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RESEARCH ARTICLE

Impact of Brick Kiln Dust on Some Leaf Epidermal Characters of Root-Knot Nematode Infected Chick Peas

Dinesh Kumar Singh and Kamal Singh
Department of Botany, D.S. College Aligarh
Email: singhdk.singh955@gmail.com, singhkamal.alg@gmail.com

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ABSTRACT

Leaf epidermal characters i.e. number of stomata, epidermal cell and trichomes were increased in brick kiln dust amended soil upto 60%. But maximum values of epidermal characters were found at 20% brick-kiln dust amendments except stomatal index. Onward to 60% there was steep decrease in the characters at 80 and 100% brick kiln dust amendments. All epidermal characters except stomatal index parameters were found greater in Rhizobium leguminosarum treated plants compared to non-treated plant but opposite result was found with Meloidogyne incognita. In joint treatment of bacteria and nematode all leaf epidermal characters were found in between (less than) root-nodule bacteria treated and more than root-knot nematode inoculated plant.

Key words: *Brick kiln dust, Meloidogyne incognita, Rhizobium leguminosarum, chick pea*

INTRODUCTION

In India, brick kiln dust are considered as major particulate problem, as several thermal power plants and brick kilns are operating throughout the country. In developing countries like ours, brick is supposed to be the basic constructing material for pucca house construction. It is dream of each and every human being to construct a pucca house for the fulfillment of basic needs of life. Most of the brick kiln industries are established on the agricultural land of the rural or peri-urban areas (Gupta and Narayan, 2010). At present, large number of brick kiln industries are running throughout the country to meet the brick demand. There are tentatively 250 brick kilns operating in Aligarh district.

Chickpea *(Cicerarietinum*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).

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. javanica*(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 the crops. The average crop yield losses are estimated to be about 25% which ranged upto 60% 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).

The main objective of the present work is the effect of particulates (i.e brick kiln dust) on epidermal character of root-knot nematode *(Meloidogyne incognita* race 1) infected and root-nodule bacteria *(Rhizobium leguminosarum)* inoculated chickpea *[Cicerarietinum*(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, *Lycopersiconesculentum*Mill (cv. Pusa Ruby) or eggplant, *Solanummelongena*L. (cv. PusaKranti) growning 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, *Cicerarietinum*(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 (66% sand, 24% silt, 8% clay, 2% OM and pH 7.7). 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 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 (J2) 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 J2 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 before sowing.

Brick kiln dust:

The experiment was conducted in the glass house fabricated at the Department of Botany, D.S. College, Aligarh (U.P.), India. The site is situated 15 km away from the source of brick kiln dust. Brick kiln dust in the experiment was obtained from the brick kiln situated at ManzoorGarhi, Aligarh. Random sampling of brick kiln was done from brick kiln dust pond. Sampled brick kiln

dust was brought to the laboratory in different gunny bags. Then a composite sample was made by mixing the separately sampled brick kiln dust. This composite mixture was sterilized by putting the mixture filled bags in the autoclave. For the experiment to be done, the soil was collected from the agricultural field of suburban Aligarh. The soil was collected from 20 to 30 cm depth, after scrapping the upper flora and litter. The soil was sandy loam field soil (66% sand, 24% silt, 8% clay, 2% OM and pH as 7.7). This field soil was filled in gunny bags and was steam sterilized in the autoclave before incorporating with different levels of brick kiln dust. The pressure of the autoclave was maintained 20 lb continuously upto 20 minutes. After drying, the autoclaved soil is ready for getting mixed with brick kiln dust. The autoclaved field soil and brick kiln dust were mixed in the following proportions to get 4000 gm (4 kg) of mixture for each treatment separately.

Brick kiln dust % level	dust	Brick kiln 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 (having 30 cm of upper diameter) were filled with 4 kg of each type of mixture. Treatment without brick kiln dust served as control. Each treatment was replicated five times. So the total 120 pots were prepared for the experiment (24 treatments x 5 replicates). Soil was analyzed from each treatment before the seed sowing.

TREATMENTS

Unamended treatments:

Plant
Plant + bacteria
Plant + nematode
Plant + bacteria + nematode

Brick kiln dust amended treatments

Plant + 20% brick kiln dust
Plant + 20% brick kiln dust + bacteria
Plant + 20% brick kiln dust + nematode
Plant + 20% brick kiln dust + bacteria + nematode
Plant + 40% brick kilndust
Plant + 40% brick kiln dust + bacteria
Plant + 40% brick kiln dust + nematode
Plant + 40% brick kiln dust + bacteria + nematode
Plant + 60% brick kilndust
Plant + 60% brick kiln dust + bacteria
Plant + 60% brick kiln dust + nematode
Plant + 60% brick kiln dust + bacteria + nematode
Plant + 80% brick kiln dust
Plant + 80% brick kiln dust + bacteria
Plant + 80% brick kiln dust + nematode
Plant + 80% brick kiln dust + bacteria + nematode
Plant + 100% brick kiln dust

Plant + 100% brick kiln dust + bacteria
Plant + 100% brick kiln dust + nematode
Plant + 100% brick kiln dust + bacteria + nematode
Each treatment was replicated five times. After the termination of experiment (120 days after sowing), epidermal character were determined as per procedure. All the data were analysed by using Fischer (1950) factorial method. At the time of analysis, the data was splitted into two factors i.e. F_1 and F_2. The L.S.D. was calculated for F_1 and F_2 separately as well as for $F_1 \times F_2$ 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 thereafter in 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).

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

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.

RESULT

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 brick kiln dust amended soil upto 60% levels compared to controls. However, maximum value of their's (epidermal characters) was found at 20% brick kiln dust amendments. Onward to 60%, there was steep decrease in the characters at 80 and 100% brick kiln dust amendments.
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).
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).

Table 1: Effect of brick kiln dust amendment on the number of stomata of lower (abaxial) and upper (adaxial) surface of the chickpea leaves (in cm²)

Treatments	Brick kiln dust							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	40.00	48.75*	45.50*	41.25ns	32.50ns	22.50ns	38.42
	Upper surface	13.25	16.00*	14.00*	13.75ns	11.20ns	8.25ns	12.74
P + R	Lower surface	46.50*	54.25*	49.75*	48.00*	34.25ns	27.75ns	43.25@
	Upper surface	16.75*	19.00*	17.25*	16.50*	10.00ns	9.50ns	14.83@
P + Mi	Lower surface	31.75ns	42.50*	32.75ns	31.50ns	22.75ns	16.25ns	29.58ns
	Upper surface	10.20ns	14.15*	11.00ns	10.05ns	7.50ns	5.25ns	9.69ns
P + R + Mi	Lower surface	38.25ns	47.50*	42.00*	39.25ns	26.00ns	21.50ns	35.75@
	Upper surface	13.50ns	16.75*	15.25*	14.00*	8.75ns	7.00ns	12.54ns
Mean	Lower surface	39.13	48.25#	42.50#	39.75ns	28.88#	22.00#	
	Upper surface	13.43	16.48#	14.38#	13.58#	9.36#	7.50#	

CD at 5% of lower surface – Brick dust (F_1) = 0.635, Treatment (F_2) = 0.778, Interaction (F_1 x F_2) = 1.556
CD at 5% of upper surface – Brick dust(F_1) = 0.218 , Treatment (F_2) = 0.267, Interaction (F_1 x F_2)= 0.535

Table 2: Effect of brick kiln dust amendment on the number of epidermal cells of lower (abaxial) and upper (adaxial) surface of the chickpea leaves (in cm²)

Treatments	Brick kiln dust							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	200.00	243.75*	205.50ns	201.25 ns	133.50ns	112.50ns	182.75
	Upper surface	66.25	82.00*	73.00*	66.75 ns	42.00 ns	31.25 ns	60.21
P + R	Lower surface	232.50*	262.25*	248.75*	233.00*	172.25ns	130.75ns	213.25@
	Upper surface	85.75*	100.00*	87.25*	84.50*	50.00 ns	45.50 ns	75.50@
P + Mi	Lower surface	159.75ns	215.50*	163.75ns	160.50 ns	113.75ns	82.25 ns	149.25@
	Upper surface	53.00ns	72.75*	58.00 ns	53.25 ns	36.50 ns	26.25 ns	49.96@
P + R + Mi	Lower surface	193.25ns	283.58*	196.00ns	194.25 ns	130.00ns	107.50ns	176.60@
	Upper surface	68.50ns	85.75*	73.25*	69.00*	42.75 ns	37.00 ns	62.71@
Mean	Lower surface	196.38	240.02#	203.50#	197.25 ns	137.38#	108.25#	
	Upper surface	68.38	85.13#	72.88#	68.38 ns	42.81#	35.00#	

CD at 5% of lower surface – Brick dust(F_1) = 3.101, Treatment(F_2) = 3.798, Interaction(F_1 x F_2) = 7.596
CD at 5% of upper surface – Brick dust (F_1) = 1.099, Treatment(F_2) = 1.346, Interaction(F_1xF_2) = 2.692

Table 3: Effect of brick kiln dust amendment on the number of trichomes of lower (abaxial) and upper (adaxial) surface of the chickpea leaves (in cm²)

Treat – ments	Brick kiln dust							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	260.50	310.00*	265.75ns	262.25 ns	225.50ns	200.00ns	254.00
	Upper surface	215.75	235.35*	220.55*	214.20 ns	180.75ns	165.70ns	205.38
P + R	Lower surface	285.25*	340.55*	292.20*	288.50*	208.00ns	190.55ns	267.51@
	Upper surface	232.00*	250.70*	235.25*	234.25*	200.65ns	183.05ns	222.65@
P + Mi	Lower surface	225.25ns	282.20*	233.00ns	224.75 ns	180.05ns	168.00ns	218.88@
	Upper surface	185.20ns	210.50*	192.60ns	185.00 ns	162.60ns	150.00ns	180.98@
P + R + Mi	Lower surface	255.40ns	325.80*	265.60ns	256.00 ns	190.40ns	178.20ns	245.23@
	Upper surface	210.20ns	232.15*	251.60*	212.00*	185.25ns	155.60ns	201.80ns
Mean	Lower surface	256.60	314.64#	264.14#	257.88 ns	200.99#	184.19#	
	Upper surface	210.79	232.18#	216.00#	211.36 ns	182.31#	163.59#	

CD at 5% of lower surface – Brick dust (F_1) = 4.032, Treatment (F_2) = 4.938,Interaction(F_1xF_2) = 9.876
CD at 5% of upper surface – Brick dust(F_1) = 3.233, Treatment(F_2) = 3.959, Interaction(F_1xF_2) = 7.918

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

Treat-ments	Brick kiln dust							
	Surface	0	20	40	60	80	100	Mean
P	Lower surface	16.67	15.13^{ns}	18.12*	17.01^{ns}	19.57*	12.86^{ns}	16.56
	Upper surface	16.66	14.78^{ns}	14.26^{ns}	17.08^{ns}	21.05*	20.88*	17.45
P + R	Lower surface	16.66^{ns}	15.28^{ns}	15.67^{ns}	16.78^{ns}	14.04^{ns}	12.39^{ns}	15.14@
	Upper surface	16.34^{ns}	14.16^{ns}	13.29^{ns}	16.33^{ns}	15.94^{ns}	14.76^{ns}	15.14@
P + Mi	Lower surface	16.57^{ns}	15.52^{ns}	15.66^{ns}	16.40^{ns}	15.87^{ns}	12.96^{ns}	15.50@
	Upper surface	16.13^{ns}	15.87^{ns}	14.86^{ns}	15.87^{ns}	19.13^{ns}	18.71^{ns}	16.76@
P + R + Mi	Lower surface	16.52^{ns}	16.02^{ns}	17.64*	16.80^{ns}	13.76^{ns}	13.07^{ns}	15.64@
	Upper surface	16.46^{ns}	16.06^{ns}	17.23^{ns}	16.86^{ns}	17.11^{ns}	16.45^{ns}	16.70^{ns}
Mean	Lower surface	16.61	15.49#	16.77^{ns}	16.75^{ns}	15.81#	12.82#	
	Upper surface	16.40	15.22#	14.91#	16.54^{ns}	18.31#	17.70#	

CD at 5% of lower surface – Brick kiln dust(F_1) = 0.252, Treatment(F_2)= 0.308, Interaction(F_1xF_2) = 0.617
CD at 5% of upper surface – Brick kiln dust(F_1)= 0.264, Treatment(F_2)= 0.323,Interaction(F_1xF_2) = 0.646
* = data significant with 0% brick kiln dust 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

DISCUSSION

Brick kiln dust is a second major particulate air pollutant after the fly ash, in India. It contains dust particles (soil + sand) and mixture of ashes (coal + wood).utilization plant nutrient are found in the blikn dust (Gupta and narayan 2010) and its addition can enrich the soil in macro and micro nutrients which may have favourable effects on the crop productivity (Rizvi and khan ,2009).increasing the crop productivity healthy plants are found.The number of stomata might have increased to favour the greater gaseous exchange so as to keep pace with high rate of metabolism in healthy plants. Healthy plants remains healthy probably through improvising the defence system. The improvement in the number of trichomes might have happened to that favour in the chickpea leaves upto 60% dust levels. Similar reasons were also asserted earlier (Khan and Khan, 1994) for 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 leaves. 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 chickpeas in the present study. 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 loss 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.

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