



ECO-FRIENDLY USE OF FLY ASH FOR THE MANAGEMENT OF ROOT-KNOT NEMATODE AND ACID RAIN IN PUMPKIN CROP

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ABSTRACT

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Fly ash (FA) production has stridently been due to unjudicious demand of electricity for fulfilling the human needs. Generation and disposal of FA has been a serious concern in the current scenario. However, FA can be used for soil amelioration that may improve physical, chemical and biological properties of the degraded soils. Simulated acid rain was prepared by adding the HNO₃ and H₂SO₄ in the ratio 3:1 and maintained the different pH levels (5.0, 4.0 and 3.0). In the present study, a pot experiment was conducted in net house during 2017-18 to evaluate the efficacy of fly ash for the alleviation of simulated acid rain (SAR) stress on pumpkin (*Cucurbita moschata*) with or without root-knot nematode, *Meloidogyne incognita* inoculations. The result indicated that the different growth, yield, biochemical variables of *Cucurbita moschata* were significantly ($P \leq 0.05$) enhance at 30% FA amended soil with lower level of SAR (pH5.0) and reduced the nematode population. After 30% of FA (40% and 50%) and all the levels of SAR (pH5.0, pH4.0 and pH3.0) were harmful to the plant growth, yield and, biochemical parameters and also reduce the nematode population as compared to control. From the result it appeared that the best level of fly ash was 30% with pH 5.0 of simulated acid rain, where lowest level of acidity (pH5.0) showed no harmful effect. However, in case of root-knot nematode the suppression was showed at all the levels of fly ash and simulated acid rain like in terms of root gall, soil population and eggmass.

Contribution/Originality: The study indicated that the lower levels of fly ash (5%, 10%, 20%, 30%) soil amendment were found beneficial for pumpkin crop even after treated with different acidity levels of simulated acid rain (5.0, 4.0 and 3.0 pH). Moreover, all the levels of fly ash and simulated acid rain were significantly reduced the root-knot nematode, *M. incognita* population.

1. INTRODUCTION

Pollution known to be irrespective of their origin cause greater perturbations in the natural ecosystem and affect the plant productivity (Khan *et al.*, 2015). Coal based thermal power plants is one of an anthropogenic source of air pollution used for the generation of electricity. In India 75% of total power obtained is from coal based thermal power plants (IEA, 2008). A huge amount of fly ash is produced (169.25 MTs) because of the use of coal in power generation and only 107.10 MTs utilized (CEA, 2017). Fly ash (FA) is generated globally by the combustion of coal and called coal combustion by-product (CCB) and its disposal is fast becoming of concern world-wide. However, FA can be used as a soil amendment that may improve the soil health quantitatively and qualitatively. Moreover, high concentration of elements (K, Na, Zn Ca, Mg and Fe) in fly ash increases the yield and growth of

various agricultural crops (Kishor *et al.*, 2010) apart from these elements fly ash also contains trace elements such as Hg, Cd, Cr, Cu, Al, Be, Pb, As, etc. (Adriano *et al.*, 1980).

Acid precipitation (acid rain), oxides of nitrogen (NO₂, NO₃, N₂O, N₂O₅) and sulfur (SO₂, SO₃) have known to be major composition of acid rain (Odiyi and Eniola, 2015). Oxides of nitrogen and sulfur react with the moisture present in the atmosphere to form wet acid in the form of rain, affects the physiology of flora and eventually reduces the plant growth yield (Pal and Kumar, 2000; Larssen *et al.*, 2006; Wondyfraw, 2014). Higher concentration of acid rain may also destroy the photosynthetic pigments and decreases the chlorophyll content in plants (Wen *et al.*, 2011). It also changes chemistry and biology of soil and due to this there is reduction in pH of soil thereby the various heavy metal active, nutrient loss and disturb the soil microbial activities (Wen *et al.*, 2013; Moharami and Jalali, 2015). Acid rain exert some visible symptoms like chlorosis and necrosis of tissues and invisible like decrease the photosynthesis rate, imbalance water potential and enzyme activity etc (Ferenbaugh, 1976; Khan and Devpura, 2004; Ni *et al.*, 2006; Rapava *et al.*, 2007a;2007b).

Root knot nematodes (RKN) are ranked first in damaging the agricultural crops (Jones *et al.*, 2013). The genus *Meloidogyne* has about 100 species, with *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* and being considered as “major” species (Elling, 2013). *M. incognita* now been seen attacking several vegetable crop including pumpkin in sub tropical region (Gheysen and Mitchum, 2011; Hajra *et al.*, 2015).

Cucurbita moschata (Duch ex. Poir) belongs to family Cucurbitaceae, commonly called as pumpkin as and more tolerant to adverse environmental comparatively (Colla *et al.*, 2006). It is one of the major vegetable crops grown in almost all arable regions of the world. They are cultivated for human consumption as vegetables and fruits, a rich source of vitamin C, thiamine, riboflavin, niacin, vitamin B6, folates, vitamin E, vitamin K, β-carotene, potassium, phosphorus, magnesium, iron, and selenium (Cerniauskiene *et al.*, 2010). However, these vegetables are suffering with number of pathogens including root-knot nematode, *M. incognita*. Therefore present study was planned to observe the effect of *M. incognita* along with FA and SAR in relation to growth and yield attributes of pumpkin under pot condition in a net house. The present study will also provide the base line for pumpkin growing in a stressed condition especially acid rain and fly ash prone area.

2. MATERIALS AND METHODS

The experiments were conducted in green house at the Department of Botany, Aligarh Muslim University, Aligarh, India during 2017-18. The fresh fly ash was collected from Thermal Power Plant, Kasimpur, Uttar Pradesh, India for the experiment. The test pathogen, *Meloidogyne incognita* was maintained on egg plants (Khan, 2008). Single egg mass culture technique was performed for pure culture. Pumpkin (*Cucurbita moschata*) Var. Nutan was selected as a test plant for the experiments. Nematode inoculum was prepared by incubating the egg masses under appropriate temperature. The freshly hatched J₂ were collected as water suspension and the number of J₂ counted in 1ml samples from the suspension. The average number of J₂ was used to represent the number of J₂ per ml of suspension. The soil used in the experiment was collected from the unpolluted agricultural field up to 20 cm depth after scrapping the surface of litters present. The collected soil was brought to the laboratory in gunny bags and autoclaved.

2.1. Experimental Set Up

For this experiment, fly ash was mixed with autoclaved soil in different proportions to prepare 5, 10, 20, 30, 40 and 50% levels. The clay pots of 12 inches height (20 cm diam.) were filled with 4 kg of soil of each type of mixture. Three seeds of pumpkin Var. Nutan were directly sown in each pot. After four leaves stage, thinning was done to retain one healthy seedling per pot. Later plants were inoculated with freshly hatched 2,000 J₂. Different doses of simulated acid rain (pH 3.0, 4.0 and 5.0) were prepared by mixing conc. 1N H₂SO₄ and 1N HNO₃ (3:1) in distilled water and pH level was maintained by measuring the solution with the help of digital pH meter. Sprayer was used

for spraying different pH (SAR) on plants twice in a week for two months. Following treatments were installed during the experimentations.

| | |
|--------------------------------|---|
| T1 = Control (soil only) | T2 = Nematode (2000 J ₂ of <i>M. incognita</i>) |
| T3 = 5% Fly ash + N + 5.0 pH | T4 = 5% Fly ash + N + 4.0 pH |
| T5 = 5% Fly ash + N + 3.0 pH | T6 = 10% Fly ash + N + 5.0 pH |
| T7 = 10% Fly ash + N + 4.0 pH | T8 = 10% Fly ash + N + 3.0 pH |
| T9 = 20% Fly ash + N + 5.0 pH | T10 = 20% Fly ash + N + 4.0 pH |
| T11 = 20% Fly ash + N + 3.0 pH | T12 = 30% Fly ash + N + 5.0 pH |
| T13 = 30% Fly ash + N + 4.0 pH | T14 = 30% Fly ash + N + 3.0 pH |
| T15 = 40% Fly ash + N + 5.0 pH | T16 = 40% Fly ash + N + 4.0 pH |
| T17 = 40% Fly ash + N + 3.0 pH | T18 = 50% Fly ash + N + 5.0 pH |
| T19 = 50% Fly ash + N + 4.0 pH | T20 = 50% Fly ash + N + 3.0 pH |

2.2. Observations

Plant growth and yield: The experiment was terminated 90 days after nematode inoculation. Plant length, fresh and dry weights of shoot and root were determined. Shoot length was taken from the point of emergence of the first root to the shoot apex and cut from this point and root length was also taken. Shoots and roots were dried in a hot air oven at 60 °C for 48 h. Yield was determined as the number of flowers and fruits per plant.

Leaf area: Leaf area determine by taking five averages sized of leaves from each treatment. Each leaf was placed on a 1 mm² graph paper. The leaf size was traced on the paper and the total area calculated based on the number of squares covered within the traced region. The formula for leaf area estimation is given as:

$$A = KLB \text{ (cm}^2\text{)}, \text{ thus: } K = A/LB$$

Where: A = Leaf area, L = Leaf length, B = Leaf width

K = Correlation coefficient which is constant

Relative water content (RWC): RWC represents a useful indicator of the state of water balance of a plant, essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation (Gonzalez and Gonzalez-Vilar, 2001). Formula enunciated by Slatyer (1967) was to determine the RWC in leaves.

$$RWC = (FW-DW)/(TW-DW) \times 100$$

Where: FW= fresh weight, DW= dry weight, TW= turgid weight

Estimation chlorophyll and carotenoids: Photosynthetic pigments were estimated by Maclachlan and Zalik (1963) method. One gram leaf sample obtained from inoculated and healthy plants were used in the estimation of chlorophyll. The leaves were grounded using mortar and pestle in 10 ml of 80% acetone. The homogenates were poured into test tubes and centrifuged for 3 min at 4,500 rpm. The supernatants were decanted and then used for chlorophyll estimation. Acetone 3 ml was used to set the blank at zero. Optical density (O.D.) was read at 645 nm and 663 nm for chlorophyll a and b and at 480 nm and 510 nm for carotenoids against 80% acetone as blank on spectrophotometer. The concentration of chlorophyll a, chlorophyll b and total chlorophyll (a + b) and carotenoids present in the given extracts were calculated according to the formulae given below.

- i) Chl a = $12.7(O.D. 663) - 2.69(O.D.645) \times V/1000 \times W$ (mg/g)
- ii) Chl b = $22.9(O.D. 645) - 4.68(O.D.663) \times V/1000 \times W$ (mg/g)
- iii) Total Chl(a+ b) = $20.2(O.D.645) - 8.02(O.D.663) \times V/1000 \times W$ (mg/g)
- iv) Carotenoids = $7.6(O.D.480) - 1.49(O.D.510) / D \times 1000 \times W$ (mg/g)

Protein and carbohydrate: Estimation of carbohydrate was done by 'Anthrone' method (Hedge and Hofreiter, 1962) and quantitative protein estimation was done by Lowry et al. (1951).

Galls, egg masses and gall index: The roots of each plant were washed under tap water and immersed in an aqueous solution of phloxin B (0.15 g/litter tap water) for 15 minutes to stain the egg masses. Then galls and egg masses per root system were counted. Gall index was done as per the scale given by Taylor and Sasser (1978).

3. RESULTS

The data presented in Figures and Tables brought about qualitative information. It was seen that plants treated with 5-30% FA + either 5.0 pH registered considerable improvement in plant growth Table 1 and yield Figure 1 parameters of pumpkin. However, on the other hand pumpkin plants treated with 40 and 50% FA with either concentration of acid rain (5.0 pH, 4.0 pH, 3.0 pH) showed negative effects on the plant growth and yield variables.

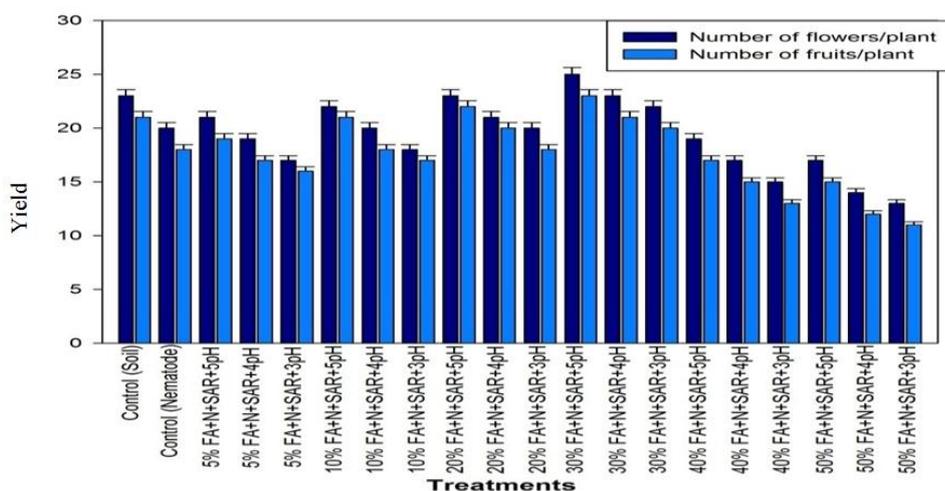


Figure-1. Graph shows the combined effect of FA, SAR and root-knot nematode on yield in pumpkin Var. Nutan.

Similar result was found in case of photosynthetic pigments Figure 2 leaf water content Figure 3 and protein content Figure 4. Similar result was found in case of photosynthetic pigments Figure 2, leaf water content Figure 3 and protein content Figure 4, carbohydrate and leaf area Table 2. The data given in Table 3 shows gall and eggmass per plant, reproduction factor and disease intensity in terms of gall and eggmass indices were significantly ($P \leq 0.05$) reduced by increasing the FA and SAR concentrations.

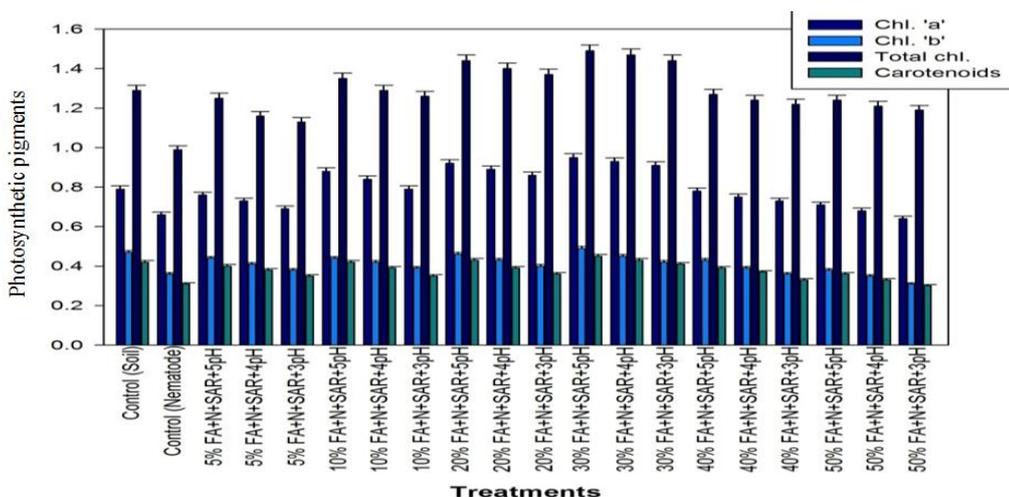


Figure-2. Graph shows the combined effect of FA, SAR and root-knot nematode on photosynthetic pigments in pumpkin Var. Nutan.

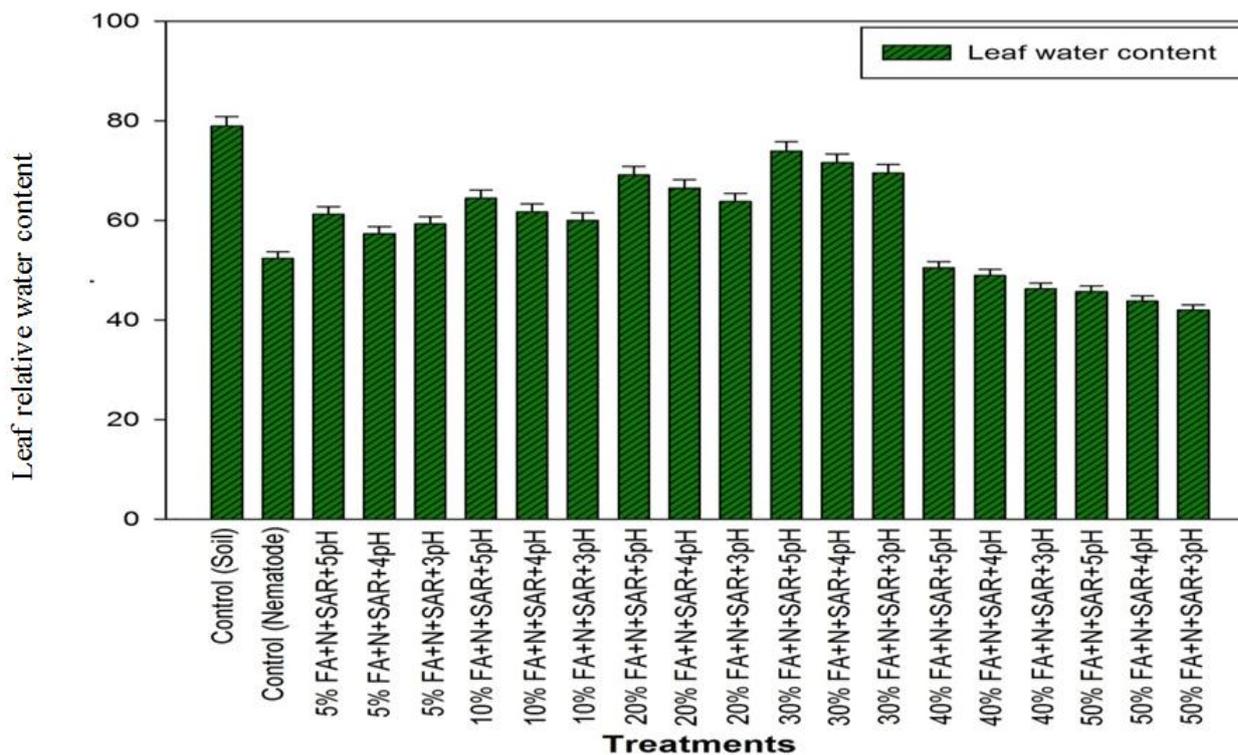


Figure-3. Graph shows the combined effect of FA, SAR and root-knot nematode leaf water content in pumpkin Var. Nutan.

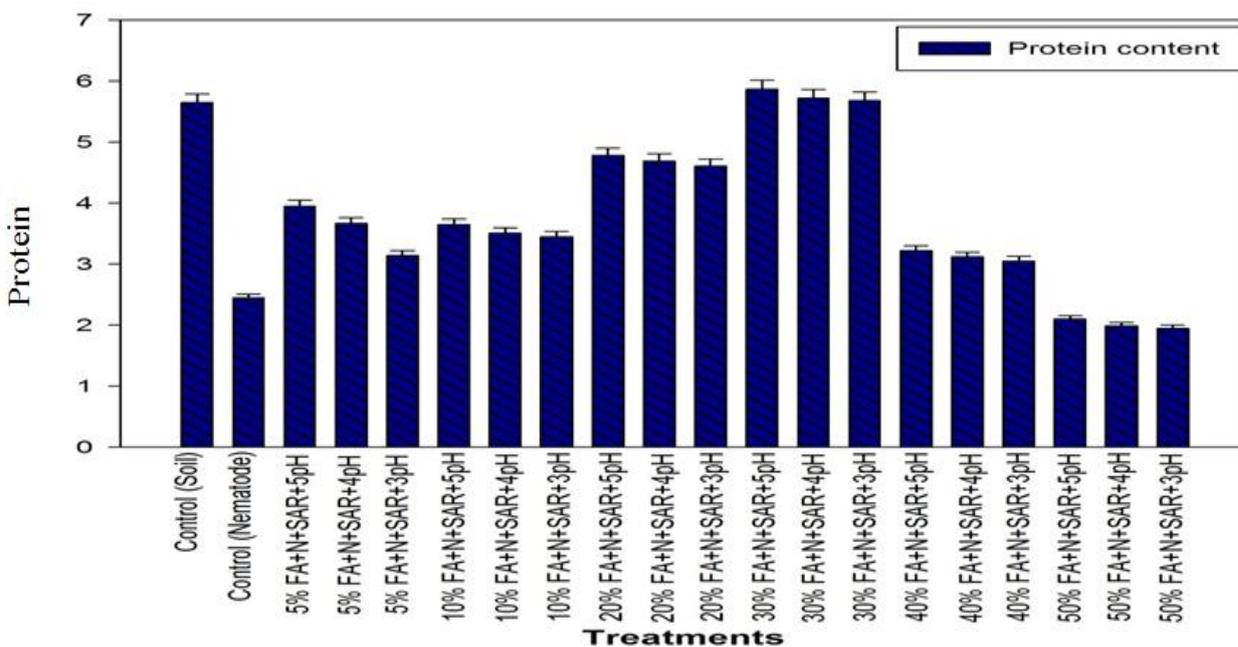


Figure-4. Graph shows the combined effect of FA, SAR and root-knot nematode leaf protein content in pumpkin Var. Nutan.

Table-1. Combined effect of fly ash, simulated acid rain and root-knot nematode (*M. incognita*) on growth of pumpkin Var. Nutan.

| Treatments (FA + SAR + N) | | Length (cm) | | Fresh weight (gm) | | Dry weight (cm) | | |
|---------------------------|-----|------------------------|------------------------|--------------------------|------------------------------|-------------------------|-----------------------------|---------------------------|
| | | Shoot | Root | Shoot | Root | Shoot | Root | |
| Control | | 285±12.89 ^a | 59±2.91 ^a | 219.4±10.67 ^a | 57±2.69 ^a | 30.2±1.45 ^a | 8.1±0.46 ^a | |
| Control (N) | | 210±11.90 ^b | 40±2.01 ^a | 198.1±9.61 ^{ab} | 51±2.90 ^{ab} | 23.0±1.21 ^{ab} | 5.3±0.26 ^a | |
| 5% FA + N | SAR | 5 pH | 212±9.75 ^c | 50±2.21 ^{ab} | 211.4±9.87 ^b | 60±2.11 ^{ab} | 27.2±1.54 ^{abc} | 6.1±0.20 ^a |
| | | 4 pH | 208±9.66 ^{cd} | 45±2.11 ^{abc} | 208.3±9.60 ^c | 58±2.05 ^{bc} | 25.6±1.60 ^{abcd} | 5.7±0.15 ^b |
| | | 3 pH | 188±8.50 ^d | 30±1.98 ^{bcd} | 195.1±9.75 ^{cd} | 45±2.08 ^{bc} | 15.9±1.50 ^{abcde} | 3.9±0.18 ^b |
| 10% FA + N | SAR | 5 pH | 245±11.01 ^d | 51±2.10 ^{bcd} | 215.2±10.45 ^{cde} | 61±2.94 ^{bc} | 30.1±1.55 ^{abcde} | 7.3±0.44 ^{bc} |
| | | 4 pH | 238±10.90 ^e | 46±2.05 ^{bcd} | 212.7±10.15 ^{def} | 59±2.99 ^{bc} | 28.4±1.48 ^{abcdef} | 6.8±0.50 ^{bc} |
| | | 3 pH | 210±9.01 ^e | 33±2.00 ^{cde} | 198.6±9.80 ^{defg} | 48±3.01 ^{bc} | 18.9±1.53 ^{bedef} | 4.9±0.42 ^{cd} |
| 20% FA + N | SAR | 5 pH | 258±10.68 ^e | 53±3.00 ^{cdef} | 226.1±10.15 ^{defgh} | 65±1.93 ^{bcd} | 33.2±1.30 ^{bcdefg} | 9.2±0.36 ^{de} |
| | | 4 pH | 253±9.55 ^f | 48±3.05 ^{cdefg} | 221.9±10.00 ^{efghi} | 61±1.85 ^{cde} | 31.5±1.25 ^{cdefg} | 8.9±0.30 ^{def} |
| | | 3 pH | 230±8.25 ^g | 35±3.15 ^{defgh} | 200.6±10.11 ^{fghij} | 50±1.90 ^{cde} | 20.7±1.15 ^{defg} | 7.5±0.38 ^{efg} |
| 30% FA + N | SAR | 5 pH | 284±11.12 ^g | 60±3.19 ^{efghi} | 245.0±6.93 ^{ghij} | 69±2.00 ^{cdef} | 35.0±1.71 ^{defgh} | 12.5±0.60 ^{efg} |
| | | 4 pH | 264±10.89 ^g | 52±2.80 ^{fghi} | 239.1±6.99 ^{hij} | 65±2.12 ^{cdef} | 33.8±1.75 ^{efgh} | 12.1±0.55 ^{efgh} |
| | | 3 pH | 238±10.76 ^g | 38±2.95 ^{ghij} | 210.3±5.80 ^{ij} | 51±1.88 ^{defg} | 22.9±1.67 ^{fghi} | 10.6±0.61 ^{fgh} |
| 40% FA + N | SAR | 5 pH | 214±8.87 ^{gh} | 48±3.01 ^{ghij} | 217.4±10.13 ^{ij} | 52±2.33 ^{efg} | 28.3±1.47 ^{ghi} | 6.2±0.30 ^{fgh} |
| | | 4 pH | 209±8.80 ^{hi} | 42±2.98 ^{ghij} | 210.5±10.02 ^{jk} | 49±2.40 ^{fg} | 26.9±1.21 ^{ghi} | 5.8±0.25 ^{fghi} |
| | | 3 pH | 180±8.85 ⁱ | 28±3.05 ^{ghij} | 194.1±9.80 ^{kl} | 40±2.30 ^{gh} | 14.5±1.45 ^{ghi} | 3.1±0.20 ^{ghij} |
| 50% FA + N | SAR | 5 pH | 200±11.30 ⁱ | 42±2.60 ^{hij} | 195.2±9.91 ^{lm} | 42±2.05 ^{hi} | 21.2±1.13 ^{ghi} | 4.5±0.27 ^{ghij} |
| | | 4 pH | 197±10.95 ⁱ | 38±2.50 ^{ij} | 191.8±9.99 ^{lm} | 38±1.95 ^{ij} | 19.6±1.15 ^{hij} | 4.1±0.21 ^{hij} |
| | | 3 pH | 169±11.05 ^j | 25±2.65 ^j | 178.3±10.01 ^m | 22±1.90 ⁱ | 10.1±1.10 ^{ij} | 2.8±0.15 ^{ijk} |

Each value in the table is the mean of five replicates (n=5), ±SDM: standard deviation of the mean.

a, b, c Means with different superscripts in the same row are statistically different (P<0.05) according to Least Significant Test (LSD).

FA- Fly ash, SAR- Simulated acid rain, N- Nematode.

Table-2. Combined effect of fly ash, simulated acid rain and root-knot nematode (*M. incognita*) on carbohydrate and proline contents of pumpkin Var. Nutan.

| Treatments (FA + SAR + N) | | | Carbohydrate (μg fresh weight) | Leaf area (cm^2) |
|---------------------------|-----|------|--|-----------------------------|
| Control (UIC) | | | 12.17 \pm 1.054 ^a | 44 \pm 2.99 ^a |
| Control (IC) | | | 9.12 \pm 1.127 ^{ab} | 32 \pm 1.65 ^b |
| 5% FA + N | SAR | 5 pH | 10.23 \pm 0.954 ^b | 30 \pm 1.67 ^{bc} |
| | | 4 pH | 09.18 \pm 0.951 ^c | 28 \pm 1.64 ^d |
| | | 3 pH | 07.14 \pm 0.953 ^d | 15 \pm 1.62 ^{de} |
| 10% FA + N | SAR | 5 pH | 10.99 \pm 0.915 ^e | 28 \pm 1.60 ^f |
| | | 4 pH | 09.96 \pm 0.916 ^e | 26 \pm 1.60 ^f |
| | | 3 pH | 07.91 \pm 0.913 ^f | 16 \pm 1.58 ^g |
| 20% FA + N | SAR | 5 pH | 11.55 \pm 1.014 ^f | 24 \pm 1.56 ^{gh} |
| | | 4 pH | 11.04 \pm 1.012 ^g | 22 \pm 1.55 ^h |
| | | 3 pH | 19.46 \pm 0.010 ^h | 18 \pm 1.53 ⁱ |
| 30% FA + N | SAR | 5 pH | 13.99 \pm 1.583 ^{hi} | 20 \pm 1.51 ^{ij} |
| | | 4 pH | 12.50 \pm 1.586 ⁱ | 18 \pm 1.49 ^{jk} |
| | | 3 pH | 10.25 \pm 1.581 ⁱ | 21 \pm 1.48 ^k |
| 40% FA + N | SAR | 5 pH | 08.19 \pm 1.890 ^{ij} | 16 \pm 1.50 ^{kl} |
| | | 4 pH | 07.15 \pm 1.888 ^j | 14 \pm 1.51 ^l |
| | | 3 pH | 06.10 \pm 1.886 ^k | 08 \pm 1.47 ^m |
| 50% FA + N | SAR | 5 pH | 04.91 \pm 0.961 ^l | 11 \pm 1.45 ⁿ |
| | | 4 pH | 8.99 \pm 0.959 ^{lm} | 09 \pm 1.43 ^o |
| | | 3 pH | 8.95 \pm 0.956 ^m | 05 \pm 1.42 ^o |

Each value in the table is the mean of five replicates (n=5), \pm SDM-standard deviation of the mean.
 A, b, c Means with different superscripts in the same row are statistically different (P<0.05) according to Least Significant Test (LSD).
 FA- Fly ash, SAR- Simulated acid rain, N- Nematode.

Table-3. Effect of different concentration of fly ash and simulated acid rain on root-knot disease of *M. incognita* in pumpkin Var. Nutan.

| Treatments (FA + SAR + N) | | Number/root system | | Number/g fresh root system | | Number of eggs/eggmass | Gall index |
|---------------------------|------|--------------------|----------|----------------------------|------------|------------------------|------------|
| Control (N) | | Galls | Egg mass | Galls | Egg masses | 210 | 5 |
| 5% FA + N | 5 pH | 225 | 150 | 5.05 | 2.75 | 170 | 5 |
| | 4 pH | 175 | 113 | 4.65 | 2.55 | 155 | 5 |
| | 3 pH | 161 | 98 | 4.39 | 2.39 | 140 | 5 |
| 10% FA + N | 5 pH | 151 | 85 | 4.18 | 2.15 | 120 | 5 |
| | 4 pH | 145 | 80 | 4.10 | 2.10 | 102 | 5 |
| | 3 pH | 130 | 75 | 3.60 | 1.90 | 87 | 4 |
| 20% FA + N | 5 pH | 95 | 69 | 3.29 | 1.75 | 70 | 4 |
| | 4 pH | 88 | 65 | 3.10 | 1.70 | 57 | 4 |
| | 3 pH | 55 | 43 | 2.88 | 1.48 | 32 | 4 |
| 30% FA + N | 5 pH | 38 | 22 | 2.24 | 1.36 | 10 | 3 |
| | 4 pH | 20 | 10 | 1.95 | 1.15 | - | - |
| | 3 pH | - | - | - | - | - | - |
| 40% FA + N | 5 pH | - | - | - | - | - | - |
| | 4 pH | - | - | - | - | - | - |
| | 3 pH | - | - | - | - | - | - |
| 50% FA + N | 5 pH | - | - | - | - | - | - |
| | 4 pH | - | - | - | - | - | - |
| | 3 pH | - | - | - | - | - | - |

Each value in the table is the mean of five replicates (n=5).
 FA- Fly ash, SAR- Simulated acid rain, N- Nematode.

Moreover, it was seen that pumpkin treated with 50% FA + SAR (5.0pH, 4.0pH, 3.0pH) caused greater reductions in plant biomass such as growth, yields, chlorophyll, carotenoids, protein, carbohydrate, leaf water content and leaf area followed by 40% FA + SAR (5.0 pH, 4.0 pH, 3.0 pH). Overall highest growth status in plant was recorded in the 30% FA + 5.0 pH inoculated with 2000 J₂ of *M. incognita*. However maximum negative effects were observed in 50% FA + SAR (5.0 pH, 4.0 pH, 3.0 pH) inoculated with 2000 J₂ of *M. incognita* treated plant. In addition, irrespective of dose levels of FA and SAR either singly or jointly when applied, caused significant reductions in nematode multiplication.

4. DISCUSSION

Several beneficial nutrients like S, B, Ca, Mg, Fe, Cu, Zn, Mn, and P helps in plant growth promotion are found in fly ash. Fly ash decreases porosity and thus increases water holding capacity which facilitates the absorption of nutrients leading to improved plant growth, physiology and biochemistry of plants. In the present study, soil amendment with fly ash was beneficial at lower levels maximum being at 30% level with pH 5.0 treatments. The plant growth was found maximum at 30% FA + N + pH 5.0 of SAR than any other combinations including controls. This shows that the toxic effect of pH 5.0 was nullified in presence of fly ash (20 and 30%) due to presence of some beneficial nutrients. On the other hand, nematodes multiplications were also significantly suppressed in 20 and 30% of FA levels along with 5.0 pH of SAR combinations. Similar results were also observed in other parameters (no of flowers and fruits, photosynthetic pigments, proline, carbohydrate, protein, leaf relative water content and the area of leaves). However the higher levels of FA (40% and 50%) and SAR (4.0 pH and 3.0pH) were significantly harmful to pumpkin.

Improved plant growth with fly ash has been observed earlier (Elseewi *et al.*, 1980; Mishra and Shukla, 1986). Due to the better health status of the plant, the yield, photosynthetic pigments, carbohydrate, protein, leaf water content and leaf area of pumpkin were also increased. The beneficial effects of FA were found from 10 to 30% levels in soil, and optimum being at 30% with 5 pH level of acid rain. Similar beneficial effects on above parameters in fly ash amendments have also been observed on a number of crops like cabbage, *Capsicum*, chickpea, collard greens, com, cucumber, *Lactuca sativa*, mustard green, radish, soybean, sunflower, tomato, *Vigna mungo*, wheat etc (Singh, 1989; Menon *et al.*, 1990; Khan and Wajid, 1996; Rengifo *et al.*, 1996; Sarangi and Mishra, 1998; Vinay and Dwivedi, 1999; Tarannum *et al.*, 2001; Upadhyay and Khan, 2002). Study showed the effect of different concentration of fly ash that changed the protein, nitrogen, proline, leghaemoglobin, chlorophyll and other biochemical parameters (Kumar and Kumar, 2017). All these were found to be favorable affected by FA. However, the responses of various crops were found to be varied. Higher levels of FA and SAR adversely affected the plant growth and other parameters of pumpkin. The adverse effects of FA and SAR at higher level of application attributed to excess of micro-nutrients (Adriano *et al.*, 1980) and toxicity (Helder *et al.*, 1982; Mishra and Shukla, 1986; Wong and Wong, 1989). On the other hand, the soil application of FA noticed the effect of *M. incognita* with respect to levels. This might be due to the excess of salts, toxic compounds and heavy metals which caused nematicidal effects on *M. incognita* either directly or within the host. Nematode might have lost its activities and later could not survive under the stress of FA. Losing the activity and not reaching the mature stage of *M. incognita* is very important for the agriculture point of view, because there will be no loss to the crop (Khan, 2007; Iram, 2010). Thus soil application of fly ash with 30% level is useful, as it suppresses the, *M. incognita* one hand, and improves the biomass of pumpkin crop on the other hand.

5. CONCLUSION

In the present study, it was observed that application of SAR (5.0 pH) + 30% FA significantly managed the damaging potential of *M. incognita* infecting pumpkin. Although, SAR (5.0 pH) may be harmful to the plant growth and its foliage but the application of FA nullified the effect of SAR (5.0 pH) significantly. Despite of this fact

combined application of 30% FA + SAR (5.0 pH) significantly increased the plant growth, yield and some biochemical content of plant. Moreover, SAR (pH 4.0, 3.0) + 30% FA application also registered greater reduction in nematodes multiplication, but this combination may not be recommended because of presence of phytotoxic elements. Conclusively, application of 30% FA + SAR (5.0 pH) may be used in the acid rain vulnerable area to manage the southern RKN disease and protecting the environment from different pollutants.

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REFERENCES

- Adriano, D., A. Page, A. Elseewi, A. Chang and I. Straughan, 1980. Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: A review 1. *Journal of Environmental Quality*, 9(3): 333-344. Available at: <https://doi.org/10.2134/jeq1980.00472425000900030002x>.
- CEA, 2017. Fly ash generation and utilization. Government of India, Ministry of Power.
- Cerniauskiene, J., J. Kulaitiene, H. Danilcenko, E. Jariene and E. Jukneviene, 2010. Pumpkin fruit flour as a source for food enrichment in dietary fiber. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 42(1): 19-23.
- Colla, G., Y. Roupheal, M. Cardarelli, D. Massa, A. Salerno and E. Rea, 2006. Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. *The Journal of Horticultural Science and Biotechnology*, 81(1): 146-152. Available at: <https://doi.org/10.1080/14620316.2006.11512041>.
- Elling, A.A., 2013. Major emerging problems with minor meloidogyne species. *Phytopathology*, 103(11): 1092-1102. Available at: <https://doi.org/10.1094/phyto-01-13-0019-rvw>.
- Elseewi, A.A., I. Straughan and A. Page, 1980. Sequential cropping of fly ash-amended soils: Effects on soil chemical properties and yield and elemental composition of plants. *Science of the Total Environment*, 15(3): 247-259. Available at: [https://doi.org/10.1016/0048-9697\(80\)90053-4](https://doi.org/10.1016/0048-9697(80)90053-4).
- Ferenbaugh, R.W., 1976. Effects of simulated acid rain on *Phaseolus vulgaris* L.(Fabaceae). *American Journal of Botany*, 63(3): 283-288. Available at: <https://doi.org/10.2307/2441572>.
- Gheysen, G. and M.G. Mitchum, 2011. How nematodes manipulate plant development pathways for infection. *Current Opinion in Plant Biology*, 14(4): 415-421. Available at: <https://doi.org/10.1016/j.pbi.2011.03.012>.
- Gonzalez, L. and M. Gonzalez-Vilar, 2001. Determination of relative water content. In: Reigosa Roger M.J. (eds) *Handbook of Plant Ecophysiology Techniques*. Dordrecht: Springer.
- Hajra, N., F. Shahina, K. Firoza and R. Maria, 2015. Damage induced by root-knot nematodes and its alleviation by vesicular arbuscular mycorrhizal fungi in roots of *Luffa cylindrica*. *Pakistan Journal of Nematology*, 33(1): 71-78.
- Hedge, J. and B. Hofreiter, 1962. *Carbohydrate chemistry* 17. Whistler, R.L. and Be Miller, J. N., Eds., New York: Academic Press.
- Helder, T., E. Stutterheim and K. Olie, 1982. The toxicity and toxic potential of fly ash from municipal incinerators assessed by means of a fish early life stage test. *Chemosphere*, 11(10): 965-972. Available at: [https://doi.org/10.1016/0045-6535\(82\)90068-6](https://doi.org/10.1016/0045-6535(82)90068-6).
- IEA, 2008. World energy outlook. International energy agency. Senapati MR. Fly ash from thermal power plants-waste management and overview. *Current Science* 2011, 100(12): 1791.
- Iram, 2010. Utilization of fly ash for the management of root-knot nematodes on some vegetable crops. Ph.D. Thesis. Aligarh Muslim University, Aligarh.
- Jones, J.T., A. Haegeman, E.G. Danchin, H.S. Gaur, J. Helder, M.G. Jones, T. Kikuchi, R. Manzanilla-López, J.E. Palomares-Rius and W.M. Wesemael, 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14(9): 946-961. Available at: <https://doi.org/10.1111/mpp.12057>.

- Khan, A., 2007. Management of root-knot nematodes by application of fly ash. Final Technical Report. DST, New Delhi (No SR/So/AS-49/2002).
- Khan, A.M., C.S. Ahmad, U. Farooq, K. Mahmood, M. Sarfraz, K.S. Balkhair and M.A. Ashraf, 2015. Removal of metallic elements from industrial waste water through biomass and clay. *Frontiers in Life Science*, 8(3): 223-230. Available at: <https://doi.org/10.1080/21553769.2015.1041187>.
- Khan, M.R., 2008. *Plant nematodes, methodology, morphology, systematics, biology and ecology*. New Jersey: Science Publishers.
- Khan, M.R. and M. Wajid, 1996. The effect of fly ash on plant growth and yield of tomato. *Environmental Pollution*, 92(2): 105-111. Available at: [https://doi.org/10.1016/0269-7491\(95\)00098-4](https://doi.org/10.1016/0269-7491(95)00098-4).
- Khan, T. and S. Devpura, 2004. Physiological and biochemical effects of simulated acid rain on phaseolus vulgaris var. HUR-15. *Environmentalist*, 24(4): 223-226. Available at: <https://doi.org/10.1007/s10669-005-0997-y>.
- Kishor, P., A. Ghosh and D. Kumar, 2010. Use of flyash in agriculture: A way to improve soil fertility and its productivity. *Asian Journal of Agricultural Research*, 4(1): 1-14. Available at: <https://doi.org/10.3923/ajar.2010.1.14>.
- Kumar, K. and A. Kumar, 2017. Effect of fly ash on some biochemical properties of vigna mungo L. *International Journal of Pharmaceutical Research and Allied Sciences*, 6(2): 1-13.
- Larssen, T., E. Lydersen, D. Tang, Y. He, J. Gao, H. Liu, L. Duan, H. Seip, R. Vogt, J. Mulder, M. Shao, Y. Wang, H. Shang, X. Zhang, S. Solberg, W. Aas, T. Okland, O. Eilertsen, L.Q. Angell V, D. Zhao, R. Xiang, J. Xiao and J. Luo, 2006. Acid rain in China. *Environmental Science & Technology*, 40: 418-425.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1): 265-275.
- Maclachlan, S. and S. Zalik, 1963. Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany*, 41(7): 1053-1062. Available at: <https://doi.org/10.1139/b63-088>.
- Menon, M., G. Ghuman, J. James, K. Chandra and D. Adriano, 1990. Physico-chemical characterization of water extracts of different coal fly ashes and fly ash-amended composts. *Water, Air, and Soil Pollution*, 50(3-4): 343-353. Available at: <https://doi.org/10.1007/bf00280634>.
- Mishra, L. and K. Shukla, 1986. Effects of fly ash deposition on growth, metabolism and dry matter production of maize and soybean. *Environmental Pollution Series A, Ecological and Biological*, 42(1): 1-13. Available at: [https://doi.org/10.1016/0143-1471\(86\)90040-1](https://doi.org/10.1016/0143-1471(86)90040-1).
- Moharami, S. and M. Jalali, 2015. Effect of acid rain on the fractionation of heavy metals and major elements in contaminated soils. *Chemistry and Ecology*, 31(2): 160-172. Available at: <https://doi.org/10.1080/02757540.2014.917173>.
- Ni, S., D. Zhao, Q. Cui, J. Li and G. Yang, 2006. Effects of simulated acid rain on growing rules of Northern wheat. *Journal of Shandong University (Natural Science)*, 41(6): 109-113.
- Odiy, B. and A. Eniola, 2015. The effect of simulated acid rain on plant growth component of Cowpea (*Vigna unguiculata*) L. Walps. *Jordan Journal of Biological Sciences*, 8(1): 51-54. Available at: <https://doi.org/10.12816/0026948>.
- Pal, S. and N. Kumar, 2000. Effects of simulated acid rain on yield and carbohydrate contents of green pepper (*Capsicum annum* L.). *Advan in Plant Science*, 13: 85-88.
- Rapava, L., E. Ketskhoveli, T. Barblishvili, N. Razmadze and I. Chichiashvili, 2007a. Physiological alterations evoked by acidification in different cultivars of common bean and maize. *Bul of the Georgian Nat Acad of Science*, 5(2): 112-118.
- Rapava, L., S. Chanishvili, G. Badridze, T. Barblishvili and S. Abramidze, 2007b. Influence of simulated acid rains on physiological indices of white and red forms of cabbage (*Brassica capitata* L.). *Bul of the Georgian Nat Acad of Science*, 5(1): 72-76.
- Rengifo, J., G. Ramírez and C. Bruzón, 1996. Importance of filter press cake as a substrate for vegetable seedling production. *Agronomic Record, National University of Colombia*, 46(1/4): 37-43.
- Sarangi, P. and P.C. Mishra, 1998. Soil metabolic activities and yield in ground nut, ladies finger and radish in fly ash amended soil. *Research Journal of Chemistry and Environment*, 2(2): 7-13.

- Singh, S., 1989. Studies on interaction of air pollutants and root-knot nematodes on some pulse crops. Ph.D. Thesis. Aligarh Muslim University. Aligarh, India.
- Slatyer, R., 1967. Plant water relationship. New York: Academic Press. pp: 73–77.
- Tarannum, A., A. Khan, I. Diva and B. Khan, 2001. Impact of fly ash hatching, penetration and development of root-knot nematode, *Meloidogyne javanica*. *Mediterranean Nematology*, 29(2): 215–218.
- Taylor, A. and J. Sasser, 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* Species).
- Upadhyay, E. and A. Khan, 2002. Physico-chemical properties of fly ash and its impact on mustard. *Environmental Biological Conservation*, 7: 85-88.
- Vinay, S. and S. Dwivedi, 1999. Effect of fly ash on seed germination, plant growth and chlorophyll content of two crops of economic importance. *Acta Botanica Indica*, 27(2): 145-150.
- Wen, K., C. Liang, L. Wang, G. Hu and Q. Zhou, 2011. Combined effects of lanthanum and acid rain on growth, photosynthesis and chloroplast ultrastructure in soybean seedlings. *Chemosphere*, 84(5): 601-608. Available at: <https://doi.org/10.1016/j.chemosphere.2011.03.054>.
- Wen, X.-j., C.-q. Duan and D.-c. Zhang, 2013. Effect of simulated acid rain on soil acidification and rare earth elements leaching loss in soils of rare earth mining area in southern Jiangxi Province of China. *Environmental Earth Sciences*, 69(3): 843-853. Available at: <https://doi.org/10.1007/s12665-012-1969-4>.
- Wondyfraw, M., 2014. Mechanisms and effects of acid rain on environment. *Journal of Earth Science & Climatic Change*, 5(6): 1-3. Available at: <https://doi.org/10.4172/2157-7617.1000204>.
- Wong, M.H. and J. Wong, 1989. Germination and seedling growth of vegetable crops in fly ash-amended soils. *Agriculture, Ecosystems & Environment*, 26(1): 23-35. Available at: [https://doi.org/10.1016/0167-8809\(89\)90035-2](https://doi.org/10.1016/0167-8809(89)90035-2).

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