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EFFECT OF SO_2 ON GROWTH, NODULATION AND ROOT-KNOT DISEASE OF PEA

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Effect of intermittent SO_2 exposure on pea (*Pisum sativum* L.) cv. Rachna was studied on plant growth, nodulation and root-knot disease under artificial treatment conditions. SO_2 at 0.1 and 0.2 ppm and root-knot nematode, *Meloidogyne javanica*, individually caused significant reductions in plant growth of pea. Greater reductions were recorded in joint treatments. Root nodule bacteria, *Rhizobium leguminosarum* improved the plant growth and provided a partial protection to plants against SO_2 and nematode. Root-knot disease caused by *M. javanica* was also found to be decreased on 0.1 and 0.2 ppm SO_2 exposures, particularly in presence of *R. leguminosarum*. Unlike others, fecundity suppressions were comparatively less in case of the roots inoculated with root nodule bacteria than their absence.

Key Words : Sulphur dioxide, *Rhizobium, Meloidogyne javanica*, pea.

Sulphur dioxide is a potential phytotoxic gaseous pollutant and is principally produced by coal burning (Wood, 1968). In urban areas, the concentration of SO_2 may vary from 0.05 to 0.4 ppm (Heagle, 1973). Sulphur dioxide readily combines with water vapour and forms acid rain that falls on ground during the atmospheric precipitation (Likens and Bormann, 1974). SO_2 after entering to leaf tissue is converted into sulphate and sulphite ions (Petering and Shih, 1975) and these ions cause disruption in various metabolic pathways and injuries to the plants. The impacts of air pollutants on crop plants are now being realised in different parts of the world (Heck *et al.* 1986). Some diseases caused by plant parasitic nematodes are also known to be influenced by air pollutants (Heagle, 1973, 1982). Reproduction of *Pratylenchus penetrans* is reported to be enhanced when the infected soyabean plants were exposed to SO_2 at 655 μgm^{-3}, but of *Heterodera glycines* and *Paratrichodorus minor* was inhibited (Weber *et al.* 1979). Recently Khan and Khan (1993) recorded severe root-knot disease caused by *Meloidogyne incognita* on tomato plants exposed to 286 $\mu gm\ SO_2\ m^{-3}$.

The discrepancies in the response of plant parasitic nematodes to air pollutants are probably due to paucity of informations on this area of research. So we designed an experiment to investigate the effect of SO_2 on plant growth, nodulation and root knot disease on pea grown in artificial treatment conditions.

MATERIALS AND METHODS

Exposure chamber : The chambers (Standard Appliances, Varanasi, India) used to expose peas were of dynamic state (Khan and Khan, 1993) and were made of transparent lucite sheets with a height of 120 cm and 8100 cm^2 cross sectronal area. The front of the chamber was provided with movable door to place the test materials inside the chamber, while the bottom plate had several perforations with nozzles for uniform inflow of SO_2. Sixty cm above the bottom plate, a meshed tray was placed in order to provide additional space to the pots. A control panel fitted to the bottom regulated the air flow from the blower into the chamber. Function of the blower was to mix SO_2 with air and inject it through the perforations for a uniform dispension inside the chamber.

SO_2 generation : SO_2 was produced in a generator by the reaction of sulphuric acid (10% H_2SO_4) and sodium sulphite (Na_2SO_3) under controlled conditions. Flow of both the reactants in the reaction chamber was controlled through the mechanical valves fitted to the capillary tubes originating from the bottles. On complete reaction, the 1 M Na_2SO_3 produces 1 M SO_2 or 126 mg Na_2SO_3 that yields 64 mg SO_2.

To determine the concentration of SO_2 during the exposure of plants, air sampling was done by placing a handy air sampler (Kimoto Electricals, Japan) in the chamber. The sampled air was analysed in laboratory according to a method given by West and Gaeke (1956) as prescribed in National Environmental Engineering Research Institute, Nagpur in its Air Quality Monitoring Coarse Manual (Anon, 1986).

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Table 1: Effect of SO_2 on shoot and root length of pea

Treatments	Shoot length (cm) ppm				Root length (cm) ppm			
	0	0.1	0.2	MM	0	0.1	0.2	MM
P	95.72	92.48	89.36	92.52	38.54	34.36	32.32	35.07
P + R	114.00	96.70	93.64	101.45	42.06	39.72	34.48	38.75
P + Mj	93.10	87.64	83.32	88.02	35.16	32.18	30.02	32.45
P + R + Mj	104.60	90.72	84.72	93.15	40.10	34.14	31.34	35.19
MM	101.86	91.89	87.76		38.97	35.10	32.04	

Cd at P = 0.05; Treatments = 1.39, SO_2 = 1.20; Treatments x SO_2 = 2.41 — Treatments = 1.22, SO_2 = 1.06; Treatments x SO_2 = NS

Table 2: Effect of SO_2 on fresh shoot and root weight of pea

Treatments	Fresh shoot weight (g) ppm				Fresh root weight (g) ppm			
	0	0.1	0.2	MM	0	0.1	0.2	MM
P	30.36	28.36	26.50	28.41	15.42	13.22	11.56	13.40
P + R	35.50	31.44	29.48	32.14	17.72	15.46	13.40	15.53
P + Mj	28.20	26.22	25.28	26.57	14.80	11.58	10.20	12.19
P + R + Mj	32.54	29.40	27.48	29.81	16.12	14.18	12.16	14.15
MM	31.65	28.86	27.19		16.02	13.61	11.83	

Cd at P = 0.05 Treatments = 1.44, SO_2 = 1.25; Treatments x SO_2 = NS — Treatments = 1.19, SO_2 = 1.03; Treatments x SO_2 = NS

Treatments and plant culture : The seeds of pea (*Pisum sativum* L.) cv. Rachna, procured from IARI, Pulse Research Section, New Delhi were water-soaked and surface sterilized (0.01% $HgCl_2$). Seeds were then sown in clay pots of 20 cm diameter. Prior to sowing, seeds of some pots were treated with the mixture of commercial *Rhizobium leguminosarum* and sugar. After twenty one days of sowing, the seedlings were inoculated with *Meloidogyne javanica* (2000 J_2/pot). Two levels of SO_2 i.e. 0.1 and 0.2 ppm were selected to expose the plants. The treatments were as follows :

Unexposed treatment (i.e. Control) :

T_1 - Plant
T_2 - Plant + *Rhizobium*
T_3 - Plant + Nematode (= *Meloidogyne javanica*)
T_4 - Plant + *Rhizobium* + Nematode

Exposed treatment :

T_5 - Plant + SO_2 (0.1 ppm)
T_6 - Plant + *Rhizobium* + SO_2 (0.1 ppm)
T_7 - Plant + Nematode + SO_2 (0.1 ppm)
T_8 - Plant + Rhizobium + Nematode + SO_2 (0.1 ppm)
T_9 - Plant + SO_2 (0.2 ppm)
T_{10} - Plant + *Rhizobium* + SO_2 (0.2 ppm)
T_{11} - Plant + Nematode + SO_2 (0.2 ppm)
T_{12} - Plant + *Rhizobium* + Nematode + SO_2 (0.2 ppm)

After the nematode inoculation, designated pots were exposed to SO_2 by putting them in exposure chamber on alternate days for three hours up to the termination of experiment (i.e. 70 days).

Each treatment was replicated five times and pots were arranged in glasshouse in complete randomised block design. At the time of harvesting, the plants were carefully uprooted and the roots were washed thoroughly under tap water to remove the sticked soil. The lengths and fresh and dry weights of shoots and roots were determined. Roots were visually examined to count rhizobial nodules. Pinkish nodules were considered as healthy i.e. functional one.

Disease intensity and reproduction of the nematode were studied according a method given by Khan and Khan (1993). Date were analysed by testing Null hypothesis (Dospekhov, 1984).

RESULTS

Plant growth (lengths, fresh and dry weights of shoots and roots) was greater in presence of *Rhizobium*. Peas inoculated with *Meloidogyne javanica* alone, showed reduction in growth parameters. Suppressions in length and weight of plants caused by *M. javanica*

Table 3: Effect of SO_2 on shoot and root weight of pea

Treatments	Dry shoot weight (g) ppm				Dry root weight (g) ppm			
	0	0.1	0.2	MM	0	0.1	0.2	MM
P	5.84	5.21	4.96	5.34	4.74	4.50	4.22	4.49
P + R	6.91	6.58	6.00	6.50	5.86	5.65	5.36	5.62
P + Mj	4.72	4.22	4.02	4.32	3.89	3.78	3.52	3.73
P + R + Mj	6.07	5.37	5.06	5.50	4.92	4.59	4.27	4.59
MM	5.89	5.34	5.01		4.85	4.63	4.34	
Cd at P = 0.05	Treatments = 0.74, SO_2 = 1.28 Treatments x SO_2 = NS				Treatments = 0.63, SO_2 = NS Treatments x SO_2 = NS			

Table 4: Effect of SO_2 on functional and total number of nodules/root system

Treatments	Number of functional nodules ppm				Number of total nodules ppm			
	0	0.1	0.2	MM	0	0.1	0.2	MM
	-	-	-	-	-	-	-	-
+ R	241.2	150.4	104.8	165.5	266.2	176.8	150.0	197.7
+ Mj	-	-	-	-	-	-	-	-
+ R + Mj	198./0	121.2	88.8	136.0	208.2	156.4	133.8	166.1
IM	291.6	135.8	96.8		237.2	166.6	141.8	
Cd at P = 0.05	Treatments = 5.3, SO_2 = 6.4 Treatments x SO_2 =9.1				Treatments = 5.6, SO_2 = 6.9 Treatments x SO_2 = 9.7			

were comparatively less in the presence of *Rhizobium*. Root nodulation also decreased in the presence of root-knot nematode. Number of galls, females and egg masses were suppressed in the presence of *R. leguminosarum*. But nodulated peas showed significant increase in fecundity.

Intermittent exposure of plants to SO_2 caused significant suppressions to lengths, fresh and dry weights of shoots and roots, being greater at 0.2 ppm than 0.1 ppm (Tables 1-3). These reductions were comparatively less in bacterized peas at either concentration of SO_2. Sulphur dioxide especially at 0.2 ppm in the presence of *M. javanica* caused highest reductions in the plant growth in comparison to other treatments. SO_2 also caused decline in the number of nodules on pea roots, which was further suppressed in the presence of *M. javanica* (Table 4).

Number of galls, females, egg masses and fecundity showed a progressive decline at 0.1 and 0.2 ppm of SO_2 (Tables 5 & 6). Like the growth parameters, the decline in the severity of root-knot was greater at 0.2 ppm. Root-knot disease (except fecundity) was suppressed to a greater extent in nodulated plants particularly at 0.2 ppm. In contrast, fecundity showed more suppression in the exposed plants in the absence of *R. leguminosarum*.

The critical difference of treatments and SO_2 were significant for all the measured parameters except dry weight of root. The interaction critical difference (i.e. CD of treatments X SO_2) was significant for only shoot length and root nodulation.

DISCUSSION

Root nodulation caused by *Rhizobium* on leguminous roots facilitates the fixation of atmosphereic nitrogen and provides additional nitrogen to plants which results in an improved plant growth and yield (Subba Rao, 1972). We also recorded better growth of pea in the presence of *R. leguminosarum*. However, suppressed plant growth of a nematode infected pea was apparently due to the nematode-induced imbalances in the conducting tissue (Endo, 1971). *M. javanica* causes impairment in absorption and translocation of water and nutrients. The decline in the nodulation might be due to the nematode-galling, thus leaving less root area (Barker and Hussey, 1976) available for the

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P	-	-	-	-	-	-	-	-
P + R	241.2	150.4	104.8	165.5	266.2	176.8	150.0	197.7
P + Mj	-	-	-	-	-	-	-	-
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MM	291.6	135.8	96.8		237.2	166.6	141.8	
Cd at P = 0.05	Treatments = 5.3, SO_2 = 6.4 Treatments x SO_2 =9.1				Treatments = 5.6, SO_2 = 6.9 Treatments x SO_2 = 9.7			

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Table 5: Effect of SO_2 on gall and female production of *Meloidogyne javanica* on pea

Treatments	Number of galls ppm				Number of females ppm			
	0	0.1	0.2	MM	0	0.1	0.2	MM
P	-	-	-	-	-	-	-	-
P + R	-	-	-	-	-	-	-	-
P + Mj	29.8	26.8	20.6	25.7	44.2	35.4	26.8	35.5
P + R + Mj	25.2	23.2	16.6	21.7	36.6	30.6	25.4	30.9
MM	27.5	25.0	18.6		40.4	33.0	26.1	
Cd at P = 0.05	Treatments = 3.6, SO_2 = 4.4 Treatments x SO_2 = NS				Treatments = 3.5, SO_2 = 4.2 Treatments x SO_2 = NS			

Table 6: Effect of SO_2 on egg mass production and fecundity of *Meloidogyne javanica* on pea.

Treatments	Number of egg mass ppm				Fecundity ppm			
	0	0.1	0.2	MM	0	0.1	0.2	MM
P	-	-	-	-	-	-	-	-
P + R	-	-	-	-	-	-	-	-
P + Mj	27.8	22.8	17.2	22.6	306.4	290.2	274.4	290.3
P + R + Mj	21.8	20.2	14.2	18.7	314.4	299.6	291.4	301.8
MM	24.8	21.5	15.7		310.4	294.9	282.9	
Cd at P = 0.05	Treatments = 1.5, SO_2 = 1.9 Treatments x SO_2 = NS				Treatments = 3.9, SO_2 = 4.8 Treatments x SO_2 = NS			

MM = Mean of means, R = *Rhizobium*, Mj = *Meloidogyne javanica*
Values in the tables are mean of five replicates.

infection and development of rhizobial nodules on pea roots (Taha, 1993).

Decrease in the number of galls, egg masses and females in bacteria inoculated plants can be seen as a protection provided by *Rhizobium* against nematode infection (Bopaiah *et al,* 1976; Khan and Husain, 1988). The increased fecundity in bacterized peas can be correlated to availability of additional nutrients for the development of healthy females, which layed greater eggs.

Greater suppressions in plant growth of peas at 0.2 ppm SO_2 than at 0.1 ppm are in support of other researches (Kumar and Yadav, 1986; Prakash *et al,* 1989). The suppression were apparently due to the interference of SO_2 in various metabolic processes related to synthesis of pigments (Sharma and Prakash, 1991; Khan and Khan, 1993). Disturbance in synthesis of pigments may reduces photosynthesis leading to decrease in overall plant growth. SO_2 readily reacts with soil moisture and cause acidity which might have been responsible for the suppression in nodulation. Low pH of soil can reduce infection and nodulation of *Rhizobium* (Shriner and Johnson, 1981).

In the present study, number of galls, females, egg masses and fecundity was reduced by SO_2. Singh (1989) and Pasha (1990) have also reported decline in galls, females and egg masses in SO_2 exposed leguminous and non-leguminous plants. The inhibitory effects on the nematode disease was due to their direct toxic effects or indirectly through the host. Soil acidity caused by SO_2 might have contributed the suppression in penetration and development of *M. javanica* (Abeles *et al,* 1971). Poor nutritional status of the SO_2-exposed plants due to altered physiology (Johnsen and Sochting, 1973; Shimazaki *et al.* 1980) can also be correlated to the suppressed development of the nematode. At either concentration of SO_2, fecundity was greater in nodulated peas than non-nodulated ones but galling, female population and egg mass production were suppressed. In *Rhizobium* inoculated plants, nematode apparently received additional nutrients as a result of symbiotic association.

Decline in the intensity of root-knot nematode was maximum in SO_2-exposed peas inoculated with *Rhizo-*

bium than the uninoculated. It indicates that the bacteria developed protection against penetration of the juveniles. From the present study, it can be predicted that damages caused by the *M. javanica* and SO_2 to crops may be reduced if treated with *Rhizobium*.

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