INTERACTION OF *MELOIDOGYNE INCOGNITA* AND COAL-SMOKE POLLUTANTS ON TOMATO

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ABSTRACT

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Seedlings of tomato cv. Pusa Ruba were placed in clay pots at 3 sites, 1 (K1) and 2 (K2) km from a coal-fired thermal power station and at a control site at the Department of Botany, Aligarh Muslim University, Aligarh, India in 1988 and 1989. Twenty pots were placed at each site and 10 were inoculated each with 2 000 juveniles of *Meloidogyne incognita* race 1. Mean concentrations of SO_2, NO_2, and suspended particulate matter were very low at the control site but at K1 were 145, 89 and 563 $\mu g/m^3$ in 1988 and 135, 83 and 553 $\mu g/m^3$ in 1989, respectively. The corresponding concentrations at K2 were 193, 107 and 329 $\mu g/m^3$ in 1988 and 181, 98 and 363 $\mu g/m^3$ in 1989, respectively. Chlorosis and browning developed on the leaves of tomatoes grown at the polluted sites, particularly at K2. Foliar injury was invariably greater on nematode-infected plants. The coal-smoke pollutants (except at K1 in 1989) and the nematodes singly caused suppressions in shoot and root growth, leaf pigments and yield. The interactive effects of the coal-smoke pollutants and the nematodes on these parameters were synergistic and mostly significant at K1 in both 1988 and 1989. The amount of sulfur in leaves was greatly enhanced in nematode infected plants, being higher at K2. The stomata formation on the leaves was suppressed by both nematode infection and exposure to coal-smoke. The length of the stomatal pores was unaffected but their widths were increased, being greater in nematode infected plants at the polluted sites. Root galling was enhanced by 9.9% at K1 and reduced by 5.2% at K2. Fecundity and egg mass production were less at K1 and K2 than the control site. Galling and egg mass production per g fresh root weight were enhanced at both the polluted sites.

Key words: leaf stomata, nematode reproduction, plant growth, root-knot disease, thermal power station, tomato.

RESUMEN

Khan, M. R. y M. W. Khan. 1995. Interacción de *Meloidogyne incognita* y poluantes del humo de carbón en tomate. Nematrópica 26:47-56.

Plántulas de tomate cv. Pusa Ruba sembradas en maceteros de barro se colocaron en tres sitios de 1988 a 1989: 1 (K1) y 2 (K2) situados a uno y dos km de una estación termoeléctrica alimentada con carbón, y el tercero en el Departamento de Botánica de la Universidad musulmana de Aligarh, en la India el cual sirvió de control. De las 20 macetas que se colocaron en cada uno de los sitios, 10 fueron inoculadas cada una con 2 000 juveniles de *M. incognita* raza 1. Las medias de las concentraciones de SO_2, NO_2, y particulas suspendidas fueron muy bajas en el sitio de control pero en K1 fueron de 145, 89, y 563 $\mu g/m^3$ en 1988 y de 135, 83 y 553 $\mu g/m^3$ en 1987, respectivamente. Las concentraciones para K2 fueron: 193, 107 y 329 $\mu g/m^3$ en 1988 y 181, 98 y 365 $\mu g/m^3$ en 1989, respectivamente. Se observó clorósis y pardeamiento del follaje de las plantas ubicadas en los sitios contaminados, particularmente en el K2. Este daño fué mayor en las plantas inoculadas con nematodos. Tanto los poluantes de carbón (excepto en K1 en 1989) como los nematodos ocasionaron supresión del crecimiento de renuevos, raíces, pigmentos foliares y producción de frutos. Efectos interactuantes entre poluantes y nematodos en los parámetros antes mencionados fueron sinérgicos y mas significativos en K1 tanto en 1988 como en 1989. La cantidad de sulfuro en las hojas fue mas favorecida en las plantas infectadas, siendo mas alta en las de K2. La formación de estomas fué suprimida tanto en las plantas infectadas de nematodos como en las expuestas a los poluantes de carbón. La longitud de los poros no se afectó pero sí su ancho, siendo este mayor en las plantas infectadas en los sitios contaminados. El agallamiento radical se favoreció por 9.9% en K1 y se redujo por 5.2% en K2. La fecundidad (número de huevos/masas de huevos) y la producción de masas de huevos fué menor en K1 y K2 que en el sitio de control. El agallamiento radical y la producción de masas de huevos por g fresco de raíz se vió favorecida en ambos sitios contaminados.

Palabras clave: agallamiento radical, crecimiento de plantas, estóma foliar, estación termoeléctrica, producción de tomate, reproducción de nematodos, tomate.

INTRODUCTION

Despite associated deleterious effects to the environment, coal continues to be used worldwide as a vital energy source. The major portion of coal is used to generate electricity resulting in the release of oxides of sulphur and nitrogen and particulate matter. Effects of these pollutants singly or jointly on crop plants include reduction in plant growth and yield with or without appearance of visible injury (Adaros *et al.*, 1991; Reinert, 1984; Sinn and Pell, 1994).

In India, vegetables usually are cultivated around large industrial areas, enabling the grower easy access to the market. This is also the case in the area around the coal-fired thermal power station located at Kasimpur about 15 km northeast of Aligarh. In 1987, during a field survey around the power station to assess the impact of air pollutants on crop plants, severe root galling caused mainly by *Meloidogyne incognita* (Kofoid and White) Chitwood was observed on tomato, egg plant, and okra.

The available literature concerning effects of air pollutants on nematode-plant relationships reports different types of interactions. The intensity of damage caused by *M. incognita* on tomato and *Pratylenchus penetrans* on soybean was enhanced as a result of intermittent exposures of the plants to SO_2 at 286 (Khan and Khan, 1993) and 655 μg/m^3 (Weber *et al.*, 1979), respectively. Reproduction and development of *Heterodera glycines* and *Paratrichodorus minor* were inhibited by O_3 singly or with SO_2, while *Belonolaimus longicaudatus* and *Aphelenchoides fragariae* remained unaffected (Weber *et al.*, 1979). Therefore, the present study was conducted to determine the effects of air pollutants emanating from a coal-fired thermal power station on the host-parasite relationship between *M. incognita* race 1 and tomato, *Lycopersicon esculentum* Mill. cv. Pusa Ruby.

MATERIALS AND METHODS

Study sites: Two wire-net houses, designated as K1 and K2, were fabricated 1 and 2 km away from the smoke stack (89 m high) of a coal-fired thermal power station located at Kasimpur in the usual windward direction (west-east). A net house at the Department of Botany, Aligarh Muslim University (AMU), Aligarh, India about 15 km south-west of the power station served as a control site. The power station burns about 3192 MT of coal daily, generating around 680 MT of fly ash. The coal is a bituminous type of slack grades B and C. Important characteristics of the coal are: moisture content 2.6-4.7%, ash content 26-33%, volatile matter 26-29%, fixed carbon

38-42%, sulphur content 0.45-0.59%, gross calorific value 4983-5674 kcal/kg and useful heat value 3878-4915 kcal/kg.

Air pollution monitoring: To monitor the ambient levels of SO_2, NO_2 and suspended particulate matter (SPM), air was sampled by a High Volume Air Sampler (APM-415, Envirotech, New Delhi, India) at the rate of 1.5 L/minute (air suction rate) for 3 hr on alternate days for 3 months (45 occasions). Of these, 15 samplings were conducted each in the morning (9 am-12 noon), noon (12-3 p.m.) and evening (3-6 pm) starting from the day the tomato seedlings were planted. For sampling SO_2 and NO_2, 20 ml of sodium tetrachloromercurate, sodium hydroxide and sodium arsenite solution were placed in separate impingers of the sampler. The SPM was determined by placing Whatman micro filter paper (GF/A grade) on the meshed face plate of the sampler (Anonymous, 1986).

Treatments: Sixty 15-cm-diam clay pots were filled with field soil (sand 76%, clay 16%, silt 8%) and compost mixed in a ratio of 3: 1 (1.5 kg/pot). The pots with soil were autoclaved at 103 kPa pressure for 3 hr. Twenty pots were placed in each of 3 net houses (K1 and K2 and the control site). One seedling of two-week-old tomato cv. Pusa Ruby was planted into each pot on 14 October, 1988, and 6 October, 1989. A week later, the seedlings in each of 10 pots were inoculated with 2 000 freshly hatched juveniles of *M. incognita* race 1 at each site. All pots were watered on alternate days (250 ml/pot). After 3 months (14 January, 1988, and 6 January, 1989, respectively), 5 of each 10 replicates of inoculated and uninoculated plants were randomly harvested for determination of dry matter and yield. The remaining plants were used to collect other data.

Disease assessment and nematode reproduction: The intensity of galling and reproduction of *M. incognita* race 1 was determined by counting the galls and egg masses on root systems stained with phloxine B solution (0.15 g/L of tap water). Fecundity (number of eggs per egg mass) was estimated by excising 20 egg masses from each of the 5 washed root systems. The egg masses from each group of 5 replicates (total 100) were blended with 500 ml of 1% NaOC1 solution for 40 sec and the resulting suspension was sieved through 100 and 400 mesh sieves. The eggs retained on the finer sieve were transferred to a beaker in 100 ml of water. One ml of the egg suspension was taken on 10 occasions to determine fecundity. Before each sampling, the suspension was carefully shaken to equalize egg distribution.

Dry matter production and yield: Plants were examined regularly for symptoms caused by the coal-smoke pollutants or *M. incognita.* Flowers that formed during the 3-month period were counted. Fruit were counted and collected at harvest and total weight and mean fruit weight determined. Uprooted plants were dried in a hot air oven at 60°C for 48 hr and dry weight of shoots and roots recorded.

Leaf pigments and sulfur: The third and fourth leaflets of the third leaf of each branch of 5 tomato plants were collected for each treatment at harvest. One g of the pooled leaves was macerated in acetone to determine carotenoid (Maclachlan and Zallk, 1963)) and total chlorophyll (Mackinney, 1941) by colorimetric methods. Whole shoot material (minus flowers and fruits) dried at 60°C was ground to pass through a 40 mesh sieve to estimate foliar sulfur, using the oxygen flask combustion method of Hunt (1980).

Leaf epidermal characters: Fully expanded leaves were fixed immediately from each treatment in formalin-acetic acid-alcohol (F.A.A.) and preserved in 70% ethanol. The peels, obtained by boiling 1 cm^2 leaf pieces with 40% HNO_3 (Ghocuse and Yunus, 1972), were washed in water and stained with iron-alum, hematoxylin and bismark brown, dehydrated in an ethanol series and mounted on glass slides in DPX mountant. The slides were microscopically examined to count stomata and to measure the length and width of pores.

Foliar deposition of particulate matter: Leaves were collected from the unharvested plants to determine the amount of particulate matter deposited on the foliage at final harvest. They were packed in soft polythene bags for transport to the laboratory. Leaf pieces of 1 cm^2 were cut and weighed. The pieces then were gently rinsed in water to remove deposited particles. Before re-weighing, the pieces were blotted to remove excess water.

Statistical analysis: Data were subjected to analysis of variance for two-factors (i.e coalsmoke at three locations and nematode) and significance for partial differences (LSD) was calculated at $P \leq 0.05$. Data on disease intensity and nematode reproduction, however, were processed for single-factor ANOVA (i.e. effect of coalsmoke on the nematode) (Dospekhov, 1984).

RESULTS

Air quality: The monitoring data collected during 1988 and 1989 indicate that the sites (K1 and K2) in the vicinity of the thermal power station were polluted with SO_2, NO_2 and SPM. Mean concentrations of SO_2, NO_2 and SPM at the K1 site were 145 (140-231), 89 (29-163) and 563 (293-749) in 1988 and 135 (39-231), 83 (29-163) and 553 (319-772) $\mu g/m^3$ in 1989, respectively. In 1988 at the K2 site, SO_2, NO_2, and SPM were 193 (83-297), 107 (51-181), and 329 (163-527) $\mu g/m^3$ in 1988 and 181 (71-307) 98 (59-174) and 363 (151-561) $\mu g/m^3$ in 1989, respectively. The concentration of gaseous pollutants was higher at K2, while SPM was greater at K1. Peak levels of gases were recorded from 12-3 p.m. and SPM from 3-6 p.m., respectively. Concentration of the pollutants at AMU was well below the recommended levels (30 $\mu g/m^3$ for SO_2 and NO_2 and 100 $\mu g/m^3$ for SPM).

Foliar injury: Chlorosis and browning of leaves was observed on the plants grown at the K2 site in both 1988 and 1989. At the K1 site, the leaves (upper surface) were blackish due to deposition of particulate matter. Accumulation of particulate matter (mean of 1988 and 1989) was 34.3, 18.1 and 0.71 $\mu g/cm^2$ leaf surface at the K1, K2 and the control site, respectively. At the K1 site, leaves showed a slight browning in 1988. The foliar injury, based on visual observations, appeared to be more severe in nematode-infected plants at the polluted sites, especially at K1.

Dry matter production and yield: At the control site, *M. incognita* caused significant growth suppression of shoots and roots of tomato (dry weights) compared to non-inoculated plants (Table 1). Growth suppression in uninoculated plants was greater at the polluted sites except at K1 in 1989 compared to the plants at the control site. Inhibition in shoot or root growth of inoculated plants was greater at the polluted sites than at the control site. Individual and interactive effects of coal-smoke and nematodes were significant in both 1988 and 1989.

No discernible effect of coal-smoke or *M. incognita* was observed on flowering (Table 1). However, other yield characters, including fruit-set, total weight of fruits per plant and mean fruit weight, were negatively impacted in noninfected plants at the polluted sites except at K1 in 1989 (Table 1). At the control site and the polluted sites, *M. incognita* caused a significant decrease in the yield in 1988 and 1989, compared to their respective controls.

Table 1. Effects of locations (L) and inoculation with *Meloidogyne incognita* race 1 (N) on growth and yield characteristics of tomato plants, 1988 and 1989.[X]

Character	Year	Inoculated[X]			Not Inoculated[X]			ANOVA effect[Z]			LSD
		C[Y]	K1	K2	C	K1	K2	L	N	LXN	$P \le 0.05$
Shoot dry weight (g)	1988	6.4	5.4	5.3	7.1	6.7	6.3	*	*	*	0.38
	1989	6.1	5.2	5.1	6.8	6.6	6.2	*	*	*	0.41
Root dry weight (g)	1988	2.7	2.2	2.2	2.9	2.7	2.5	*	*	ns	0.10
	1989	2.3	1.9	1.8	2.5	2.4	2.3	*	*	*	0.13
Number of fruit/plant	1988	15	13	13	17	16	15	*	*	ns	1.00
	1989	14	13	12	15	15	13	*	ns	*	0.93
Weight of fruit/plant (g)	1988	511	392	382	633	577	519	*	*	*	12.5
	1989	523	400	341	612	591	502	*	*	*	11.8
Mean fruit weight (g)	1988	34.0	30.1	29.4	37.2	36.0	34.6	*	*	*	1.23
	1989	37.3	30.8	28.4	40.8	39.4	38.6	*	*	*	1.33

[X]Inoculated with 2 000 juveniles per plant or not inoculated.
[Y]C = Control site, the Department of Botany, AMU, K1 = polluted site 1 km away from the stack of a coal-fired thermal power station, K2 = polluted site 2 km away from the stack.
[Z]Effects from analysis of variance (ANOVA) significant at $P \le 0.05$ (*) or not significant (ns).

Interactive effects of coalsmoke and the root-knot nematode were significant for total weight, mean fruit weight/plant and fruit-set in 1989.

Leaf pigments and sulphur: Nematodes singly or jointly with the air pollutants caused decreases in carotenoid and chlorophyll content of leaves compared with the respective controls (Table 2). Coal-smoke pollutants at the K2 site in the absence of the nematodes also caused a suppression in both pigments but at the K1 site the reduction was significant only for chlorophyll in 1988. Foliar sulfur was increased in the plants grown at the polluted sites, being greater at K2 (except at K1 in 1988) (Table 2). At the polluted sites, percentage of foliar sulfur was increased significantly in infected plants compared with non-infected plants. Individual effects of coal-smoke and *M. incognita* were significant for leaf pigments and sulfur except with the nematode for foliar sulphur (Table 2). The interactive effects were not different for carotenoids in both 1988 and 1989.

Leaf stomata: The nematode and air pollutants caused a decline in the number of stomata, compared to their respective controls in both 1988 and 1989 (Table 2). No discernible effect of nematode or coal-smoke was observed on stomatal pore length, but their widths increased (Table 2). The nematode caused a slight increase in the stomatal pore width but the increase was only significant at the polluted sites, being greater at K2. The stomatal pores of nematode-infected plants at the polluted sites were wider compared with the non-infected plants at the same sites in both 1988 and 1989. Individual effects of coal-smoke were significant for number of stomata and stomatal pore widths while effects of the nematodes were significant for stomata number. The interactive effects were significant for number of stomata and stomatal pore width.

Disease assessment and nematode reproduction: Meloidogyne incognita caused severe galling on tomato roots at the control site, which was further enhanced at the polluted sites (Table 3). Galling (mean for 1988 and 1989) was enhanced by 9.9% at K1 but suppressed by 5.2% at K2 compared with the control. The number of galls per g fresh root weight was, however, enhanced by 37 and 25% at K1 and K2, respectively. Corresponding values for number of egg masses were 34 and 15%, respectively. The number of egg masses per root system was enhanced by 9.3% at K1 and suppressed by 14.5% at K2, respectively. Fecundity was reduced by 8.6 and 29% at K1 and K2, respectively.

DISCUSSION

The concentration of SO_2, NO_2 and SPM at K1 and K2 near the power station usually exceeded the safe levels (100 µg/m^3 for SO_2 and NO_2, 500 µg/m^3 for SPM) recommended by the Central Pollution Board, India, for industrial areas. At the control site, the concentrations of the pollutants were very low. Chlorosis and browning appearing on the leaves of the tomato plants at the polluted sites was probably due to bleaching and phaeophytinization of leaf pigments (Rao and Lablanc, 1966). Suspended particulate matter (SPM) and its subsequent deposition on foliage was considerably greater at K1, the site nearest the power station and was visible as a thin layer of black particles on the upper leaf surface. Accumulation of particulate matter on leaves (Krajikova and Mejstrik, 1984) and nematode infection of plants might have increased the rate of transpiration which in turn could have accelerated the diffusion of SO_2 and NO_2 inside the leaf tissues (Mjuge and Estey, 1978). Higher intake of the gases resulted in greater injury due to

Table 2. Effects of locations (L) and inoculation with *Meloidogyne incognita* race 1 (N) on pigments, sulphur and density of stomata and their pore width of leaves of tomato plants, 1988 and 1989.

		Inoculated[X]			Not Inoculated			ANOVA effect[Z]			LSD
Character	Year	C[Y]	K1	K2	C	K1	K2	L	N	LXN	$P \leq 0.05$
Carotenoid μg/g leaf	1988	6.6	6.1	6.0	7.1	6.8	6.5	*	*	ns	0.51
	1989	6.7	6.3	6.1	7.3	7.2	6.7	*	*	ns	0.57
Chlorophyll μg/g leaf	1988	1082	911	870	1194	1084	1023	*	*	*	108
	1989	1187	1025	942	1268	1195	1109	*	*	*	121
Foliar sulphur (%)	1988	0.108	0.144	0.151	0.103	0.117	0.121	*	ns	*	0.017
	1989	0.115	0.134	0.156	0.109	0.121	0.129	*	ns	*	0.015
Stomata/cm^2 leaf	1988	35813	35307	34865	38316	36880	35211	*	*	*	781
	1989	35344	34621	33907	37002	35010	34782	*	*	*	694
Pore width (μm)	1988	5.26	5.60	5.64	5.16	5.30	5.41	*	ns	*	0.13
	1989	5.60	6.10	6.17	5.51	5.61	5.74	*	ns	*	0.10

[X]Inoculated with 2 000 juveniles per plant or not inoculated.

[Y]C = Control site, the Department of Botany, AMU, K1 = polluted site 1 km away from the stack of a coal-fired thermal power station, K2 = polluted site 2 km away from the stack.

[Z]Effects from analysis of variance (ANOVA) significant at $P \leq 0.05$ (*) or not significant (ns).

Table 3. Effects of locations on root galling and reproduction of *Meloidogyne incognita* race 1 on tomato plants.[X]

Character	Year	Locations[Y]			ANOVA effects[Z]	LDS $P \leq 0.05$
		C	K1	K2		
Galls/root system	1988	118	130	112	*	11.2
	1989	113	124	107	*	10.5
Galls/g fresh root	1988	8.2	11.8	10.0	*	0.82
	1989	8.6	11.2	10.7	*	0.79
Egg masses/root system	1988	115	125	101	*	9.4
	1989	108	119	89	*	10.1
Egg masses/g fresh root	1988	8.3	11.2	9.6	*	0.83
	1989	8.0	10.6	9.2	*	0.85
Eggs/egg mass	1988	319	289	223	*	21.4
	1989	283	261	204	*	2.27

[X]Inoculated with 2 000 juveniles per plant.
[Y]C = Control site, the Department of Botany, AMU; K1 = polluted site 1 km away from the stack of a coal-fired power station; K2 = polluted site 2 km away from the stack.
[Z]Effects from analysis of variance (ANOVA) significant (*) at $P \leq 0.05$.

pollution in the nematode-infected plants. Moreover, the observation that pores of stomata of nematode-infected plants are wider also indicates that the plants transpired more, which in turn led to increase entry of the pollutants. An increase in leaf sulfur in the nematode-infected plants at the polluted sites is also an indication of greater entry of SO_2 in the leaf.

At the K1 site, inhibition of shoot or root growth in 1988 may have been caused by the slightly higher levels of the pollutants compared with 1989 or by an other unknown factor. Nematodes caused significant suppression of plant growth and yield of tomato at the control site. Coal-smoke pollutants and *M. incognita* interacted positively, resulting in a synergistic growth reduction of tomato, being most significant at K1.

Penetration and development of the nematodes were favored at the polluted sites, which is evident by an increased root galling at K1. Greater diffusion of SO_2 and NO_2 and higher galling may have resulted in synergistic suppressions in tomato plants at K1 site. At K2, nematode development and reproduction may have been impeded by poor plant health. Also, the foliar deposition of particulate was lower at this site which could not accelerate diffusion of the gases as much as at K1. Lesser formation of galls and egg masses at K2 site can be attributed to unavailability of roots for feeding and development of the nematode. A decrease in fecundity at K1 indicates that root nutrients were sufficient to support development and egg mass production of the nematodes but not to an extent that they could lay eggs to their full capacity. At K2 site, tomato roots were so nutritionally deficient that many of the juveniles which formed galls could not produce egg masses and those that did deposited fewer eggs.

In a previous study (Khan and Khan, 1984), we reported significant enhancement in root galling and egg mass production of *M. incognita* race 1 on tomatoes intermittently exposed to SO_2 at 286 μg/m^3. In the present study, however, root galling and egg mass production were suppressed at 193 and 181 μg ug/m^3 SO_2. This variable response of the same nematode to SO_2 could be caused by differences in the ambient conditions and the presence of NO_2 and SPM.

The inhibitory effect of coal-smoke pollutants and *M. incognita* race 1 on development of stomata (number and size) can be seen as a morphological adaptation of tomato to control the entry of SO_2 and NO_2 and loss of water. SO_2 can injure guard cells and cause their collapse in the open position in a number of plants like maize and barley (Black and Unsworth, 1980). A wider stomatal aperture has been observed (Majernik and Mansfield, 1970). A direct correlation was reported between percent reduction in the plant growth and yield characters and degree of widening of the stomatal pore.

This paper shows that root-knot disease and nematode reproduction were enhanced at sites with higher levels of SO_2, NO_2, and SPM, and the nematode-infected plants suffered greater damage from the pollutants.

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