45% and 60% fly ash concentrations, over the IC plants (Table- 4).

On comparing inoculated control plants with the plants having fly ash treatments, showed the reduction in number of mature females in the root was noticed. There was a non-significant decrease in T1 plants (at 15% fly ash), significant ( $P \leq 0.05$ ) decrease in T2 plants, in the soil amended with 30%

**Table 4: Effect of different concentrations of fly ash on the development of nematode infecting *Vigna radiata*.**

| Treatments              | No. of galls | Size of galls (mm <sup>2</sup> ) | No. of egg sacs root system <sup>-1</sup> | No. of mature females g <sup>-1</sup> root | Length of female (μm) | Width of female (μm) |
|-------------------------|--------------|----------------------------------|---|--|-----------------------|----------------------|
| C (Control)             | -            | -                                | -   | -  | -                     | -                    |
| IC (Inoculated Control) | 124.39       | 13.32                            | 201.86                                    | 91.25                                      | 792.42                | 424.50               |
| T1 (J2+15%)             | 118.43       | 12.85                            | 192.34                                    | 86.12                                      | 787.45                | 415.35               |
| T2 (J2+30%)             | 111.81       | 11.80                            | 180.52                                    | 78.50                                      | 773.35                | 410.45               |
| T3 (J2+45%)             | 101.62       | 10.00                            | 168.86                                    | 72.35                                      | 761.50                | 398.51               |
| T4 (J2+60%)             | 85.75        | 9.50                             | 150.34                                    | 61.56                                      | 752.35                | 393.76               |
| <b>LSD P≤0.05</b>       | <b>9.57</b>  | <b>1.33</b>                      | <b>21.34</b>                              | <b>12.48</b>                               | <b>17.20</b>          | <b>12.01</b>         |
| <b>LSD P≤0.01</b>       | <b>13.11</b> | <b>1.83</b>                      | <b>28.18</b>                              | <b>17.10</b>                               | <b>23.57</b>          | <b>16.46</b>         |

Each value is a mean of five replicates

**J2**= 800 second stage juveniles of *Meloidogyne incognita* per pot

**FA**= Fly ash

fly ash. At the higher concentration of fly ash (45% and 60%), there was a significant ( $P \leq 0.01$ ) decrease in T3 and T4 plants, respectively over inoculated control (IC) plants (Table- 4).

The length and width of mature females were decreased in all the treatments from T1 to T4 when compared with inoculated control (IC) plants. In T1 plants, grown at 15% fly ash level, the reductions in the length and width of the mature females were non-significant, whereas at 30% fly ash level in T2 treatment, reduction was significant ( $P \leq 0.05$ ). In T3 and T4 plants, grown at 45% and 60% fly ash levels, the reduction in the length and width of mature females was higher and significant ( $P \leq 0.01$ ) when compared with nematode inoculated plants grown in un-amended soil (IC) (Table- 4).

## DISCUSSION

The root knot nematode, *Meloidogyne incognita* impaired the plant growth and reduced the yield of *Vigna radiata* at all the initial inoculum levels. Changes in the amount of inoculum levels 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 reduction in fresh and dry weight of the roots and the shoots. The number of leaves and the leaf area were also decreased. From the finding it might be inferred that *V. radiata* 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 noticed by several workers like Ibrahim and Lewis (1985) on soybean; Fazal *et al.*, (1996a) on black gram; Singh and Goswami (2000) on cow pea; Hisamudddin *et al.*, (2005a) on *Phaseolous mungo*. Besides stunted growth, poor pod formation, lesser yield and lesser Rhizobium nodules were found commonly associated with the root-knot disease of mung bean. The nematodes may feed directly on the nodules or indirectly by affecting surface area of plant root available to bacterium for interaction (Kanhwar *et al.*, 1988).

Different concentrations of fly ash used in the present study for amendment of soil, were analyzed for their effects on plant and nematode. The plants responded differently to different concentrations of fly ash, as is evident from the data. A downward trend was observed from T1 to T4 in plant growth parameters. Highest increase was witnessed at 15% of fly ash level, on the other hand, further increase caused progressive decrease in all the growth and yield parameters. At higher concentrations (30%, 45% and 60%), porosity of soil was decreased which resulted in increased water holding capacity. Higher pH, higher concentration of mineral elements decreased porosity and increased water holding capacity became unfavourable for plant growth and development that resulted in reduction in plant growth and other parameters. These findings were in accordance with other reports (Mishra and Shukla, 1986; Kalra *et al.*, 1998; Parveen *et al.*, 2003; Singh *et al.*, 2005), in which adverse effects of higher concentration of fly ash on plant growth and yield in different crops were ascertained. Pasha *et al.*, 1990 observed that 10% and 25% fly ash enhanced growth of cucumber plants but higher levels (50-100%) were proved to be toxic leading to suppressed plant growth and decreased chlorophyll contents in the leaves.

Nematode development was adversely affected at all the fly ash concentrations (15%, 30%, 45% and 60%) resulted in the subsequent decrease in number of galls and gall size in the roots of infected plant. This reduction might be due to higher concentration of carbonates and bicarbonates (Khan and Khan, 1996; Siddiqui *et al.*, 2004) and pH above neutral (Khalil and Shah, 1979), produced detrimental effects on nematode penetration and subsequently disease development. Some other toxic compounds *viz.* Dibenzofuran and dibenzo-p-dioxine mixtures (Helder *et al.*, 1982; Sawyer *et al.*, 1983), metallic elements like arsenic, cadmium, copper lead, nickel, selenium and Zinc (Khan *et al.*, 1997) in fly ash, might have played a major role in killing the nematode juveniles directly in the soil.

Excessive uptake of certain elements especially B, K and P, and their subsequent accumulation in the plant enhances natural defence against nematodes (Kirkpatrick *et al.*, 1964; Francois, 1984). 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 caused abnormal development of nematode juveniles (Singh 1993).

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