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INDIVIDUAL AND COMBINED EFFECTS OF SO_2 AND O_3 ON ROOT KNOT NEMATODE MULTIPLICATION

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ABSTRACT

Both (sulphur dioxide and ozone) gases have been proved harmful to all the living organisms. In the present study, root-knot nematode (*Meloidogyne incognita* Race 1)infected green gram (*Vigna radiata* cv.T-44) plants were exposed with SO_2 and O_3 individually or jointly in presence or absence of root-nodule bacteria (*Rhizobium leguminosarum* bv trifolli). Both SO_2 and O_3 individually reduces the fecundity, number of females and juveniles(J2&J3+J4), population(root,soil and total population) and reproduction factor (Rf) of root-knot nematode.However, O_3 is proved to be more toxic to all these parameters, compared to any SO_2 level. Although, the reductions were enhanced in SO_2 and O_3 joint exposures. The reductive trend in the studied parameters can be arranged as:SO_2<O_3<SO_2+O_3.The reductions were furthered by *Rhizobium* inoculations except fecundity. Nematode multiplication, in terms of their population andRf, was reduced gradually in gradual SO_2 and/or O_3 pollutant/s combinations. Thus reduction was SO_2 and/or O_3 concentration dependent. More reduction occurred in higher than lower SO_2 and O_3 joint combinations. Maximum reductions were occurred in 0.3ppm of each of SO_2 and O_3 mixture, particularly in presence of root-nodule bacteria.

KEYWORDS : Root-knot nematode, Root-nodule bacteria, Nematode multiplication, reduction

Amongst gaseous air pollutants, SO_2 and O_3 are considered to be the main atmospheric pollutantand may present either as individual or in combination, in the ambient conditions. SO_2 is mainly produced by coal burning power plants, where fuel is burnt in a huge amount.O_3 is mainly produced by automobile exhaust. O_3 concentration is comparatively less compared to SO_2 at the ground level. But its concentration increases by baking the primary pollutants (NOx) together with some volatile organic compounds (VOC's) under the impact of direct sunlight.

Root-knot nematode (*Meloidogyne* species) is an obligate parasite and attacks on several kind of crops all over the world. They are highly destructive plant pathogen and causeworld wide loss exceed 125 billion annually (Chitwood, 2003). The average crop yield losses are estimated to be ranges from 25% to 60%. *Meloidogyne incognita* (Kofoid and White) Chitwood,is one of the important amongst the major root-knot nematode species.This is considered to be the most prevalent species with approximate distribution of 75% in agricultural soils (Adegbite and Adesiyan, 2005). SO_2 and / or O_3 are also reported to affect the reproduction and multiplication of the root-knot nematode (Singh and Khan, 1999). However, different SO_2 and O_3 exposure causes varied responses with regard to reproduction and multiplication of five phytonematodes on begonia andsoyabean (Weber et al., 1979). No exact systematic mechanism is available so far on the impact of SO_2 and O_3 on multiplication of the root-knot nematode. So the present study has been conducted in order to assess the effect of individual or joint SO_2 and O_3 exposures on the nematode multiplication on the green gram plants. This assessment would become more interested in presence of root-nodule bacteria presence, since the test crop is in important leguminous crop.

MATERIALS AND METHODS

SO_2 and/or O_3 Generation and Exposure

SO_2 and O_3 were generated by SO_2 and O_3 generators respectively (Khan and Khan, 1993a). Polyvinylchloride (PVC) tube originated from SO_2 or O_3 generator, was connected to inlet of blower assembly of the different exposure chambers in order to expose the plants individually.Similarly for SO_2 and O_3 joint exposures, PVC tube originated from their respective generators were connected to the two different inlets of blower assembly of the same exposure chamber.

The exposure of the potted green gram seedlings, designated to receive SO_2 + O_3 exposures, was started immediately after the nematode inoculation (i.e. simultaneous inoculation exposure). Three week-old seedlings were placed in the exposure chamber for doing

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exposure by0.1, 0.2 and 0.3 ppm of SO_2 and O_3 or by their all possible combinations, for 3 hours on every alternate day up to 80 days from sowing (i.e. termination period).During exposure process, sampling of the exposure chamber was done by two handy air samplers in order to assess and maintain the above said concentration of the pollutant/s. For determining SO_2 concentration, first handy air sampler was placed inside the chamber by taking KTMC (Potassium Tetra ChloroMercurate) as the absorbing media in its impinger. Likewise, O_3 concentration was determined by placing another handy air sampler with KI (potassium iodide) as the absorbing media in its impinger. For estimation of SO_2 + O_3 concentrations,both samplers were placed in the exposure chamber by taking KTMC and KI as the absorbing media in different impingers. Onward analysis was done in the laboratory through calorimetry.

Plant Culture

Seedlings of green gram (*Vigna radiata*) (L) Wilczek cv. T-44 were grown in clay pots of 30 cm diameter from surface sterilized seeds. Prior to sowing seeds were soaked in water for 24 h and then surface sterilized with 0.01% mercuric chloride ($HgCl_2$) for 15 min. Such surface sterilized seeds were sown in the pots already filled with autoclaved sandy loam field soil (66% sand, 24% silt, 8% clay, 2% OM and pH 7.7).

Root-Nodule Bacteria Culture

Pure culture of *Rhizobium leguminosarum* bvtrifolli was procured from the Agriculture Farm House, Quarsi, Civil Lines, Aligarh, U.P. (India). This commercial and pure culture of bacteria was used in the experiment. Prior to sowing, seeds were treated with sugar + water + *R. leguminosarum*, followed by drying in shade for half an hour before sowing.

Root-Knot Nematode Culture

Meloidogyne incognita (Kofoid and White) Chitwood Race 1 of root-knot nematode was used in the experiment. Field population of *M. incognita* wasinitially first raised on tomato, *Lycopersicone sculentum* Mill (cv. Pusa Ruby). Tomato roots infected with root-knot nematode were collected from the field through survey and the species present in the collected samples were identified on the basis of the characteristics of perineal patterns of the females (Eisenbacket et al., 1981). The race of nematode was determined by conducting the North Carolina Host Test. Roots infected with *M. incognita* were chopped and added to the pots containing moistened sterilized field soil.

Thereafter seedling (3-4 week old)of tomato plants, raised from surface sterilized seeds in autoclaved soil, were transplanted in the pots. Single egg mass culture of the nematode, obtained from the root of tomato plants maintaining the field population of *M. incognita*, was added near each seedling root in the pots. The root-knot nematode culture was ready to use after 50 days. Subculturing was done by inoculating new tomato seedlings by inoculating atleast 15 egg masses after every 2-3 months, in order to maintain the sufficient inoculum.

Inoculum and Inoculation

Second stage juveniles (J_2) of the root-knot nematode were used as inoculum in the experiment. J_2 were obtained by incubating egg masses collected from the roots of tomato plants. Egg masses were incubated in coarse sieve fitted with double layer tissue paper and placed on Baerman funnel containing water. The sieves were then placed in an incubator (temp. 25±2°C). After 72 h, hatched J_2 were collected in a beaker and number of juveniles per ml was standardized by counting the juveniles (in a counting dish) from ten, 1 ml samples. Inoculation, to 3-4 week old green gram seedling was done by injecting the J_2 suspension through syringe of micropipette controller to the holes made in soil. The holes were covered immediately with the soil scraped from the same pot. Number of J_2 inoculated / pot were 1500 (i.e. initial inoculum level).

Treatments

Control Treatments

1. Plant + Root-knot nematode
2. Plant + Root-knot nematode + Root-nodule bacteria

SO_2 and / or O_3 exposed treatments

Both the control treatment set were exposed separately with 0.1, 0.2, and 0.3 ppm of SO_2 or O_3 and with all joint possible combinations of SO_2 and O_3 with their concentration range from 0.1 to 0.3 ppm each.

Statistical analysis was done through two factorial method.Two factors were created i.e factor one (f_1) for SO_2 and / or O_3 and factor two .(f_2) for nematode and /or bacterial

treatments. Separate LSD was calculate for these factors along with their interactive LSD at P = 0.05.

Fecundity

Roots after harvesting were washed under the tap water and were immersed in an aqueous solution of Phloxin B (0.15 g/l tap water) for 15 minutes to stain the egg masses. Fecundity (i.e. number of eggs per egg mass) was measured by shaking vigorously 10 egg masses in 5.25, NaOCl solution. The eggs were separated from egg masses and collected over 500 mesh sieve. From the sieve the eggs were transferred into a beaker and 0.35% acid fuchsin was added into 20 to 25 ml of suspension with boiling for 2 minute for staining the eggs. The eggs were counted after cooling and the number eggs per egg mass were calculated to find out the fecundity.

Nematode Population and Reproduction Factor

Root population of the nematode was obtained by summating the number of J_2, $J_3 + J_4$ and mature females. Root from each replicate was weighed and cut into pieces of 1 cm length. One gram of root pieces werestained with acid fuchin and lactophenol. The root pieces were placed between two slides and examined under stereoscopic microscopefor favour of counting the number of J_2 and $J_3 + J_4$. Total number of J_2 and $J_3 + J_4$ for the whole root system of each replicate was calculated and an average of five replicates of a treatment was then calculated.

For counting the numbers of females, 1 gm of root pieces were transferred in 5% nitric acid and incubated at 25°C. After 72 h, root pieces were gently teased to release the females. The number of females/g root were counted and total number of female for whole root system was calculated. The means of replicates was then calculated.

Soil population (J_2 + male) of the nematode was estimated by modified Cobb's sieving and decanting technique (Southey, 1986). The total final population of the nematode (Pf) was determined by computing the soil and root population and number of eggs per egg mass and an averagewas calculated. The reproduction factor (Rf) was determined according to R_F = Pf / Piformula, where numerator Pf represents the final population and denominator Pi represents the initial population (J_2 used as initial inoculum level) of the root-knot nematode.

RESULTS

Fecundity, Females and Juveniles

Fecundity was enhanced by root-nodule bacteria but reverse happened to number of females and juveniles (i.e. J_2 & $J_3 + J_4$). However, gradual reductions to them were occurred in progressive increasing level of SO_2 from 0.1 ppm to 0.3 ppm. Reductions were furthermore increased in O_3 exposures. From amongst the O_3 exposed treatments, they were the 0.3 ppm exposed green gram plants which received minimalnumber of eggs/eggmass, females and juveniles. Thus reductionwas occurred by both SO_2 and O_3 simultaneously but later was proved relatively more toxic compared to former with regard to all above mentioned parameters. Reductions were, however, greater in presence of *R. leguminosarum* except fecundity.

SO_2 and O_3 in combined treatments,suppressed them furthermost. SO_2 and O_3 jointly reduced them to a greater extent than what they did individually. If arrangement is done for the studied parameters to show off their progressive reductions in SO_2 and/or O_3 treatments, then they can be arranged in the following fashion with respect to their gradual increase in reductions: $SO_2 < O_3 < SO_2 + O_3$. However, within each of them, higher exposure level/swere proved more injurious than lower level/s with respect to considered root-knot disease parameters. So obviously 0.2 and 0.3 ppm of $SO_2 + O_3$, at all possible cross combinations, decreased them utmostly than rest of the mixture treatments (i.e. 0.1 and 0.2 ppm combinations). Maximum reduction, with regard to fecundity, females and juveniles, was occurred in 0.3 ppm SO_2 and 0.3ppm O_3 combination exposure (table 1).

Nematode Population and Reproduction Factor

Root-knot nematode multiplication, in terms of population (Soil, root and total population) and reproduction factor (Rf), was recorded less in presence of root-nodule bacteria. Highest population was recorded in those control green gram plants set which were not inoculated root-nodule bacteria. Nematode population was decreased gradually with progressive increase of SO_2 level. Lowest population was recorded in 0.3ppm SO_2 exposed plants particularly in presence of root-nodule bacteria. Similar but greater reductions to nematode population were

Table 1 : Effect of SO_2 and/or O_3 on Number of Second, Third and Fourth Stage Juveniles (i.e. J_2 & J_3+J_4) Eggs/Eggmass (i.e. Fecundity) and Females of *Meloidogyne incognita* Race 1 on Green Gram

Treatments → / Pollutant ↓ mixture (SO_2+O_3)	Number of J_2			Number of J_3 + J_4			Number of egg/eggmasses			Number of females		
	P+N	P+N+R	MM	P+N	P+N+R	MM	P+N	P+N+R	MM	P+N	P+N+R	MM
0.0+0.0	1800.00	1675.60	868.90	405.00	355.40	190.10	434.40	445.20	219.90	80.40	74.60	38.75
0.1+0.0	1600.00	1500.40	775.10	360.40	351.20	177.90	426.00	431.40	214.35	65.00	59.20	31.05
0.2+0.0	1589.20	1405.00	748.55	354.20	338.00	173.05	411.20	420.00	207.80	56.00	51.20	26.80
0.3+0.0	1494.00	1364.60	714.65	341.00	325.00	166.50	400.60	408.20	202.20	45.20	41.00	21.55
0.0+0.1	1584.00	1420.20	751.05	351.00	343.00	173.50	418.00	426.00	211.00	55.40	52.00	26.85
0.0+0.2	1565.20	1398.40	740.90	348.40	330.60	169.75	398.60	413.00	202.90	49.20	45.00	23.55
0.0+0.3	1479.00	1350.20	707.30	335.00	319.80	163.70	385.00	401.00	196.50	39.60	35.20	18.70
0.1+0.1	1525.00	1398.40	730.85	340.00	337.00	169.25	405.20	419.00	206.05	53.20	49.00	25.55
0.1+0.2	1445.00	1304.60	687.40	308.00	297.60	151.40	365.60	351.20	179.20	42.00	40.00	20.50
0.1+0.3	1415.00	1274.60	672.40	291.00	264.00	138.75	339.00	335.00	168.50	36.20	36.00	18.05
0.2+0.1	1498.00	1356.00	713.50	328.20	325.60	163.45	392.00	398.00	197.50	48.60	45.80	23.60
0.2+0.2	1421.20	1295.20	679.10	298.00	284.00	145.50	350.20	347.60	174.45	39.80	38.20	19.50
0.2+0.3	1360.00	1224.00	646.00	263.20	234.60	124.45	318.00	305.00	155.75	31.00	32.00	15.75
0.3+0.1	1465.80	1319.20	696.25	317.60	309.00	156.65	380.20	384.60	191.20	45.00	42.20	21.80
0.3+0.2	1394.00	1256.00	662.50	284.00	251.00	133.75	328.00	321.00	162.25	34.00	34.60	17.15
0.3+0.3	1342.00	1215.80	639.45	244.60	225.20	117.45	309.00	295.20	151.05	29.80	29.20	14.75
MM	1498.59	1359.89		323.10	305.69		378.81	381.34		46.90	44.08	
LSD at 5%												
Pollutant mixture (F_1) =		13.66		3.02		3.640		0.452				
Treatments (F_2) =		27.32		6.04		7.279		0.905				
Interaction (F_1 x F_2)		54.64		12.07		14.558		1.810				

occurred at same level O_3 than SO_2. From amongst them, they were the 0.3 ppm O_3 exposed plants which showed minimum nematode populationparticularly onnodulated green grams.

Joint SO_2 and O_3 exposures suppressed the population furthermore and as usual the suppressive effects were greater in *R. leguminosarum* inoculated thanuninoculated plants. Different possible SO_2 (0.1-0.3ppm) and O_3 (0.1-0.3ppm) combinations, used in exposures, were proved important determinant for the suppressions that happened to the nematode population. Nematode population was suppressed in the increasing order as: 0.1+0.1, 0.2+0.1, 0.1+0.2, 0.2+0.2, 0.3+0.1, 0.1+0.3, 0.3+0.2, 0.2+0.3, 0.3+0.3 in SO_2 +O_3 combinations (Table - 2). Thus greatest suppressions to population were occurred at 0.3+0.3 ppm SO_2 and O_3.

Since reproduction factor (Rf) is equal to the ratio of total final nematode population to the initially used population in the experiment, so the impacts of SO_2 and/or O_3 and root-nodule bacteria on Rf were more or less similar as happened to total final population of the nematode. The numerical value of initial nematode population remains constant throughout the experiment (i.e. 1500 J_2 per pot). But it was theonly final population of the nematode which varies according to the treatments. So the value of Rf is directly proportional to the value of final nematode population i.e. it increases with increase in final population and vice-versa. So according variations were occurred in the numerical values of Rf in the different treatments (table 2).

DISCUSSION

Presence of *R. leguminosarum* adversely affectsthe number females and juvenilesbut not fecundityof *M. incognita*. Green gram plant showed good health status in presence of *R. leguminosarum* which might have induced resistance against nematode juvenile penetration (Bird et al., 2003). Additional nitrogen fixed by the root-nodule bacteria, probably caused the inhibition in juvenile (i.e. J_2) penetration and their further development into J_3 + J_4. Since J_3+J_4 transcended into females through hatching, so the female production would also perturbed significantly.In sequential inoculations, (Singh, 2011) also observed reduced disease intensity due to poor penetration of juveniles. That was tentatively the way through which overall nematode population might have decreased. Comparatively less ingression of juveniles in suchnodulatedand healthy plants might have reduced the competition amongst them in the host root. Due to such advancement, the juveniles could have transformed into healthy females which laid ultimately more eggs. This could be advanced as a meddlingly reason behind the fecundity improvement.

Multiplication of the root-knot nematode wassuppressed by SO_2. Fecundity female and juvenile production including overall nematode population showed a gradual decline in different SO_2 exposures. SO_2 may affect the nematode directly through reacting with soil solution or indirectly through host mediated effects. Female energy demandincreasedgreatly during the oviposition (Melakeberhan and Webster, 1993). Less infection sites (due to poor root growth) and nutrient deficiency on SO_2 stressed green gram plants might have adversely affected the egg production and population of *M. incognita*. A number of workers have already been reported the altered physiologyand biochemistry (Singh and Singh, 2003; Singh, 2011) of the exposed plants. Such physiologically altered plants could not remain capable to provide sufficient and / or nutritious food to the developing nematodes and thereby disturb root population. Soil acidity increased due to SO_2 reaction with soil water to increase the H^+ and SO_4^{--} ionswhich probably had direct toxic effects on J_2 and males as they were mostly inhabited in the soil. Ions formed in the soil solution might have create hindrance in the free movement of J_2 and therefore chances of their infection diminishes. Above advocations, individually or mutually, could be held responsible for nematode population suppression in the soil and thus the total population in their parasitic and non-parasitic phase. According reflections could be observed in the reproduction factor as it directly proportional to the total final population of the nematodes. The reduction happened to nematode multiplication were SO_2 concentration dependent as they were greater at 0.3

Table 2 : Effect of SO_2 and/or O_3 on Root, Soil and Total Population and Reproduction Factor of *Meloidogyne incognita* Race 1 on Green Gram

Treatments → / Pollutant ↓ mixture (SO_2+O_3)	Root population			Soil population			Total population			Reproduction factor		
	P+N	P+N+R	MM	P+N	P+N+R	MM	P+N	P+N+R	MM	P+N	P+N+R	MM
0.0+0.0	2282.00	2102.60	1096.15	6641.00	5517.00	3039.50	9357.40	8064.80	4355.55	3.74	3.23	1.74
0.1+0.0	2019.00	1900.80	979.95	5846.20	5420.60	2816.70	8291.20	7752.80	4011.00	3.32	2.86	1.55
0.2+0.0	1998.40	1793.20	947.90	5346.00	4087.60	2358.40	7755.60	6300.80	3514.10	3.10	2.52	1.41
0.3+0.0	1879.20	1724.60	900.95	4945.00	3887.60	2208.15	7224.80	6020.40	3311.30	2.89	2.41	1.33
0.0+0.1	1989.40	1814.20	950.90	5608.20	4227.00	2458.80	8015.60	6467.20	3620.70	3.21	2.59	1.45
0.0+0.2	1954.80	1772.20	931.75	5141.00	4038.00	2294.75	7494.40	6223.20	3429.40	3.00	2.49	1.37
0.0+0.3	1849.60	1703.20	888.20	4741.00	3540.00	2070.25	6975.60	5644.20	3154.95	2.79	2.26	1.26
0.1+0.1	1911.20	1773.40	921.15	5545.00	4124.20	2417.30	7861.40	6316.60	3544.50	3.14	2.53	1.42
0.1+0.2	1789.00	1640.20	857.30	5242.00	3618.20	2215.05	7396.60	5609.40	3251.50	2.96	2.24	1.30
0.1+0.3	1740.20	1572.60	828.20	5135.00	2997.60	2033.15	7214.20	4905.20	3029.85	2.89	1.96	1.21
0.2+0.1	1873.80	1725.40	899.80	5448.00	4038.20	2371.55	7713.80	6161.60	3468.85	3.08	2.46	1.39
0.2+0.2	1754.00	1611.40	841.35	5167.00	3015.20	2045.55	7271.20	4974.20	3061.35	2.91	1.99	1.23
0.2+0.3	1651.20	1484.60	783.95	4913.00	2208.00	1780.25	6882.20	3997.60	2719.95	2.75	1.60	1.09
0.3+0.1	1821.40	1669.40	872.70	5326.00	3998.60	2331.15	7527.60	6052.60	3395.05	3.01	2.42	1.36
0.3+0.2	1710.00	1542.80	813.20	5000.20	2873.00	1968.30	7038.20	4736.80	2943.75	2.82	1.89	1.18
0.3+0.3	1615.40	1461.20	769.15	4830.80	2118.60	1737.35	6755.20	3875.00	2657.55	2.70	1.55	1.06
MM	1864.91	1705.74		5304.71	3731.84		7548.44	5818.90		3.02	2.31	

LSD at 5%

Pollutant mixture (F_1) =	17.077	44.53	64.996	0.026
Treatments (F_2) =	34.154	89.07	129.991	0.104
Interaction (F_1 x F_2)	63.308	178.14	259.982	0.104

- P = Green gram plant, N = *Meloidogyne incognita* race 1
  R = *Rhizobium leguminosarum*, MM = Mean of means
- Values in the table are mean of five replicates.

ppm followed by 0.2 and then by 0.1 ppm of SO_2.Higher doses of SO_2 might have made so much so greater impact at the soil and/or host that they could not remain fit to provide proper space and sufficient food to the developing nematodes.(Singh and Khan,1999) also observed similar impacts of SO_2 on the nematode multiplication.

Like SO_2, O_3 also reduced nematode multiplication but to a greater extent. Quality and quantity of the host nutrients are important factors for the nematode hatching and their further development, which may be altered due to O_3 exposures. More changes to quantity and quality could have occurred to host plant at higher than lower O_3 concentrations which were reflected back in the form of higher nematode multiplication reduction. O_3 usually reduces root more than shoot (Pasqualini, 2003). So greater reduction in roots would obviously have lesser availability chance for juvenile penetration to the host. Culmination of this can be seen as greater subsequent reductions to J_3+J_4, females and total population of the nematode. Greater reduction in nematode population was also recorded (Singh and Khan, 1999).

O_3 did greater reductions to nematodes than SO_2. This can bejustified through greater reductions occurred to growth and yield under the physiological stress due to O_3 than same level SO_2 (Singh and Singh, 2003). Such relative lower and higher adverse impacts of SO_2 and O_3 on plants would subsequently have similar respective impacts on the root-knot multiplication, as the nematode reclines upon the plants for their energy requirements. Thus O_3 exposed plants could became more inhospitable to nematode parasitism than SO_2 exposed plants. That can be accounted as a good reason to interpret the poor root-knot nematode multiplication on O_3 than SO_2 stressed plants.

Furthermore reductions to nematode multiplication were observedin joint SO_2 and O_3 treatments compared to SO_2 or O_3 alone treatments SO_2 and O_3 acts synergistically in reducing thegrowth and yield in the joint treatments (Weber et. al.,1979). This synergism could be reflected back in the form of reduced nematode multiplication. Such reduction to studied nematode parameters were happened by SO_2 and/or O_3 irrespective of the presence or absence of the root-nodule bacteria.(Singh and Khan, 1999) also experimented on the performance of nematode on SO_2 and O_3 stressed plants and found that the suppressions to reproduction and development of the nematode were pollutant concentration dependent as they were reported greater at higher than lower SO_2 + O_3 combinations.

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