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Effects of Fly Ash Amendments, *Ralstonia solanacearum, Meloidogyne incognita* and *Phomopsis vexans* on the Growth of *Solanum melongena*

M. KHAN and Z. A. SIDDIQUI*

Department of Botany, Aligarh Muslim University, Aligarh-202002, India

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Effects of fly ash amendments in soil (0%, 25% and 50% vol/vol), *Ralstonia solanacearum, Meloidogyne incognita* and *Phomopsis vexans* were observed on the growth, chlorophyll and carotenoid contents of eggplant. Addition of 25% fly ash in soil caused a significant increase in plant growth, chlorophyll and carotenoid contents over plants grown without fly ash. However, amendments of 50% fly ash in soil had an adverse effect on the growth, chlorophyll and carotenoid contents of eggplant. Inoculation of the pathogens caused a significant reduction in growth, chlorophyll and carotenoid contents. Inoculation of *R. solanacearum* caused the greatest reduction followed by *P. vexans* and *M. incognita*. Root galling and nematode multiplication was reduced with the increase in fly ash. Wilting and blight indices were 3 in plants grown in 0% and 25% fly ash amended soil while 4 in 50% fly ash amended soil.

Keywords: Egg plant, root knot nematode, wilt, blight.

Eggplant or brinjal (*Solanum melongena* L.) is an important solanaceous crop of sub-tropics and tropics. Eggplant has been cultivated in India for the last 4,000 years and produces about 7.676 M mt from an area of 0.472 M ha with an average productivity of 16.3 mt/ha *(*www.ncpahindia.com/eggplant.php). Eggplant is known to suffer from many diseases and among them fruit and leaf blight caused by *Phomopsis vexans* (Sacc and Syd.) Harter, bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al. and root knot disease caused by *Meloidogyne incognita* (Kofoid and White) Chitwood are common. Root-knot nematodes are particularly damaging vegetables in tropical and subtropical countries of the world and cause losses up to 80% in heavily infested fields (Sikora and Fernandez, 2005). *Ralstonia solanacearum* is a Gram-negative, rod-shaped, strictly aerobic bacterium. *R. solanacearum* causes wilt and is one of the most important plant pathogenic bacterium responsible for great economic losses worldwide (Hayward, 1991). Similarly, *Phomopsis vexans* (teleomorph: *Diaporthe vexans* Gratz) is also a major constraint in the production of eggplant. Fruit rot and leaf blight disease caused by *P. vexans* is a major concern, reduces yield and marketable value of the crop nearly 20–30% (Jain and Bhatnagar 1980; Kaur et al., 1985).

* Corresponding author; e-mail: zaki_63@yahoo.co.in

In India alone, more than 112 million tons of fly ash is generated annually, and the production is projected to exceed 170 million tons per year (Sarkar et al., 2012). Burning of coal releases oxides of nitrogen and sulphur and enormous quantity of fly ash gets deposited on the soil and the plants. Fly ash is major solid waste as a coal combustion residue and recycling or safe disposal of solid industrial wastes has become a prime environmental concern throughout the world. Hence, many potential applications have been identified for the utilization of fly ash including soil amendments in agricultural fields.

Heavy metals (Fe, several micronutrients and toxic metals) are abundant in fly ash, with the deficiency of fixed C- and N-compounds (Martens et al., 1970; Parab et al., 2012). Several heavy metals are useful in minute quantities to plants and classified as micronutrients, which nonetheless are toxic at higher levels. Moreover, metal contents in fly ash are known to cause the enhancement of crop growth in amended soils. Fly ash has been used in amending soils supporting several crops (Korcak, 1995, Mishra et al., 2005, Mishra et al., 2007; Samy et al., 2010), as a method of sustainable waste disposal and soil fertilization, worldwide (Dick et al., 2000).

The present study was carried out to observe the effect of fly ash amendments on the growth, chlorophyll and carotenoid contents of eggplant both in the presence and absence of *R. solanacearum, M. incognita* and *P. vexans*.

Materials and Methods

Fly ash used in this experiment was collected from the thermal power plant (530 MW capacity) located at Kasimpur, Aligarh, India. This power plant consumes 3192 ton of bituminous coal per day and produces 650 ton per day of fly ash. The field soil (from Aligarh) used in the experiment was loam, containing 71%, 18% and 11% sand, silt and clay particles, respectively. Field soil as collected was autoclaved at 137.9 kPa for 20 minutes while fly ash was dried in the sun for 10 days and mixed (vol:vol) in 3 proportions with soil, i.e. 0% (100% soil), 25% and 50% fly ash. Clay pots (30 cm dia.) which were previously lined with polythene were filled with 3.5 kg of the ash-soil mixtures.

Physico-chemical analyses

The physico-chemical characters of the soils used in the treatments were determined before sowing the seeds of eggplant cultivar Navkiran (Table 1). The soil samples were passed through a 2 mm sieve before analyses and the following properties were determined: porosity and water holding capacity by hydrometry; pH, conductivity and cation exchange capacity (CEC) using soil:distilled water in pH and conductivity meters. Organic carbon was estimated by Nelson and Sommers (1972). Sulphur, zinc, manganese, copper and iron by analytical methods (Chopra and Kanwar, 1982); nitrogen by Kjeldahl digestion (Nelson and Sommers, 1972); and phosphorus by phosphomolybdic blue colorimetry (Jackson, 1958).

Table 1

Physico-chemical characteristics of soil mixed with fly ash

Characteristics	Fly ash in field soil			C.D. $P = 0.05$
	0%	25%	50%	
pH	5.9[c]	6.7[b]	7.5[a]	0.4
CEC (mmhos/cm)	0.997[c]	1.213[b]	1.522[a]	0.071
Water holding capacity (%)	47.2[c]	51.2[b]	55.3[a]	3.4
Organic carbon (%)	0.52[a]	0.40[b]	0.19[c]	0.05
N (kg/ha)	201.4[a]	176.2[b]	142.1[c]	4.6
P (kg/ha)	18.0[a]	14.5[b]	12.6[c]	0.7
K (kg/ha)	220.05[c]	345.15[b]	508.5[a]	8.3
S ($mg \times kg^{-1}$)	10.86[c]	13.69[b]	16.12[a]	0.6
Zn ($mg \times kg^{-1}$)	5.82[a]	3.64[b]	2.94[c]	0.24
Fe ($mg \times kg^{-1}$)	4.80[a]	4.27[b]	4.06[c]	0.14
Mn ($mg \times kg^{-1}$)	2.71[c]	2.86[b]	3.04[a]	0.08
Cu ($mg \times kg^{-1}$)	0.42[c]	0.55[b]	0.63[a]	0.6

*Values in a row followed by the different letters are significantly different at $P \leq 0.05$ using Duncan's multiple range test. Multiplication factors for converting mg of mineral per kg of soil to kilograms per hectare (kg/ha) were calculated for a 30-cm soil layer
*C.D. = Critical difference

Plant culture

Five seeds of eggplant cultivar Navkiran were sown separately in the three soil–fly ash mixtures after surface sterilization by 0.1% sodium hypochlorite. Two weeks after germination thinning was done and a healthy seedling was maintained in each pot. Plants grown in each type of soil were inoculated with *R. solanacearum, M. incognita*, *P. vexans* and control (without pathogen). Each treatment was replicated five times ($12 \times 5 = 60$ pots). The pots were arranged on a glasshouse bench at 30 °C and watered regularly. The experiment was terminated 120 days after inoculation.

Fungus inoculum

Phomopsis vexans was isolated from infected eggplant leaf having blight symptoms. *P. vexans* was characterized as it produces only one type of conidia in its pycnidia, which are hyaline, one celled, sub-cylindrical and $5–9 \times 2–2.8$ µ in size during summer months, which gradually changed into the beta form (Islam et al., 2010). Inoculation of host plants with beta conidia caused intraveinal necrosis, which progressed towards the leaf base and resulted in premature defoliation, thus indicating their role in pathogenesis. Fungus was grown in Petri dishes containing potato dextrose agar (PDA) medium at

25 °C for 15 days. For obtaining sufficient inoculum, *P. vexans* was cultured on Richard's liquid medium (Riker and Riker, 1936) having the following composition:

Potassium nitrate	10.00 g
Potassium dihydrogen phosphate	5.00 g
Magnesium sulphate	2.50 g
Ferric chloride	0.02 g
Sucrose	50.00 g
Distilled water	1000 ml

The medium was prepared and filtered through muslin cloth, sterilized in an autoclave at 103.4 kPa for 15 minutes in 250 ml Erlenmeyer flasks each containing 80 ml liquid medium. The fungus was inoculated in each flask with the help of an inoculation needle. Inoculated flasks were incubated at 25 ± 1 °C for about 15 days. After sufficient growth of the fungus, liquid medium was filtered through Whatman filter paper No. 1. The mat of fungal mycelium was washed in distilled water and any excess of water and nutrients were removed with the help of blotting paper. The inoculum was prepared by mixing 10 g fungal mycelium in 100 ml of distilled water and blending for 30 seconds in a Waring blender. Twenty ml of this suspension, containing 2 g fungus, was inoculated.

Bacterial inoculum

Eggplants showing typical symptoms of bacterial wilt were collected from eggplant fields. Stems of infected eggplants were cut obliquely at the base and placed in sterile distilled water. The stem pieces showing milky white ooze in water were selected for isolation of the pathogen. The pathogen was isolated on nutrient agar medium. Nutrient agar plates were streaked separately with a pure colony of *R. solanacearum* and incubated at 30 °C for 24 h. Single colonies from a 24-h-old pure culture of either *R. solanacearum* were inoculated separately into nutrient broth liquid medium (peptone 5 g / L; meat extract 1 g / L; yeast extract 2 g / L; sodium chloride 5 g / L; pH 7.0) in flasks and incubated at 30 °C for 72 h. Bacterial cell density in the concentrated suspension was determined following Sharma (2001) and 1.2×10^5 colony-forming units (c.f.u.)/ml was adjusted by adding required amount of sterilized water calculated mathematically. Twenty ml bacterial suspension was inoculated per pot.

Nematode inoculum

Meloidogyne incognita was collected from the eggplant roots and multiplied on the roots of egg plants using single egg mass. Large numbers of egg masses from heavily infected eggplant roots were hand-picked with the help of sterilized forceps. The egg masses were washed with distilled water and placed in a small sieve (9 cm diameter with 1-mm pore size) containing crossed layers of tissue paper. The sieve was placed in a Petri dish containing distilled water deep enough to contact the egg masses. A number of these assemblies were kept in an incubator running at 25 ± 1 °C in order to obtain the required

number of second-stage juveniles for inoculation. The hatched second-stage juveniles were collected from the Petri dishes every 24 h, fresh water was added, and the process was repeated. For counting nematode juveniles, an average of 5 counts was made to determine the density of nematodes in the suspension. The volume of nematode suspension was so adjusted that each ml may contain 200 ± 5 nematodes. Twenty ml of this suspension (i.e. 4000 freshly hatched juveniles) was added to each pot around a eggplant seedling.

Experimental design

Soil around the roots was carefully removed and suspensions of *M. incognita*, *R. solanacearum* and *P. vexans* were poured around the roots uniformly and soil replaced. In control pots, water was poured in similar amount to inoculum suspension. Three types of soil were used, viz. 0:100 (% v/v) fly ash:soil; 25:75 fly ash:soil; 50:50 fly ash:soil, respectively. These soil mixtures were inoculated with *M. incognita*, *R. solanacearum* and *P. vexans* alone and a control (Table 2). There were 12 treatments, i.e. 3 types of soil

Table 2

Effects of fly ash, *Ralstonia solanacearum, Meloidogyne incognita* and *Phomopsis vexans* on the growth of eggplant

Treatment		Plant length (cm)	Plant fresh weight (g)	% increase (+) or decrease (–) in shoot dry weight over control	Shoot dry weight (g)	Root dry weight (g)
Control	0% Fly ash	76.8^{b}	62.32^{b}	–	13.58^{b}	2.34^{b}
	25% Fly ash	82.7^{a}	65.61^{a}	+10.16	14.96^{a}	2.81^{a}
	50% Fly ash	72.4^{c}	60.43^{c}	–10.08	12.21^{c}	1.93^{c}
R. solanacearum	0% Fly ash	55.4^{hi}	31.63^{i}	–	8.16^{h}	1.13^{fg}
	25% Fly ash	57.9^{g}	33.62^{h}	+5.26	8.59^{g}	1.40^{ef}
	50% Fly ash	54.2^{i}	29.61^{j}	–9.06	7.42^{i}	1.00^{g}
M. incognita	0% Fly ash	63.7^{e}	42.72^{d}	–	9.80^{e}	1.57^{de}
	25% Fly ash	66.8^{d}	44.23^{d}	+12.44	11.02^{d}	1.77^{cd}
	50% Fly ash	60.7^{f}	39.92^{e}	–0.81	9.72^{e}	1.47^{e}
P. vexans	0% Fly ash	58.2^{g}	35.82^{g}	–	9.08^{f}	1.32^{ef}
	25% Fly ash	61.2^{f}	37.92^{f}	+6.93	9.71^{e}	1.51^{de}
	50% Fly ash	56.8^{gh}	33.22^{hi}	–9.58	8.21^{gh}	1.28^{efg}
C.D. at 5%	Pathogen (P)	1.092	0.806	–	0.238	0.152
	Soil type (S)	0.945	0.931	–	0.206	0.132
	Interactions (P×S)	1.897	1.612	–	0.412*	0.264*

Values in a column followed by the different letters are significantly different at $P \leq 0.05$ using Duncan's multiple range test
C.D. = Critical difference

mixtures × 4 treatments including control. Each treatment was replicated five times, i.e. 12 × 5 = 60 pots and the experiment was conducted in bi-factorial design.

Evaluation of experiment

The plants were harvested 120 days after inoculation. Data on plant length, fresh plant weight, plant dry weight, number of galls, nematode population, chlorophyll and carotenoid contents were recorded. Wilt and blight indices and number of galls, nematode population were also recorded. The length of plants was recorded in cm from the top of the first leaf to end of the root. Excess water was removed by blotting before weighing the plant for fresh weight. The plants were cut with a knife above the base of the root emergence zone to separate shoot and root. Shoots and roots were kept in envelopes at 60 °C for 2–3 days before weighing for dry weight. A 250 g subsample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting technique followed by Baermann funnel extraction (Southey, 1986). Nematode suspension was collected after 24 h and the numbers of nematodes were counted in five aliquots of 1 ml of suspension from each sample. The means of five counts were used to calculate the population of nematodes in 3.5 kg soil. To estimate the number of juveniles, eggs and females inside the roots, a 1 g subsample of roots was macerated in a Waring blender and counts were made from the suspension thus obtained. Numbers of nematodes present in roots were calculated by multiplying the number of nematodes present in 1 g of root by the total weight of root. Wilt and blight indices were determined by scoring the severity of disease on a scale ranging from 0 to 5 where 0 = no disease (no wilt/blight symptoms observed); 1 = wilt/blight symptoms up to 12.5 % on shoot; 2 = wilt/blight symptoms 12.6 to 25% on shoot; 3 = wilt/blight symptoms 25.1 to 37.5% on shoot; 4 = wilt/blight symptoms 37.6 to 50% on shoot, and 5 = more than 50% wilt/blight symptoms on shoot. The chlorophyll and carotenoid contents in fresh leaves were estimated following the method of Mackinney (1941).

Statistical analysis

Data obtained were analysed statistically http://hau.ernet.in/about/opstat.php (for off campus users) using two factorial analysis of variance (ANOVA) (i.e. pathogens × types of soil). Effects of types of soil and pathogens and their interaction were calculated. Critical differences (C.D.) were calculated at $P = 0.05$, Duncan's multiple range test was later employed to denote significant differences between the treatments.

Results

Physico-chemical characteristics

Addition of fly ash resulted in the increase of soil pH, CEC, water holding capacity, potassium, sulphur, manganese and copper (Table 1). Amendment of 50% fly ash caused

greater increase in pH, CEC, water holding capacity, potassium, sulphur, manganese and copper than the addition of 25% fly ash. However, organic carbon, nitrogen, phosphorus, zinc and iron were reduced with the addition of fly ash. Amendment of 50% fly ash caused greater reduction than caused by 25% fly ash (Table 1).

Plant growth

Plants grown in 25% fly ash amended soil showed a significant increase in shoot dry weight as compared to plants grown in soil without fly ash (Table 2). However, addition of 50% fly ash to the soil caused a reduction in shoot dry weight. Inoculation of *M. incognita*/*R. solanacearum* or *P. vexans* caused a significant reduction in shoot dry weight in all types of soil mixtures compared to their respective control. Inoculation of *R. solanacearum* caused the greatest reduction in shoot dry weight followed by *P. vexans* and *M. incognita*. Addition of 25% fly ash to the soil caused 10.16% increase in shoot dry weight over plants grown without fly ash amended soil. However, application of 50% fly ash in the soil resulted in 10.08% reduction in shoot dry weight compared to plants grown in soil without fly ash. After inoculation with *R. solanacearum*, shoot dry weight was 5.26% higher in plants grown in 25% fly ash amended soil compared to plants grown without fly ash amendments. However, inoculation of *R. solanacearum* to plants grown in 50% fly ash amended soil resulted in 9.06% reduction in shoot dry weight compared to plants inoculated with *R. solanacearum* and grown without fly ash amendment. Increase in shoot dry weight was 12.44% in plants inoculated with *M. incognita* and grown with 25% fly ash amended soil compared to plants inoculated with *M. incognita* and grown without fly ash amendments. However, reduction in plant growth was 0.81% in plants inoculated with *M. incognita* and grown in 50% fly ash amended soil compared to plants inoculated with *M. incognita* and grown without fly ash. Increase in shoot dry weight was 6.93% in plants inoculated with *P. vexans* and grown with 25% fly ash amendment compared to plants inoculated with *P. vexans* and grown without fly ash amendments. However, reduction in shoot dry weight was 9.58% in plants inoculated with *P. vexans* when grown in 50% fly ash amended soil compared to plants inoculated with *P. vexans* and grown without fly ash (Table 2).

Plant length, fresh weight and root dry weight were also increased with the addition of 25% fly ash to the soil (Table 2). However, addition of 50% fly ash to the soil had adverse effect on plant length, fresh weight and root dry weight. Inoculation of *M. incognita*/*R. solanacearum* or *P. vexans* caused a significant reduction in plant length, fresh weight and root dry weight in all types of soil mixtures compared to their respective control. Inoculation of *R. solanacearum* caused the greatest reduction in plant length, fresh weight and root dry weight followed by *P. vexans* and *M. incognita* (Table 2).

Chlorophyll and carotenoid contents

Plants grown in 25:75 fly ash:soil mixture showed a significant increase in chlorophyll and carotenoid contents compared to plants grown in the field soil (Table 3). However, 50% amendment with fly ash resulted in the reduction of chlorophyll and carote-

noid contents compared to the plants grown in the soil without fly ash. Inoculation of *M. incognita*/*R. solanacearum* or *P. vexans* caused a significant reduction in chlorophyll and carotenoid contents as compared to their respective control. The results indicate that *R. solanacearum* caused the greatest reduction in chlorophyll and carotenoid contents followed by *P. vexans* and *M. incognita* in all fly ash and soil mixtures. (Table 3).

Root galling and nematode multiplication

Root galling and nematode multiplication was greater in plants grown in the soil without fly ash ash compared to plants grown in fly ash amended soil (Table 3). We found that galling and nematode population decreased with increasing fly ash content of the soil. The least galling intensity and nematode multiplication was observed in plants grown in 50% fly ash amended soil.

Table 3

Effects of fly ash, *Ralstonia solanacearum, Meloidogyne incognita* and *Phomopsis vexans* on chlorophyll and carotenoid contents of eggplant

Treatment		Chlorophyll (mg/g)	Carotenoid (mg/g)	Number of galls/root	Nematode population	Wilt / blight index
Control	0% Fly ash	1.848^{b}	0.052^{b}	–	–	–
	25% Fly ash	2.035^{a}	0.058^{a}	–	–	–
	50% Fly ash	1.717^{c}	0.046^{cd}	–	–	–
R. solanacearum	0% Fly ash	0.970^{h}	0.045^{d}	–	–	3
	25% Fly ash	1.135^{g}	0.048^{c}	–	–	3
	50% Fly ash	0.901^{i}	0.039^{e}	–	–	4
M. incognita	0% Fly ash	1.477^{d}	0.050^{bc}	228^{a}	45400^{a}	–
	25% Fly ash	1.850^{b}	0.052^{b}	164^{b}	32980^{b}	–
	50% Fly ash	1.397^{e}	0.039^{e}	104c	19700^{c}	–
P. vexans	0% Fly ash	1.390^{e}	0.050^{b}	–	–	3
	25% Fly ash	1.458^{d}	0.051^{b}	–	–	3
	50% Fly ash	1.269^{f}	0.045^{d}	–	–	4
C.D. at 5%	Pathogen (P)	0.029	0.001	–	–	–
	Soil type (S)	0.025	0.001	–	–	–
	Interactions (P × S)	0.051	0.002	16.2	1174.2	–

Values in a column followed by the different letters are significantly different at $P \leq 0.05$ using Duncan's multiple range test
C.D. = Critical difference

Disease indices

Wilt and blight indices were 3 when *R. solanacearum* and *P. vexans* inoculated plants were grown in soil without fly ash and also in 25% fly ash amended soil (Table 3). The disease indices were 4 when *R. solanacearum* and *P. vexans* were inoculated into 50% fly ash amended soil.

Discussion

The improved growth, chlorophyll and carotenoid contents of eggplant in 25% fly ash amended soil was apparently due to the availability of a greater amount of utilizable plant nutrients as revealed by the chemical analysis of the soil. A greater amount of potassium, sulphur, manganese and copper etc. present in the fly ash amended soil have been absorbed by the roots and utilized by the plant that led to improved growth of egg plant. The nutrients from fly ash have been reported to be beneficial for the plant growth and yield of rice (Sarangi et al., 1997), wheat, chickpea (Dubey et al., 1982) and tomato (Khan and Khan, 1996). However, plants grown in 50% fly ash amended soil showed a decrease in plant growth, chlorophyll and carotenoid contents, probably due to excess of plant nutrients or to a build-up of certain toxic elements (Wadge and Hutton, 1987) at a level that became phytotoxic and inhibited plant growth.

After inoculation with *R. solanacearum* and *P. vexans* or *M. incognita*, a considerable increase in plant growth, chlorophyll and carotenoid contents were observed in plants grown in 25% fly ash amended soil compared to plants grown in the soil without fly ash. Greater availability of nutrients in fly ash-amended soil enabled the plants to grow better and may inhibit invasion of *M. incognita.* Higher uptake of boron and potassium etc. helped the plants in building natural defences against the nematode (Kirkpatrick et al., 1964; Francois, 1984; Khan et al., 1997). Therefore, galling and nematode multiplication was reduced with the addition of fly ash. Moreover, excess of salts, i.e. chlorides, carbonates, bicarbonates, sulphates etc. (Khan and Khan, 1996), nutrients (Elseewi et al., 1981), heavy metals (Wadge and Hutton, 1987) and/or poly-chlorinated compounds (Helder et al., 1982; Sawyer et al., 1983) with the amendment of 50% fly ash caused toxic effect on *M. incognita* either in soil or within the host. The substantial decline in the galling and nematode population indicates that the fly ash caused direct inhibitory effect on the survival and multiplication of *M. incognita.*

Amendment of 25% fly ash had no apparent effect on the blight and wilt disease indices of eggplant. Moreover, there was no substantial decline in the soil population of the *P. vexans* or *R. solanacearum* (data not shown) with the addition of fly ash indicates that the fly ash caused no direct inhibitory effect on the survival and multiplication of the fungal/bacterial pathogens in the soil. However, amendment of 50% fly ash caused an increase in blight and wilt disease indices without increase in the soil population of the *P. vexans* or *R. solanacearum*. Plants grown in 50% fly ash amended soil were poor in growth as compared to plants grown in 25% fly ash amended soil and plants grown in the soil without fly ash. Plants grown in 50% fly ash amended soil had more severe disease

symptoms than those grown in 25% fly ash amended soil because plants grown in 50% fly ash amended soil with severe disease symptoms were poor in growth. It is a general observation that more vigorous plants suffer less damage from pathogens as compared to plants restricted in growth (Agrios, 2005) which is also indicated by increased wilt and blight indices.

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