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SINGLE AND CONCOMITANT EFFECTS OF SULPHUR DIOXIDE AND *MELOIDOGYNE JAVANICA* ON PUMPKIN

by

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Summary. Intermittent exposures of SO_2 at 200 and 300 µg m^{-3} caused chlorosis of the leaves of pumpkin whether or not infected with *Meloidogyne javanica*. At 100 µg SO_2 m^{-3}, a mild chlorosis appeared only in the infected plants. SO_2 at 200 or 300 µg m^{-3} and root-knot nematode, separately caused significant suppression of plant growth, dry matter production, flowering, fruit setting and chlorophylls. In the combined treatments of SO_2 and the nematode, the suppression were relatively greater. The interactive effects of the nematode and SO_2 were synergistic at 100 µg m^{-3} and antagonistic or additive (slightly synergistic/antagonistic) at 200 and 300 µg m^{-3}. Root galling and egg mass production were enhanced by about 11% and 6% at 100 µg m^{-3} and declined by 23% and 24% at 300 µg m^{-3}, respectively. The numbers of galls and egg masses remained unaffected at 200 µg SO_2 m^{-3}. The fecundity was 16% lower at 300 µg m^{-3}, whereas at rest of the concentrations it was more or less equal to the control.

Sulphur dioxide (SO_2) is a phytotoxic air pollutant which is released into the atmosphere as a result of the burning of fossil fuels for industrial and domestic purposes. Sulphur dioxide causes an appreciable amount of damage to plants.

Air pollutants can influence the parasitism of plant parasitic nematodes. Weber *et al.* (1979) reported that 655 µg SO_2 m^{-3} enhanced the reproduction of *Pratylenchus penetrans* on soybean. However, exposure of infected soybean plants to O_3 and O_3-SO_2 mixture inhibited reproduction and development of *Heterodera glycines* and *Paratrichodorus minor*, but *Belonolaimus longicaudatus* and *Aphelenchoides fragariae* remained unaffected. Shew *et al.* (1982) found that a mixture of 0.2 µl O_3 and 0.8 µl SO_2 per litre of air enhanced the reproduction of *P. penetrans* on tomato. The infected-exposed plants have often shown more discernible symptoms of SO_2 than the non-infected plants exposed to the same concentration of the gas. The SO_2-induced foliar injury was considerably higher on tomato plants inoculated with *Pratylenchus penetrans* or *Meloidogyne incognita* (Shew *et al.*, 1982; Khan and Khan, 1993). The effects of air pollutants on host-parasite interactions involving the nematodes have not been thoroughly studied and there is no satisfactory explanation about the variable responses of nematodes to pollutants.

The interaction of SO_2 and root-knot nematode was investigated using concurrent inoculation-exposure treatment to determine the single and combined effects of SO_2 (100, 200 and 300 µg m^{-3}) and *Meloidogyne javanica* (Treub) Chitw. on plant growth, flowering, fruit-setting and photosynthetic pigments of pumpkin, *Cu-*

curbita moschata L. The effect of SO_2 on root-knot disease intensity and reproduction of the nematode was also examined.

Materials and methods

The exposure system (Khan and Khan, 1993) consisted of three chambers each 90 x 90 x 120 cm dimension. Exposure chambers were made up of transparent fibre glass. Sulphur dioxide was produced in a generator by the reaction of dilute sulphuric acid and sodium sulphite.

Five water-soaked seeds of pumpkin were sown in 40 clay pots each containing 2.5 kg autoclaved soil (field soil + compost 3:1). One week later, the germinated seeds were thinned to one in each pot. A week later, 20 pots were inoculated each with 2000 freshly hatched juveniles of *M. javanica.* Immediately after inoculation, 5 inoculated pots and 5 uninoculated pots were exposed to 100, 200 or 300 μg SO_2 m^{-3} for 3 hours every third days for 60 days. Five pots each with and without nematode were not exposed to SO_2 and served as control. Each treatment had five replicates. All the pots were placed on glass-house benches at 30 °C in a complete randomized block design. The pots were brought out of the glasshouse only for exposure to SO_2. All plants were irrigated with tap water before the exposure. Plants were harvested at 60 days after the start of first exposure and lengths and fresh and dry weights of shoots and roots were determined. Plants were examined regularly for symptoms attributable to SO_2. Flower buds formed during the course of the experiment and total number of fruits at the time of termination were counted.

Before drying the plants, galls (disease intensity) and egg masses (reproduction of the nematode) per root system were counted. For fecundity, 10 egg masses of similar size and colour were excised from each of the five roots of a treatment. The egg masses were blended in an electric blender to determine number of eggs per egg mass (Khan and Khan, 1994).

To estimate the amount of chlorophyll a and chlorophyll b, 1g fresh leaves from interveinal areas were ground with acetone (80%) for each treatment. The clear suspension was filtered through two Watman filter Papers (No. 1) in a Buchner funnel and used to determine the percent transmittance in a spectrophotometer at 645 and 663 nm (Mackinney, 1941).

The data were subjected to the analysis of variance (ANOVA) for two factors i.e., SO_2 (0, 100, 200 and 300 μg m^{-3}) and nematode (0, 2000 nematode juveniles/pot) and critical differences (C. D.) were calculated to identify the significant effects at $P \leq 0.05$. Data on disease intensity and reproduction were processed for a single factor ANOVA (Dospekhov, 1984).

Results and discussion

Sulphur dioxide at 200 and 300 μg m^{-3} induced chlorotic patches on pumpkin leaves both in the presence and absence of root-knot nematodes. The inoculated plants exposed to 100 μg SO_2 m^{-3} exhibited a mild chlorosis which was not discernible in the absence of *M. javanica.*

Root-knot nematodes and SO_2 (except 100 μg m^{-3}) separately caused significant suppressions of lengths and fresh and dry weights of shoots and roots of pumpkin compared with uninoculated-unexposed plants (Table I). Combined treatments of *M. javanica* and SO_2 had significant negative effects on plant growth at all concentrations of the gas. The growth parameters of inoculated plants were more or less equal at 200 and 300 μg SO_2 m^{-3}. According to the ANOVA, F values of individual effects of SO_2 and *M. javanica* were significant ($P \leq 0.05$) for all the considered parameters of plant growth. Their interactive effects were, however, significant for dry weights of shoots and roots (Table I).

TABLE I - *Individual and joint effects of SO_2 and root-knot nematode on plant growth and dry matter production of pumpkin.*

Nematode juveniles/pot	SO_2 µg m^{-3}	Length cm Shoot	Length cm Root	Fresh weight g Shoot	Fresh weight g Root	Dry weight g Shoot	Dry weight g Root
0	0	134	51	216	62	29.4	8.1
0	100	127	46	201	59	29.0	8.1
0	200	110*	42*	194*	55*	26.3*	7.2*
0	300	102*	39*	181*	52*	25.1*	6.7*
2000	0	117*	46*	193*	57*	27.1*	7.5*
2000	100	98*	42*	181*	55*	24.8*	7.0*
2000	200	94*	39*	172*	48*	24.2*	6.6*
2000	300	91*	38*	163*	48*	23.9*	6.6*
C.D. $P \leq 0.05$		14.7	5.6	17.8	4.1	1.8	0.43
F value SO_2 (df=3)		21.3*	27.8*	18.3*	16.6*	19.1*	18.5*
Nematode (df=1)		16.5*	14.2*	18.9*	17.1*	18.4*	17.9
SO_2 x Nematode (df=3)		NS	NS	NS	NS	5.2*	5.6*

* Significantly different from uninoculated-unexposed plants (control) at $P \leq 0.05$, or significant at $P \leq 0.05$.

The flower development of pumpkin was influenced by neither the nematode nor SO_2, but fruit-setting was inhibited (Table II). The number of fruits declined in all the treatments (except 100 µg SO_2 m^{-3} in the absence of nematode), being lowest at 300 µg SO_2 m^{-3} in inoculated plants. Overall individual and interactive effects of SO_2 and *M. javanica* were significant for number of fruits/plant (Table II).

The synthesis of chlorophyll a was significantly suppressed as a result of exposure of the plants to SO_2 at 200 and 300 µg m^{-3} (Table III). Reduction in chlorophyll b was significant only at 300 µg SO_2 m^{-3}. Root-knot nematodes, however, caused a significant decrease in both the pigments. The combined treatments of SO_2 and the nematode had negative significant effects also on both the pigments, but chlorophyll a was relatively more affected than chlorophyll b. F values for the individual and joint effects were significant for chlorophyll a, whereas for chlorophyll b it was significant for SO_2 alone (Table III).

M. javanica induced severe galling in the roots of pumpkin, which was significantly enhanced at 100 µg SO_2 m^{-3} and suppressed at 300 µg m^{-3} (Table III). The egg mass production was enhanced by 5.6% (non-significant) at 100 µg while significantly inhibited at 300 µg m^{-3}. The galling and egg mass production was little influenced at 200 µg SO_2 m^{-3}. When number of galls and egg masses were calculated per g fresh root, a significant increase was recorded at 100 and 200 µg SO_2 m^{-3} and decrease at 300 µg (Table III). Fecundity (number of eggs/egg mass) was marginally increased at 100 µg m^{-3}. A gradual decline in number of eggs was recorded for the remaining concentrations that was significant for galls, egg masses and fecundity.

Interaction of *M. javanica* with SO_2 was consistently synergistic at 100 µg m^{-3} and antagonistic or additive (slightly synergistic or antag-

TABLE II - *Individual and joint effects of SO_2 and root-knot nematode on flower production, fruit-setting and foliar chlorophylls of pumpkin.*

Nematode juveniles/pot	SO_2 µg m^{-3}	Number/plant Flower	Fruit	Chlorophyl µg/g fresh leaf a	b
0	0	23	23	581	317
0	100	24	23	569	311
0	200	21	18*	540*	295
0	300	21	17*	482*	267*
2000	0	22	21*	551*	304*
2000	100	22	19*	522*	283*
2000	200	21	17*	489*	278*
2000	300	21	16*	454*	267*
C.D. $P \leq 0.05$		2.2	1.8	29.3	23.8
F value SO_2 (df=3)		NS	22.7*	27.8*	12.5*
Nematode (df=1)		NS	16.5*	21.2*	NS
SO_2 x Nematode (df=3)		NS	5.1*	5.9*	NS

* Significantly different from the uninoculated-unexposed plants; (control) at $P \leq 0.05$, or significant at $P \leq 0.05$.

TABLE III - *Effect of intermittent exposures of SO_2 on root-knot disease development and reproduction of* Meloidogyne javanica.

Nematode juveniles/pot	SO_2 µg m^{-3}	Number/root system Galls	Egg masses	Number/g fresh root Galls	Egg masses	Number of eggs/ egg mass
2000	0	238	161	4.17	2.82	218
2000	100	264*	170	4.8*	3.14*	223
2000	200	245	157	5.0*	3.20*	209
2000	300	182*	122*	3.71*	2.49*	182
C.D. $P \leq 0.05$		29.8	16.4	0.32	0.13	21.6
F value (df=3)		43.5*	12.8*	57.1*	59.4*	11.8*

* Significantly different from unexposed plants (control) at $P \leq 0.05$, or significant at $P \leq 0.05$.

onistic) at 200 and 300 µg m^{-3}. The synergistic effects at 100 µg m^{-3} on the considered plant growth parameters can be justified that probably the nematode infection enhanced the SO_2 up take through higher transpiration rates and SO_2 exposures lowered the soil pH to a level optimum for nematode development. The antagonistic interaction might have resulted due to adverse effects of SO_2 on the nematode through its various developmental stages from penetration to egg laying.

The present investigation demonstrated that root-knot nematodes may become more pathogenic in the areas where air is contaiminated with SO_2 at the concentration the tolerance level (i.e., 120 µg m^{-3}).

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