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PLANT GROWTH AND ROOT-KNOT DISEASE OF SOYBEAN UNDER THE STRESS OF SO_2 AND O_3 GASES MIXTURE

by

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Summary. Plant growth and root-knot disease of soybean was evaluated under the stress of SO_2 + O_3 gases in artificial treatment conditions. *Meloidogyne javanica* suppressed plant growth significantly but negative effects of nematodes were masked by *Bradyrhizobium japonicum* to some extent. Plants inoculated with *B. japonicum* and *M. javanica* jointly, achieved growth less than *B. japonicum* inoculated but more than *M. javanica* inoculated plants. All the considered parameters in this experiment showed remarkable suppressions in different SO_2+O_3 mixtures, being highest at 0.2+0.2 ppm, with or without root-knot nematodes and/or root-nodule bacteria. SO_2 and O_3 interacted synergistically in causing damages to the plant growth particularly in presence of *M. javanica*.

SO_2 and O_3 are reported to influence the effect of nematodes on plant growth both positively or negatively (Weber *et al.*, 1979; Khan and Khan, 1993) through altered physiology and biochemistry of the exposed plants. In nature, air pollutants generally occur as a mixture and are known to act synergistically in their effect on plants. Their effect on some plant pathogen has also been examined but the effect of root-knot nematode on plants under the stress of air polutant mixture has not been determined properly. The experiment reported here was undertaken to determine the impact of root-knot nematode *Meloidogyne javanica* (Treub) Chitw. on soybean (*Glycine max* L.) grown under the stress of a mixture of SO_2 and O_3 in artificial conditions.

Materials and methods

Seeds of soybean var. PK. 327 were surface sterilized by submerging them in 0.01% $HgCl_2$ for 15 minutes and sown in clay pots (5 seeds/pot) containing autoclaved soil (field soil + compost in proportion of 3:1). In some of the pots *Bradyrhizobium japonicum* Jordan was incorporated. Two weeks later, the plants were thinned to retain one healthy seedling per pot. A week after thinning, 1500 freshly hatched second stage juveniles of *M. javanica* were added to some of the pots. Immediately after nematode inoculations, all the pots were exposed to the mixture of all possible combinations of 0.1 and 0.2 ppm of SO_2 and O_3.

SO_2 and O_3 were generated in two separate generators. SO_2 was produced by the reaction of sodium sulphite (Na_2SO_3) and sulphuric acid (10% H_2SO_4) whereas O_3 was generated under the silent electric discharge i.e. UV ozone generator. Both the generators were connected through PVC pipes to an exposure chamber of $90 \times 90 \times 120$ cm^3 dimensions. The chamber was made from transparent fibre glass and provided with a blower assembly at the bottom and

exhaust duct at the top. Actual concentrations of SO_2 and O_3 were determined with the help of a handy air sampler (Kimoto, Japan) and following the colorimetric method of West and Gaeke (1956). The exposure system has been described elsewhere (Khan and Khan, 1993). The seedlings of soybeans were exposed to the mixture of SO_2 and O_3 (Table I) for 3 hours on alternate day for 55 days.

After 28 exposures, the plants were harvested and brought to the laboratory where lengths and fresh weight of shoot and root were measured. Roots were also examined for the presence of nodules, the number of functional (pink and healthy appearance) and total nodules per plant were counted. At the termination of the experiment, harvested roots were washed in tap water and examined for the presence of galls. Number of galls per root system was counted. Roots were immersed in a aqueous solution of phloxin B (0.15 g/lit. tap water) for 15 minutes to stain the egg masses. Egg masses were released from the roots by vigorously shaking them in 5.25% NaOCl solution. The eggs were separated from egg masses and collected on a 500 mesh sieve and then transferred to a beaker and 0.35% acid fuchsin (in 25% lactic acid) was added into 20 to 25 ml of suspension with boiling for one minute for staining the eggs. After cooling, the eggs were counted and the fecundity was calculated.

For counting number of females, 1 g of root pieces were transferred into 5% HNO_3 and incubated at 25 °C. After 72 hours, root pieces were gently teased to release the females. The number of females/g of root was counted and total number of females for the whole root system was calculated.

The possible combinatons of 0.1 and 0.2 ppm of SO_2 and O_3, with or without *Bradyrhizobium japonicum* and/or nematode are given in all the tables. Each treatment (exposed and unexposed) was replicated five times and pots were arranged in a completely randomized block design. Data were subjected to ANOVA and critical difference (CD) calculated.

Results and conclusions

Plant growth in terms of lengths and fresh weight of shoot and root showed significant improvement in the presence of *B. japonicum* but the reverse was true with *M. javanica*. However, the suppression caused by the nematode was less in the presence of *B. japonicum* (Tables I and II). Root nodulation caused by *B. japonicum* was also significantly suppressed by *M. javanica* (Table III). Root-galling, egg mass

TABLE I - *Effect of SO_2 + O_3 mixture on length of shoot and root of soybean.*

Treatments	Shoot length (cm)						Root length (cm)					
	SO_2 + O_3 (ppm)						SO_2 + O_3 (ppm)					
	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x
None	60.4	50.0	47.2	49.3	45.2	50.4	34.7	26.6	23.2	24.6	22.4	26.3
Bradyrh.	71.8	51.8	49.3	51.2	47.3	54.3	40.4	29.3	25.0	27.8	24.4	29.4
Nematode	56.1	49.9	46.8	48.6	44.4	49.2	31.5	25.7	22.3	24.4	20.5	24.9
Bradyrh. +Nematode	63.1	50.2	47.4	49.3	46.6	51.3	36.7	27.5	24.3	26.6	23.5	27.7
X	62.8	50.5	47.6	49.6	45.9		35.8	27.3	23.7	25.8	22.7	
CD at P = 0.05	Treatments = 0.71, SO_2 + O_3 = 0.79 Treatments x SO_2 = 1.59						Treatments = 0.74, SO_2 + O_3 = 0.82 Treatments x SO_2 + O_3 = 1.65					

TABLE II - *Effect of SO_2 + O_3 mixture on fresh weight of shoot and root of soybean.*

Treatments	Shoot weight (g)						Root weight (g)					
	SO_2 + O_3 (ppm)						SO_2 + O_3 (ppm)					
	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x
None	22.3	15.9	12.1	13.4	11.1	15.0	13.4	10.4	9.5	10.0	9.1	10.5
Bradyrh.	27.2	17.9	15.1	16.5	14.5	18.2	17.7	11.2	10.4	10.8	10.2	12.0
Nematode	20.2	12.8	11.4	12.5	10.5	13.5	10.8	9.6	8.7	9.3	8.1	9.3
Bradyrh. +Nematode	24.4	16.3	13.2	15.4	12.3	16.3	15.9	10.8	9.8	10.0	9.6	11.2
X	23.5	15.7	13.0	14.4	12.1		14.4	10.5	9.6	10.0	9.2	
CD at P = 0.05	Treatments = 0.09, SO_2 + O_3 = 1.09 Treatments x SO_2 + O_3 = NS						Treatments = 0.17, SO_2 + O_3 = 0.19 Treatments x SO_2 + O_3 = 0.33					

TABLE III - *Effect of SO_2 + O_3 mixture on functional and total number of nodules per plant of soybean.*

Treatments	Number of functional nodules						Number of total nodules					
	SO_2 + O_3 (ppm)						SO_2 + O_3 (ppm)					
	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x
None	–	–	–	–	–	–	–	–	–	–	–	–
Bradyrh.	199.6	54.8	43.8	49.2	40.4	77.5	222.4	81.6	68.6	73.2	62.2	101.6
Nematode	–	–	–	–	–	–	–	–	–	–	–	–
Bradyrh. +Nematode	155.0	51.6	36.6	39.0	29.6	62.3	187.2	69.8	57.8	66.0	56.4	87.4
X	172.3	53.2	40.2	44.1	35.0		204.8	75.7	63.2	69.6	59.3	
CD at P = 0.05	Treatments = 1.31, SO_2 + O_3 = 2.07 Treatments x SO_2 + O_3 = 2.92						Treatments = 3.24, SO_2 + O_3 = 5.12 Treatments x SO_2 + O_3 = 7.25					

production and number of females were found decreased significantly by *B. japonicum* inoculation of soybean but the reverse was true with fecundity (Tables IV and V).

Exposure of the plants to a mixture of SO_2 and O_3 caused significant reductions in length of shoot and roots, their fresh weight and nodulation, irrespective of the presence of *B. japonicum* and/or *M. javanica*. Suppression of all growth parameters and root-knot disease was in the following order: Unexposed < 0.1+0.1 < 0.2+0.1 < 0.1+0.2 < 0.2+0.2 ppm pf SO_2 and O_3. The plants infected with *M. javanica* showed greater suppression of growth at all levels of exposure than uninoculated plants. In the presence of *B. japonicum* and *M. javanica*, plant growth was better than that with the plants inoculated with nematode alone. Galls and egg masses in presence or absence of root-nodule bacteria were found to be at par in 0.2+0.1 ppm and 0.2+0.2 ppm of SO_2 and O_3. Thus SO_2 and O_3 mixture at 0.2+0.2 ppm was most effective in reducing the considered plant growth and root-knot disease parameters.

The data of the experiment indicate that SO_2 and O_3 interacted synergistically in causing damage to plant growth particularly in the presence of root-knot nematode. Also, at the same ti-

TABLE IV - *Effect of SO_2 + O_3 mixture of number of egg masses and fecundity of* Meloidogyne javanica *on soybean.*

Treatments	Number of egg masses						Fecundity					
	SO_2 + O_3 (ppm)						SO_2 + O_3 (ppm)					
	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x
None	–	–	–	–	–	–	–	–	–	–	–	–
Bradyrh.	–	–	–	–	–	–	–	–	–	–	–	–
Nematode	34	11	7	8	5	13	403	371	349	360	344	365
Bradyrh. +Nematode	30	9	6	8	5	12	414	383	367	372	362	379
X	32	10	6.5	8	5		408	377	358	366	353	
CD at P = 0.05	Treatments = 0.92, SO_2 + O_3 = 1.30 Treatments x SO_2 + O_3 = 1.84						Treatments = 7.84, SO_2 + O_3 = 12.39 Treatments x SO_2 + O_3 = NS					

TABLE V - *Effect of SO_2 + O_3 mixture on gall and female production of* M. javanica *on soybean.*

Treatments	Number of galls						Number of females					
	SO_2 + O_3 (ppm)						SO_2 + O_3 (ppm)					
	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x	0+0	0.1+0.1	0.1+0.2	0.2+0.1	0.2+0.2	x
None	–	–	–	–	–	–	–	–	–	–	–	–
Bradyrh.	–	–	–	–	–	–	–	–	–	–	–	–
Nematode	56	32	25	28	20	32	74	40	34	38	32	44
Bradyrh. +Nematode	44	28	20	25	17	27	64	33	28	32	26	37
X	51	30	22	26	18		69	38	31	35	29	
CD at P = 0.05	Treatments = 1.52, SO_2 + O_3 = 2.40 Treatments x SO_2 + O_3 = 3.39						Treatments = 1,41, SO_2 + O_3 = 2.23 Treatments x SO_2 + O_3 = NS					

me, root-knot nematode disease parameters were suppressed by SO_2 and O_3 combination, particularly at 0.2+0.2 ppm. These effects of SO_2+O_3 and/or nematode were masked by *B. japonicum* as it prevented damage to plant growth to some extent.

Acknowledgements. Senior author is grateful to CSIR for providing financial assistance as Research Associateship.

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Accepted for publication on 28 June 1996.