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Original Research Article

Utilization of Fly Ash to Improve the Growth and the Management of Root-Knot Nematode on Carrot

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Abstract

Excessive use of chemical fertilizers in agriculture causes degradation of soil and vegetation health. The consistent use of such agrochemical products shows harmful impacts on the environment. Therefore, urgent needs an eco-friendly substance which replace the chemical fertilizers. Thus, in the present study, the main objective was to evaluate the effect of fly ash to improve the growth and the management of root-knot nematode *(Meloidogyne incognita)* of carrot (*Daucus carota* L.). A greenhouse experiment was conducted to observe the effect of different levels of fly ash concentrations (10%, 20%, 30%, 40% and 50% w/w) with normal agriculture soil on plant growth, photosynthetic pigments and against to disease intensity of nematode. Plant growth parameters and photosynthetic pigments were increased significantly from 10% to 30% fly ash levels as compared to control (0% fly ash). Maximum growth and photosynthetic pigments were found at 30% level of fly ash. But at higher levels of fly ash (40% and 50%), plant growth was reduced significantly. However, in the experiment of nematode management the number of galls, egg masses and eggs/egg mass gradually suppressed as the levels of fly ash increased from 10% to 50% and promote the growth of plant at 30% concentration of fly ash as compared to untreated inoculated control (0% fly ash with nematode).

Keywords: Carrot, fly ash, growth, nematode, photosynthetic pigments.

INTRODUCTION

On a global scale, coal fly ash is a solid waste which produces several problems after generating in large amounts from power plants based on coal-fired [1, 2]. The coal fly ash is widely available and is the most economical waste material which is responsible for the recovery of deteriorated soils [3]. The number of fly ash dump sites and the continuous increase in the area are a cause of concern in the whole world because of their possible toxic effects; heavy metals are released in nature [4]. The total quantity of waste generated around 15-30% during the combustion of coal is fly ash, also known as "pulverized fuel ash". In fly ash, some of the beneficial applications are evaluated to reduce the waste, minimize the cost of ejection and provide many value-added products for humanity. The generation rate of fly ash in present is approximately 131.09 million tons per year and usage rate is about 73.13 million tons every year [5]. Now, fly ash generation in India is the expectation to be 300-400 million tons per annum by 2016-2017 [6]. In India, during from 1996-1997 to 2010-2011, the unmapped fly ash's backlog thrown in fly ash pond is approximately 922.95 million tons, the total amount of fly ash [7]. These unaccustomed fly ash will impose an adversely impact on natural quality of the environment and ecosystem. The results of many studies suggest the tremendous potential to improve farming, degraded/wasteland and mining-affected soil by the use of fly ash for forestry and agricultural [8]. Due to its useful physical-chemical properties, with appropriate amount of macro-nutrients (P, S, Ca, K, and Mg) and micro-nutrients (Zn, Cu, Fe, and Mn), the use of coal fly ash has been advocated for the last three years [9-13].

Root-knot nematodes (RKNs) are microscopic and plant-parasitic roundworms found in field crops and horticultural also. RKN (*Meloidogyne* spp.) occur all over the world. RKN (*Meloidogyne* spp.) are most commonly seen and harmful plant-parasitic nematodes in the production of vegetable [14]. First-stage of the juveniles matures within the eggs and after the hatching juveniles of the second-stage from the eggs infect the roots of the various host plants [15]. RKNs especially harmful to vegetables in the tropical and sub-tropical countries of the world, and cause till 80% damage in the heavily infected areas [16]. RKNs are globally grave pathogens in carrot crop [17-20]. In 15th and 16th

decades, the carrot was widely grown in Europe and was moved to North America for the first time during the same period. In industry and market, carrot plays a beneficial role for both processing and freshness. After potatoes, carrots are the most important vegetables. Carrots are eaten in the different variety of ways.

MATERIALS AND METHODS

Collection of fly ash

Fly ash was assembled in gunny bags from the fly ash ponds of Thermal Power Plant, which are located in Kasimpur 20 Km away from Aligarh. Fly ash was brought to the Botany Department, A.M.U., Aligarh for the experiments.

Chemical properties of soil and fly ash

The chemical properties of soil and fly ash were analyzed before showing by the following method. For the plant growth concentration of hydrogen in soil solution was estimated use of pH meter, which calibrated by the standard buffer of known pH (4.0, 7.0 and 9.2) on the basis of [21] method. Nitrogen content in soil and fly ash was determined by the micro Kjeldahl method, as given by [21]. Analysis of Phosphorus from the samples extract was done by the use of calorimetrically after the digestion of perchloric acid (HClO4) with the help of method given by [22]. Estimation of Potassium and Sodium from soil and fly ash samples was done through the flame photometer. Calcium content in the soil and fly ash was analyzed by EDTA and atomic absorption methods. Magnesium was measured by the method of [23]. The soluble sulphate from the soil was determined by the method of [24]. Chloride content in soil and fly ash was done with the help of [21] method. Total carbonate and bicarbonates were determined by the common procedure of [22].

Table-1: Chemical characteristic of soil and fly ash

Characteristics Texture	Soil Sandy loam	Fly ash
pH	$7.6^{b} \pm 0.19$	$8.2^{c} \pm 0.14$
Nitrogen (g kg^{-1} soil)	$0.259^{b} \pm 0.014$	$0.061^{c} \pm 0.004$
Phosphorous (g kg^{-1} soil)	$0.139^{b} \pm 0.009$	$0.03^{c} \pm 0.013$
Potassium (mg l^{-1})	$19.81^{b} \pm 1.03$	$14.96^{c} \pm 0.51$
Magnesium (mg l^{-1})	$30.92^{b} \pm 0.99$	$17.88^{c} \pm 0.52$
Calcium (mg l^{-1})	$17.67^{b} \pm 0.91$	$19.31^{b} \pm 0.42$
Sodium (mg l^{-1})	$10.7^{c} \pm 0.39$	$15.91^{b} \pm 0.54$
Carbonate (mg l^{-1})	$76.81^{b} \pm 0.92$	$68.73^{c} \pm 0.99$
Bicarbonate (mg l^{-1})	$17.85^{b} \pm 0.37$	$12.68^{c} \pm 0.44$
Sulphate (mg l^{-1})	$15.79^{c} \pm 0.38$	$24.57^{b} \pm 1.2$
Chloride (mg l^{-1})	$24.96^{b} \pm 1.18$	$17.5^{c} \pm 0.31$

All determinations in mg l^{-1}, except pH or as specified
Each value is a mean of three replicates ± standard deviation (SD)
Different letters indicate significant between treatments

Collection of field soil

For experimental work, agriculture field was the source of collection of soil after removing the debris. The sandy loam soil was containing 2% organic matter, 8% clay, 24% silt, 66% sand and pH 7.7. Before utilization, agriculture field soil was collected in gunny bags and autoclaved at 20 lb pressure for 20 min. After drying, the soil was mixed with fly ash to obtain different levels 10-50%. The clay pots of 6 inches height (15cm) were filled with 1 kg of each type of mixture.

For the present study, fly ash as one of the major particulate air pollutants, *M. incognita* as a test pathogen and carrot *(Daucus carota)* var. Lali as test plant were selected and procured from Chola Beej Bhandar (Agro company), Aligarh (UP) India.
The treatments were given as below:

T1 = Control (only autoclaved soil)
T2 = 10% fly ash
T3 = 20% fly ash
T4 = 30% fly ash
T5 = 40% fly ash
T6 = 50% fly ash

Inoculum preparation

For the collection of root samples from plants infected with root-knot nematodes, several fields were visited in and around Aligarh. Samples of infected roots were collected in the bag and very carefully brought to the laboratory. The population was established originally by roots infected with root-knot nematode collected in the fields of tomatoes and eggplant and identified with the help of perineal patterns [26]. For the preparation of inoculation, egg masses were picked from the roots by the use of sterilized forceps. Inoculums were prepared by incubating egg masses in distilled water at 27 ± 2 °C. The freshly hatched second stage juveniles (J2) were collected as water suspension and number of the J2 per ml were standardized by counting the number of J2 in ten 1ml samples from the suspension. At four leaves stages of carrot inoculated with freshly hatched juveniles of the nematode which were designated to receive *M. incognita* (2000 J_2/pot). The treatments were given as below:

T1 = Control (only autoclaved soil)
T2 = Autoclaved soil + J_2 (Second stage juvenile)
T3 = 10% fly ash + J_2
T4 = 20% fly ash + J_2
T5 = 30% fly ash + J_2
T6 = 40% fly ash + J_2
T7 = 50% fly ash + J_2

After proper mixing of soil with fly ash, 1 kg of each fly ash mixture was filled in 15cm clay pots. Total 60 pots (12 treatments x 5 replicates) were

prepared for the experiments. Control was same of both experiments. Plants were harvested at 3 months after inoculation; number of galls was counted by the following scale which was proposed by [27].

Number of galls	Galling index
0	0
1-2	1
3-10	2
11-30	3
31-100	4
>100	5

Photosynthetic Pigments

The chlorophyll pigments were estimated by the method of [28]. 1g fresh leaves were collected before one week of harvesting, for the observation of photosynthetic pigments in the leaf tissue. Photosynthetic pigments were determined by grinding with the help of pestle and mortar in 20 ml acetone (80%). The suspension was filtered through Whatman filter paper (No.1) in the flask. The filtrate was used to record the percent transmittance in spectrophotometer, optical density (O.D.) at 645nm and 663nm for chlorophyll and 480nm and 510nm for carotenoid content. The chlorophyll content was calculated with the help of following formulae.

i) Total Chl. = $20.2\ (O.D._{645}) + 8.02\ (O.D._{663}) \times (V/1000 \times W)$

ii) Carotenoid = $7.6\ (O.D._{480}) - 1.49\ (O.D._{510}) \times (V/1000 \times W)$

Where, V = Final volume of chlorophyll extract in 80% acetone (ml)

W = Fresh weight of leaf tissue (g)

O. D. = Optical density at given wave length viz. 645 nm, 663 nm, 480 nm and 510 nm

Statistical analysis

Data of experiment were analyzed statistically with the help of SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Mean values were compared statistically according to the Duncan Multiple Range Test at $P \leq 0.05$. In the tables standard deviation of each treatment was calculated and each variables graph was graphed with the help of Sigma plot.

RESULTS AND DISCUSSION

Fly ash has a great potentiality in agriculture due to its efficiency to modify soil health and crop performance. The nutrients of fly ash are said to be beneficial for plants through soil application [29]. The results observed from this work show that 30% Fly ash level improves both plant growth as well as photosynthetic pigments in carrot. This improvement is because of the presence of some essential plant nutrients like Ca, Mg, K and Fe in fly ash [30, 31]. In addition to nutrient enrichment, it also improves the pH of soil and makes it more favorable for plant growth [32]. The amino acid content in soybean *(Glycine max)* has been reported to increase when grown in fly ash amended soils [33]. The plant growth parameters (Length of shoot, length of root, fresh weight of shoot, fresh weight of root, dry weight of shoot and dry weight of root) significantly increased than the control with increasing the level of fly ash up to 30% levels. However, the highest plant growth was observed at 30% level of fly ash. After that all growth parameters were gradually decreased in 40% and 50% levels of fly ash (Table 2).

Table-2: Effect of different levels of fly ash on plant growth of carrot cv. Lali

Treatment	Plant length(cm)		Fresh weight(gm)		Dry weight (gm)	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	20.15±2.01bc	11.00±1.11cd	04.67±0.48c	26.00±2.61ab	1.16±0.12c	06.50±0.66ab
FA 10%	20.92±2.01bc	12.04±1.21bc	04.98±0.51c	26.89±2.69ab	1.24±0.13c	06.72±0.68ab
FA 20%	23.06±2.31ab	13.23±1.33ab	06.54±0.66b	28.31±2.84ab	1.63±0.17b	07.07±0.71ab
FA 30%	25.52±2.51a	15.14±1.52a	08.01±0.80a	30.44±3.05a	1.90±0.20a	07.61±0.77a
FA 40%	17.58±1.71cd	9.78d±0.98e	04.36±0.44c	24.21±2.42bc	1.09±0.11c	06.05±0.61bc
FA 50%	16.24±1.61d	08.18±0.82e	03.31±0.34d	20.35±2.04c	0.83±0.09d	05.08±0.51c

FA- Fly ash

Each value is the mean of five replicates.

SD- Standard deviation. Means in each column with different letters denote significant difference according to Duncan's Multiple Range Test at $P \leq 0.05$

The adverse effects of fly ash have been observed at higher levels, as it contains some toxic heavy metals. [34] found that higher levels of fly ash showed reduced growth of nodulation, chlorophyll, carotenoids, proteins, nitrate reductase activity and accumulation of elements Fe, Ze, Cu and Mn in the plants were in large amounts. Result given in table-3, show that plant growth parameters were increased at all the nematode and fly ash mixed soil combinations as compared to UIC (Untreated inoculated control), maximum being at 30%. However, all growth parameters were decreased at 40% and 50% fly ash amended soil inoculated with nematode as compared to UUC (Untreated Uninoculated control). In addition, the use of fly ash can enhance the crop yields and also improve food security where the conditions of soil are subject to inherent structural and limited nutrition [35]. Fly ash applied to the soil in higher proportions (75%

and 100%) was considered harmful for plant growth and tomato yield [36]. In the present study, similar results were obtained that low levels were beneficial, while high levels were harmful to carrot.

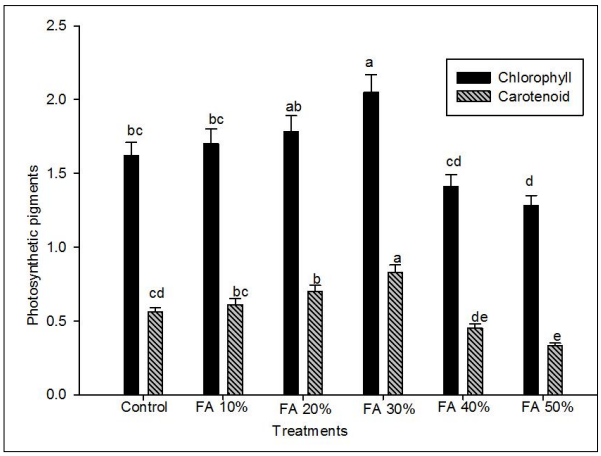
Table-3: Combined effect of fly ash and *Meloidogyne incognita* on plant growth of carrot cv. Lali

Treatment	Plant length(cm)		Fresh weight(gm)		Dry weight (gm)	
	Shoot	Root	Shoot	Root	Shoot	Root
FA 10%+N	18.91±1.81bc	09.46±0.95bc	04.50±0.46ab	25.81±2.51ab	1.12±0.11ab	06.45±0.65ab
FA 20%+N	19.74±1.91ab	10.37±1.01b	04.58±0.46ab	25.89±2.51ab	1.14±0.11ab	06.47±0.65ab
FA 30%+N	22.46±2.21a	13.12±1.31a	05.26±0.53a	27.67±2.71a	1.31±0.13a	06.91±0.70a
FA 40%+N	17.31±1.71bc	08.06±0.81c	04.42±0.45b	23.53±2.31abc	1.10±0.11b	05.9±0.60abc
FA 50%+N	16.11±1.61cd	07.97±0.80c	03.29±0.32c	22.18±2.21bc	0.82±0.08c	05.54±0.56bc
UIC	13.15±1.31d	05.48±0.91d	2.51±0.26d	19.11±1.91c	0.62±0.06d	04.82±0.49c
UUC	20.15±2.01ab	11.00±1.11b	4.67±0.47ab	26.00±2.61ab	1.17±0.11ab	06.50±0.66ab

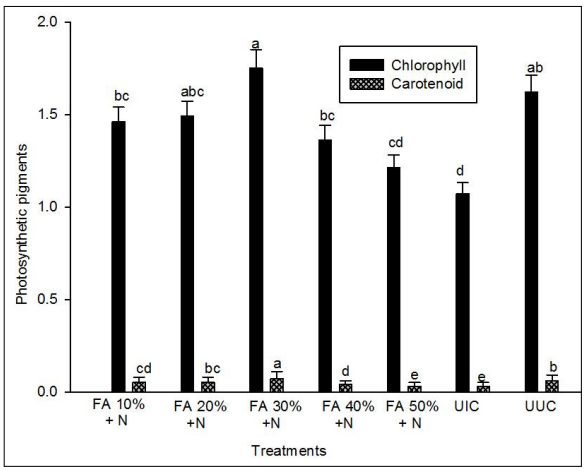
FA- Fly ash, N- Nematode *(M.incognita)*

Each value is the mean of five replicates. Means in each column with different letters denote significant difference according to Duncan's Multiple Range Test at $P \leq 0.05$

The photosynthetic pigments (chlorophyll and carotenoid) were significantly increased at 10%, 20% and 30% levels of fly ash as compared to control. However, there was a gradual declining both pigments at 40% and 50% levels of fly ash (Graph 1). Maximum photosynthetic pigments were shown at 30% level of fly ash. Data presented in graph 2, show that photosynthetic pigments were increased in 10% to 50% fly ash levels with nematode combinations as compared to UIC (Untreated inoculated control). Maximum Photosynthetic pigments were shown at 30% level of fly ash. If compare with UUC (untreated uninoculated control) 10%, 20%, 40% and 50% levels of fly ash decreased the photosynthetic pigments.



Graph-1: Effect of fly ash on chlorophyll and carotenoid content in the leaf of carrot. Error bars indicate standard errors. Different letters show significant difference on the basis of Duncan's Multiple Range Test at $P \leq 0.05$



Graph 2: Effect of fly ash and *M. incognita* on chlorophyll and carotenoid content in the leaf of carrot. Error bars indicate standard errors. Different letters show significant difference on the basis of Duncan's Multiple Range Test at $P \leq 0.05$

In addition to the improvement in plant growth, this work also reports the nematicidal effects of fly ash (Table 4). As fly ash contains various toxic elements, it becomes detrimental for root-knot nematodes to survive thus results in their death. The substantial decrease in the egg masses and galling shows that the fly ash has caused a direct inhibitory response on the survival and multiplication of *M. incognita*. [37] argued that the application of fly ash can be detrimental to soil microbes. Similarly, [38] observed that the soil amendment with different levels of fly ash inhibits the penetration of the *M. incognita* juveniles in the roots of pumpkin. Root-knot disease in terms of root gall index, no. of egg masses and eggs/egg mass was highest in UIC (Untreated inoculated control). In fly ash + nematode combinations, galls were formed but less than inoculated control which gradually decreased to 10%, 20% and 30% levels of fly ash. However, galls were completely absent at 50% level of fly ash (Table 4).

Table-4: Effect of fly ash against *Meloidogyne incognita* on carrot cv. Lali in relation to nematode infestation parameters

Treatment	No. of egg masses/root	Eggs/egg mass	Galling index
FA 10%+N	19.34±1.91b	261.09±27.15b	3.4±0.31b
FA 20%+N	10.31±1.01c	189.89±19.97c	3.0±0.31b
FA 30%+N	02.12±0.46d	140.06±15.1d	2.0±0.21c
FA 40%+N	01.29±0.13d	73.73±8.47e	1.4±0.15d
FA 50%+N	0±0.00	0±0.00	0±0.00
UIC	27.08±2.71a	314.83±32.90a	4.0±0.41a
UUC	0±0.00	0±0.00	0±0.00

FA- Fly ash, N- Nematode (*M. incognita*)

UIC-Untreated inoculated control. UUC-Untreated uninoculated control

Each value is the mean of five replicates. Means in each column with different letters denote significant differences according to Duncan's Multiple Range Test at $P \leq 0.05$. Data with same letters do not differ significantly.

COUNCLUSION

From the results it has been concluded that fly ash improve properties of soil due to the presence of plant nutrients. Fly ash is a kind of waste which can be used eco-friendly in agriculture. Therefore, fly ash improves growth, yield and photosynthetic pigments in carrot. Fly ash also has the nematicidal activity for the management of root-knot nematode. So, it is the better option to replace chemical fertilizers.

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