Proc. Indian natn. Sci. Acad. B 45 No. 3 pp 261-268 (1979)

Persistence of Aretan in Soil and its Effect on Soil Bio-ecosystems and their related Biochemical activity

A P SINHA,* V P AGNIHOTRI and KISHAN SINGH
Indian Institute of Sugarcane Research, Lucknow (U.P.)

(Communicated by Professor D D Pant)

(Received 17 August 1978)

Addition of aretan (a.i. 2.5, 5, 10 and 20 ppm) to fresh soil affected the different bio-ecosystems of soil and their related biochemical activities.

Bioassay studies using *Myrothecium verrucaria* as test fungus showed extremely low persistence of aretan in soil and at 40 ppm it was fully degraded in 18 days. The fungicide markedly reduced the fungal propagules in the early part of the experiment and thereafter fungal numbers gradually increased. Lower doses of aretan reduced the number of *Fusaria*, while higher concentrations were lethal. Aretan, in general, stimulated the total population of bacteria and actinomycetes for 45 days and 15 days, respectively. However, the population of *Rhizobia* and *Azatobacter* decreased for first 15 days.

Aretan also affected growth and reproduction of 14 common soil fungi. It was more effective in curtailing growth of pathogenic fungi as compared to saprophytic ones.

The toxicant adversely affected soil respiration, and initial depression in CO_2 production was directly proportional to aretan concentration applied. Similarly, nitrification was adversely affected for varying periods of time, depending on the aretan concentration. Ammonification process was stimulated after an intial depression.

Introduction

Organo-mercurial toxicants because of their broad-spectrum fungicidal and bactericidal activity are widely used for seed and soil treatments (Nene 1971). They are also used for controlling damping-off, seedling blights and for treating bulbs, planting stocks and nursery beds (Vaartaja 1968).

The consumption of aretan (methoxy ethyl mercury chloride), a common organo-mercurial fungicide, is increasing every year in sugar crops (e.g. sugarcane and sugarbeet), but limited attention has been devoted to the side effects of this fungicide on soil microbes and their related biochemical activities. Thus, there is an urgent need to explore the possible microbial changes that occur in soil after aretan treatment because microflora, within broad limits, affect the crop-producing ability of the soil.

**Present address* : Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar (Nainital), U.P.

The objects of the present study were to determine (i) the persistence of aretan in soil, (ii) its influence on different groups of soil microbes, and (iii) its effects on three biochemical activities, namely, respiration, nitrification and ammonification.

Materials and Methods

Composite soil samples were collected from a depth of 15 cm from the Farm of the Indian Institute of Sugarcane Research, Lucknow. Based on active ingredient, aretan was applied to soil on an oven-dried basis (o.d.b.), at the rate of 2.5, 5, 10 and 20 ppm.

1. *Microbial study*

The dilution plate technique was used for estimating different microbial populations. Fungi were assessed on peptone rose-bengal agar medium (Martin 1950), *Fusaria* on Nash and Snyder's medium (Nash & Snyder 1962), *Azatobacter* on Jensen's agar medium (Jensen 1951), and *Rhizobia* on yeast-mannitol agar medium (Allen 1957). Total bacteria were assessed on Thornton's medium, as modified by Agnihotri (1971) by adding 50mg pentachloronitrobenzene, 40mg actidione and 35mg pimaricin. Actinomycetes were estimated on water agar medium (Lingappa & Lockwood 1962). To this medium 40mg actidione and 20mg pimaricin (Agnihotri 1971), were added to prevent the development of unwanted fungal colonies. The effect of aretan on radial growth and sporulation of the common soil fungi (table 7) was studied on potato dextrose agar. Method of inoculation, measurement of radial growth, and the method for calculating ED50 were the same as described by Singh et al. (1973a).

2. *Biochemical studies*

The apparatus designed by Peterson (1926) was employed for studying soil respiration. Evolved carbon dioxide was estimated at an interval of 4 days by the method of Pramer and Schmidt (1964). For nitrification experiment, 500g soil (o.d.b.) was mixed with different concentrations of aretan and ammonium sulphate (100 ppm N). The soil moisture was adjusted and maintained at 50% water-holding capacity (W.H.C.). The containers were incubated at $28\pm1°C$ and each treatment was replicated thrice. Nitrate nitrogen was determined by phenoldisulphonic acid method of Harper (1924). For ammonification study, peptone (100 ppm N) and required amounts of aretan were thoroughly mixed with soil and ammonia was estimated by Nesslerization (Jackson 1962).

3. *Bioassay studies*

The persistence of aretan in the soil was studied by the technique of Munnecke (1958), with slight modification. The spore concentration of *Myrothecium verrucaria* used was standardized using Systronic photometric colorimeter type 101, and at 60% transmission maximum clear inhibitory zone was recorded.

Results and Discussion

1. *Changes in microflora*

Aretan markedly reduced the number of fungal propagules, particularly at 5, 10 and 20 ppm, during the early part of the experiment. On the 15th day, the fluctuation in population was non-significant and thereafter the fungal population gradually increased in aretan amended soils as compared to the untreated check (table 1). The increase in fungal counts was mostly due to *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. terreus* and *Trichoderma viride* propagules. Two factors may have contributed for the greater number of fungi occurring in aretan amended soil : (i) the cell material of killed microbes offering a readily available energy source for surviving microbes or those which become re-established first, and/or (ii) the surviving organisms or those first to become re-established reached higher numbers in a less competitive environment. Aretan at 2.5 and

5.0 ppm drastically curtailed the population of *Fusaria*, while its higher concentrations (10 and 20 ppm) were lethal (table 2).

All concentrations of aretan increased the total bacterial population and it remained so throughout the experiment but significant stimulation was recorded only at 10 and 20ppm —more so at 10 ppm (table 3). The population of actinomycetes was appreciably increased in the early part of the experiment

Table 1 *Effect of aretan on the population of fungi in soil*

Treatments (ppm)	Number × 10^4/g soil on dry weight basis (mean of 3 replications)				
	Days of incubation				
	0	7	15	30	45
0.0	20.00	21.22	24.00	25.77	18.22
2.5	20.00	19.55	23.55	22.00	23.00
5.0	20.00	18.77	23.00	29.77	34.00
10.0	20.00	16.00	21.00	29.00	32.00
20.0	20.00	14.33	17.00	27.33	30.00
C.D. at 5%	—	3.36	—	5.08	3.95

Table 2 *Effect of aretan on the population of Fusaria in soil*

Treatment (ppm)	Number × 10^3/g soil on dry weight basis (mean of 3 replications)				
	Days of incubation				
	0	7	15	30	45
0.0	10.00	12.55	13.00	15.55	16.00
2.5	10.00	4.00	6.00	7.11	9.00
5.0	10.00	2.00	3.11	4.22	6.00
10.0	10.00	0.00	0.00	0.00	0.00
20.0	10.00	0.00	0.00	0.00	0.00
C.D. at 5%	—	2.36	2.34	3.40	1.99

Table 3 *Effect of aretan on the population of bacteria in soil*

Treatment (ppm)	Number × 10^6/g soil on dry weight basis (mean of 3 replications)				
	Days of incubation				
	0	7	15	30	45
0.0	30.00	37.00	39.11	35.55	33.00
2.5	30.00	40.00	48.55	46.11	44.22
5.0	30.00	43.22	59.00	56.00	48.00
10.0	30.00	50.66	68.00	73.00	60.44
20.0	30.00	53.22	61.77	68.22	56.77
C.D. at 5%	—	10.01	9.04	11.21	8.47

Table 4 *Effect of aretan on the population of actinomycetes in soil*

Treatment (ppm)	Number × 10^6/g soil on dry weight basis (mean of 3 replications)				
	Days of incubation				
	0	7	15	30	45
0.0	25.88	30.11	33.33	42.00	35.99
2.5	25.88	81.00	90.00	47.11	49.00
5.0	25.88	79.00	87.11	45.00	45.99
10.0	25.88	73.22	78.22	40.55	29.99
20.0	25.88	51.00	60.44	35.33	27.00
C.D. at 5%	—	9.83	11.93	—	9.64

Table 5 *Effect of aretan on the population of* Azatobacter *in soil*

Treatment (ppm)	Number × 10^3/g soil on dry weight basis (mean of 3 replications)				
	Days of incubation				
	0	7	15	30	45
0.0	21.99	26.11	28.00	24.22	22.33
2.5	21.99	20.55	22.00	21.11	23.22
5.0	21.99	23.00	23.55	24.55	25.11
10.0	21.99	17.33	19.22	18.88	18.55
20.0	21.99	15.33	18.11	20.00	20.66
C.D. at 5%	—	6.89	6.55	—	—

Table 6 *Effect of aretan on the population of Rhizobia in soil*

Treatment (ppm)	Number × 10^3/g soil on dry weight basis (mean of 3 replications)				
	Days of incubation				
	0	7	15	30	45
0.0	28.00	31.00	32.66	29.33	27.44
2.5	28.00	24.22	28.66	27.77	28.66
5.0	28.00	23.00	27.11	28.00	29.00
10.0	28.00	20.00	24.33	26.00	26.44
20.0	28.00	17.44	21.00	23.00	25.55
C.D. at 5%	—	6.83	6.99	—	—

(table 4) at all concentrations tried. The population of *Azatobacter* and *Rhizobia* (table 5 and 6) in soils containing 10 and 20 ppm aretan was significantly lower than the untreated check for first 15 days and there after the fluctuation in population was non-significant. The deleterious effect of organo-mercurials on *Rhizobia* has been demonstrated earlier (Golebiowska 1965, Muthusamy 1973).

Table 7 *Percentage reduction in the growth of some common soil fungi by aretan*

Organism	Concentration of aretan (ppm)							
	2.5	5	10	20	40	80	160	ED/50
Alternaria tenuis	59.67	67.21	70.49	78.36	100.00			1.60
Aspergillus flavus	1.98	5.78	7.89	30.78	46.05	51.63	75.00	65.00
Aspergillus fumigatus	25.45	27.27	33.33	54.54	66.31	74.84	100.00	17.70
Aspergillus niger	3.44	13.79	24.13	34.48	62.06	71.26	100.00	31.00
Aspergillus terreus	10.66	20.00	28.00	62.66	68.00	100.00		16.50
Curvularia sp.	44.04	83.82	100.00					2.83
Fusarium moniliforme	62.03	78.66	100.00					1.19
Helminthosporium sp.	4.83	16.00	24.00	37.34	65.05	100.00		29.50
Penicillium sp.	16.12	46.23	51.61	100.00				8.60
Pythium aphanidermatum	33.01	43.01	100.00					5.63
Rhizoctonia bataticola	69.89	84.62	100.00					1.15
Rhizoctonia solani	90.00	100.00						0.71
Sclerotium rolfsii	100.00							—
Trichoderma viride	31.61	52.47	83.54	100.00				4.69

Table 8 *Effect of aretan on* CO_2 *evolution*

Treatment (ppm)	CO_2 (mg) evolved/100 g oven-dry soil (mean of 3 replications)									
	4	8	12	16	20	24	28	32	36	40
0.0	21.78	16.28	10.26	6.90	6.16	5.63	4.53	4.50	3.40	3.11
2.5	21.06	16.18	10.09	6.84	6.24	5.56	4.46	4.43	3.47	3.26
5.0	20.35	16.06	9.93	6.86	6.33	5.50	4.49	4.40	3.50	3.22
10.0	18.70	15.51	9.82	6.85	5.72	5.36	4.38	4.28	3.63	3.17
20.0	18.15	14.40	9.60	6.16	6.11	5.70	4.74	4.56	3.71	3.31
C.D. at 5%	1.03***	0.91**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S., Non significant **Highly significant ***Very highly significant

2. *Effect of Aretan on Growth and Sporulation*

Fourteen fungi were grown on potato-rdextose agar in the presence of different concentrations of aretan (table 7). Aretan curtailed the growth of most fungi and the degree of inhibition was dependent on the initial concentrations of the fungicide and the organism involved. In general, pathogenic fungi like *Pythium aphanidermatum*, *Rhizoctonia solani*, *R. bataticola*, *Fusarium moniliforme*, *Curvularia* sp. and *Sclerotium rolfsii* were more susceptible to aretan than the common saprophytes like, *Aspergilli* and *Trichoderma viride*.

Presence of aretan in the medium also affected the sporulation of some fungi. For example, both *Rhizoctonia solani* and *R. bataticola* produced abundant sclerotia in the control (minus aretan) but not in the presence of aretan. Similarly, in aretan enriched medium *Helminthosporium* sp. produced short conidiophores with light brown spores, while in the control, normal conidiophores and typical black spores were produced.

3. *Biochemical Activity*

(i) *Carbondioxide Production*

All concentrations of aretan adversely affected CO_2 production and this trend continued for 16 days, although changes became non-significant from the 12th day (table 8). This was probably due to the efficient decomposition of organic matter by microorganisms. Maximum production of CO_2 in the control soil occurred on the 4th day and thereafter it decreased, indicating depletion of food material for rapid proliferation of soil microbes.

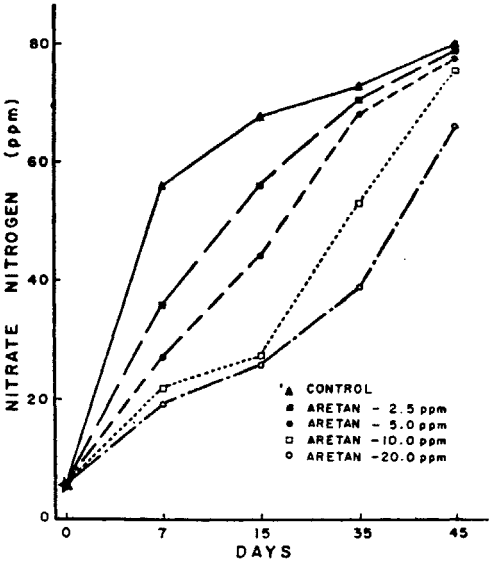

Figure 1 Nitrate nitrogen in the soil after various times of incubation in the presence of 100 ppm nitrogen and different concentrations of aretan.

(ii) *Nitrification*

The data on nitrification are presented in Figure 1. All concentrations of aretan tested significantly suppressed nitrification for first 30 days as treated soils contained less NO_3-N as compared to the control soil. On 45th day, significant differences occurred only from 5 to 20 ppm of aretan. Almost similar results have been reported by Van Faassen (1973).

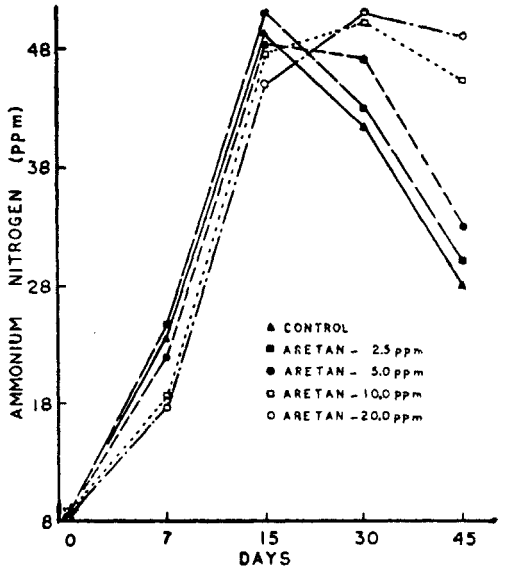

Figure 2 Ammonical nitrogen in the soil after various times of incubation in the presence of 100 ppm nitrogen and different concentrationsof aretan.

(iii) *Ammonification*

Application of aretan (except 2.5 ppm) produced detrimental effect on ammonification process only in the initial stages of the experiment (figure 2), and thereafter the rate of ammonification was stimulated over that of the control. The temporary inhibition of ammonification process by aretan was perhaps due to the direct toxic effect of the compound, while increase in NH_3-N after 30 days may be due to the re-establishment of bacteria regulating this process.

(iv) *Bioassay Studies—Persistence of aretan in soil*

When dosage-response curves (DRC) were plotted from the data obtained by measuring inhibition zone from soil plugs having different concentrations of aretan, a linear relationship was found (figure 3). By using this DRC, it was possible to quantify the concentration of fungicide and/or its toxic

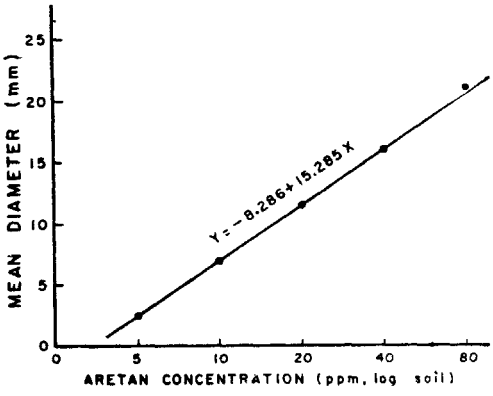

Figure 3 Mean diameter of clear inhibition zones surrounding plugs of soil in the presence of different concentrations of aretan.

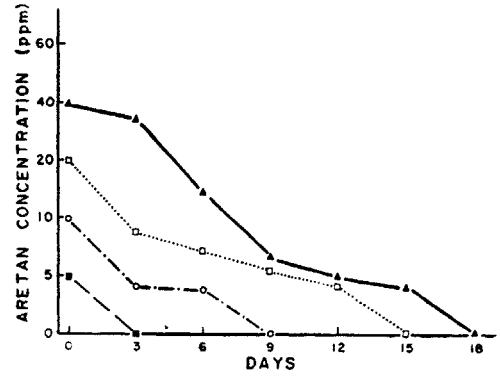

Figure 4 Persistence of different concentrations of aretan in soil.

degradation product. The results of the persistence of aretan are shown in figure 4. Degradation of aretan in soil was related to its initial concentration. At 40 ppm, it was completely degraded in 18 days. The present finding lends support to the observations of Munnecke and Moore (1967) and Indulkar and Grewal (1970), who worked with various organo-mercurials.

Data from the present study clearly demonstrate that fungitoxicity of aretan causes both chemical and microbial changes in soil. The general increase in the total population of bacteria and actinomycetes with simultaneous decrease in the population of pathogenic fungi supports the hypothesis of indirect biological control (Gram & Vaartaja 1967, Vaartaja 1957). Therefore, it can safely be surmised that extraordinary success of aretan in controlling seed borne (Singh et al. 1973) and seedling diseases (Sen et al. 1974) of sugarbeet is not solely due to its fungicidal properties.

References

Agnihotri V P 1971 Persistence of Captan and its effects on microflora, respiration, and nitrification of a forest nursery soil; *Can. J. Microbiol.* **17** 377–383

Allen O N 1957 *Experiments in Soil Bacteriology. 3rd rev.* ed. Burgess Publ. Co., Minneapolis, Minn. (U.S.A.).

Golebiowska J 1965 Effect of fungicides on the symbiosis of leguminous plants with bacteria; *Pam. Pulawaski* **18** 367–382

Gram W H and Vaartaja O 1957 Rate and timing of fungicidal soil treatments; *Phytopathology* **47** 169–173

Harper H J 1924 The accurate determination of nitrates in soils. Phenoldisulphonic acid method; *Indi. J. Engg. Chem.* **16** 180–183

Indulkar A S and Grewal J S 1970 Studies on chemical control of *Sclerotium rolfsii*; *Indian Phytopath.* **23** 455–458

Jackson M L 1962 *Soil Chemical Analysis.* Engle Wood. Cliffs, N J : Prentice-Hall.

Jensen H L 1951 Notes on the biology of *Azotabacter*; *Proc. Soc. appl. Bact.* **14** 89–94

Lingappa Y and Lockwood J L 1962 Chitin media for selective isolation and culture of actinomycetes; *Phytopathology* **52** 317–323

Martin J P 1950 Use of acid, rosebengal and streptomycin in the plate method for estimating soil fungi; *Soil Sci.* **60** 215–232

Munnecke D E 1958 A biological assay of non-volatile diffusible fungicides in soil; *Phytopathology* **48** 61–63

Munnecke D E and Moore B J 1967 Fungicidal activity in soil in relation to time, concentration, and *Penicillium* population; *Phytopathology* **57** 823 (Abstr.)

Muthusamy S 1973 Effect of seed dressing and soil fungicides on the growth of *Rhizobium* of

groundnut; *Pesticides* **7** 27-28

Nash S M and Snyder W C 1962 Quantitative estimation by plate counts of propagules of the been root rot *Fusarium* in field soils; *Phytopathology* **52** 567–572

Nene Y L 1971 *Fungicides in Plant Disease Control* Bombay : Oxford. and IBH Publishing Co.

Pramer D and Schmidt E L 1964 *Experimental Soil Microbiology*. Minneapolis, Minn. (USA. : Burgess Publishing Co.

Peterson E J 1926 Forholdet mellem jordens kusyreproduction Kemiske tilstands form og microbiologische aktivitet; *Tidsskr. Plateavl* **32** 625–674

Sen C, Srivastava S N and Agnihotri V P 1974 Seedling diseases of sugarbeet and their chemicontrol; *Indian Phytopath.* **27** 596–602

Singh K, Agnihotri V P, Srivastava S N and Misra S R 1973a *Rhizoctonia bataticola* (Taub.) Butler causing root rot of sugarbeet (*Beta vulgaris* L.); *Indian J. agric. Sci.* **43** 361–365

Singh K, Srivastava S N, Sen C and Agnihotri V P 1973b Seed mycoflora of sugarbeet (*Beta vulgaris* L.) and its fungicidal control; *Proc. Indian natn, Sci. Acad.* **39** 695–700

Vaartaja O 1964 Chemical treatment of seed beds to control nursery diseases; *Bot. Rev.* **30** 1–91

——1968 *Pythium* and *Mortierella* in soils of Ontario forest nurseries; *Can. J. Microbiol.* **14** 265–269

Van Faassen H G 1973 Effect of mercury compounds on soil microbes; *Pl. Soil* **38** 485–487