EFFECT OF FLY ASH ON GROWTH, YIELD AND ROOT-KNOT DISEASE OF SOYBEAN

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Summary. An experiment was conducted to assess the potential of different levels (25-100%) of fly ash on growth and yield of soybean and on the root-knot nematode, *Meloidogyne javanica*. Low levels (25 and 50%) of fly ash enhanced plant growth and yield of soybean, which were greater at the 50% level. Further improvements in growth and yield were noticed at 50% of fly ash in the presence of *Bradyrhizobium japonicum*. At higher levels (75 and 100%) fly ash was phytotoxic. The presence of *B. japonicum* suppressed the numbers of galls, females and egg masses of the nematode but increased its fecundity. Root galling increased at 25 and 50%, but decreased at the 75 and 100% fly ash rates. Gradual suppression in egg mass production and fecundity were, however, observed at all levels of fly ash. The number of females of the nematode was increased at 25-50% levels, but at 75 and 100% levels fly ash was inhibitory for the nematode and no females developed at the highest level.

Key words: *Bradyrhizobium japonicum*, control, *Glycine max*, *Meloidogyne javanica,* soil amendment.

Fly ash is the major particulate waste in India. It is mainly produced by the thermal power plants and other industries using coal as fuel. It contains plant nutrient elements such as P, K, Ca, Mg, Mn, Cu, Zn, carbonates, bicarbonates, sulphate and chloride. Porosity, water-holding capacity, pH, conductivity and cation exchange capacity are also increased in fly ash amended soil (Adriano *et al*., 1980; Khan *et al*., 1997).

Hence, fly ash has a vast potential for use in agriculture as a soil amendment. It has been found beneficial for the growth of many plants (Mishra and Shukla, 1986; Khan and Khan, 1996; Raghav and Khan, 2002; Rizvi and Khan, 2009a). Recently, fly ash has also shown inhibitory effects on root-knot nematodes (Khan *et al*., 1997; Tarannum *et al*., 2001; Rizvi and Khan, 2009b). Therefore, it was planned to evaluate the potential of fly ash as an inorganic amendment of the soil for the control of the root-knot nematode, *Meloidogyne javanica* (Treub) Chitw., on soybean [*Glycine max* (L.) Merr.].

MATERIALS AND METHODS

Fly ash used in the experiment was obtained from the Thermal Power Plant, Kasimpur. The soil, collected from the field, was sandy loam containing 66% sand, 24% silt, 8% clay, 2% organic matter and of pH 7.7. It was kept in gunny bags and steam sterilized in an autoclave at 20 lb pressure for 20 minutes. After drying, the soil was mixed with fly ash to obtain different levels of fly ash of 0, 25, 50, 75 and 100%. Clay pots (30 cm diam.) were each filled with 2 kg of each type of amended soil. Five seeds of soybean cv. 327 were sown in each pot and seedlings were thinned to one per pot when they reached the four leaf stage. All pots were kept in a glasshouse at 27-23 °C day/night temperature.

The following treatments were provided at each fly ash rate: 1) no inoculation; 2) inoculation with the rhizobacterium *Bradyrhizobium japonicum* Jordan procured from Agriculture Farm, Aligarh; 3) inoculation with *M. javanica*; 4) inoculation with both *B. japonicum* and *M. javanica*. Seeds for pots designated to receive *B. japonicum* (Bj) were inoculated with the root-nodule bacteria before sowing. Three-week-old seedlings of soybean designated to receive *M. javanica* (Mj) were inoculated with freshly hatched second stage juveniles of the nematode (1500 J_2/pot). The juveniles were obtained from egg masses of a pure culture of *M. javanica* incubated in sterile water in an incubator at 25 °C for 72 h. Each treatment was replicated five times and pots were arranged according to a randomized block design. At the end of the experiment (75 days after sowing), the variables mentioned hereafter were recorded.

Plant growth and yield. At the end of the experiment, length and fresh weight of shoots and roots and numbers of pods per plant were recorded.

Root nodules. The roots of the soybean plants were washed thoroughly under tap water and examined for the presence of nodules. The numbers of functional and total nodules per plant were determined. The pinkish healthy nodules were considered functional.

Galls and egg masses. The roots of each plant were washed under tap water and immersed in an aqueous solution of phloxin B (0.15 g/litter tap water) for 15 minutes to stain the egg masses. Then galls and egg masses per root system were counted.

Fecundity. Fecundity, i.e. eggs per egg mass, was measured by shaking vigorously 10 egg masses in a 0.5% NaOCl solution and pouring the egg suspension onto a 500 mesh sieve over which water was gently sprayed to eliminate excess NaOCl (Hussey and Barker, 1973). The eggs retained on the sieve were transferred into a beaker and few drops of 0.35% acid fuchsin solution (in 25% lactic acid) were added to 20 to 25 ml of the egg suspension and boiled for 1 minute to stain the eggs and make it easier to count them. The eggs were counted in a counting dish containing 1 ml of egg suspension. From the counts, the numbers of eggs per egg mass were calculated.

Females. The roots from each replicate were weighed and cut into pieces of 1 cm length. One gram of root pieces was stained with acid fuchsin and lactophenol (Byrd *et al.*, 1983). Then, 1 g of root was transferred into 5% HNO_3 and incubated at 25 °C. After 72 h, root pieces were gently teased under a binocular microscope to release the females. The number of females/g of root was counted and the total number of females for the whole root system was calculated.

Statistical analysis. Statistical analysis of the data was done according to the Fisher (1950) factorial method.

RESULTS

Plant growth and yield. All plant growth (length and fresh weight of shoot and root) and yield (number of pods) components were increased at the 25 and 50% fly ash levels and were greatest at the 50% fly ash level in the presence of *B. japonicum*. However, at 75 and 100% of fly ash these variables were suppressed. The least growth and yield were recorded in the pots inoculated with *M. javanica* at all levels of fly ash. All the above mentioned variables were slightly greater in Mj + Bj treatments at all fly ash levels as compared to control and nematode alone treatments (Tables I).

Root nodules. Root nodulation (functional and total nodules) was affected by fly ash. All the levels of fly ash suppressed root nodulation significantly and suppression gradually increased with the increase of fly ash in the soil. No nodule was observed at the 100% fly ash level. Nodulation was decreased further in the presence of the nematode (Table II).

Root-knot disease. Numbers of galls and females of the nematode increased at the 25 and 50% fly ash levels. However, at 75% of fly ash a sharp decline was observed. No gall or female of the nematode was observed at the 100% fly ash level. Also, the numbers of egg masses and fecundity of the nematode decreased at the 25 and 50% fly ash levels and egg formation was completely prevented at the 75 and 100% levels. In the

Table I. Effect of different fly ash rates on length and fresh weight of shoot and root and numbers of pods per plant of soybean inoculated with *Bradyrhizobium japonicum* and *Meloidogyne javanica*, singly or in combination.

Treatment	Shoot length (cm)					Root length (cm)					Shoot weight (g)					Root weight (g)					No. of pods				
	Fly ash (%)					Fly ash (%)					Fly ash (%)					Fly ash (%)					Fly ash (%)				
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
Control	60	69	72	57	33	35	38	39	28	23	22	26	30	18	12	13	16	19	9	7	46	59	63	36	9
Bj	72	72	75	57	34	40	40	42	30	22	27	31	36	24	12	18	19	22	10	8	53	63	65	39	10
Mj	56	67	69	54	32	32	34	37	25	20	20	23	28	16	12	11	15	18	9	7	40	56	57	32	7
Bj + Mj	63	70	74	57	32	37	38	41	29	21	24	29	32	20	12	16	18	21	9	7	46	58	63	36	8
MM	63	69	73	56	33	36	38	40	28	22	24	27	32	19	12	15	17	20	9	7	46	59	62	36	9
CD at P=0.05	Treat. = 1.01, FA = 1.13					Treat. = 1.09, FA = 1.22					Treat. = 1.10, FA = 1.23					Treat. = 1.09, FA = 1.21					Treat. = 1.73, FA = 1.94				
	Treat. _FA = 2.26					Treat. _FA = 2.43					Treat. _FA = 2.45					Treat. _FA = 2.42					Treat. _FA = NS				

Bj = *Bradyrhizobium japonicum*, Mj = *Meloidogyne javanica*, MM = Mean of means, Treat. = Treatment, FA = Fly ash %
Each value is the mean of five replicates.

Table II. Effect of fly ash rates on functional and total number of bacterial nodules/plant of soybean inoculated with *B. japonicum* and *M. javanica*, singly or in combination.

Treatment	Number of functional nodules						Total number of nodules					
	Fly ash (%)						Fly ash (%)					
	0	25	50	75	100	MM	0	25	50	75	100	MM
Control	-	-	-	-	-	-	-	-	-	-	-	-
Bj	199.60	152.20	96.60	11.80	0	92.04	222.4	181.4	102.4	32.4	0	107.72
Mj	-	-	-	-	-	-	-	-	-	-	-	-
Bj + Mj	155.00	124.80	84.80	9.60	0	74.84	187.2	142.8	97.6	23.4	0	90.20
MM	177.30	138.50	90.70	10.70	0		204.8	162.1	100.0	27.9	0	
CD at P=0.05	Treat. = 1.12, FA = 1.77, Treat. _ FA = 2.50						Treat. = 3.40, FA = 5.38, Treat. _ FA = 7.61					

Bj = *Bradyrhizobium japonicum,* Mj = *Meloidogyne javanica,* MM = Mean of means, Treat. = Treatment, FA = Fly ash %.
Each value is the mean of five replicates.

Table III. Effect of fly ash rates on numbers of galls, egg masses and females and the fecundity of *M. javanica* on soybean inoculated or not with *B. japonicum*.

Treatment	Number of galls					Number of egg masses					Number of females					Fecundity				
	Fly ash (%)					Fly ash (%)					Fly ash (%)					Fly ash (%)				
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bj	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mj	56	65	86	12	0	34	13	8	0	0	74	75	95	23	0	403	369	358	0	0
Bj + Mj	46	59	80	10	0	30	9	6	0	0	64	73	89	17	0	414	383	360	0	0
MM	51	62	83	11	0	32	11	7	0	0	69	74	92	20	0	409	376	359	0	0
CD at P=0.05	Treat. = 1.18, FA = 1.87, Treat. _ FA = 2.65					Treat. = 0.65, FA = 1.03, Treat. _ FA = 1.46					Treat. = 1.80, FA = 2.85, Treat. _ FA = 4.03					Treat. = NS, FA = 7.63, Treat. _ FA = NS				

Bj = *Bradyrhizobium japonicum*, Mj = *Meloidogyne javanica,* MM = Mean of means, Treat. = Treatment, FA = Fly ash %, Fecundity = eggs per egg mass of the nematode.
Each value is the mean of five replicates.

presence of *B. japonicum*, the numbers of galls, egg masses and females and the fecundity of the nematode decreased at all fly ash levels when compared to corresponding pots inoculated with the nematode alone (Table III).

DISCUSSION

Growth and yield of soybean were enhanced in soils amended with 25 and 50% of fly ash. Addition to the soil of fly ash can enrich the soil in macro- and micronutrients (Druzina *et al.*, 1983), neutralize soil acidity and increase ion exchange capacity, water holding capacity and pore size (Adriano *et al.*, 1980; Elseewi *et al.*, 1981). All this may result in improvement of plant growth and yield (Singh and Siddiqui, 2003). Plant growth and yield did increase at the 25 and 50% levels but further increase suppressed these variables. This indicates that, up to the 50% level, the changes brought about by fly ash in the physico-chemical characteristics of the soil were close to optimal. Higher levels of fly ash (75 and 100%) were harmful for plant growth, perhaps because the elements present in fly ash at higher levels exceeded threshold limits for soybean, so causing adverse effects on plant growth and yield.

At the 25 and 50% fly ash levels, growth and yield of soybean inoculated with root nodule bacteria were relatively better than those of non-nodulated plants, supporting observations made on pea plants (Siddiqui and Singh, 2005). This improvement was comparatively less in the presence of *M. javanica*. Soybean inoculated jointly with root-knot nematode and root-nodule bacteria, at 25 and 50% of fly ash, showed a significant increase in plant growth and yield as compared to the inoculated plants grown in non-amended soils. At 75% and 100% of fly ash, the differences in plant growth and yield components were not significant in the nematode- and bacteria-inoculated plants. Recently, similar results were also obtained by Siddiqui *et al.* (2005) on pea and Singh *et al.* (2010) on cowpea. Thus, the effects of adding *B. japonicum* and *M. javanica* together were adversely affected by fly ash, especially at 100% fly ash. The reduction in the number of nodules could be due to increased soil alkalinity (Adriano *et al.*, 1980).

Improved plant growth in the presence of *B. japonicum* may have been due to induced resistance against juvenile penetration of the nematode (Tu, 1980), leading to reduced root galling (Fazal *et al.*, 1992) and egg mass formation. Nitrogen plays an important role in disease resistance of plants. Increased numbers of eggs per egg mass may have been due to the low number of nematode juveniles in the roots and a consequent reduced competition among them, leading to better female development and the release of more eggs per egg mass. Increased soil porosity, which can facilitate the movement of juveniles in the soil (O'Bannon and Reynolds, 1961), and increased water holding capacity (Van Gundy, 1985) are suggested to be responsible for increased penetration of juveniles into the roots. Increased juvenile penetration into the roots (up to 50% fly ash) would have subsequently led to an increase in number of females of *M. javanica* and greater root galling. Unlike root galling, egg mass production and fecundity were suppressed at all levels of fly ash and no egg mass was formed at 100% of fly ash. This inhibitory effect of the fly ash can probably be attributed to excess amounts of some elements.

According to our findings, where fly ash is easily available it could be used as a soil amendment to control nematodes and improve plant growth and yield. This would also help in getting rid of this waste material.

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