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Degradation of Selected Insecticides by Bacteria Isolated from Soil

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In semi-arid tropics, monocropping of groundnut, involving high-yielding varieties, has led to the outbreak of serious insect pests causing major losses in crop yield (Reddy and Ghewande 1986). As a consequence, there has been the use, both extensively and intensively, of organophosphorus insecticides, particularly monocrotophos and quinalphos, for an effective control of the insects (Patel and Vora 1981). Also, there is a steady increase in the application of large quantities of synthetic pyrethroids, mainly cypermethrin and fenvalerate, in groundnut pest management (Das 1988).

The entry of such widely used insecticides into soil might have far-reaching consequences because it would disturb the delicate equilibrium between a microorganism and its environment, both involved in an important biological process. It has now been well established that the major or fequently the only means of degradation for several pesticides in the environment is microbial. Also, the data available in literature indicate that soil bacteria may be more important in the degradation of certain pesticides (Tu and Miles 1976). No information is, however, available on degradation of monocrotophos, quinalphos, cypermethrin and fenvalerate by soil bacteria. The present study has, therefore, been aimed at assessing the capability of pure cultures of bacteria, isolated from insecticide-treated soil by enrichment culture technique, in degradation of insecticides commonly used in groundnut cultivation.

MATERIALS AND METHODS

To isolate soil bacteria, capable of degrading the selected insecticides, enrichment culture technique was followed. One millilitre aliquots of 500 ppm aqueous solutions prepared from commercial formulations of the four insecticides (Table 1) were added separately to 10 g portions of a black soil, collected from a fallow groundnut field to provide a final concentration of 5 kg ha^{-1}. The insecticide-treated soil samples were maintained at 60% water-holding capacity and incubated at room temperature (28 ± 4°C). Ten days after four such additions, at

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Table 1. Insecticides used in the present study

Registered/ Trade name	Commercial and chemical name	Chemical class	Commercial formulations	Technical grade (% purity)	Source for commercial and/ or technical samples
Nuvacron	Monocrotophos: Dimethyl (E)-1-methyl-2-methyl carbomoylvinyl phosphate	Organophosphate	36 EC	74.2	*Hindustan Ciba Geigy Ltd., Bombay
Ekalux	Quinalphos: O, O-Dimethyl O-quinoxaline-2-yl-phosphorothioate	Organophosphate	25 EC	86.7	*Sandoz (India) Ltd., Bombay
Cyperkill	Cypermethrin: ∝-Cyano-3-phenoxy-phenyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate	Synthetic pyrethroid	25 EC	92.2	*Bharat Pulveris-Mills (Pvt.) Ltd., Bombay
Fenkem	Fenvalerate: Cyano (3-phenoxyl-∝-methyl 4-chloro- (1-methyl) ethyl) benzeneacetate	Synthetic pyrethroid	20 EC	93.7	New Chemie Industrial Pvt. Ltd.,Bombay *Rallis India Ltd., Bombay

*The Sources from which technical grade insecticides were obtained as gift samples

10-day intervals, of each insecticide, duplicate soil samples were withdrawn for isolation of either Azospirillum sp., employing the semi-solid malate medium (Rangaswamy et al. 1989), or other predominant heterotrophic bacteria following the serial dilution agar plate technique with the nutrient agar medium. The heterotrophic bacteria thus isolated were raised to axenic cultures and attempts were made to identify them to generic level.

The abilities of the above bacteria to degrade the four insecticides were tested following the method adopted by Venkateswarlu and Sethunathan (1984 and 1985). Aliquots from stock solutions, prepared in acetone, or the technical grade insecticides were added to 250 ml sterilized Erlenmeyer flasks to provide a final concentration of 40 ug ml^{-1}, keeping in view the toxic levels of the selected insecticides to Azospirillum sp. (Ranaswamy et al. 1989). The carrier solvent was completely evaporated to dryness and 50 ml portions of steam sterilized mineral salts medium (Venkateswarlu and Sethunathan 1984), were introduced into each flask under aseptic conditions. The residues were then equilibrated for a day to obtain aqueous solutions of the insecticides. One millilitre of aqueous cell suspension from the cultures of A. lipoferum and other bacteria were used to inoculate the medium in each flask. Portions of uninoculated medium with an insecticide served as controls. All the culture flasks including controls were incubated at 37°C. Triplicate samples were withdrawn after 7 and 14 days of incubation for solvent extraction and estimation of the parent compounds.

The residues of the parent compounds and products of bacterial degradation from the samples were extracted three times with chloroform-diethyl ether (1:1) mixture (Megharaj et al. 1987). The solvent fractions were pooled and after evaporation of the solvent at room temperature, the residues were redissolved in 1 ml acetone for estimation of parent compounds by gas-liquid chromatography (GLC). The metabolites of monocrotophos and quinalphos, if any, formed during bacterial degradation, were qualitatively analyzed by thin-layer chromotography (TLC) (Megharaj et al. 1987).

The residues of the parent insecticides, redissolved in acetone, were analyzed in a microprocessor-controlled chemito gas chromatograph (Model 3865), equipped with a flame-ionising detector (FID). The spiral column (0.312 cm o.d., 1.5 m length) was packed with 5% SE 30 on chromosorb W (HP). The flow rates of different gases were: nitrogen (carrier gas) 30 ml min^{-1}; hydrogen, 30 ml min^{-1}; and air, 130 ml min^{-1}. The respective temperatures of injector, column and detector were: monocrotophos - 230°C, 210°C, and 240°C; quinalphos - 250°C 230°C, and 250°C; and cypermethrin and fenvalerate - 290°C, 280°C and 300°C. Under these operating conditions, the retention times, in seconds, of parent insecticides were:

monocrotophos, 90.5; quinalphos, 106.6; cypermethrin, 215.2; and fenvalerate, 284.5. Amounts of the selected insecticides as low as 0.01 ug L^{-1} could be detected accurately and reproducibly, and recoveries above 90% were routine. The data obtained at each sampling for a chemical were subjected to analysis of variance, and means were compared by the Duncan's new multiple range test at 5% level (Megharaj et al. 1987).

The residues of monocrotophos and quinalphos, redissolved in acetone, were spotted along with technical samples on chromatoplates coated with silica gel G, 300 um thick. The plates were developed for a distance of 15 cm with hexane-chloroform-methanol (7:2:1, v/v/v). The air-dried plates were sprayed with 0.25% 4-(p-nitrobenzyl)-pyridine (NBP) in redistilled acetone, followed by heating at 150°C for 15 min in a hot-air oven and then spraying lightly with 10% tetra ethylene pentamine in redistilled acetone until the development of intense blue spots against a white back ground (Megharaj et al. 1987). The Rf values of the metabolites, if any, were recorded.

RESULTS AND DISCUSSION

Four cultures of predominant heterotrophic bacteria, besides Azospirillum lipoferum, capable of degrading the selected insecticides, were isolated from the enrichment cultures. Basing on some of the biochemical and physiological characteristics (Buchanan and Gibbons 1974), the four isolates were tentatively identified as distinct species of Bacillus.

The per cent initial (0-day) recoveries, with the complex extraction and analytical procedures employed, of monocrotophos, quinalphos, cypermethrin and fenvalerate, immediately after their application to the culture medium were 74.94, 78.29, 73.06 and 78.27 respectively. There was an appreciable decrease in the levels of both monocrotophos and quinalphos during the incubation period even in uninoculated controls (Table 2). Thus, by the end of 14 days, about 40% of added monocrotophos was lost from the uninoculated medium as against to 16% loss of quinalphos from the corresponding uninoculated samples during this period. The chemical degradation of these two insecticides in uninoculated medium with pH of 6.8 could be expected because of the fact that organophosphate ester insecticides are highly susceptible to hydrolysis and are inherently unstable, decomposing slowly even at normal temperatures (Brown et al. 1966).

Even by the end of 7 days, about 40% of monocrotophos (in relation to the per cent recovery in uninoculated samples) supplemented to the mineral salts medium was degraded by A. lipoferum and species of Bacillus. Although there was a significant decrease after 14 days of incubation in the amount of monocrotophos, recovered from medium inoculated with the bacteria, no appreciable degradation of insecticide occurred

Table 2. Degradation of monocrotophos, quinalphos, cypermethrin and fenvalerate by bacteria isolated from soil

Organism	Per cent of initial recovery, after incubation							
	Monocrotophos		Quinalphos		Cypermethrin		Fenvalerate	
	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
Uninoculated	80.75a	60.27a	92.10a	83.86a	85.37a	78.32a	83.54a	52.50a
Azospirillum lipoferum	38.73b	37.14b	52.01b	27.55b	56.69b	32.48b	51.25b	7.81b
Bacillus sp. 1*	37.19b	20.84b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bacillus sp. 2*	n.d.	n.d.	50.46b	7.62c	n.d.	n.d.	n.d.	n.d.
Bacillus sp. 3*	n.d.	n.d.	n.d.	n.d.	36.57b	8.59c	n.d.	n.d.
Bacillus sp. 4*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33.12b	0

Insecticide added to 50 ml medium, 2 mg. *The insecticide-degrading capacity of a *Bacillus* sp. isolated from soil treated with an insecticide was tested only with the respective insecticide. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) according to the Duncan's multiple range test.

n.d. = not determined

during this period. Quantitative analysis of the residues of monocrotophos in organic solvent extract of the medium by TLC showed the presence of two unidentified metabolites with Rf values of 0.48 and 0.75 together with the parent compound (Rf, 0.17) in samples inoculated with A. lipoferum as against to the occurrence of one metabolite in samples inoculated with Bacillus sp. This again clearly agreed with the gas chromotograms obtained from samples incubated for 14 days. Evidently, these two metabolites are the resultant products of hydrolysis since it has been established that the initial degradation of monocrotophos in soils is likely to involve only hydrolytic reactions mediated by microorganisms to form dimethyl phosphate or O-desmethyl monocrotophos together with N-methyl acetoacetamide (Beynon et al. 1973). However, no attempt was made to identify the degradation products by cochromatography due to the nonavailability of authentic compounds of the metabolism.

Both A. lipoferum and Bacillus sp., isolated from quinalphos-treated soils, degraded quinalphos rapidly even by the end of 7 days of incubation (Table 2). Further, the metabolism of quinalphos was more pronounced by 14 days. Thus, besides 16% loss of quinalphos by chemical decomposition, nearly 56 and 76% was degraded by A. lipoferum and Bacillus sp., respectively. Qualitative analysis of resideues in the organic solvent fraction by TLC revealed the presence of only quinalphos (Rf, 0.89). Admittedly, the metabolites likely to be formed during bacterial degradation must have been utilized by the test organisms.

Megharaj et al. (1987) also demonstrated the rapid disappearance of monocrotophos and quinalphos in culture media inoculated with Chlorella vulgaris, Scenedesmus bijugatus, Synechococcus elongatus, Phormedium tenue and Nostoc linckia, all isolated from a black cotton soil. The results of the present investigation clearly indicate that quinalphos is more susceptible to microbial metabolism than monocrotophos, thus conforming to the report of Megharaj et al. (1987) related to the algal degradation of these two insecticides.

As with the organophosphates, the selected pyrethroids were subjected to chemical decomposition in mineral salts medium during the incubation. The disappearance in uninoculated samples was thus accounted for nearly 22 and 47% for cypermethrin and fenvalerate, respectively. A. lipoferum metabolized cypermethrin slowly but steadily. However, the degradation of fenvalerate by the diazotroph was more rapid as compared to metabolism of cypermethrin. Only 8% of added fenvalerate was recovered after 14 days of incubation. Bacillus sp., isolated from cypermethrin-treated soil, exhibited greater activity in metabolizing cypermethrin when compared with the strain of A. lipoferum. Thus, about 91% of added cypermethrin was degraded by Bacillus sp. after 14 days

as against to 32% recovery of the insecticide from A. lipoferum culture during this period. It is interesting to note that the isolate of Bacillus sp. from fenvalerate-treated soil effected complete degradation of fenvalerate by 14 days after incubation. Also, it may be mentioned that bacterial degradation of fenvalerate is more rapid than that of cypermethrin. In the absence of a suitable chromogenic reagent, it could not, however, be possible to analyze the metabolites of the pyrethroids qualitatively by TLC.

The results of the present investigation clearly indicate that the selected insecticides are prone to chemical decomposition, but microbial degradation is more pronounced and rapid. Also, the susceptibility to bacterial degradation of the insecticides followed the order: Monocrotophos > cypermethrin > quinalphos > fenvalerate.

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REFERENCES

Beynon KI, Hutson DH, Wright AN (1973) The metabolism and degradation of vinyl phosphate insecticides. Residue Rev 47: 55-142.

Brown NPH, Foster AS, Furmidge CGL (1966) Stability of agricultural chemicals. I. Hydrolytic and thermal stabilities of phosphorylated crotonamides. J Sci Food Agric 17: 510-517

Buchanan RE, Gibbons NE (1974) Bergey's Manual of Determinative Bacteriology, 8th ed., Williams and Wilkins, Baltimore

Das BB (1988) Efficacy of some synthetic pyrethroids and conventional insecticidal spray against groundnut pest complex. Pesticides 22 (1): 10-12

Megharaj M, Venkateswarlu K, Rao AS (1987) Metabolism of monocrotophos and quinalphos by algae isolated from soil. Bull Environ Contam Toxicol 39: 251-256

Patel BR, Vora VJ (1981) Efficacy of different insecticides for the control of groundnut jassid Empoasca kerri Pruthi. Pesticides 15 (10): 33-34

Rangaswamy V, Charyulu PBBN, Venkateswarlu K (1989) Effect of monocrotophos and quinalphos on soil population and nitrogen-fixing activity of Azospirillum sp. Biomed Environ Sci 2: 305-311

Reddy PS, Ghewande MP (1986) Major insect pests of groundnut and their management. Pesticides 20 (5): 52-56

Tu CM, Miles JRW (1976) Interaction between insecticides and soil microbes. Residue Rev 64: 17-66

Venkateswarlu K, Sethunathan N (1984) Degradation of carbofuran by Azospirillum lipoferum and Streptomyces spp. isolated from alluvial soil. Bull Environ Contam Toxicol 33: 556-560

Venkateswarlu K, Sethunathan N (1985) Enhanced degradation of carbofuran by *Pseudomonas cepacia* and *Nocardia* sp. in the presence of growth factors. Plant Soil 84: 445-449

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