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RESEARCH ARTICLE

Root-Knot Nematode Impact on Some Leaf Epidermal Characters of Fly Ash Stressed Chick Peas

Dinesh Kumar Singh and Kamal Singh
Section of Environmental Sciences,
Department of Botany, D.S. College, Aligarh (U.P.)
Email: singhdk.singh955@gmail.com, singhkamal.alg@gmail.com

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ABSTRACT

All epidermal characters except stomatal index were found greater in Rhizobium leguminosarum treated than non-treated plants but opposite result was found with Meloidogyne incognita. In joint treatment of bacteria and nematode, all studied leaf epidermal characters were found stranded in between the root-nodule bacteria treated and root-knot nematode inoculated plants. Leaf epidermal characters i.e. number of stomata, epidermal cells and trichomes were increased in fly ash amended soil upto 40%, compared to controls. But maximum values of such characters were found at 20% fly ash amendments except stomatal index. Onward to 40% there was steep decrease in the characters at 80 and 100% fly ash amendments.

Key words: *Fly ash, Meloidogyne incognita, Rhizobium leguminosarum, chick pea*

INTRODUCTION

Root-knot nematodes are the major biological constraints that reduce per capita growth and yield of leguminous and non-leguminous crops (Rehman *et al.,* 2011). Out of more than 90 known species, four species of root-knot nematode viz. *Meloidogyne incognita* (Kofoid and White) Chitwood, *M. javanicav* (Treub) Chitwood, *M. arenaria* (Neal) chitwood and *M. hapla* chitwood, are considered as the dominating species due to their worldwide distribution, extensive host range and the damage quantified to different host crops. The average crop yield losses are estimated to be about 25% which ranged upto 40% in the individual fields (Sasser, 1980; Sasser and Carter, 1982). Root-knot nematodes also have a tendency to develop a relationship with root-nodule bacteria on the leguminous plants (Singh *et al.,* 1996; Siddiqui*et al.,* 2001).

Chickpea *(Cicer arietinum* L.) is the world's third most important leguminous crop (Dhar and Gupta, 1998). World production of chick pea has averaged about 8 million metric tons in the recent years (Anonymous, 2007). It is a source of high quality protein, and is known as "a poor man's meat" (Isabel and Garmen, 2003; Rincon *et al.,* 1998). Chickpea is rich source of complex carbohydrates, vitamins and minerals (Wang *et al.,* 2010).

The particulate air pollutants are major problem for the developing countries. Main particulate are pollutants are coal dust, metallic dust, lime dust, pesticide dust and fly ash. They are originated by natural or anthropogenic means (Das,1986). In India, fly ash are considered as major particulate problem, as several thermal power plants and fly ash are operating throughout the country. Most of the thermal power stations are coal based, which are consuming bituminous and sub-bituminous types of coal ash a fossil fuel. These varieties of fuel produce approximately 30-35% of fly ash after complete combustion. Currently, 100 million tons of fly ash is being generated annually in India with 65,000 acres of land occupied by ash ponds.

The main objective of the present work is to assess the effects of particulates (i.e. fly ash) on epidermal characters of root-knot nematode *(Meloidogyne incognita* race 1) infected and root-

nodule bacteria *(Rhizobium leguminosarum)* inoculated chickpea *[Cicer arietinum* (L.) cv. P-391] plants.

MATERIALS AND METHODS

Root-knot nematode culture:

Meloidogyne incognita (Kofoid and White) chitwood race 1, is one of the commonest root-knot nematode species in the Aligarh and the adjoining area. This major species of root-knot nematode was used in the experiment for experimental purpose. Roots of tomato or egg plant were surveyed in the agriculture fields for the root-knot nematode infection. The root-knot nematode infected roots were collected from the field and brought to the laboratory by putting their in polypacks in order to not to allow them (root) to dry. The species of root-knot nematode present in the collected samples were identified on the basis of the characteristics of the perineal patterns of the females. After species identification, roots infected with *M. incognita* were chopped and added to the pots containing seedlings of tomato, *Lycopersicon esculentum* Mill (cv. Pusa Ruby) or eggplant, *Solanum melongena* L. (cv. PusaKranti) growing in steam sterilized field soil.

Seeds of tomato or eggplant were first of all surface sterilized by putting in 0.01% $HgCl_2$ for 15 min and washed thoroughly in sterilized water afterward. Thereafter, surface sterilized seeds were sown in autoclaved soil filled clay pots having 30 cm diameter, in order to get adequate number of seedlings for further experimental use. Three to four days old seedlings were carefully uprooted from the parental stock and transplanted to the clay pots duly filled with autoclaved field soil. Single egg mass of the nematode, obtained from the roots of plants maintaining pure population of *M. incognita,* was injected in the soil by making a hole near the roots of each seedling in the pot. This way, single egg mass culture of *M. incognita* was established within 45 to 50 days after inoculation. Subculturing was done in the similar fashion after every 2 to 3 months by inoculating new tomato or eggplant seedlings with at least 15 egg masses per pot, each obtained from a single egg mass culture in order to maintain sufficient inoculum for further experimental studies.

Plant Culture and nematode inoculation:

Seeds of chickpea, *Cicer arietinum* (L.) cv. P-391 were procured from Chola Seed Centre, G.T. Road, Aligarh, U.P., India. Seeds were soaked in water for 24 h and then surface sterilized by 0.01% mercuric chloride ($HgCl_2$ for 15 minutes. Five seeds of chickpea were sown separately in each clay pot (having upper diameter as 30 cm.) after surface sterilization. But prior to seeding, the clay pots were filled with autoclaved sandy loam field soil, having the physico-chemical properties as referred below under the heading of brick kiln dust. Seedlings of chickpea were thinned to one in order to maintain single healthy seedling per pot which were used for further experimental study. The pots were arranged on the glasshouse benches of botanical garden of D.S. College, Aligarh at 27±2°C and watered regularly after certain time intervals. The plants were harvested 120 days after sowing.

For inoculation of *M. incognita,* the soil around the roots was carefully moved aside without damaging the roots. The nematode suspension containing the second stage juveniles (J_2) of *M. incognita,* was taken in micropipette controller and poured around the roots of seedlings. After the addition of juvenile suspension, the soil was replaced. Inoculum density was 2000 J_2 per pot. The inoculation was done after two weeks of seed germination.

Root-nodule bacteria:

Commercial culture of *Rhizobium leguminosarum*strain Jordan, obtained from the Agriculture Farm House, Quarsi, Ramghat Road, Aligarh (U.P.), was used in the experiment. Prior to sowing, seeds of chickpea were treated with a mixture of sugar, water and *R. leguminosarum* culture, followed by drying in shade for half an hour.

Fly ash:

Fly ash used in the present experiment was collected from the thermal power plant, Kasimpur, a place nearly 12 Km away from the Aligarh,(U.P.),India. The thermal power plant at Kasimpur is of 530 megawatt capacity and consumes tentatively 3192 metric tons of bituminous type of coal daily as fuel. The field soil (contains 66% sand, 24% silt, 8% clay, 2% organic matter and pH as 7.7) and fly ash were mixed in requisite quantities to obtain 0,20,40.60.80 and 100% (w/w) levels of fly ash. The field soil without fly ash was taken as check or control. The mixture of soil and fly ash were filled in clay pots. The pots were filled with each of the above 4 Kg mixture separately and were allowed to be autoclaved by putting than in a autoclave. The pressure of the autoclave was maintained at 20 lb for 20 minutes in order to sterilize the soil and /or fly ash were mixed in the following proportions to get 4000 gm (4 Kg) of mixture for each treatment separately.

Fly ash % level		Fly ash weight		Field soil weight	Total mixture weight
0%	=	0.000 g	+	4000 g	4 kg
20%	=	800 g	+	3200 g	4 kg
40%	=	1600 g	+	2400 g	4 kg
60%	=	2400 g	+	1600 g	4 kg
80%	=	3200 g	+	800 g	4 kg
100%	=	4000 g	+	0.000 g	4 kg

After proper mixing, clay pots were filled with 4 kg of each type of mixture. Treatment without fly ash served as control. Each treatment was replicated five times. So the total 120 pots were prepared for the experiment (24 treatments x 5 replicates). The following were the fly ash unamended (4 set with five replicates) and amended (20 set with five replicates) treatments.

TREATMENTS

Fly ash unamended (controlled) treatments:

Plant
Plant + bacteria (= *R. leguminosarum*)
Plant + nematode (= *M. incognita*)
Plant + bacteria + nematode

Fly ash amended treatments:

Plant + 20% fly ash
Plant + 20% fly ash + bacteria
Plant + 20% fly ash + nematode
Plant + 20% fly ash + bacteria + nematode
Plant + 40% fly ash
Plant + 40% fly ash + bacteria
Plant + 40% fly ash + nematode
Plant + 40% fly ash + bacteria + nematode
Plant + 60% fly ash
Plant + 60% fly ash + bacteria
Plant + 60% fly ash + nematode
Plant + 60% fly ash + bacteria + nematode
Plant + 80% fly ash
Plant + 80% fly ash + bacteria
Plant + 80% fly ash + nematode
Plant + 80% fly ash + bacteria + nematode
Plant + 100% fly ash
Plant + 100% fly ash + bacteria

Plant + 100% fly ash + nematode
Plant + 100% fly ash + bacteria + nematode

After the termination of experiment (120 days after sowing), epidermal character were determined as per the procedure described below. All the data were analysed by using the Fischer (1950) factorial method. At the time of analysis, the data was splitted into two factors i.e. F_1 and F_2. The factor with fly ash treatment is considered as F_1 and that with different nematode and/or bacteria is considered as F_2. The L.S.D. was calculated for F_1 and F_2 separately as well as for F_1 x F_2 collectively.

EPIDERMAL CHARACTER

Freshly collected mature leaves from the unharvested plants were fixed and preserved in formalin-aceto alcohol (FAA) at the end of the experiment (Johansen, 1940).

Leaf peelings were prepared according to Ghouse and Yunus (1972) method. Preserved leaf pieces were boiled in 40% HNO_3 for 2 to 3 minutes. When epidermis of both the surfaces of leaves had separated, epidermal peelings were washed three times with water. The peeling was transferred to 20% KOH thereafter, for 15 minutes. The function of the KOH is to neutralize the HNO3. Now the leaf peeling was ready for staining.

Put the washed epidermal peelings for 10 min in 30% alcohol and transferred to 50% alcohol for 5 minutes. The peelings were then stained with bismark brown (prepared in 50% alcohol) for 12 hours. After 12 h, the peelings were washed thrice with 50% alcohol, and after 5 min interval, passed them (peelings) through a series of 70%, 90% and absolute alcohol + xylene and xylene. The peelings were finally mounted in Canada Balsam.

The slides of peelings were ready for the observation of stomata and trichomes. The slides were examined under light microscope. The number of stomata, epidermal cells and trichomes were counted on both (i.e. upper and lower) the surfaces of leaves and calculated in per cm^2 leaf surface.

After the calculation of number of stomata and epidermal cell, following formula was employed for the calculation of stomatal index (SI).

$$\text{Stomatal index} = \frac{\text{Number of epidermal cells}}{\text{Number of epidermal cells + number of stomatal cells}} \times 100$$

With the help of this formula, stomatal index (S.I.) was calculated for both (i.e. upper and lower) the surfaces of the leaves separately.

Table 1: Effect of fly ash amendment on the number of stomata of lower (abaxial) and upper (adaxial) surface of the chickpea leaves (in cm^2)

Treatments	Fly ash (%)							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	40.00	45.40*	51.20*	43.60*	30.80ns	23.78ns	39.13
	Upper surface	13.25	16.00*	20.00*	15.40*	10.25ns	6.08ns	13.50
P + R	Lower surface	46.50*	50.60*	55.80*	47.40*	35.70ns	27.00ns	43.83@
	Upper surface	16.75*	19.60*	22.25*	18.25*	13.40ns	9.80ns	16.68@
P + Mi	Lower surface	31.75ns	38.60ns	43.75*	33.64ns	26.50ns	18.65ns	32.15@
	Upper surface	10.20ns	13.37ns	17.50*	11.00ns	8.40ns	6.40ns	11.15@
P + R + Mi	Lower surface	38.25ns	44.00*	49.64*	39.75ns	29.10ns	22.60ns	37.22@
	Upper surface	13.50ns	17.50*	20.65*	15.60*	11.40ns	7.80ns	14.41@
Mean	Lower surface	39.13	44.65#	50.10#	41.10#	30.53#	23.01#	
	Upper surface	13.43	16.62#	20.10#	15.06#	10.86#	7.52#	

CD at 5% of lower surface– Fly ash (F_1) = 0.645, Treatment (F_2) = 0.795, Interaction (F_1 x F_2) = 1.590
CD at 5% of upper surface– Fly ash (F_1) = 0.247, Treatment (F_2) = 0.302, Interaction (F_1 x F_2)= 0.640

Table 2: Effect of fly ash amendment on the number of epidermal cells of lower (abaxial) and upper (adaxial) surface of the chickpea leaves (in cm^2)

Treatments	Fly ash (%)							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	200.00	254.50*	278.40*	224.20*	185.60ns	160.80ns	217.25
	Upper surface	66.25	92.25*	120.20*	80.25*	55.35 ns	48.40 ns	77.12
P + R	Lower surface	232.50*	280.38*	300.16*	250.00*	218.40*	190.80ns	245.37@
	Upper surface	85.75*	118.75*	145.05*	98.60*	70.62*	56.56 ns	95.89@
P + Mi	Lower surface	159.75ns	210.00*	235.60*	170.50 ns	140.40ns	125.20ns	173.58@
	Upper surface	53.00ns	70.86*	100.20*	60.70 ns	35.50 ns	27.80 ns	58.01@
P + R + Mi	Lower surface	193.25ns	230.50*	269.80*	210.00*	182.30ns	150.20ns	206.01@
	Upper surface	68.50ns	91.40*	120.10*	80.80*	55.20 ns	39.60 ns	75.93ns
Mean	Lower surface	196.38	243.85#	270.99#	213.68#	181.68#	156.75#	
	Upper surface	68.38	93.32#	121.39#	80.09 #	54.17#	43.09#	

CD at 5% of lower surface– Fly ash (F_1) = 3.494, Treatment (F_2) = 4.280, Interaction (F_1 x F_2) = 8.559
CD at 5% of upper surface– Fly ash (F_1) = 1.416, Treatment (F_2) = 1.734, Interaction (F_1xF_2) = 3.468

Table 3: Effect of fly ash amendment on the number of trichomes of lower (abaxial) and upper (adaxial) surface of the chickpea leaves (in cm^2)

Treat – ments	Fly ash (%)							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	260.50	312.40*	380.48*	250.80 ns	220.50ns	200.10ns	270.80
	Upper surface	215.75	250.35*	260.42*	210.75 ns	175.80ns	150.95ns	210.67
P + R	Lower surface	285.25*	350.50*	390.40*	288.50*	218.75ns	180.55ns	285.66@
	Upper surface	232.00*	270.60*	280.95*	214.65ns	180.90ns	160.65ns	223.29@
P + Mi	Lower surface	225.25ns	292.20*	300.25*	225.40 ns	180.25ns	162.60ns	230.99@
	Upper surface	185.20ns	220.50ns	240.75*	175.10 ns	162.60ns	150.00ns	189.03@
P + R + Mi	Lower surface	255.40ns	325.75*	345.60*	255.40 ns	190.75ns	171.91ns	257.47@
	Upper surface	210.20ns	245.50*	260.60*	190.40ns	170.62ns	155.81ns	205.52@
Mean	Lower surface	256.60	320.21#	354.18#	255.03 ns	202.56#	178.79#	
	Upper surface	210.79	246.74#	260.68#	197.73#	172.48#	154.35#	

CD at 5% of lower surface– Fly ash (F_1)= 4.406, Treatment (F_2)= 5.397, Interaction (F_1xF_2)= 10.793
CD at 5% of upper surface– Fly ash (F_1)= 3.381, Treatment (F_2)= 4.141, Interaction (F_1xF_2)= 8.283

Table 4: Effect of fly ash amendment on the stomatal index (SI) of lower(abaxial) and upper (adaxial) of the chickpea leaves.

Treat-ments	Fly ash (%)							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	16.67	15.13ns	15.53ns	17.41*	14.23ns	12.86ns	15.31
	Upper surface	16.66	14.78ns	14.26ns	16.10 ns	15.62ns	11.22ns	14.77
P + R	Lower surface	16.66 ns	15.28 ns	15.67 ns	15.93 ns	14.04ns	12.39ns	15.00@
	Upper surface	16.34 ns	14.16 ns	13.29 ns	16.61 ns	15.94ns	14.76ns	15.02@
P + Mi	Lower surface	16.57 ns	15.52 ns	15.66 ns	16.47 ns	15.87ns	12.96ns	15.51ns
	Upper surface	16.13ns	15.87ns	14.86ns	15.34 ns	19.13*	18.71*	16.67@
P + R + Mi	Lower surface	16.52ns	16.02ns	15.53ns	15.91 ns	13.76ns	13.07ns	15.14ns
	Upper surface	16.46ns	16.06ns	14.67ns	16.18ns	17.11ns	16.45ns	16.16@
Mean	Lower surface	16.61	15.49#	15.60#	16.43 ns	14.48#	12.82#	
	Upper surface	16.40	15.22#	14.27#	15.81 #	16.95#	15.29#	

CD at 5% of lower surface– Fly ash (F_1) = 0.203, Treatment (F_2)= 0.293, Interaction(F_1xF_2) = 0.585
CD at 5% of upper surface – Fly ash (F_1)= 0.244, Treatment(F_2)= 0.299,Interaction(F_1xF_2) = 0.599

* = data significant with 0% fly ash and at P treatment only at P = 0.05
NS= Not significant
@ = data significant within a column at P=0.05
# = data significant in a raw at P = 0.05
P = chickpea plant, R = *Rhizobium leguminosarum,* Mi = *Meloidogyne incognita* race 1

RESULT

All the epidermal characters were found greater in *Rhizobium leguminosarum* treated plants compared to non-treated plants. But opposite trend was found with the *Meloidogyne incognita*. All the epidermal characters in joint bacteria and nematode treatments were found in between (less than) root-nodule bacteria treated and (more than) root-knot nematode inoculated treatments (table 1-3). Since, stomatal index (SI) happens to be the percentage ratio of number of the epidermal cells to the total number of epidermal plus stomatal cells, so not much impact could be seen on indices (table 4).

When both surfaces of the chickpea leaves were examined for the different epidermal characters (i.e. number of stomata, epidermal cells, trichomes and stomatal index). They all were present in greater numbers on the upper than lower surfaces of the leaves (table 1-4).

All the epidermal characters were increased in fly ash amended soil upto 40% levels compared to controls. However, maximum value of their's (epidermal characters) was found at 20% fly ash amendments. Onward to 40%, there was steep decrease in the characters at 80 and 100% fly ash amendments.

DISCUSSION

The development of greater trichomes and/or hairs on the plant leaves. Leaf epidermal characters in terms of number of stomata, epidermal cells and trichomes were also increased significantly. Besides this, root-nodule bacteria also increased the photosynthetic pigments of the chickpea leaves, accompanied by amelioration of seed proteins and leaf nitrogen. These favourable effects were apparently due to root nodulation and symbiotic nitrogen fixation, which were beneficial for the plants (Fyson and Sperent. 1982; Singh and Prakash, 2008). Improved plant growth due to *R. leguminosarum* was reflected in all the considered epidermal parameters. Healthy leaves of chickpea plants, in presence of root-nodule bacteria, were probably need greater amount of gases for exchange so as to keep pace with high rate of metabolism. May be due to this fact, the leaves of plant might have developed greater number of stomata and thereby the surrounding epidermal cells. Healthy chickpea plants remains healthy probably by way of improvising the resistance power which a reflected back in the form of improved number of trichomes on both surfaces of the leave. Some workers have earlier reported the development of trichomes in favour of resistance development (Khan and Khan, 1994). Root-knot nematode attacks on several kind of crops and results enormous reduction to plant growth and yield (Sasser and Carter, 1982;. Koenning, *et al*., 1999). *M. incognita* also suppressed the plant growth of chickpea in the present sturdy. Reduction in plant growth due to M. incognita may have been happened by dysfunctioning of the absorption and supply of water and minerals to the infected plants because of various anatomical and biochemical transformations induced by the nematodes (Wilcox and Loria, 1987). Such plants, which were already under water stress, could not afford to bear with high number of stomata on their leaves and/or otherwise they will lose a huge quantity of water through transpiration. Such a factorial reason might have helped to trigger the mechanism in chickpea plants which favours the water conservation. Similar impact, like stomatal number, would obviously be gone on to the number of epidermal cells. Such nematode infected chickpea plants, which were in a poor state of health, could not develop high number of trichomes on their leaf surfaces and thereby show the weak resistant power against the biotic and abiotic stresses Fly ash application in the agriculture which is a fast emerging and promising field of research, does the dual function. On one hand, its

addition to soil improves the growth and yield of growth plants (Kalra *et al*., 1998; Rizvi and Khan ,2009; Singh et al., 2011) and on the other it controls the root-knot nematodes in their parasitic phase (Siddiqui and Singh, 2005). It acts as fertilizer if used judiciously (Varshney and Mathur, 2011). Chickpea Plants showed improved growth and yield in 20 and 40% fly ash amended soils. Fly ash contains utilizable plant nutrients (Druzina *et al*., 1993; Tejasvi, 2011) and its addition can enrich the soil in macro- and micronutrients which may have favourable effect on crop productivity (Martens and Beahm , 1978; Singh and Siddiqui , 2003). Different Plant Species have 'shown the luxuriant growth in fly ash amended soils (Singh et al., 2010; Varshney and Mathur, 2011). Addition of fly ash to soil can nutrilize the soil acidity and can increase the ion exchange capacity, Water holding Capacity and pore size (Elseewi et al,.1981; Siddiqui and Singh, 2005) which May ameliorate the plant growth and yield. Similar factors might have played some key role in improving the growth and biomass of the Chickpea plants. Improvement in plant yield leaf epidermal character, leaf pigments and seed proteins was recorded at 20,40 and 60% level, being maximum at 40% level. Further increase in fly ash level caused suppressions to all these parameters.

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