



Research article

Interaction of a fly ash and root-knot nematode pathogens on Pumpkin (*Cucurbita moschata* Duch. ex Lam.)

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Abstract: In the present investigation it was tried to find out the interactive effect of various level of fly ash (abiotic) and root-knot nematode, *Meloidogyne incognita* (biotic) on plant growth, yield and some biochemical contents in pumpkin (*Cucurbita moschata*). The experiments were conducted in pots with different level of fly ash and soil mixture in the green house at Department of Botany, Aligarh Muslim University, Aligarh, India. Seeds of pumpkin (F1 hybrid-Nutan) were grown in pots filled with different level of fly ash (5, 10, 20, 30, 40 and 50%). At four leaves stage, seedlings were inoculated with 2000 larvae (J2 stage) of *M. incognita*. Plants grown in soil (uninoculated and inoculated sets) serve as control for comparing with different level of fly ash amended soil. Plant growth and yield parameters were increased significantly in 10 to 30% fly ash amended soils. However, at higher level of fly ash (40% and 50%), plant growth and yield were reduced significantly. The suppression in plant growth and yield were maximum at 50% fly ash level. Similarly the chlorophyll, carotenoids, carbohydrate and proline contents were also increased at 10–30% levels, then increased in 40 and 50% of fly ash level compared to control (uninoculated set). All the parameters; growth, yield, chlorophyll, carotenoids, carbohydrate and proline content of plants were better for all the fly ash level as compared to nematode inoculated sets.

Keywords: Carotenoids - Carbohydrate - Chlorophyll - Fly ash - Nematodes - Pumpkin.

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INTRODUCTION

Fly ash (FA) is the waste product of coal combustion process for generation of electricity and is accepted as an environmental pollutant. Fly ash contains fine, powder-like particles that are spherical in shape, solid or hollow, and mostly amorphous in nature. Depending on the quantity of unburned carbon in fly ash the color may vary by gray to black. The surface area of FA may vary from 170 to 1000 m².kg⁻¹ while the specific gravity ranges by 2.1 to 3.0. Basically four types of coal, each of which varies in terms of its chemical composition, ash content, value of heating and geological origin and are anthracite, bituminous, sub-bituminous, and lignite. Alumina, Silica, Iron oxide and Calcium, with variable amounts of carbon are the components of bituminous fly ash. Higher concentrations of calcium and magnesium oxide and low percentages of iron oxide and silica and carbon contents are characters of lignite and sub-bituminous coal fly ashes.

Due to fly ash application, there is an increase in the availability of major nutrients in the soil was reported by Ram *et al.* (2011). Except K, the increase in the availability major nutrients concluded by Dey *et al.* (2012). There is an increase in organic carbon by the application of fly ash and farm-yard manure was reported by Karmakar *et al.* (2009). As an amendment, fly ash is used in agriculture especially due to the presence of macro and micro-nutrients (Wong & Wong 1986, Raghav & Khan 2002, Rizvi & Khan 2009). Fly ash has been found beneficial for the growth of many plants (Mishra & Shukla 1986, Singh 1989, Pasha *et al.* 1990, Khan & Khan 1996, Raghav & Khan 2002, Rizvi & Khan 2009).

Root-knot nematodes (*Meloidogyne* spp.) are microscopic roundworms found in a broad range of habitats and agro-ecosystems. Almost they complete their life cycle in the roots of the host plants; in spite of they can

survive in the soil as eggs or as second-stage juveniles. The juveniles of first-stage develop within the eggs while juveniles of second-stage hatch from the eggs and infect the roots of the various host plants. Giant cells develop where they obtain nutrients from one permanent site in the root, called *sedentary endoparasitism*. For the development of root-knot nematodes, the optimum soil temperature should be 25–28 °C. The test plant was pumpkin is commonly applied to any plant in the taxonomically diverse Cucurbita genus and colour of fruit varies yellow to orange. Pumpkin cultivars may belong to one of the several species: *Cucurbita pepo* L., *C. maxima* Duch., *C. moschata* Duch. and *C. mixta*. Cv. This species, *C. moschata* has been considered to have many functions for human health as antimicrobial and to preserve kidney function (Caili *et al.* 2006, El-Aziz & El-Kalek 2011). Because of the high content of carbohydrates and fibre, this vegetable plant has been performed as a food with an esteemed source of dietary fiber in human nutrition (Hussain *et al.* 2010) may decrease the serum cholesterol level, the risk of coronary heart disease, and hypertension. Apart from, the seeds of *Cucurbita moschata* have high amount of zinc, used in treatment of the early stages of prostate problem (Pandya & Rao 2010). Fly ash has shown its inhibitory effect on root-knot nematodes (Tarannum *et al.* 2001, Raghav & Khan 2002, Rizvi 2008, Rizvi & Khan 2009) however their combined effects on cucurbits so far.

MATERIAL AND METHODS

The experiments were conducted in glass houses at the Department of Botany, Aligarh Muslim University, Aligarh. The fresh fly ash was collected from Thermal Power Plant, Kasimpur for the experiment. The test pathogen, *Meloidogyne incognita* Chitwood was maintained in pure form. Pumpkin (*Cucurbita moschata*) var. NUTAN (F1 hybrid) was selected as a test plant for the experiments. Inoculum was prepared by incubating the egg masses of pure *M. incognita* in distilled water. The freshly hatched second stage (J2) juveniles were collected as water suspension and the number of J2 counted in ten 1ml samples from the suspension. The average numbers of J2 were used to represent the number of second juveniles (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.

For this experiment, fly ash was mixed with autoclaved soil in different proportion to prepare 10, 20, 30, 40 and 50% levels. The clay pots of 12 inches height (20 cm diam.) were filled with 2 kg of each type of mixture. Ten days old plants were inoculated with 2,000 juveniles. The treatments were given as below,

- T1 = Control
- T2 = Nematode (2000 J2 of *Meloidogyne incognita*)
- T3 = 10% Fly ash + N
- T4 = 20% Fly ash + N
- T5 = 30% Fly ash + N
- T6 = 40% Fly ash + N
- T7 = 50% Fly ash + N

Fly ash effect on development of the nematode juvenile

Fly ash and autoclaved soil were mixed as above proportion. Pots containing only autoclaved soil served as controls. A total of 140 pots (7 treatments × 4 weekly observations × 5 replicates) were prepared. A 15-day-old seedling of pumpkin at the four leaves stage was kept on a glasshouse bench at 25–27 °C and each seedling was inoculated with 2000 juveniles of *M. incognita*. Five seedlings were harvested from each treatment at different intervals (1st, 2nd, 3rd and 4th week) and roots were cut and gently wash under tap water. After cutting, the roots were taken into test tube with distilled water and boil under spirit lamp for 2 minutes. After boiling 2–3 drops of acid fuschin add in each tube for staining and then roots were kept on slide. After this, different developmental stages of the nematode were counted under a stereoscopic microscope.

Plant growth and yield

The plant growth (length, fresh weight and dry weight of root and shoot/plant) and yield (flower/plant; fruit/plant) were taken after termination of experiments. Shoot length was taken from the point of emergence of the root to the shoot apex. While root length was recorded from root emergence to longest root and both were recorded in centimeter (cm). Fresh weight of roots and shoots were recorded in gram (g). After taking fresh weight, roots and shoots were dried in a hot air oven at 80°C for 48 hrs and their dry weights were recorded.

Photosynthetic pigments

Photosynthetic pigments were estimated by Maclachlan & Zalik (1963) method. After 120 days of planting, www.tropicalplantresearch.com

one g fresh leave was ground in 80 % acetone with the help of mortar and pestle. The suspension was filtered through the Whatman filter paper No. 1 to the 100 ml volumetric flask and made to the known volume by adding 80% acetone. 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 extract were calculated according to the formulae given below,

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

Carbohydrate content

Carbohydrates are dehydrated with concentrated H_2SO_4 to form “Furfural”, which condenses with anthrone to form a green color complex which can be measured by using colorimetrically at 620 nm (or) by using a red filter. Anthrone reacts with dextrans, monosaccharides, disaccharides, polysaccharides, starch, gums and glycosides. But they yields of color where is to form carbohydrate. *Anthrone reagent*: Dissolve 200 mg of anthrone reagent in 100ml of concentrated H_2SO_4 .

To take 0.2 to 1ml of working standard solution of five different test tube and add water to bring the volume to 1ml in each test tube add 4ml of anthrone reagent and mix the contents as well and cover the test tube with bath for 10 min then cool the test tube to the room temperature and measure the optical density in a photoelectric colorimeter at 620 nm (or) by using a red filter. Simultaneously prepare a blank with 1ml of distilled water and 4 ml of anthrone reagent. Construct a calibration curve on a graph paper, by plotting the glucose concentration (10 to 100 mg) on x-axis and absorbance at 620 nm on the y-axis. Compute the concentration of the sugar in the sample from the calibration curve. While calculating the sugar concentration in the unknown sample, the dilution factor has to be taken into account. For the calculation of total carbohydrate content following formula are required-

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \text{mg of glucose/Volume sample} \times 100$$

Proline estimation

Proline is very soluble and can be readily extracted by heating explants or aliquots of ground plant material for 20 min in pure ethanol as well as in water. Proline can also be extracted together with total amino acids, pigments, soluble sugars by heating plant material twice with 80% ethanol and once with 50% ethanol as described by Cross *et al.* (2006), which results into a 70:30 ethanol: water mixture (v/v). Proline and total amino acids may also be extracted using a cold extraction procedure by mixing 20–50 mg fresh weight aliquots with 0.4–1.0 ml of ethanol : water (40:60 v/v). The resulting mixture is left overnight a 4°C , and then centrifuged at 14000 g (5 min). The cold extraction procedure can be repeated on the pellet and supernatants pooled and used for the analyses. The first extraction, however, already allows a recovery > 93% (Carillo *et al.* 2008).

Calculation

$$\mu \text{ moles per g tissue} = \mu\text{g proline/ml} \times \text{ml toluene}/115.5 \times 5/\text{g sample}$$

Where, 115.5 is the molecular weight of proline

RESULTS

The development of juveniles of *meloidogyne incognita* in the roots of pumpkin shows in table 1 was also significantly suppressed by all fly ash and soil mixtures. The J2 developed to J3 /J4 stages at all levels of fly ash amendment, but their number were less than in the control and decreased with the increase of the fly ash up to 40% soil mixture. At the end of the first week, neither premature nor mature females were found. During the second week, J2 developed to older stages. However, while pre-mature females occurred in all roots only a few mature females occurred in the control and at the 5–10% levels of fly ash and none at larger proportions of the amendment. During the third week, the juveniles that had penetrated into the roots developed further. However, numbers of pre-mature females were significantly suppressed by all proportions of fly ash while mature females were significantly suppressed at 5–10% of this amendment and were still absent at larger proportions. After 4 weeks, all the J3 /J4 had developed further but pre-mature females were still significantly less than in the control

at all proportions of the amendment and a few mature females were observed only up to 20% of the amendment, with none at all at greater proportions.

Table 1. Effect of different levels of fly ash on developmental stages of *Meloidogyne incognita* in roots of pumpkin (*Cucurbita moschata*), after 1, 2, 3, and 4 weeks from inoculation of nematode juveniles.

Fly ash level (%)	Developmental stages One week				Developmental stages Tow week				Developmental stages Three week				Developmental stages Four week			
	J2	J3/J4	P	M	J2	J3/J4	P♀	M♀	J2	J3/J4	P♀	M♀	J2	J3/J4	P♀	M♀
	Control	155	405	-	-	138	156	118	32	-	168	201	71	-	-	98
5	166	378	-	-	132	138	98	11	-	148	175	24	-	-	82	46
10	201	310	-	-	164	110	75	-	-	142	171	16	-	-	40	15
20	229	260	-	-	175	98	53	-	-	132	161	10	-	13	30	-
30	274	190	-	-	188	79	36	-	-	105	137	-	16	69	21	-
40	235	105	-	-	134	95	17	-	108	160	89	-	21	102	14	-
50	187	75	-	-	96	73	-	-	88	139	28	-	27	130	09	-
LSD at 5%	14.2	23.7			2.1	5.3				7.0	9.1				8.1	

The data given in table 2 shows that the growth (length of shoot and root, fresh and dry weight of shoot and root) and yield (flowers/plant and fruits/plant) parameters of pumpkin were increased significantly in 5, 10, 20 and 30% levels of fly ash and *M. incognita* combinations compared to uninoculated (only soil) control set. The maximum increment in all above parameters was observed at 30% level of fly ash and *M. incognita* combination. But plant growth and yield parameters in higher levels (40% + *M. incognita* and 50% + *M. incognita* combinations) were reduced significantly (P=0.05) compared to control set and maximum being at 50% FA and Mi level.

Table 2. Effect of different doses of fly ash and root-knot nematodes (*Meloidogyne incognita*) on growth and yield parameters of pumpkin (*Cucurbita moschata*).

Concentration of fly ash	Length (cm)		Fresh weight (gm)		Dry weight (gm)		Number/Plant	
	Root	Shoot	Root	Shoot	Root	Shoot	Flower	Fruits
C (Soil)	60±2.96	281±12.91	62.4±2.79	223.4±10.67	9.1±0.46	32.2±1.45	25±1.23	24±0.95
C (Nematode)	42±2.00	216±11.99	54.6±2.95	201.1±9.61	6.3±0.26	25.0±1.21	23±1.47	20±0.87
5% FA + N	52±2.25	232±9.75	59.6±2.11	214.4±9.87	7.1±0.20	29.2±1.54	21±0.88	20±0.87
10% FA + N	54±2.18	253±11.05	63.4±2.94	220.2±1.45	8.3±0.44	33.1±1.55	22±1.21	21±1.06
20% FA + N	57±3.00	265±11.68	68.2±1.93	231.1±10.15	10.2±0.36	35.2±1.30	24±1.39	22±1.18
30% FA + N	61±3.29	282±12.12	71.0±2.00	250.0±6.93	13.5±0.60	38.0±1.71	26±1.05	25±0.87
40% FA + N	50±3.00	224±8.85	54.6±2.33	220.4±10.13	7.2±0.30	29.3±1.47	21±1.00	17±0.85
50% FA + N	45±2.65	202±11.38	47.6±2.05	198.2±9.91	5.5±0.27	23.2±1.13	18±0.87	14±0.87
LSD-P≤0.05	6.5	20.8	4.4	19.2	1.32	2.1	2.3	2.0

Note: C, Control; Each value is a mean of five replicates, ± values shows the standard deviation with mean.

Table 3. Effect of different doses of fly ash and root-knot nematode (*Meloidogyne incognita*) on some biochemical parameters of pumpkin (*Cucurbita moschata*).

Concentration (%) of Fly ash	Chlorophyll (mg.g ⁻¹ fresh leaf)			Carotenoids (mg.g ⁻¹ fresh leaf)	Carbohydrate (µg fresh weight)	Proline (µmol.g ⁻¹ fresh weight)
	a	b	Total			
Control	0.82±0.091	0.49±0.015	1.31±0.038	0.46±0.105	14.17±1.054	23.3±1.952
Nematodes	0.68±0.079	0.39±0.007	1.07±0.069	0.35±0.098	10.02±1.127	18.58±2.100
5%+N	0.81±0.088	0.46±0.009	1.27±0.096	0.44±0.130	11.23±0.954	19.02±2.032
10%+N	0.91±0.124	0.47±0.012	1.39±0.059	0.45±0.121	11.98±0.915	19.99±1.638
20%+N	0.95±0.048	0.52±0.010	1.47±0.046	0.46±0.136	12.05±1.014	21.22±1.947
30%+N	0.98±0.022	0.55±0.006	1.53±0.082	0.47±0.156	15.05±1.583	24.77±2.947
40%+N	0.81±0.188	0.48±0.007	1.30±0.103	0.43±0.182	11.99±1.890	22.32±2.629
50%+N	0.79±0.037	0.46±0.011	1.28±0.008	0.42±0.103	10.95±0.961	20.87±2.722
LSD-P≤0.05	0.016	0.008	0.009	0.007	0.83	1.34

Note: Each value is a mean of five replicates, ± values shows the standard deviation with mean.

Similar pattern of increase/decrease in photosynthetic pigments (chl. a, chl. b, chl. a+b and carotenoids), carbohydrate and proline content of pumpkin were also observed in all fly ash + *M. incognita* treatments when compared to control one shows in table 3. However, maximum increment was found at 30% fly ash + *M. incognita*. When growth, yield and photosynthetic pigments, carbohydrate and proline content of pumpkin in different fly ash and *M. incognita* combinations were compared to inoculated set (nematode alone), it was

observed that all parameters were increased significantly ($P=0.05$). And all above biochemical parameters decreased significantly at 40% FA + Mi and 50% FA + Mi combinations and maximum at 50% level of FA and nematode.

DISCUSSION AND CONCLUSION

Several beneficial nutrients including S, B, Ca, Mg, Fe, Cu, Zn, Mn, and P, which are responsible for plant growth, found in fly ash. It is known that plants take up nitrogen in the form of nitrate (NO_3^-) because nitrates are more quickly available to plants as they move through the roots and as such lesser content of nitrate in 5% and 10% fly ash containing fields may be due to more hydraulic absorption because of higher water holding capacity in the fly ash amended soil. Fly ash decreases porosity and thus increases water holding capacity. This would facilitate the absorption of nutrients as well as photosynthetic activity. Similar findings have been reported by (Thetwar 2007). In the present study, soil amendment with fly ash was harmful to the nematode at all levels. The alkaline nature of fly ash may have directly affected the juveniles, leading to less penetration into the roots and subsequently delayed development. Edongali (1982) stated that juvenile penetration is affected by the concentration of different elements, irrespective of the type of element in the soil solution. We found higher chlorophyll a and b concentration in *cucurbita moschata* plant could be due to the micronutrients available in fly ash than the control. Similar reports have been made by (Niyaz & Singh 2006, Hisamuddin & Singh 2007). Pumpkin plant grows in soil with different doses of fly ash and did not show any visible injury and the lower at 30% level of fly ash was found beneficial to plant growth, yield, photosynthetic pigments, carbohydrate and proline contents of pumpkin.

The soil application of fly ash ameliorated plant growth of pumpkin and suppressed the *M. incognita* in pots. Improved plant growth with fly ash has been observed earlier (Elsewi *et al.* 1980, Mishra & Shukla 1986). Due to the better health status of the plant, the yield, photosynthetic pigments, carbohydrate, proline contents of pumpkin were also increased. The beneficial effects of fly ash were found from 10 to 30% levels in soil, and optimum being at 30%. Similar beneficial effects on above parameters 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 & Khan 1996, Rengifo *et al.* 1996, Sarangi & Mishra 1998, Sahu & Dwivedi 1999, Tarannum *et al.* 2001, Upadhyay & Khan 2002, Upadhyay 2004). However, the responses of various crops were different to different levels of fly ash (10–50%). Higher level adversely affected the plant growth and other parameters of Pumpkin. The adverse effects of fly ash at higher level of application are attributed to excess of micro-nutrients (Adriano *et al.* 1980) and toxicity of compounds like dibenzofuron and dibenzo-p-dioxine as well as heavy metals found in fly ash (Helder *et al.* 1982, Mishra & Shukla 1986, Wong & Wong 1986). Harmful effects of higher levels above 50% have been observed on *Brassica juncea*, chickpea, cucumber, lentil, *Linum usitatissimum*, maize, potato, soybean and tomato (Mishra & Shukla 1986, Pasha *et al.* 1990, Raghav 2006). On the other hand, the soil application of fly ash noticed the effect of *M. incognitai* 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 fly ash. 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 Pumpkin crop on the other hand.

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