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FLY ASH EFFECT ON HATCHING, MORTALITY AND PENETRATION OF ROOT-KNOT NEMATODE (*Meloidogyne incognita*) IN PUMPKIN ROOTS

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Abstract

An experiment was conducted to observe the effect of fly ash on hatching, mortality and penetration of root-knot nematode (*Meloidogyne incognita*) in pumpkin roots. For hatching experiment different fly ash-extract concentrations (5, 10, 20, 30, 40, and 50%) were prepared. Hatching was significantly reduced in all concentrations, maximum being at 50% concentration. The mortality (%) of juveniles was observed in 1, 2, 3, 4, 5, 6 and 7th days with different levels (5, 10, 20, 30, 40 and 50 %) of fly ash-extract. All the levels were found harmful to juveniles. As the level was increased, the killing percentage of juveniles was also increased. Highest mortality was observed in 7th day with 50% level.

For the penetration experiment, fly ash was mixed with soil to prepare different concentrations (5, 10, 20, 30, 40, and 50%). Seeds of pumpkin were grown in coffee cups filled with different mixtures. At two leaf stage, seedlings were inoculated with 2000 larvae. The penetrated larvae in roots were observed after 1, 2, 3, 4, 5, 6 and 7 days. Root penetration was found inversely proportional to concentration. Significant results in the suppression of nematode penetration were noted up to 40% concentration. However, none of the juveniles was penetrated at 50% concentration.

Keywords: Fly ash, Hatching, *Meloidogyne incognita*, Mortality, Penetration, Pumpkin

Introduction

Pollution is one of the major problem of whole over the world, increasing with every passing year and causing very serious and irremediable damage to the earth. There are five basic types of environmental pollution like soil, air, water, light and noise (Anonymous, 2016). It emerged as a serious problem in the past few decades. By increasing air pollution is seen in lung cancer, asthma, allergies, and various breathing problems along with severe and irreversible damage to plants and animals. Environmental pollution defined as unfavorable alterations in our surroundings resulting from industrialization and modern lifestyle.

Air pollution is by far the most harmful form of pollution in our environment. Air pollution is caused by the injurious smoke emitted by cars, buses, trucks, trains, and factories. There are two types of air pollutants like gaseous and particulate air pollutants namely, sulphur dioxide, carbon monoxide and nitrogen oxides, fly ash, cement dust, respectively. The particulate air pollutants include fly ash, brick kiln, dust of lime, pesticides, cement, metals, textile, fumes, mist, vapors etc. and are released by both natural and anthropogenic sources. Various ecological and environmental problems are caused by these particulate air pollutants, however at low level, fly ash has been found beneficial to plants. In thermal power plants fly ash is wastage of coal by combustion and coal is used to generate the electricity. In India the Indian coal constitutes of 30-40% fly ash (Kumar et al., 2000) and fly ash is being produced 100 million tons per year from the thermal power plants. It is likely to exceed 140 million tons by the year 2020. Fly ash has been found beneficial to plant growth because it includes several nutrients (Adriano *et. al.,* 1980). At 40% level of fly ash in soil, it increases growth and yield of tomato, maize, okra, wheat, cucumber and potato (Khan and Khan, 1996; Raghav and Khan, 2002; Kausar, 2007; Khan, 2007.)

Plant-parasitic nematodes are one of the major agricultural pathogens, attack plants and causes crop loss all over the world. Root-knot nematode is the most damaging plant-parasitic nematode. It occurs throughout the world but are found more frequently and in greater numbers in areas with hot and warm climates and short and mild winters. There are about 107 species at present included in the genus *Meloidogyne* i.e. *M. incognita (*Kofoid and White) Chitwood, *M. javanica* (Trueb) Chitwood, *M.arenaria* (Neal) Chitwood and *M. hapla* Chitwood are recognized as four major species of *Meloidogyne* as they are most common and damaging (Taylor et al. 1982). *M. incognita* is very common on vegetables in and around Aligarh district in Uttar Pradesh, India (Khan, 2007). For controlling this nematode with non-conventional method is a big task. However fly ash has been found effective against root-knot nematodes (Tarannum et al., 2001; Iram, 2006; Rizvi and Khan, 2009). Therefore, it was planned to observe the effect of fly ash on hatching, mortality and penetration of juveniles in pumpkin roots.

Materials and Methods

Fly ash was selected as major particulate air pollutant for present study, *M. incognita* as a test pathogen and pumpkin as a test plant. For conducting different experiments, fly ash was collected in gunny bags from Thermal Power Plant, Kasimpur, situated 18 km away from Aligarh and brought to the laboratory of Department of Botany, A.M.U.

Preparation of inoculums

Collection of pure population, inoculums was prepared by incubating the egg masses of pure *M. incognita* in distilled water. The freshly hatched second stage (J_2) juveniles of *M. incognita* were collected as water suspension and the number of J_2 counted in ten 1ml samples from the suspension. The average numbers of J_2 were used to represent the number of second juveniles (J_2) per ml of suspension.

Hatching

For hatching experiment, fly ash-extract was prepared by adding two liters distilled water (D.W.) to one kg of fly ash for overnight. After filtration, following dilutions were prepared from the standard extract obtained.

F1= 100 ml distilled water (D.W.) (0%conc.) Control
F2= 5 ml fly ash-extract + 95 ml D.W. (5% conc.)
F3= 10 ml fly ash-extract + 90 ml D.W. (10% conc.)
F4= 20 ml fly ash-extract + 80 ml D.W. (20% conc.)
F5= 30 ml fly ash-extract + 70 ml D.W.(30% conc.)
F6= 40 ml fly ash-extract + 60 ml D.W. (40% conc.)
F7= 50 ml fly ash-extract + 50 ml D.W. (50% conc.)

Five average sized egg masses of *M. incognita* obtained from pure population were placed in 5 cm diameter petridishes containing 10 ml of these different dilutions. Each treatment was replicated five times. Petridishes containing only distilled water served as control. The petridishes were kept at room temperature 25-27C. Hatched juveniles were counted at different intervals (1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} days) under stereoscopic microscope. Mean was taken and percent inhibition over control was calculated. Data were analyzed statistically for significance.

Mortality

For mortality experiment, fly ash-extract was prepared as in case of hatching experiment. However, the different dilutions prepared from the standard extract were of double strength.

F1= 100 ml distilled water (D. W.) (0% conc.) Control
F2= 10 ml fly ash-extract + 90 ml D.W. (For 5% conc.)
F3= 20 ml fly ash-extract + 80 ml D.W. (For 10% conc.)
F4= 40 ml fly ash-extract + 60 ml D.W. (For 20% conc.)
F5= 60 ml fly ash-extract + 40 ml D.W. (For 30% conc.)
F6= 80 ml fly ash-extract + 20 ml D.W. (For 40% conc.)

F7= 100 ml fly ash-extract only (For 50% conc.)

5 ml nematode suspension containing about 100 juveniles of *M. incognita* were transferred separately to each 5 ml dilution (double strength) placed in 5 cm diameter petridishes. Each treatment was replicated five times. Petridishes containing only distilled water served as control. Dead juveniles were counted at different intervals (1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} day) under stereoscopic microscope and mortality percentage was calculated. Data were analyzed statistically for significance.

Penetration of juveniles

For the penetration, fly ash was mixed with autoclaved soil to obtain following levels (w/w).

F1= Control (only autoclaved soil)

F2= 5% fly ash + 95% autoclaved soil

F3= 10% fly ash + 90% autoclaved soil

F4= 20% fly ash + 80% autoclaved soil

F5= 30% fly ash + 70% autoclaved soil

F6= 40% fly ash + 60% autoclaved soil

F7= 50% fly ash + 50% autoclaved soil

Disposable cups of 7cm size were filled with different fly ash mixtures. Total 140 cups (7 treatments, 5 replicates, 4 intervals) were prepared. Seeds of pumpkin were directly sown to each cup. 2000 freshly hatched juveniles of *M. incognita* were inoculated in 15 days old seedlings. In glasshouse cups were placed on bench at 26-27ºC.Five seedlings from each treatment were harvested carefully from the cups at different intervals (1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} days) and the penetrated juveniles were observed. For avoiding the soil particles roots of the seedling were thoroughly washed under tap water. The roots were cut and boiled gently in acid fuchsin (0.1%) + lactophenol solution. Each root was observed under stereoscopic microscope and penetrated juveniles were counted. Then percent penetration was calculated for each treatment. Data were analyzed statistically for significance.

Results

Data presented in table 1 shows that all the concentrations of fly ash-extract significantly impaired the hatching of juveniles as compared to control at all-time intervals. The lowest hatching inhibition was recorded 10.0 and highest 97.8 after 7^{th} day at 5% and 50% concentrations, respectively. All concentrations of fly ash-extract were harmful to *M.incognita*. As the concentration of the extract increased, the hatching of juveniles were decreased. Thus, hatching was inversely proportional to the concentration as well as with the number of days.

Tables 1: Effect of different concentrations of fly ash-extract on hatching of *M.incognita* juveniles

Concentration (%)	Percent inhibition over control													
	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day	
	H	I	H	I	H	I	H	I	H	I	H	I	H	I
C	315	-	390	-	470	-	570	-	455	-	490	-	550	-
5	260	17.7	295	24.4	320	31.9	370	35.9	400	12.9	425	13.8	490	10.0
10	220	30.2	250	35.9	280	40.4	325	42.9	345	24.9	390	20.4	400	27.8
20	180	42.9	210	46.2	230	51.6	255	55.3	295	35.7	275	43.9	285	48.9
30	130	58.7	140	64.1	160	65.9	170	70.9	189	58.5	153	68.8	135	75.5
40	170	77.8	80	79.5	83	82.3	90	84.2	95	79.2	78	84.0	60	89.0
50	20	93.6	24	93.9	26	94.5	28	95.9	25	94.5	20	95.9	15	97.8
$LSD_{0.05}$	24.23		26.63		28.91		32.1		35.34		37.32		39.42	

H-Hatching, I-Inhibition, C-control

Each value is mean of five replicates in the above table.

The mortality of *M.incognita* juveniles was observed at different levels as shown in table 2. All the levels were found harmful to juveniles. As the concentration of extract was increased the killing percentage was also increased. Thus the mortality of juveniles was directly proportional to the concentration as well as number of days.

Table 2: Effect of different concentrations of fly ash-extract on mortality of *M. incognita* juveniles

Concentration (%)	Mortality percent						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
control	4.20	5.40	7.40	9.40	11.00	15.77	20.58
5	6.60	8.60	10.00	12.00	16.96	30.86	38.96
10	8.40	10.80	12.60	13.20	24.27	41.00	51.03
20	9.80	11.20	13.20	16.00	35.08	55.04	63.01
30	12.40	13.20	16.40	20.00	41.79	68.30	78.31
40	17.20	19.00	24.40	28.00	53.66	75.90	89.99
50	25.20	27.80	30.80	34.00	66.09	88.54	99.08
$LSD_{0.05}$	4.65	3.01	3.15	3.81	4.65	5.08	5.38

Each value is mean of five replicates in the above tables.

Fly ash was found to be toxic to the nematodes at all the levels in soil. Soil mixture of fly ash considerably suppressed the penetration of *M.incognita* juveniles in pumpkin roots compared

to the control, at all-time intervals. Root penetration was inversely related to fly ash concentrations in soil. At 40%, penetrations by the juveniles in roots were greatly suppressed. At 50% root penetration of *M.incognita* juveniles was very close to total suppression as shown in table 3.

Table 3: Effect of different concentrations of fly ash on penetration percent of *M. incognita* juveniles in pumpkin roots

concentration (%)	Penetration of juveniles (%)													
	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day	
	M	P (%)	M	P (%)	M	P (%)	M	P (%)	M	P (%)	M	P (%)	M	P (%)
control	219	21.9	260	26.0	313	31.3	386	38.6	419	41.9	460	46.0	491	49.1
5	178	17.8	196	19.6	209	20.9	201	20.1	204	20.4	225	22.5	366	36.6
10	176	17.6	183	18.3	198	19.8	249	24.9	288	28.8	315	31.5	349	34.9
20	146	14.6	156	15.6	195	19.5	234	23.4	256	25.6	298	29.8	321	32.1
30	128	12.8	149	14.9	166	16.6	155	15.5	198	19.8	215	21.5	242	24.2
40	110	11.0	125	12.5	138	13.8	166	16.6	176	17.6	182	18.2	199	19.9
50	98	9.8	108	10.8	124	12.4	141	14.1	158	15.8	163	16.3	178	17.8
$LSD_{0.05}$	10.10		11.56		12.15		10.2		12.2		10.1		11.8	

M=Mean, P=Penetration,

Each value is mean of five replicates in the above tables.

Discussion

Although, fly ash is considered as particulate air pollutant, however, in recent years, it is used in field for production as well as friendly control measures for soil pathogens including nematodes (Adriano et al., 1980; Iram, 2010). Root-knot nematodes are the most damaging pathogen to the plants especially vegetable crops throughout the world.

In the present study, it was attempted to evaluate the potential of fly ash effect on hatching, mortality and penetration of root-knot nematode (*M.incognita*) in pumpkin roots. The observations showed that all the fly ash-extract levels inhibited the hatching of *M. incognita*. This might be due to presence of some toxic constituents and changes in pH level (Helder et al., 1982; Khan, 2007) which were harmful to juveniles. Similar results were also obtained earlier by Tarannum et al., 2001; Iram (2006) on *M. javanica* and *M. incognita* Race 1 respectively. Rizvi (2008), also observed the inhibition in hatching of *M. javanica* juveniles when exposed to fly ash and brick kiln dust-extracts levels. Present study also showed the inhibition in hatching of juveniles against the extract.

In the present study all the levels of fly ash-extract were found to have potential to kill the juveniles. The rate of the juveniles killing was directly proportional to concentration and time

intervals. This might be due to presence of toxic compounds i.e. dibenzofuron, dibenzo-p-dioxine and sulphur and chlorides (Helder et al., 1982). The rise in pH (9.18) level also may be one of the reasons for mortality. Similar results were observed by Singh et al. (2011) in *M. incognita*. Recently, Kausar (2007) has also reported the harmful effect of fly ash-extract on juvenile's mortality of seed gall nematode (*Anguina tritici*). Rizvi and Khan (2009) have also observed the mortality of *M. javanica* in different levels of fly ash and brick kiln dust-extracts.

The penetrations of juveniles of *M. incognita* in the roots of pumpkin were suppressed greatly under the influence of fly ash amended soils. As the levels of fly ash increased, the penetration was decreased. This might be due to toxic effect of fly ash to nematodes. Similar results have also been observed by Tarannum et al. (2001) on *M. javanica* in chickpea roots and by Rizvi (2008) in brinjal roots. Edongali et al. (1982) stated that juvenile's penetration is affected by the concentration of the different elements, perhaps the type of element present in the soil solution.

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