Nematol. medit. (2001), 29: 215-218

Plant Pathology and Plant Nematology Laboratories, Department of Botany, Aligarh Muslim University, Aligarh-202002, India

IMPACT OF FLY ASH ON HATCHING, PENETRATION AND DEVELOPMENT OF ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

by

A. TARANNUM, A. A. KHAN, I. DIVA and B. KHAN

Summary. Fly ash extract significantly impaired the hatching of *Meloidogyne javanica* juveniles, the inhibition in hatching being proportional to the concentration of the extract. Soil application of fly ash in different doses (0.0%, 25%, 50% and 100%) inhibited root penetration by juveniles, with penetration being inversely related to fly ash ratios. Penetration was completely suppressed at 100% concentration. All the rates suppressed the development of juveniles in chickpea roots. At lower levels (25%, 50%) of mixtures low numbers of J_2 developed to the mature female stage.

Fly ash is a major particulate air pollutant, generated during combustion of coal in coal-fired power plants. Fly ash emanating from the stack of thermal power plants settles on plant surfaces and soil. Its concentration in soil gradually increases and over a period of time the colour and soil texture are changed. Recently fly ash has been used as a fertilizer in cultivated fields (Khan *et al.*, 1997). It appears to suppress plant nematode activity such as reproduction and disease intensity depending on its concentration (Singh *et al.*, 1994; Khan *et al.*, 1997). Experiments were conducted to determine the effect of fly ash on juvenile hatching, root-penetration and post-penetration development of *Meloidogyne javanica* (Treub) Chitw. in the roots of chickpea (*Cicer arietinum* L.).

Materials and methods

For the hatching experiment, 250 g of fly ash were added to one litre of distilled water and kept overnight in the laboratory. This suspension was filtered to obtain the standard fly ash extract. Different dilutions were prepared from the standard extract (Table I). Ten ml of each of the different dilutions were poured into 5 cm diameter Petri dishes. Five similar sized egg masses of *M. javanica* obtained from root cultures were added to Petri dishes kept at room temperature (25-27 °C). Treatments were replicated five times. Petri dishes receiving distilled water only served as controls. After five and seven days hatched juveniles were counted using a stereoscopic microscope. Means of replicates of the various treatments were calculated and per cent inhibition compared to the control were determined.

Egg masses of *M. javanica* were collected from the roots of tomato in a glasshouse and incubated in sterile water in an incubator at 25 °C for 72 h. Hatched juveniles were collected in a water suspension and their number/ml were standardized by counting ten, 1 ml samples.

For the root penetration study different ratios of fly ash were mixed with autoclaved field soil (Tables II and III). The different percentages of

TABLE I - *Effect of different fly ash-extract concentrations on hatch of* Meloidogyne javanica *in Petri dishes.*

Fly ash extract concentration (%)	Hatching after		% Inhibition compared to controls after	
	5 days	7 days	5 days	7 days
0	485	765	–	–
25	307	387	37	49
50	75	80	85	90
75	10	12	98	98
100	0	0	100	100
L.S.D. (P=0.05)	48	81	–	–
L.S.D. (P=0.01)	80	135	–	–

TABLE II - *Effect of different levels of fly ash on percent penetration of* M. javanica *juveniles in chickpea roots in pots.*

Fly ash extract concentration (%)	Time (day)			
	1	2	3	4
0	20.2	30.4	36.0	40.1
25	10.6	13.3	17.8	20.2
50	8.3	9.4	12.6	15.3
75	0.0	0.0	0.5	1.2
100	0.0	0.0	0.0	0.0
L.S.D. (P=0.05)	1.37	2.26	2.73	4.23
L.S.D. (P=0.01)	2.00	3.25	3.98	6.16

fly ash 25, 50, 75, 100 and field soil were mixed in 7 cm disposable cups. Cups containing field soil only served as controls. Three surface sterilized (dipped in 0.01% $HgCl_2$ for 15 min.) seeds of chickpea, cv. BG-383, were sown per cup. There were 20 cups for each treatment and a total cups (5 treatments x 4 times x 5 replicates). After emergence seedlings were thinned to one seedling/cup. One week later each seedling was inoculated with 500 freshly hatched *M. javanica* juveniles. Cups were placed on a glasshouse bench at 25-27 °C. Five seedlings from each treatment were harvested at different time intervals (Table II). Roots were thoroughly washed with tap water to remove soil particles. Roots were cut into 10 mm segments and gently boiled in a solution of acid fuchsin (0.1%) + lactophenol. Root pieces were observed separately with the aid of a stereoscopic microscope for the presence of juveniles. Per cent penetration was calculated for each treatment.

Fly ash and autoclaved field soil were mixed and one kg mixtures were prepared and placed in 15 cm clay pots. Pots containing field soil only served as control. There were 20 pots for each treatment and a total 100 pots (5 treatments x 4 weeks x 5 replicates). Pots were maintained on a glasshouse bench at 25-27 °C. Five surface sterilized chickpea seeds were sown in each pot. After emergence seedlings were thinned to one seedling per pot. Five days after thinning, each pot was inoculated with 1000 *M. javanica* juveniles obtained from single egg mass population. Five plants from each treatment were harvested at different time intervals to examine nematode development (Table III). Roots were washed, cut into 10 mm pieces and boiled in 0.1% acid fuchsin + lactophenol solution. Different stages of juveniles present in the root pieces were counted using a stereoscopic microscope. All data were statistically analysed.

TABLE III - *Effect of different levels of fly ash on the development of* M. javanica *on chickpea in pots.*

Time (week)	Concentration (%)	Stage of development			
		J_2	J_3/J_4	Premature ♀	Mature ♀
One	0	125	370	–	–
	25	260	102	–	–
	50	236	53	–	–
	75	20	0	–	–
	100	–	–	–	–
L.S.D. (P=0.05)	–	60.0	22.3	–	–
L.S.D. (P=0.01)	–	99.5	37.0	–	–
Two	0	104	125	203	–
	25	128	145	45	–
	50	135	152	2	–
	75	21	5	–	–
	100	–	–	–	–
L.S.D. (P=0.05)	–	11.3	8.9	6.5	–
L.S.D. (P=0.01)	–	18.8	14.7	10.8	–
Three	0	–	139	175	105
	25	37	185	63	7
	50	86	145	15	2
	75	25	12	3	–
	100	–	–	–	–
L.S.D. (P=0.05)	–	21.1	10.6	8.2	9.8
L.S.D. (P=0.01)	–	35.0	17.7	13.7	16.2
Four	0	–	–	72	207
	25	–	125	94	28
	50	17	120	73	19
	75	5	21	7	–
	100	–	–	–	–
L.S.D. (P=0.05)	–	–	11.3	12.5	13.0
L.S.D. (P=0.01)	–	–	18.8	20.4	21.6

Results and discussion

All fly ash extract concentrations significantly (P=0.01) reduced juvenile hatch. Numbers of hatched juveniles were less as compared to control at both time intervals. Inhibition in juvenile hatching in the treatments compared to controls was directly proportional to the extract concentration at both time intervals. However, 100% fly ash extract completely suppressed juveniles hatching (Table I). Toxic compounds like dibenzofuran, dibenzo-p-dioxime and heavy metals are reported to be present in fly ash (Helder *et al.*, 1982) which may have suppressed hatching.

Fly ash was found to be toxic to the nematodes at all concentrations used in the soil. Soil mixtures of fly ash significantly suppressed penetration of *M. javanica* juveniles in chickpea roots compared to the control, at all time inter-

vals. Root penetration was inversely related to fly ash concentrations in soil. At 75%, root penetration by the juveniles was greatly suppressed. At 100% complete root penetration inhibition of *M. javanica* juveniles occurred (Table II).

Post-penetration development of *M. javanica* was adversely affected by the fly ash in soil. All ratios of fly ash caused suppression in post-penetration development of *M. javanica* J_2 in chickpea roots. Root penetration was also initially delayed as fewer juveniles penetrated roots in the first week. It gradually increased in subsequent weeks. Penetration was significantly less in all treatments compared to controls. The higher concentrations of fly ash completely suppressed root penetration and/or development of juveniles to mature females (Table III). The J_2 developed to J_3/J_4 stage at 25%, 50%, concentrations in the first week, however, numbers were significantly less compared to controls. In 75% fly ash ratio, J_2 did not develop into J_3/J_4 stages. In the second week J_2 developed to premature females through J_3/J_4 stages in control, while at lower levels (25%, 50%) of mixtures only few premature females were observed. At high fly ash concentration (75%), very low number of juveniles had penetrated the roots (Table III). In the third week, juveniles that penetrated roots were transformed into either J_3/J_4 stage or premature/mature female stages in control. Few juveniles reached the premature and mature stages at lower concentrations of fly ash (25% and 50%) and the remaining juveniles were either in J_2 or J_3/J_4 stages. At higher concentrations, there were no subsequent development of juveniles. In the fourth week, all J_3/J_4 that developed into premature stages were comparatively less than premature to mature stages in controls. Moreover, half of them were still at J_2 or J_3/J_4 stages (Table III). The highest level of fly ash (100%) was found to be highly toxic to the nematode juveniles.

Acknowledgement. The authors wish to dedicate this paper to our Beloved Research Supervisor, Late Prof. M. Wajid Khan, ex-chairman and Section Incharge of Nematology, who left to all of us on 30.1.2001. We pray to "Almighty Allah" to give him eternal peace and better place in Heaven.

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Accepted for publication on 19 June 2001.