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Effect of Indar on Soil Microflora and their Nitrification and Ammonification Activities*

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Treatments of soil with Indar (4-n-butyl-1, 2, 4-triazole) at 0.5 and 1.0 ppm (active ingredient) had an appreciable disruptive effect on soil microbes, particularly on fungi, bacteria and actinomycetes for 52, 37 and 37 days of incubation, respectively.

Addition of Indar affected the physiological activity of soil microbes. The fungicide at the above rates impaired the process of nitrification throughout the experimental period i.e. 67 days while ammonification rate was enhanced.

Introduction

The experimental systemic fungicide Indar, 4-n-butyl- 1,2,4-triazole (RH-124 or Dithane R-24, Rohm and Haas Co.) has shown extraordinary promise in combating brown rust (*Puccina recondita* Rob. ex. Desm. f, sp. *tritici*) of wheat, when applied through soil (Singh & Singh 1975). Although extensive field studies have been made on the control of brown rust of wheat by Indar, no information is, however, available regarding its possible effects on soil microbial population and their physiological activities that play key roles in soil fertility.

There is a danger that soil fertility may be reduced by increased use of toxicants through their effects on the soil microorganisms (Bollen 1961, Gaur 1973). Attention should, therefore, be focused on the possible effects of pesticides on soil microorganisms and their activities. Among several soil microbiological processes; nitrification or oxidation of ammonia to nitrate and ammonification or liberation of ammonia from proteinaceous matter play conspicuous role in making nitrogen readily available to plants.

The present paper reports the results on the effect of Indar on the population of fungi, fusaria, bacteria and actinomycetes and on nitrification and ammonification in soil.

Materials and Methods

A composite soil sample was collected from a depth of 15 cm from plots at the Crop

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Research Centre, Pantnagar. Since air drying of soil induces both microbial and chemical changes (Chandra & Bollen 1961), it was promptly sieved and used the same day. Based on active ingredient, Indar was incorporated in soil at the rate of 0.25, 0.50 and 1.00 ppm on oven dry weight basis (o.d.b.). These rates approach 0.5, 1.0 and 2.0 kg/ha, respectively.

Nitrification studies

Nitrification experiment was performed in 500 ml Erlenmyer flasks, each of which contained 500 g soil mixed with different rates of Indar. Ammonium sulphate was added at the rate of 100 ppm N. The moisture content of the soil was adjusted to 50% water-holding capacity (WHC) and was maintained throughout the experiment. Water lost by evaporation was restored by adding sterile distilled water at required intervals. The flasks were incubated at 25°±1°C and each treatment was replicated thrice.

Ten grammes of soil was first suspended in 50 ml distilled water. Approximately 0.5 g calcium hydroxide and 1.0 g magnesium carbonate were added to the lechate to flocculate colloidal matter. Nitrate-N was determined by the phenol-disulphonic acid method of Harper (1924).

Ammonification studies

Peptone (100 ppm N) and required amount of Indar were thoroughly mixed with the soil and ammonia was estimated by nesslerization (Jackson 1962).

Population of soil microflora—Dilution plate technique was employed for estimating the number of soil microbes. The total population of fungi was assessed on Peptone rose bengal agar medium (Martin 1950); Fusaria on a selective medium (Nash & Snyder 1962); bacteria on soil-extract agar medium (Allen 1957); and of actinomycetes on starch ammonium agar medium (Kuznetsov & Arjuna Rao 1972). The population of soil microorganisms was determined over a period of 67 days.

Results

In general, all the concentrations of Indar had inhibitory effect on the population of fungi. However, higher concentrations (0.5 and 1.0 ppm) could significantly reduce the fungal propagules from 22 to 52 days. Thereafter the fungal population gradually increased. At 67th day, the number of fungi was higher in treated soils as compared to the check (table 1). Whenever the population of fungi increased in Indar treated soil, this was attributable mainly to species of

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

Treatments (ppm)	Number × 10^4/g soil on dry weight basis (average of 3 replications)					
	Days of incubation					
	0	7	22	37	52	67
0.00	21.50	16.42	14.75	21.42	16.83	13.67
0.25	21.50	13.84	13.50	19.17	17.25	11.17
0.50	21.50	12.75	11.59	12.09	12.34	15.92
1.00	21.50	13.59	8.00	12.75	10.17	17.92
C.D. at 5% level	—	N. Sig.	Sig. 3.99	Sig. 2.91	Sig. 3.88	Sig. 2.47

N. Sig. = Non-significant
Sig. = Significant

Apsergillus and *Penicillium*. The application of Indar did not indicate any detrimental effect on the population of fusaria throughout the experiment (table 2).

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

Treatments (ppm)	Number × 10^3/g soil on dry weight basis (average of 3 replications)					
	Days of incubation					
	0	7	22	37	52	67
0.00	24.50	25.84	28.42	20.00	22.50	16.58
0.25	24.50	25.66	23.00	23.00	23.66	14.66
0.50	24.50	22.10	25.20	17.80	19.83	16.83
1.00	24.50	21.66	28.20	21.16	20.00	15.83
C.D. at 5% level	—	N. Sig.	N. Sig.	N. Sig.	N. Sig.	N. Sig.

N. Sig. = Non-significant

The population of bacteria and actinomycetes decreased in relation to the concentration of Indar initially applied. However, significant effect of the fungicide on bacteria and actinomycetes was discernible only up to 37 days, thereafter the differences were non-significant and at 67th day populations seemed to increase (table 3 and 4).

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

Treatments (ppm)	Number × 10^6/g soil on dry weight basis (average of 3 replications)					
	Days of incubation					
	0	7	22	37	52	67
0.00	34.00	29.67	26.09	24.00	31.00	26.59
0.25	34.00	28.59	22.17	23.09	30.25	30.92
0.50	34.00	17.09	13.17	14.84	27.92	23.84
1.00	34.00	14.34	14.67	14.92	25.42	29.16
C.D. at 5% level	—	Sig. 4.04	Sig. 5.12	Sig. 5.38	N. Sig. —	Sig. 3.88

N. Sig. = Non-significant
Sig. = Significant

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

Treatments (ppm)	Number × 10^5/g soil on dry weight basis (average of 3 replications)					
	Days of incubation					
	0	7	22	37	52	67
0.00	30.00	26.59	23.67	21.09	26.17	19.09
0.25	30.00	25.34	22:67	23.00	28.59	18.58
0.50	30.00	17.42	11.67	13.92	27.92	26.67
1.00	30.00	13.09	12.92	13.00	23.25	32.92
C.D. at 5% level	—	Sig.	Sig.	Sig.	N. Sig.	Sig.
		4.55	2.75	3.00	—	5.63

N. Sig. = Non-significant
Sig. = Significant

Higher concentrations (0.5 and 1.0 ppm) of Indar retarded the rate of nitrification for 67 days and the inhibition was more with the increase in the concentration of fungicide. The lower rate (0.25 ppm) of Indar significantly increased the nitrification rate at 52 and 67 days of incubation period. In the absence of Indar, ammonium sulphate was rapidly transfered into nitrate nitrogen (table 5). Approximately 70% of the added nitrogen was nitrified within 67 days indicating the presence of nitrifying microorganisms in soil. Higher concentrations (0.5 and 1.0 ppm) of Indar significantly enhanced the ammonification rate for 67 days of incubation period (table 6).

Table 5 *Effect of Indar on nitrification in soil*

T reatments (ppm)	ppm nitrate and ammonium nitrogen (average of 3 replications)											
	Days of incubation											
	0		7		22		37		52		67	
	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_3–N	NO_3–N	NH_4–N
0.00	6.34	105.12	13.19	82.49	50.74	35.03	62.29	5.47	72.56	1.42	76.67	0.00
0.25	6.34	105.12	12.99	82.14	47.49	36.61	61.13	6.34	75.52	1.53	78.96	0.00
0.50	6.34	105.12	11.72	86.78	42.08	47.18	57.51	11.33	67.46	4.88	73.61	4.59
1.00	6.34	105.12	11.43	98.16	40.15	52.22	51.45	13.32	62.95	7.44	68.98	9.17
C.D. at 5% level	—	—	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
			1.59	2.65	1.47	1.54	1.70	0.90	0.87	0.73	1.26	1.79

Sig. = Significant

Table 6 *Effect of indar on ammonification in soil*

Treatments (ppm)	ppm ammonium and nitrate nitrogen (average of 3 replications)											
	Days of incubation											
	0		7		22		37		52		67	
	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N
0.00	8.09	15.02	21.29	52.82	12.43	67.59	11.55	79.29	9.85	88.40	8.97	93.51
0.25	8.09	15.02	21.99	52.37	12.54	66.69	12.43	78.51	9.79	88.40	8.91	93.96
0.50	8.09	15.02	23.05	48.82	16.13	63.33	18.00	77.48	11.25	87.63	9.85	92.15
1.00	8.09	15.02	25.16	47.91	21.29	58.35	22.00	65.39	14.07	81.49	10.79	90.92
C.D. at 5%	—	—	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
level			1.24	1.01	0.99	2.67	0.92	1.16	0.78	2.72	0.93	1.98

Sig. = Significant

Discussion

In the present study, Indar (0.5 and 1.0 ppm) significantly reduced the population of fungi, bacteria and actinomycetes in soil for 52, 37 and 37 days, respectively. This indicates to the possible inhibitory effects of Indar on these soil microflora. Though toxic effects of fungicides on soil microflora have been observed (Pugashetty & Rangaswami 1969, Agnihotri 1974), Indar is reported to be highly specific to leaf rust (*Puccinia recondita* Rob. ex. Desm. f. sp. *tritici*) of wheat (Von Meyer et al. 1970). The populations of the micro-organisms were, however, enhanced at 67 days. Several factors may contribute to the greater number of microbes occurring in soils after application of toxicants. These are : (1) the cell material of killed micro-organisms offers a readily available food source for surviving micro-organisms or those which become re-established first after treatment, (2) the residual chemical may serve as a carbon and energy source for certain soil micro-organisms (Audus 1964), and (3) the surviving organisms or those first to become re-established reach higher numbers in a less competitive environment (Garrett 1956).

Indar (0.5 and 1.0 ppm) retarded the rate of nitrification for 67 days. While lower concentration (0.25 ppm) did not produce any detrimental effect on nitrifiers. This indicates that soil bacteria that oxidise NH_3 to NO_2 and NO_2 to NO_3 are highly sensitive to Indar. This is because they are usually non-spore formers (Waksman & Starkey 1923). Similar detrimental effect was also observed by Agnihotri (1974) for thiram. Ammonification rate was significantly stimulated by the application of Indar in soil. The magnitude of ammonia buildup in soil was related to the concentration of Indar applied initially. This was expected because ammonification in soil is brought about by a large number of microorganisms that degrade protein molecules both under aerobic and anaerobic conditions with the concomitant formation of ammonia. Furthermore, most ammonifying bacteria are spore formers and thus difficult to kill (Bollen 1961). Enhanced ammonification rate was observed with thiram (Agnihotri 1974).

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