

Evaluation of Coal Fly-Ash in Growth, Yield and Physiological Improvement of Groundnut (*Arachis hypogaea* L.) Crop and Mitigation of Root-Knot Nematode (*Meloidogyne arenaria*) Stress

Sandeep Kumar Yadav*¹ and Kamal Singh²

¹⁻² Department of Botany, Dharam Samaj College (Affiliated to Dr. Bhim Rao Ambedkar University, Agra - 282 004, Uttar Pradesh), Aligarh - 202 001, Uttar Pradesh, India

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Abstract

Chemical nematicides have negative impact on both soil and its microbes hence alternatives are required to replace them. Thus, the current study was conducted to illustrate the fly-ash's nematicidal and plant-growth-promoting abilities in eco-friendly manner. Pot experiments were performed during the period of, June 2024 to October 2024. Seeds of groundnut (*Arachis hypogaea* L.) were sown in clay pots containing fly-ash (10, 20, 30, 40 and 50%) amended soil. Plants were inoculated with *Meloidogyne arenaria*, identified by perineal pattern. Results revealed that application of fly-ash improved the soil physicochemical properties. It has been found that *M. arenaria* inoculation significantly reduced the growth, yield and pigment content of groundnut plants compared to the untreated and un-inoculated plants. Plants cultivated in 30% fly-ash (70:30 w/w soil:fly-ash) the growth and yield substances had significantly ($P \leq 0.05$) improved as compared to untreated and inoculated plants. Moreover, the physiological attributes were also enhanced in 30% fly-ash amended soil. However, fly-ash amended soil not only increased growth and yield but also reduced the nematode disease on groundnut plants. Therefore, our results demonstrated that Fly ash at 30% level can be utilized to manage *M. arenaria* in eco-friendly manner and enhance growth, yield and resistance of plants.

Key words: Groundnut, *M. arenaria*, Fly-ash, Growth, Yield, Biochemical components, Eco-friendly management

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown during kharif season in India, belongs to family Fabaceae. It is mostly grown on low fertility soils of marginal rainfed areas. Possess food, oils, kernels and rich in protein (25–28%), oil (48–50%) several antioxidants, vitamins, biologically active polyphenols, isoflavones and flavonoids [1]. It also contains minerals like calcium (Ca), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), iron (Fe), zinc (Zn) and phosphorus (P) among other vital elements.

From last decade, stress causing biotic agents infecting plants as plant-parasitic nematodes (PPNs) has reduced the groundnut production. Root-knot nematodes (*Meloidogyne* sp.), one of the most dangerous PPNs, reduced the groundnut production by forming multinucleate, big cells (called galls) in roots [2-3]. Second-stage juveniles (J2s) as infective stage of root-knot nematodes enter the plant's root and migrate to vascular tissue before moving towards neighbouring cells to establish feeding sites for long duration [4]. PPNs alter the protein machinery of the host plant, causing denaturation of proteins and altering several metabolic pathways [5]. Galls formation, aberrant water and mineral uptake and transport are the primary signs of PPNs infection, which reduce the plant's nutrients and water absorption that result in chlorosis [6]. Additionally, it is important to utilize suitable eco-friendly nematicides against nematodes that decrease the groundnut

production. To control nematodes, a number of control strategies have been developed. Nematicides are also quite successful for controlling nematodes, but their regular usage pollutes the soil and environment and endangers human health [7]. Fly-ash (FA), a fine waste produced as a byproduct of burning coal in thermal power plants, has been proposed by researchers as a possible therapy for nematodes management on a variety of crops such as pumpkin [8], brinjal [9], carrot [10] and tomato [11]. In India, up to 280.82 million tons of FA was produced, but only 259.86 million tons were consumed [12-13]. FA possessing minerals that enhancing growth of plants including potassium (K), sodium (Na), calcium (Ca), silica (Si) and has trace amount of heavy metals like lead (Pb) and arsenic (As), few necessary elements like zinc (Zn), copper (Cu) and iron (Fe) [14].

FA also enhanced the soil's electrical conductivity (EC), pH, water-holding capacity (WHC) and soil porosity [12, 15-17]. Furthermore, hazardous metals viz., As and Pb are present in trace in FA and do not affect plant growth or soil quality when apply to soil at optimum level [18], [14]. Therefore, FA can be useful in both agricultural fields to preventing the reproduction and growth of root-knot nematodes and enhancing the plant yield if used at its optimum level [19-21]. Several researchers explore the soil-improving qualities of FA in a variety of agricultural crops such as castor bean [22], mung

*Correspondence to: Sandeep Kumar Yadav, E-mail: kumaryadav2701@gmail.com; Tel: +91 8439508583

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bean [20], okra [14], wheat [23], Indian jujube [24], Indian wild rice [25], safed musli [26], pumpkin [16], *Capsicum annum* [17], pea [21], radish [27] and mustard [28]. While, role of FA on groundnut crop remains unconfirmed. Thus, the present study explored the antinemic potential of FA and influencing growth, yield and biochemical performance of groundnut crop.

MATERIALS AND METHODS

Identification of collected RKN species

Infected galled root samples were collected from groundnut fields and taken into laboratory for the identification of species. Nematode species was identifying by traditional method (known as perineal pattern) given by Eisenback *et al.* [29]. In this method, RKN female (pear in shape) was dissected from infected root and slides were made using microscope (dissecting): the posterior part of female body was cut with the help of needle and sharp blade and cut portion was trimmed into square shape and vied under light microscope. Species was identified as *M. arenaria*, on the basis of characters described by Eisenback *et al.* [29] (Fig 1).

Maintenance and preparation of *M. arenaria* inoculum

After identification, the *M. arenaria* in pure form was maintained on brinjal plants. Furthermore, egg mass was

extracted from brinjal roots and kept in petri dishes having distilled water (DW) to favor egg hatching at 27 ± 2 °C temperature. Freshly hatched J2s were collected from suspension, and DW was added to petri dishes. The freshly hatched J2s at 2000 J2s/pot were used as inoculum for pot experiments while remaining J2s were stored at 4 -10 °C for further use (Fig 2).

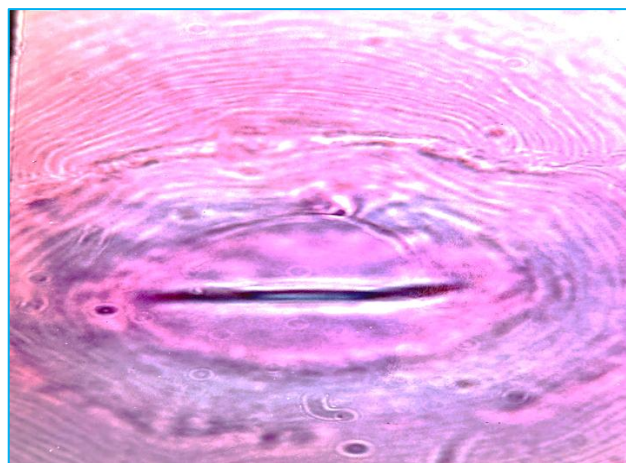


Fig 1 Microscopic image of perineal pattern of *M. arenaria*

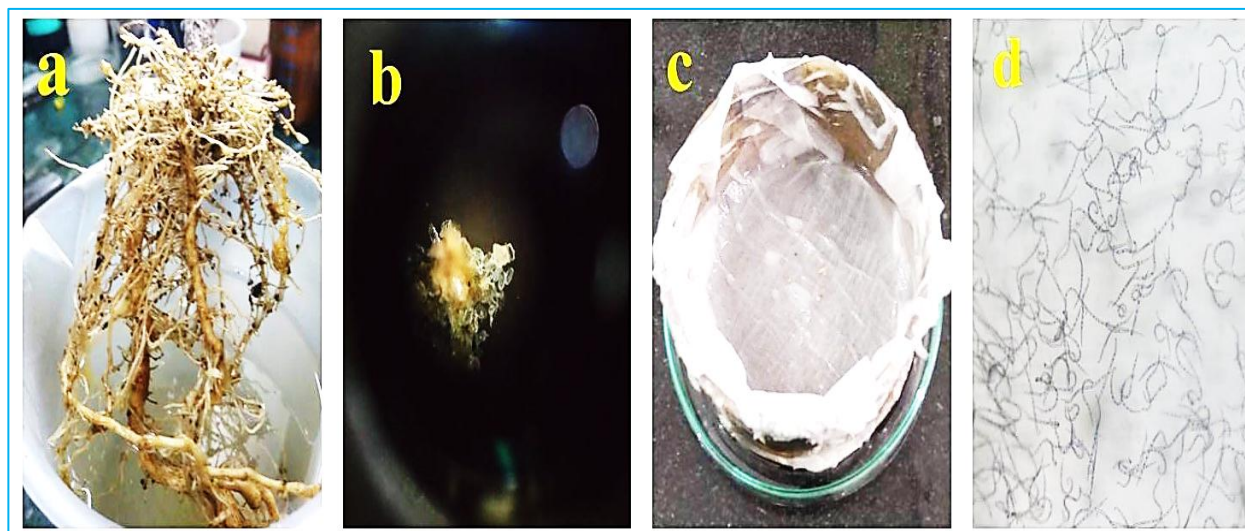


Fig 2 Figure shows the preparation of *M. arenaria* inoculum, (a) infected eggplant root, (b) egg mass, (c) coarse sieve with eggs in petri plate, (d) freshly hatched J2s



Fig 3 Figure shows the fly ash as eco-friendly material used for the management of *M. arenaria* on groundnut crop

Aligarh, UP, India (Fig 3). While soil was collected from agriculture field up to 20 cm of depth, after removing the unnecessary litters and soil particles. Soil and FA texture was determined by the rubbing of moist soil/fly-ash between the fingers and thumb.

In vitro experiments

Preparation of fly-ash extracts

Extracts of FA were prepared by using Tarannum *et al.* [30] method. According to this method FA at 1 kg was soaked in 2000 ml DW after soaking, it was filtered by using Whatman filter paper (No.1). As a result, a stock solution of FA extracts was obtained, which was then diluted with DW to make various FA extracts concentrations (0-30% FA). Extract with 0% FA concentration is considered DW only.

Egg hatching inhibition test

For hatching inhibition, fresh egg masses (5 in number) were isolated from highly infected root of brinjal plant. Such

Collection of fly-ash and soil

For the experimental work, fresh FA was obtained from Harduaganj Thermal Power Plant, located at Kasimpur,

egg masses were kept in petri dishes having 10 ml solution of each FA extract. Five replicates were taken for each treatment and petri dishes with DW were considered as control having 0% FA. Incubation was done at 28 °C temperature for each petri dish. After 5 days of incubation the hatched J2s were counted using a binocular microscope. The percent hatching inhibition was determined using the below formula:

$$\% \text{ Hatching inhibition} = \frac{\text{Total No. of J2s in control} - \text{Total No. of J2s in treatment}}{\text{Total No. of J2s in control}} \times 100$$

Juvenile's mortality test

J2s mortality was examined in various concentrations of FA extract. J2s (100 in number) were transferred to all the petri dishes for each treatment. Petri dishes with only DW were considered as control (0% FA). After duration of 24, 48 and 72 hours, all the petri dishes were carefully observed under binocular microscope. Living J2s appeared in curved shape while dead J2s straight in shape or immobile. Such assessment of living and dead J2s (Fig 4a-b) was also done by touching the J2s with fine brush under microscope [31]. The percent mortality of J2s was calculated by the following formula:

$$\% \text{ Mortality} = \frac{\text{No. of J2s in control} - \text{No. of J2s in treatment}}{\text{No. of J2s in control}} \times 100$$



Fig 4 In vitro examination of the second stage juveniles (J2s) of *M. arenaria*, (a) living J2s in control (distill water), (b) dead J2s treated with fly ash extract

Plant observations and data collection

Plants were gently uprooted after 90 days of J2s inoculation and water was used to rinse the adhering soils. Data were collected including plant growth, yield, photosynthetic pigments such as chlorophyll a, b and carotenoids and disease performance viz., egg masses/root system, root-knot index (RKI) and nematode population (NP).

Growth and yield performance

Growth parameters such as length (shoot and root), fresh and dry weight (shoot and root), while yield parameters like number and area of leaves, number of pods and flowers were measured. Weighing balance was used to measure the fresh and dry weight followed by oven drying at 80 °C for 2-3 days.

Physiological performance

Photosynthetic pigments estimation

Fresh groundnut plant leaves were examined for photosynthetic pigments such as chlorophyll (a and b) and

Pot experiments and experimental design

All the pot experiments were performed in clay pots (3 kg soil/pot) in Net House, D. S. College, Aligarh, Uttar Pradesh under natural conditions and arranged in a randomized complete block design. Collected soil was autoclaved at 15 lb. pressure for 20 minutes before experiment. After sterilizing the soil, the experiment was categorized into seven groups with five replicas of each (n=5). The first group consider as: untreated and un-inoculated control (UUC) means the plants not treated with FA and not inoculated with *Meloidogyne arenaria*. In second group: untreated and inoculated control (UIC) means plants not treated with FA while inoculated with *Meloidogyne arenaria* J2s. In third group: 10% FA + *Meloidogyne arenaria*. Fourth group: 20% FA + *Meloidogyne arenaria*. Fifth group: 30% FA + *Meloidogyne arenaria*. In sixth group: 40% FA + *Meloidogyne arenaria*; and the seventh group: 50% FA+ *Meloidogyne arenaria*. All the seeds were surface sterilized in 1% NaOCl solution before sowing to the pots (5 in each). After seedling emergence, thinning was done in all pots to maintain a healthy seedling. Then, inoculation was done with freshly hatched J2s of *Meloidogyne arenaria* at 2000 J2s/pot on the basis of experimental design. Every pot containing groundnut seedlings in holes received 5 ml of nematode suspension. The majority of the J2s swiftly approached the roots since three to five holes were carefully drilled without causing any harm to the root structure. To prevent dry conditions, all of the plants received frequent irrigation.

carotenoids using the Mackinney [32] method. According to this method, fresh leaf of groundnut (1g) was crushed into a powder form and then mixed with 20 ml acetone (80%). For chlorophyll estimation absorbance was measured at wavelength of 645 and 663 nm and for carotenoid at 480 and 510 nm against acetone (80%) as blank using spectrophotometer (UV 1700, Shimadzu, Japan).

Following formulae were considered for chlorophyll and carotenoid estimation:

$$\text{Chl. 'a'} = 12.7 (A_{663}) - 2.69 (A_{645}) \times (V/(W \times 1000))$$

$$\text{Chl. 'b'} = 22.9 (A_{645}) - 4.68 (A_{663}) \times (V/(W \times 1000))$$

$$\text{Carotenoids} = 7.6 (A_{480}) - 1.49 (A_{510}) \times (V/(W \times D \times 1000))$$

Where;

A = Absorbance, W = Weight of leaf sample, V = final volume taken, D = Path length of light

Disease performance

Number of egg masses

Egg mass number in the infected roots of groundnut plants were measured according to method proposed by Holbrook et al. [33]. According to this method, roots were washed with water and stained with Phloxine B (0.15 g/L water) for 5 – 10 minutes, extra stain washed and egg masses number were count manually.

Nematode population

Cobb's [34] sieve and decanting method, followed by Baermann's funnel [35] technique, was used to determine the nematode population from 250 g of soil per treatment. Five replicates per treatment were employed, and aliquots of 1 ml of the prepared culture were sampled under a stereomicroscope to determine the number of nematodes after 72 hours.

Root-knot index

Taylor and Sasser [36] method was used for the measurement of RKI by applying the 0-5 scale i.e., 0 = No galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls and 5 = more than 100 galls per root system.

Data analysis

The data for both *in vitro* and pot experiments are the mean of five replicates. Analysis was done by analysis of variance (ANOVA) using SPSS-17.0 statistical software (SPSS Inc., Chicago, IL, USA) to determine the significant at $P \leq 0.05$. Significant differences between treatments were analyzed by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

In vitro experiments

Effect of fly-ash on egg hatching and J2s mortality

The hatching and mortality of *M. arenaria* J2s were recorded in aqueous extract of FA under *in vitro* experiments. Results given in (Table 1) showed that hatching significantly decreased ($P \leq 0.05$) with increase in FA extract concentrations. The minimum egg hatching inhibition (39.82%) was found in 10% FA concentration, while maximum inhibition (81.31%) was found in 50% FA concentration after 5 days of exposure compared to control.

Table 1 Effect of different concentrations of fly-ash extract on the egg hatching and juvenile mortality of *M. arenaria* *in vitro*

Treatments	Number of hatched J2s after 5 days	J2s mortality after different exposure hours		
		24 h	48 h	72 h
DW	450.00 ^a (0)	0.00 (0)	0.00 (0)	0.00 (0)
10% FA	270.79 ^b (39.82%)	23.83 ^e (23.83%)	29.87 ^d (29.87%)	34.89 ^e (34.89%)
20% FA	175.41 ^c (61.02%)	41.36 ^d (41.36%)	46.77 ^c (46.77%)	51.90 ^d (51.90%)
30% FA	111.11 ^d (75.30%)	60.64 ^c (60.64%)	67.70 ^b (67.70%)	72.89 ^c (72.89%)
40% FA	103.21 ^d (77.06%)	68.36 ^b (68.36%)	70.79 ^b (70.79%)	79.86 ^b (79.86%)
50% FA	84.07 ^e (81.31%)	79.67 ^a (79.67%)	81.89 ^a (81.89%)	89.88 ^a (89.88%)

Each value is the mean of five replicates. Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. J2s – Second stage juveniles; DW – Distilled water (control); FA – Fly-ash; h – Hours

Similarly, a clear relationship between the FA extracts and the J2s mortality of *M. arenaria* was given in (Table 1). Mortality of J2s of *M. arenaria* significantly increased with increasing FA extract concentrations. The highest mortality

(89.88%) was found in 50% FA concentration after 72 hours of exposure time, the lowest mortality (23.83%) was found in 10% FA concentration after 24 hours of exposure time, while mortality was recorded zero in control (DW) (Fig 4b-5).

Table 2 Effect of different fly-ash concentrations on *M. arenaria* in relation to plant growth and yield performance of groundnut crop

Parameters	Treatments						
	UUC	UIC	10% FA + N	20% FA + N	30% FA + N	40% FA + N	50% FA + N
Shoot length (cm)	48.22 ^a	17.32 ^f	28.12 ^e	34.51 ^c	43.55 ^b	35.61 ^c	30.11 ^d
Root length (cm)	25.22 ^a	10.21 ^e	13.31 ^d	17.55 ^c	22.31 ^b	19.37 ^c	14.61 ^d
Shoot fresh weight (g)	44.35 ^a	20.69 ^f	27.55 ^e	34.19 ^c	41.33 ^b	36.75 ^c	30.83 ^d
Root fresh weight (g)	10.59 ^a	5.55 ^f	6.45 ^e	7.89 ^c	9.13 ^b	8.49 ^c	7.05 ^d
Shoot dry weight (g)	10.11 ^a	3.71 ^f	5.11 ^e	7.84 ^c	9.57 ^b	8.11 ^c	6.51 ^d
Root dry weight (g)	3.68 ^a	1.05 ^e	1.70 ^d	2.25 ^c	3.11 ^a	2.55 ^b	2.08 ^d
Number of leaves (/plant)	84.51 ^a	38.89 ^g	51.59 ^f	66.73 ^d	78.74 ^b	70.55 ^c	60.47 ^e
Leaf area (cm ² /plant)	12.51 ^a	5.59 ^f	7.21 ^e	8.55 ^d	10.59 ^b	9.41 ^c	8.02 ^d
Number of flowers	14.11 ^a	6.15 ^g	8.11 ^f	10.82 ^d	13.13 ^b	11.11 ^c	9.49 ^e
Number of pods (/plant)	15.27 ^a	6.19 ^f	8.78 ^e	9.91 ^d	12.11 ^b	10.78 ^c	9.11 ^d

Each value is the mean of five replicates. Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. UUC – Untreated uninoculated control; UIC – Untreated inoculated control; FA – Fly-ash, N – Nematodes (*M. arenaria*)

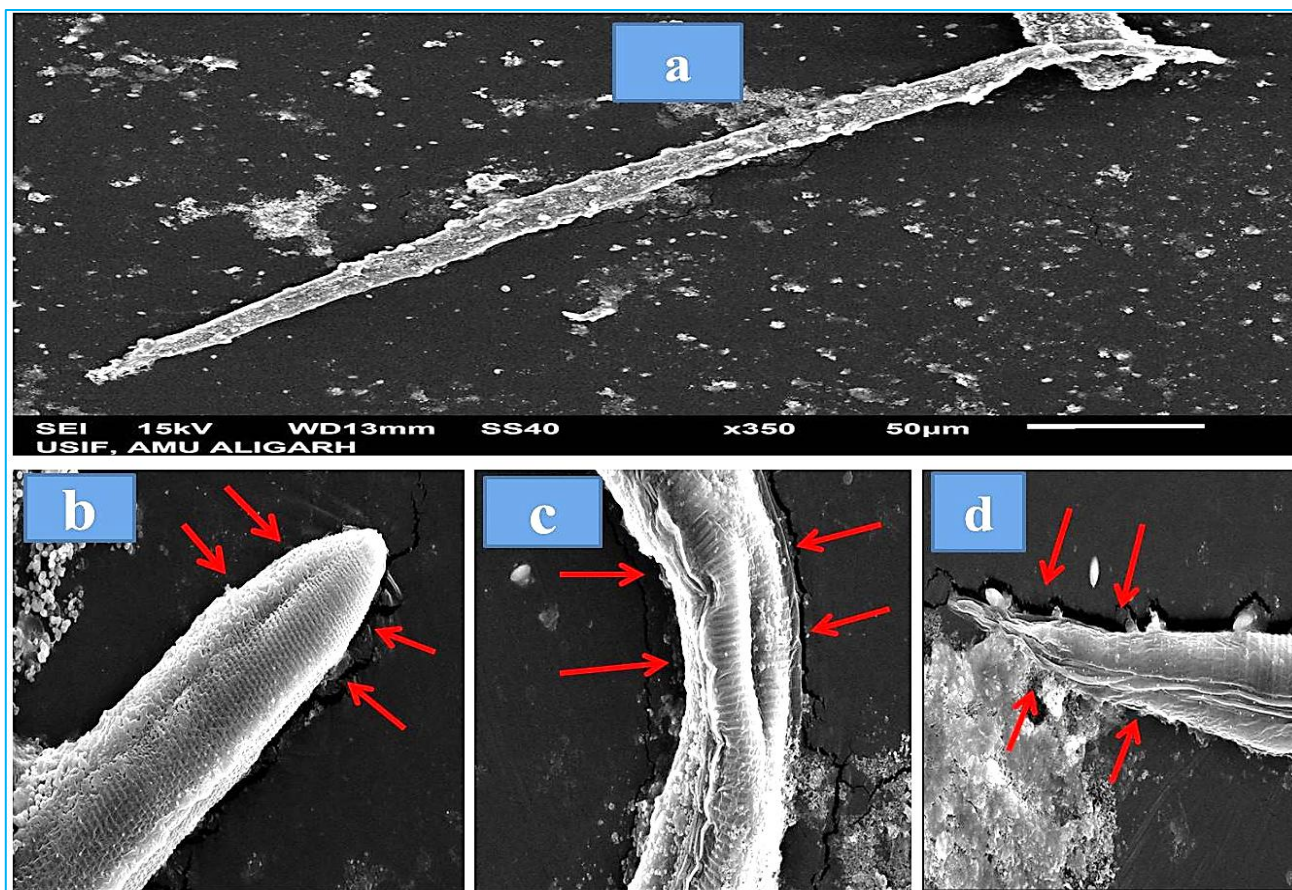


Fig 5 Scanning electron microscopic (SEMs) image of the dead J2 after treated with fly ash extract (a) the complete damaged J2 with disturbed cuticle, (b) the anterior portion of J2 with ruptured cuticle, (c) the middle portion of J2 with damage cuticle, (d) the posterior portion of J2 with rupture cuticle



Fig 6 Figure shows the interactive effect of fly ash and *M. arenaria* on the growth of groundnut plants. UUC=Untreated uninoculated control; UIC=Untreated inoculated control with *M. arenaria*; FA = Fly ash; N = Nematodes

Pot experiments

Effects of FA on growth and yield attributes of groundnut crop

Results presented in (Table 2) showed that growth and yield (shoot and root length, shoot and root fresh and dry weight, leaf area, number of leaves and pods) of groundnut plants under the influence of *M. arenaria* are increased by the soil application of FA from 10 to 50%, as compared to untreated and inoculated control (UIC). The results revealed that at 30% FA, the significant increase in growth and yield of groundnut plants in terms of shoot and root length (43.55 and 22.31 cm), shoot and root fresh weight (41.33 and 9.13 g), shoot and root dry weight (9.57 and 3.11 g), number of leaves (78.74), leaf area

(10.59 cm²/plant), number of flowers and pods (13.13 and 12.11) of groundnut crop, as compared to UIC (Table 2, Fig 6).

Effects of FA on physiological attributes of groundnut crop

Results given in (Table 3) demonstrated that all the physiological attributes like chlorophyll 'a', 'b' and carotenoids of groundnut plants under the influence of *M. arenaria* are increased by the soil amendment with FA from 10 to 50%, as compared to UIC. The results revealed that at 30% FA, the significant increase in physiological attributes like chl. a (1.70 mg/g), chl. b (0.81 mg/g) and carotenoids (0.78 mg/g) of groundnut crop, as compared to UIC (Table 3).

Table 3 Effect of different fly-ash concentrations on *M. arenaria* in relation to physiological attributes of groundnut crop

Treatments	Physiological attributes		
	Chlorophyll 'a' (mg/g FW)	Chlorophyll 'b' (mg/g FW)	Carotenoids (mg/g FW)
UUC	1.85 ^a	0.90 ^a	0.84 ^a
UIC	0.80 ^f	0.42 ^e	0.40 ^f
10% FA + N	0.95 ^e	0.56 ^d	0.50 ^e
20% FA + N	1.40 ^c	0.67 ^c	0.64 ^c
30% FA + N	1.70 ^b	0.81 ^b	0.78 ^b
40% FA + N	1.48 ^c	0.70 ^c	0.68 ^c
50% FA + N	1.20 ^d	0.59 ^d	0.56 ^d

Each value is the mean of five replicates. Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. UUC – Untreated uninoculated control; UIC – Untreated inoculated control; FA – Fly-ash; N – Nematodes (*M. arenaria*); FW – Fresh weight

Table 4 Effect of different concentrations of fly-ash on root-knot disease caused by *M. arenaria* in groundnut crop

Treatments	Pathological attributes		
	Number of egg masses/root system	Nematode population/250g of soil	Root-knot index (RKI)
UUC	0.00	0.00	0.00
UIC	109.34 ^a	1911.22 ^a	5.79 ^a
10% FA + N	70.43 ^b	1121.31 ^b	2.90 ^b
20% FA + N	26.11 ^c	840.22 ^c	1.77 ^c
30% FA + N	1.12 ^d	101.11 ^d	1.01 ^d
40% FA + N	0.00	0.00	0.00
50% FA + N	0.00	0.00	0.00

Each value is the mean of five replicates. Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. UUC – Untreated uninoculated control; UIC – Untreated inoculated control; FA – Fly-ash; RKI – Root-knot index; N – Nematodes (*M. arenaria*)

Effects of FA on disease performance of *M. arenaria*

The results given in (Table 4) demonstrated that the untreated and inoculated plants had the highest disease infestation in terms of number of egg mass, nematode population and root-knot index. However, the FA levels from 10 to 50% significantly decreased the disease infestation (number of egg masses, nematode population and root-knot index) in compared to UIC. Moreover, the minimum reduction in disease was found at 10% FA treated plants while complete suppression of disease was found at 40% and 50% FA concentrations as compared to UIC. Our results showed that the disease on groundnut plants significantly decreased with increasing FA concentrations.

Soil amendment with FA is an emerging area of research in agriculture as it has been utilized as fertilizer to enhance development and productivity of several crops [8, 10]. Rich in important nutrients like K, Mg, and S [37]. The high proportion of inorganic components in FA and the increased quantity of soluble micro and macro-nutrients as well as metals released with ash to soil may be the cause of the increase in the aforementioned physical characteristics in EC [38]. Soil application of FA affects the soil properties by increasing WHC because it contains salts and trace elements [39]. In compare to non-amended soil, the FA amendment to soil enhances water content in the soil by 14-33% and its hollow silt-sized particles enable the soil to retain water for long time [40].

Soil with 30% FA rich in nutrients such as K, P, N, Mg, Mn and Zn compared to 50% FA. Tripathy and Sahu [41] and Brahmchari *et al.* [42] also found that 30% FA possess more nutrients that beneficial for agricultural crops. Bradshaw and Chadwick [43] found that FA possesses low level of N because of a volatilization of the N present in coal during combustion.

Previous studies also suggest that K and P in the soil were increased with increasing FA and Mn, Mg and Zn were also increased because of FA contains an adequate amount of these nutrients. Similarly, cereals, pulses, oilseeds and vegetables showed a comparable positive impact when treating with FA [44-47]. However, distinct crop reactions differed according to the various FA levels i.e., 10–50%. For example, the better growth of ladyfinger, groundnut and radish was obtained at 15% [48], chickpea at 40% [49], *Vigna mungo* and okra at 25% and 50% [50], *Coccinia cordifolia* at 30% [51], mustard at 40% [52] and carrot at 20% [10] and eggplant at 20% [53].

In vitro study demonstrated that all the concentrations of FA-extract possess nematicidal potential for egg-hatching inhibition and mortality of J2s of *Meloidogyne arenaria*. Although, 50% FA extract was found most effective for egg-hatching inhibition and J2s mortality (Table 1). This might be due to the presence of toxic compounds such as S, dibenzo-p-dioxine, dibenzofuron and chlorides [54]. However, the nematicidal action of FA-extract was found to be directly proportional to the extract concentrations, higher the concentration, the greater the nematicidal action and vice versa. These results are comparable to those of Ahmad *et al.* [16], who similarly obtained a significant decrease in *M. incognita* egg hatching and J2s mortality following *in vitro* application of FA extracts. Bhat *et al.* [55] found that J2s mortality and egg hatching of root-knot nematodes can be raised by modification in soil EC. Moreover, FA as nematicides due to the presence of heavy metals toxic to PPNs [56-57]. These findings are also in accordance with Forghani and Hajihassani [58] and Khan and Siddiqui [9].

Pot study revealed that the growth and yield of groundnut plants were increased up to 30% FA level, after that there was

a gradual reduction in both yield and growth of plants. The increased groundnut crop development and production seen in soil amended with 30% FA that lead to large soil pores which facilitate root penetration and increased soil nutrient uptake [59]. The negative effects of FA have been linked to greater concentrations of micronutrients and the toxicity of substances such as dibenzofuran, and dibenzo-p-dioxin [54], [60]. Similar studies were also performed on chickpea, *Brassica juncea*, cucumber, lentil, *Linum usitatissimum*, wheat maize, potato, soybean and tomato suggest that FA at high level 50% were toxic to all the tested crops [61-62]. FA contains minerals like B, Se and Mo are harmful to plants while Mg, Ca, Fe and K essential for plant growth. It has also been employed as an adsorbent to lower the P that is soluble in the soil solution [63]. The crop productivity increased up to 11.6–29.2% at the low level of FA (i.e., < 25% of soil FA) and decreased up to 45.8% at the high level of FA (i.e., 50–100% FA) because of metal toxicity [64-66].

Experimental findings suggest that FA 10–30% levels, there were significant ($P \leq 0.05$) increases in growth and yield as compared to untreated and nematode inoculated plants. Similarly beneficial effect of FA was also observed in vegetables [47], cereals [44], pulses and oilseeds [45-47]. However, the responses of various crops were found to be different at different levels of FA i.e., 10–50%. For instance, ladyfinger, radish and groundnut found to grow and yield better at 15% FA level [48]; okra and *Vigna mungo* at 25% and 50% [50], eggplant at 20% [53], *Coccinia cordifolia* at 30% [51], mustard at 30% [67], chickpea at 40% [49], and carrot at 20% [10]. While FA at 40 and 50% levels, affect growth and yield parameters negatively. Similar results were also found in case of chickpea, *Brassica juncea*, cucumber, lentil, *Linum usitatissimum*, maize, soybean, potato, tomato, and wheat cultivated in high level of FA [61-62], [68].

Data given in (Table 2) showed that the highest improvement in growth and yield of groundnut plants infected with *M. arenaria* was observed at 30% FA. The improvement in plant growth and yield as well as in soil qualities like WHC, EC and pH is due to the increase in soil nutrient status with essential nutrients like Al, Fe, Cu, Mg, K and Ca etc., [10]. The pH of the soil is one of the important factors that aids plants obtaining the nutrients they need from the soil [69]. The findings of this investigation are also similar with those of Jezek *et al.* [70] and Thor [71], who found that Ca plays a vital role in plant development through its involvement in numerous physiological processes, including metabolism and secondary messenger functions. Hence, several nutrients in FA viz., Fe, Mg, K, Mn and Cu improve growth and yield performance of groundnut crop.

Similarly, the reduction in chlorophyll content of leaves was the primary cause of decline in photosynthetic rate at high FA levels. The study revealed that significant improvement in

photosynthetic pigment like chlorophyll a, b and carotenoids was obtained at the 30% FA level inoculated with *Meloidogyne arenaria* (Table 3). Similar findings were also obtained that the beneficial effect of FA at low levels (10–30%) on several crops like tomato, wheat, chickpea, potato and pumpkin [62], [72-73]. Shakeel *et al.* [67] also found that different FA concentrations influenced the photosynthetic pigments of *Brassica juncea* and the highest was found at 30% FA level. More significant improvement in photosynthetic pigments such as chlorophyll and carotenoids was found at 30% while more reduction at 50% FA amended soil. These findings are similar with those of Mishra and Shukla [68] found that the high levels of FA reduced the amount of photosynthetic pigments in soybean and corn crops.

Pot study also suggests that the different FA concentrations from 10 to 50% exhibit the nematicidal potential against the *Meloidogyne arenaria* in terms of different disease parameters (Table 4). In this study, FA amended to soil at FA 10 to 50% resulted in reducing nematode disease in terms of number of egg masses/root system, nematode population/250 g of soil and root-knot index (RKI) caused by *Meloidogyne arenaria*. However, the complete reduction of disease was obtained at 40% and FA 50% concentration. Our results conformed to those of Ahmad *et al.* [8] and Haris *et al.* [10].

CONCLUSION

The current study examined the effect of various fly ash (FA) concentrations amended in soil to determine the performance of groundnut crops inoculated with *Meloidogyne arenaria*. Study revealed that 30% FA concentration was optimal and effective. The parameters like growth, yield and physiology of groundnut crop were improved at 30% FA concentration. On the other hand, gradual reduction in root-knot disease in terms of egg masses, nematode population and root-knot index were also seen when FA concentrations increased from 10 to 50% on the groundnut plants. Also, inhibition in egg hatching and J2s mortality was shown when FA concentration increases from 40 to 50%. Thus, the utilization of FA as a nematocide-cum-fertilizer is an effective approach toward the sustainable and eco-friendly use of FA in agro ecosystems. Hence, this study suggests that 30% FA level to the soil is the most effective for managing *Meloidogyne arenaria* on groundnut plants.

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