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MORPHOLOGICAL AND BIOCHEMICAL RESPOSES OF COW PEA (CV. PUSA BARSATI) GROWN ON FLY ASH AMENDED SOIL IN PRESENCE AND ABSENCE OF *MELOIDOGYNE JAVANICA* AND *RHIZOBIUM LEGUMINOSARUM*

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ABSTRACT

Plant growth, yield, pigment and protein content of cow-pea were increased significantly at lower levels (20 and 40%) of fly ash but reverse was true at higher levels (80 and 100%). Soil amended by 60% fly ash could cause suppression in growth and yield in respect to 40% fly ash treated cow-pea plants but former was found at par with control (fly ash untreated plants). Maximum growth occurred in plants grown in soil amended with 40% fly ash. Nitrogen content of cow-pea was suppressed progressively in increasing levels of fly ash. Moreover, *Rhizobium leguminosarum* influenced the growth and yield positively but *Meloidogyne javanica* caused opposite effects particularly at 20 and 40% fly ash levels. The positive effects of *R. leguminosarum* were marked by *M. javanica* at initial levels. However, at 80 and 100% fly ash levels, the positive and negative effects of *R. leguminosarum* and/or *M. javanica* did not appear as insignificant difference persist among such treatments.

Key words: *Meloidogyne javanica*, *Rhizobium leguminosarum*, fly ash, growth, yield.

INTRODUCTION

Fly ash is produced by thermal power plants and other industries using coal as fuel. Their deposition on the soil and foliage causes varied responses of the plants depending upon its level of deposition. Except a few studies conducted at Aligarh (Khan and Khan 1996), none of the information regarding the growth and yield of nodulated and non-nodulated cow-pea in presence or absence of root-knot nematode in fly ash stressed condition, is available to make the generalization. So this piece of work was done in order to assess cow-pea growth and yield in presence or absence of root-knot nematode and/or root-nodule bacteria grown in soils amended with fly ash at different levels.

MATERIALS AND METHODS

The autoclaved field soil and fly ash (obtained from thermal power plant, Kasimpur, Aligarh, India) were mixed with field soil in order to obtain 20, 40, 60, 80 and 100% levels. These mixtures were filled in 30 cm diameter clay pots and surface sterilized seeds (dipped in 0.01% $HgCl_2$ for 15 minutes) of *Vigna sinensis* cv. Pusa Barsati (cow pea) were sown in the pots and simultaneously

inoculated with *Rhizobium leguminosarum*. Three week-old seedlings were inoculated with freshly hatched 2nd stage juveniles of *Meloidogyne javanica*. The following were the treatments in the experiment.

Control

Plant + 1000g soil + Nil fly ash

Plant + 1000 g soil + *R. leguminosarum*

Plant + 1000 g soil + Nematode (=*M. javanica*)

Plant + Rhizobium + Nematode

Fly ash treatment

Plant + Fly ash (20%) = 200g fly ash + 800g soil

Plant + Fly ash (20%) + Rhizobium

Plant + Fly ash (20%) + Nematode

Plant + Fly ash (20%) + Nematode + Rhizobium

Plant + Fly ash (40%) = 400g fly ash + 600g soil

Plant + Fly ash (40%) + Rhizobium

Plant + Fly ash (40%) + Nematode

Plant + Fly ash (40%) + Nematode + Rhizobium

Plant + Fly ash (60%) = 600 g fly ash + 400g soil

Plant + Fly ash (60%) + Rhizobium

Plant + Fly ash (60%) + Nematode

Plant + Fly ash (60%) + Nematode + Rhizobium

Plant + Fly ash (80%) = 800g fly ash + 200g soil

Plant + Fly ash (80%) + Rhizobium

Plant + Fly ash (80%) + Nematode

Plant + Fly ash (80%) + Nematode + Rhizobium

Plant + Fly ash (100%) = 1000g fly ash + Nil soil

Plant + Fly ash (100%) + Rhizobium

Plant + Fly ash (100%) + Nematode

Plant + Fly ash (100%) + Nematode + Rhizobium

Each treatment was replicated five times. After the termination of the experiment (95 days after sowing), lengths and dry and fresh weights of shoot and root were determined as per procedure. Number of flowers and fruits were counted at 10 day intervals in the last two months of the experiment and an average was calculated. Amount of chlorophyll (a, b and total) and carotenoid contents were calculated by using the method given by MacKinney (1941) and MacLachlan and Zalik (1963), respectively. Moreover, protein (soluble, insoluble and total) and nitrogen contents were determined by employing Lowry *et al*. (1951) and Linder (1944) methods, respectively.

All the data were analysed by using Fischer (1950) factorial method. Treatments with fly ash were considered as factor one (F_1) and treatments with root-knot nematode and/or root-nodule bacteria were considered as factor two (F_2). This way three CD (i.e., for F_1, F_2 and $F_1 \times F_2$) were calculated by putting the data to Analysis of Variance Table.

RESULTS AND DISCUSSION

Fly ash variably affected the plant growth (length, fresh and dry weights of shoot and root), yield (flowering and fruiting), pigments (chlorophyll a, b and total and carotenoids), protein (soluble, insoluble and total) and nitrogen contents of cow-pea. Cow-pea plants showed enhanced plant growth and yield in soils amended with 20 and 40% fly ash (Tables 1-4). Utilizable plant nutrients are found in fly ash (Druzina *et al*. 1983) and its addition can enrich the soil in macro and micro-nutrients which may have favorable effect on crop productivity (Martens and Beahm 1978). Addition of fly ash to soil can neutralize soil acidity and can increase ion exchange capacity, water holding capacity and pore size (Elseewi *et al*. 1981), which may ameliorate plant growth and yield. These factors may have played some role in improving the growth and biomass of the cow-pea plants. Improvements in plant leaf pigments (chlorophylls and carotenoids) and protein of seeds were also recorded at 20 and 40%, being maximum at 40% level (Tables 5-7). Further increase in the fly ash level caused suppression in growth, yield

and pigments of cow-pea. It is indicated that the changes exerted by fly ash at 40% level in the physico-chemical characteristics of soil were optimal for cow-pea crop which was evident from the improved plant growth and yield and enhanced leaf pigments and protein contents.

Higher levels of fly ash (60, 80 and 100%) were harmful for plant growth of cow pea. However, at 60% fly ash level, suppression to growth and other parameters occurred in comparison to 40% treatment but still the parameters were at par to the control (i.e., fly ash untreated plants). Fly ash contains some toxic compounds like dibenzofuran and dibenzo-p-dioxime (Helder *et al*. 1982). At 60, 80 and 100% fly ash level, concentration of these substances may have exceeded threshold limit for cow-pea, causing adverse effects on plant growth, yield, leaf pigment and protein contents of seeds. High alkalinity and excess of salts and nutrients in soil (Adriano *et al*. 1980) may also have contributed towards the poor performance of cow-pea at 60, 80 and 100% fly ash levels.

Plant growth and other characters of cow-pea with root nodule bacteria at 20 and 40% levels were relatively better than non-nodulated plants. This improvement was comparatively less in the presence of *M. javanica*. Cow-pea plants inoculated with *R. leguminosarum* and *M. javanica* in fly ash treatments (20 and 40%) showed a significant enhancement in all the parameters as compared to the inoculated plants grown in non-amended soils. These effects were, however, reduced at 60% fly ash level. The differences in plant growth, yield etc. of the nematode and bacteria inoculated plants in 100% fly ash level were non-significant. So, it is obvious that mutual effects of *R. leguminosarum* and *M. javanica* were adversely affected by higher levels of fly ash particularly at 100% fly ash. Higher levels of fly ash, due to accumulation of toxic substances, may have suppressive effect for microbial activity (Wong and Wong 1986) like root nodule bacteria and root-knot nematode (Singh *et al*. 1994).

Nitrogen content of leaves of cow-pea were progressively decreased with an increase in fly ash level (Table 8). Adverse effect on nitrogen content may have resulted from the absence of nitrogen in fly ash (Mishra and Shukla 1986). The reduction can also be indirectly correlated to inhibited root nodulation as reported by Khan *et al*. (1988) and accumulation of heavy metals (Eiceman and Vandiver 1983) in soils amended with fly ash. Plant growth, yield, leaf pigments and seed protein might be affected adversely through poor nitrogen availability in fly ash amended soils.

Table 1. Effect of fly ash (P) on length of shoot and root of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Shoot length (cm)							Root length (cm)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	41.40	47.78	50.89	42.74	37.44	33.42	42.23	28.10	30.14	31.94	28.90	22.94	24.54	28.52
P + R	50.72	52.74	55.24	52.37	43.39	34.10	48.09	31.80	33.40	35.60	31.42	29.44	24.34	31.00
P + MJ	35.84	43.24	47.80	35.82	35.34	33.14	38.53	24.30	29.10	30.10	25.30	23.15	24.62	26.10
P + R+MJ	39.55	47.80	52.70	41.67	37.90	33.19	42.14	26.50	30.10	31.00	27.82	25.57	24.44	22.57
MM	41.88	47.82	51.66	43.15	38.52	33.46		27.68	30.69	32.16	28.36	26.53	24.49	

CD at P=0.05 Treat = 0.70, Fly ash = 0.89, Treat × Fly ash = 1.79 Treat = 0.88, Fly ash = 1.14, Treat × Fly ash = 2.26

Observations taken on 95th day of after sowing.

Table 2. Effect of fly ash (P) on fresh weight of shoot and root of cow-pea in presence and absence of *M. javanica* (MJ) and R. *leguminosarum (R).*

Treatments	Shoot fresh weight (g)							Root fresh weight (g)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	30.21	31.47	34.19	31.47	28.70	25.54	30.26	18.90	19.74	20.48	19.21	18.20	17.84	19.86
P + R	41.72	43.40	44.24	42.04	36.74	27.04	39.20	22.67	23.04	24.47	22.87	21.90	18.10	22.18
P + MJ	23.75	25.67	28.79	24.17	23.70	23.54	24.94	16.37	18.42	19.22	16.94	16.24	16.80	17.32
P + R+MJ	32.20	33.17	33.98	31.87	28.72	25.14	30.85	19.12	19.20	19.68	19.22	18.04	17.17	18.74
MM	31.97	33.43	35.30	32.39	29.47	25.32		19.27	20.10	20.96	19.56	18.57	17.48	
CD at P=0.05	Treat = 1.71, Fly ash = 2.28, Treat × Fly ash = 4.54							Treat = 0.13, Fly ash = 0.17, Treat × Fly ash = 0.34						

Table 3. Effect of fly ash (p) on dry weight of shoot and root of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Shoot dry weight (g)							Root dry weight (g)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	7.18	8.10	9.47	8.14	7.04	6.98	7.82	3.98	4.77	5.54	4.04	3.77	3.12	4.20
P + R	8.93	9.94	10.88	9.17	8.03	6.67	8.94	5.23	6.17	7.15	5.94	4.98	3.74	5.54
P + MJ	6.12	7.87	8.07	9.04	5.99	5.74	7.24	3.10	4.10	5.04	3.97	3.01	3.00	3.70
P + R+MJ	7.86	7.97	8.98	7.09	6.72	5.82	7.56	4.14	4.82	5.47	4.01	3.90	3.18	4.25
MM	7.52	8.47	9.35	8.59	6.95	6.30		4.11	4.97	5.80	4.49	3.92	3.26	
CD at P=0.05	Treat = 0.23, Fly ash = 0.31, Treat × Fly ash = 0.59							Treat = 0.22, Fly ash = 0.27, Treat × Fly ash = 0.56						

Table 4. Effect of fly (P) ash on number of flowering and fruiting of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Flowering							Fruiting						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	75.80	80.20	90.60	78.20	75.50	73.30	78.93	36.0	41.8	44.0	37.30	34.7	33.0	37.8
P + R	114.70	120.40	125.40	115.50	97.80	84.70	109.25	71.8	77.8	79.2	70.8	63.9	56.7	70.03
P + MJ	60.30	78.40	84.40	69.40	63.40	60.30	69.37	24.6	33.4	40.1	26.2	25.4	24.2	28.98
P + R+MJ	71.40	81.50	89.90	70.30	67.30	65.40	74.30	33.2	37.8	43.2	33.9	32.0	31.0	35.17
MM	80.55	90.13	97.58	83.35	76.00	70.93		41.4	47.7	51.6	42.0	39.0	36.2	
CD at P=0.05	Treat = 2.37, Fly ash = 3.05, Treat × Fly ash = 6.11							Treat = 1.54, Fly ash = 1.99, Treat × Fly ash = NS						

Table 5. Effect of fly ash (P) on chlorophyll a and b of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Chlorophyll a (mg/g)							Chlorophyll b (mg/g)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	0.984	1.042	1.242	1.110	0.924	0.813	1.019	0.847	0.913	1.132	0.820	0.788	0.673	0.871
P + R	1.124	1.492	1.567	1.217	0.998	0.819	1.203	0.974	1.182	1.312	0.994	0.923	0.694	1.013
P + MJ	0.718	0.897	1.047	0.813	0.765	0.755	0.833	0.629	0.713	0.994	0.694	0.610	0.610	0.708
P + R+MJ	0.887	0.446	1.204	0.900	0.889	0.823	0.950	0.834	0.911	1.130	0.873	0.784	0.613	0.858
MM	0.928	1.107	1.265	1.010	0.894	0.803		0.857	0.930	1.142	0.858	0.776	0.646	
CD at P=0.05	Treat = 0.24, Fly ash = 0.031, Treat × Fly ash = 0.062							Treat = 0.009, Fly ash = 0.012, Treat × Fly ash = 0.041						

Table 6. Effect of fly ash (P) on total chlorophyll and carotenoid content of leaves of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Total chlorophyll (mg/g)							Carotenoid (mg/g)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	1.930	2.134	2.413	2.113	1.984	1.467	2.007	0.487	0.513	0.710	0.489	0.430	0.392	0.504
P + R	2.197	2.718	2.918	2.317	1.993	1.432	2.263	0.528	0.621	0.698	0.571	0.509	0.417	0.557
P + MJ	1.452	1.712	2.134	1.924	1.417	1.431	1.678	0.365	0.417	0.521	0.392	0.351	0.319	0.394
P + R+MJ	1.821	2.104	2.410	2.104	1.615	1.417	1.912	0.417	0.503	0.700	0.419	0.410	0.392	0.474
MM	1.850	2.167	2.470	2.115	1.752	1.437		0.449	0.514	0.657	0.468	0.425	0.380	
CD at P=0.05	Treat = 0.004, Fly ash = 0.007, Treat × Fly ash = 0.011							Treat = 0.003, Fly ash = 0.003, Treat × Fly ash = 0.006						

Table 7. Effect of fly ash (p) on soluble and insoluble protein contents of seeds of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Soluble protein (%)							Insoluble protein (%)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	11.19	11.74	11.87	11.16	10.84	10.08	11.15	13.62	14.12	14.94	13.68	12.60	11.98	13.41
P + R	12.24	12.36	12.57	12.12	11.93	10.10	11.89	14.67	14.96	15.38	14.70	13.14	11.87	14.12
P + MJ	10.79	11.04	11.53	10.82	10.07	10.02	10.71	12.91	13.90	14.14	12.97	12.21	11.83	12.99
P + R+MJ	11.04	11.00	11.21	11.08	10.80	10.10	10.87	13.66	14.43	15.99	13.69	12.58	11.90	13.71
MM	11.32	11.54	11.80	11.30	10.91	10.08		13.72	14.35	15.11	13.76	12.63	11.90	
CD at P=0.05	Treat = 0.05, Fly ash = 0.05, Treat × Fly ash = 0.11							Treat = 0.07, Fly ash = 0.09, Treat × Fly ash = 0.17						

Table 8. Effect of fly ash (P) on total protein content of seeds and nitrogen content of leaves of cow-pea in presence and absence of *M. javanica* (MJ) and *R. leguminosarum* (R).

Treatments	Total protein (%)							Nitrogen (%)						
	0	20	40	60	80	100	MM	0	20	40	60	80	100	MM
P	24.60	25.86	26.81	24.84	23.44	22.06	20.13	4.48	3.98	3.18	2.98	2.68	2.50	3.30
P + R	26.91	27.32	27.95	26.82	25.07	21.97	26.01	5.92	4.92	4.04	3.93	3.10	2.58	4.08
P + MJ	23.70	24.94	25.67	23.79	22.28	21.85	20.37	4.34	3.88	3.10	2.90	2.60	2.39	3.20
P + R+MJ	24.70	25.43	27.20	24.77	23.38	22.00	24.58	5.04	4.14	3.34	3.06	2.73	2.40	3.45
MM	24.98	25.89	26.91	25.06	23.54			4.95	4.23	3.42	3.22	2.78	2.47	
CD at P=0.05	Treat = 0.09, Fly ash = 0.11, Treat × Fly ash = 0.23							Treat = 0.03, Fly ash = 0.03, Treat × Fly ash = 0.03						

Reduction in nitrogen content of cow-pea leaves was comparatively less in the presence of root-nodule bacteria. The reverse was true in presence of *M. javanica*. Root nodule bacteria and root-knot nematode both together could cause more reduction in nitrogen content than in plants inoculated with *R. leguminosarum*. Singh *et al*. (1994) have also reported that in soyabean, nitrogen content was decreased gradually with an increase in the fly ash concentration.

The study showed that fly ash amendment of soil was beneficial for plant growth and yield of cow-pea at 20 and 40% being maximum at 40% level, in presence or absence of *M. javanica* and/or *R. leguminosarum*. At the initial (20 and 40%) levels, the beneficial effects of fly ash were suppressed by the presence of *M. javanica* but reverse was true with root-nodule bacteria. However, fly ash at the higher levels (80 and 100%) suppressed the positive effects of *R.*

leguminosarum and also increased the negative effects of *M. javanica*.

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