Toxicol. Int.Vol. 16, No. 2, 2009 pp 127-132

Biodegradation of selected insecticides by *Bacillus* and *Pseudomonas* sps in ground nut fields

R. Jaya Madhuri* **and V. Rangaswamy**[1]

Department of Applied Microbiology, Sri Padmavathi Mahila University, Tirupathi-517502

[1]Department of Microbiology, Sri Krishnadevaraya University, Anantapur-515003

Abstract

Bacterial cultures isolated by selective enrichment technique were identified as species of *Bacillus* and *Pseudomonas*. These isolates were tested for their ability to degrade the respective insecticides in mineral salts medium. Within 7 days of incubation, nearly 75% of chlorpyrifos and phorate and 50% of dichlorvos, methyl parathion and methomyl were degraded by cultures of soil bacteria. Qualitative analysis of chlorpyrifos and methyl parathion residues by gas chromatography revealed the formation of one unidentified metabolite in inoculated samples. Whereas no metabolite formation was detected in case of other insecticides inoculated samples. Moreover, dichlorvos and phorate were completely degraded by soil isolates at the end of 14 days. Order of microbial degradation of selected insecticides in the present experiment is as follows: phorate > dichlorvos > methyl parathion > chlorpyrifos > methomyl.

Keywords: *Bacillus,* Organophosphates, Carbamate, Groundnut soils, *Pseudomonas*,

Introduction

Groundnut (*Arachis hypogaea L.)* is one of the principal cash crops grown in dry land of India (Dharne *et al.*, 2001). In Andhra Pradesh, it covers a major area of 7.5 m/ha with production of 6.4 million tonnes in Kharif season (Sawashe *et al.*, 2003). Within the State, Anantapur district, a semi-arid region occupies a predominant place in groundnut cultivation (Anonymous, 2003). The present day agriculture involves abundant cultivation of the crop because of its vital role in edible oil seeds production (Kori *et al.*, 2002). This condition led to inherent risk of yield loss due to drought, diseases and pests (Galgunde and Kurundkar, 2002). More than 120 species of insect pests are deported to attack groundnut crop at various stages of growth impending potential crisis for peanut growers (Weeks and Well, 2003). The percentage of crop loss attributed to the pests is 50% (Ramesh Babu *et al.*, 2002).

The progressive increase of pest problem and demand for agricultural food production necessitated the application of agrochemicals that ensure high quality and yield (Graebing *et al.*, 2002). Of these, particularly chlorpyrifos, dichlorvos, methyl parathion, phorate and methomyl have been proved effective in eradicating groundnut pests (Bhandari *et al.*, 2003 and Marois and Wright, 2003). The introduction of these pesticides into the soil environment raises concern as to their effect on ecological balance in terms of soil fertility (Graebing *et al.*, 2002). In general, pesticide degradation in soil is influenced by a number of abiotic factors. Even though the pesticide degradation is under the impact of various physico – chemical parameters, literature survey reveals that the major pathway governing degradation and ecotoxicity of these compounds is microbial mediated (Shah and Thakur, 2003). Microbes transform the applied xenobioties by utilizing them as energy sources. Otherwise, microbes develop new metabolic strategies and adapt to changing environment through mutation, induction or by selective enrichment technique (Raymond *et al.*, 2003). Despite the wealth of information on the behavior of pesticides in soils of temperate environment, scientific data available on the fate of such chemicals in tropical environments was limited. Hence, the present investigation has been aimed to isolate bacterial axenic cultures involved in insecticide metabolism and to elucidate

Corresponding author: Mob.: 9885895974,
E-mail: ravuri_jayamadhuri@rediffmail.com

biodegradation potential of bacterial isolates of the selective insecticides.

Materials and Methods

Isolation of insecticide degrading bacteria from soils

To isolate soil bacteria capable of degrading the selected insecticides, enrichment culture technique was opted. One ml aliquots of 500 ppm aqueous solutions were prepared from commercial formulations of the insecticides. The treatments were added separately to 10g portions of the soil samples to provide a final concentration of 5 kg ha^{-1} and maintained at 60% water holding capacity and incubated at room temperature. After four such additions, at 10-day intervals, of each insecticide, duplicate soil samples were withdrawn for isolation of soil bacteria following the serial dilution agar plate technique with the use of nutrient agar medium. These bacteria were further raised to axenic cultures and attempts were made to identify them.

Degradation of insecticides by pure cultures of bacteria

The ability of the above bacteria to degrade the selected insecticides was tested following the method adopted by Rangaswamy and Venkateswarlu (1992) with certain modifications. Aliquots from stock solutions, prepared in acetone of the technical grade insecticides were added to 250 ml sterilized Erlenmeyer flasks to provide a final concentration of 40 mg/ml keeping in view of their toxic levels to bacteria .The carrier solvent was completely evaporated to dryness and 50 ml portions of steam sterilized mineral salts medium ($K_2H\ PO_4$-0.1 g, $MgSo_4.7H_2O$-0.2 g, $Fe_2So_4.7H_2O$ -0.001g, Distilled water-1000 ml) were introduced into each flask under aseptic conditions.

The residues were then equilibrated for a day to obtain aqueous solutions of the insecticides. One ml aqueous cell suspension of bacterial cultures was used to inoculate medium in each flask. Uninoculated media with an insecticide served as control. All the culture flasks including controls were incubated at 37°C. Triplicate soil samples were withdrawn after 7 and 14 days of incubation for solvent extraction and estimation of the parent compound.

Extraction of insecticide residues

The residues of the parent compound from the media were extracted 3 times with chloroform – diethyl ether (1:1) mixture. The solvent fractions were pooled and after evaporation of the solvent at room temperature, the residues were dissolved in 1 ml acetone for estimation of parent compounds by gas liquid chromatography (Cement *et al.*, 1995) and by HPLC for methomyl (Zabik *et al.*, 2000).

Quantification of insecticide residues by gas liquid chromatography

Insecticides residues redissolved in acetone were analyzed at Indian Institute of Horticultural Research, Bangalore, in a microprocess controlled Chemito Gas Chromatograph (Model 3800) equipped with a flame ionizing detector. The spiral megabore column is long made up of stainless steel of 13 cm length packed with 10% OV – 101 TSD. Gas flow rates were maintained as follows: Nitrogen (Carrier gas) flow - 30 ml/min, Air flow - 175 ml/min and Hydrogen flow - 4.5 ml/min

	Injector Temp. (°C)	Column Temp. (°C)	Detector Temp. (°C)
Chlorpyrifos	220	260	280
Dichlorvos	180	250	280
Methly parathion	220	260	280
Phorate	180	250	300

HPLC conditions for methomyl quantification

Flow rate	-	1 ml/min
Aceto nitrile injected	-	0.4ml
Water	-	0.6ml
Wave length	-	254 nm

Column: Phenomenex Bond clone 10C 18 Silicone bonded 300 x 3.9 mm, Particle size 10 μ.

Detector: Variable wavelength JASCO 4V Dec 100 UV detector.

Under these conditions, the retention times of

parent insecticides in minutes are: chlorpyrifos: 5.36; dichlorvos: 1.51; methyl parathion: 4.22; phorate: 5.98 and methomyl: 2.84 mins.

Data was analyzed for significant differences ($P \leq 0.05$) between insecticide treated and untreated soils using Duncans Multiple Range test (Megharaj *et al.*, 1999).

Results

Cultures of predominant heterotrophic bacteria efficient in degrading the selected insecticides were isolated by enrichment culture technique. Based on some of the biochemical and physiological characteristic features, bacterial isolates were tentatively identified as distinct species of *Bacillus and Pseudomonas* (Table 1). As the cultures were not performed RNA sequencing, the species was not mentioned, even though identified tentatively based on the Bergeys manual. Appreciable decrease in concentration of selected insecticides even in uninoculated controls was observed during incubation period. Thus, about 24-38% of insecticide level has been mineralized at the end of 14 day incubation period in controls.

At the end of 7 days, 71-77% of chlorpyrifos was degraded by *Bacillus* and *Pseudomonas sps*. (Fig. 1). After an incubation period of 14 days, the concentration of the parent compound was further decreased. Gas chromatographic analysis revealed the presence of extra peak indicating metabolite formation from the parent compound in the inoculated samples. This may be the resultant microbial hydrolysis product. However, no attempt has been made to identify the transformation product due to non-availability of authenticated compound of the metabolite. Regarding degradation of dichlorvos, about 26-29% of degradation was observed in inoculated samples at 7 days (Fig. 1). At the end of 14 days, both the cultures degraded the parent compound to an appreciable level and only trace amount of pesticide residue was detected (Fig. 1). Gas chromatograph analysis revealed the presence of only one peak representing parent compound. Intermediate metabolites formed might have been utilized further as nutrient source by the cultures.

Table 1: Some biochemical and physiological parameters of the bacteria, isolated from insecticide treated soil samples

Insecticide treatment	Isolate	Catalase activity	Starch hydrolysis	Nitrate reduction	Gelatin hydrolysis	Casein hydrolysis	Lipase activity	Oxidase test	Acid production	Urease	IM ViC	H_2S
Chlorpyrifos	*Bacillus sps1*	+	+	+	+	+	+	+	Glucose +ve	+	---+	-
	Pseudomonas sps1	+	+	+	-	+	+	+	+	+	---+	-
Dichlorvos	*Bacillus sps2*	+	+	+	-	+	+	+	-	+	---+	-
	Pseudomonas sps2	+	-	+	-	+	+	+	+	+	---+	-
Methyl parathion	*Bacillus sps3*	+	+	+	+	-	+	-	+	+	---+	-
	Bacillus sps4	+	-	+	+	+	+	+	+	+	---+	-
Phorate	*Bacillus sps5*	+	-	+	-	+	+	-	-	+	---+	-
	Pseudomonas sps3	+	+	+	-	-	+	+	+	+	---+	-
Methomyl	*Pseudomonas sps4*	+	-	+	+	+	-	+	+	+	---+	-
	Pseudomonas sps5	+	+	-	-	+	+	+	+	+	---+	-

+ Positive response; - Negative response

Results presented in Fig. 1 indicate that both cultures treated with methyl parathion exhibited rapid degradation even at 7-day incubation period. Thus, 43-52% of insecticide was metabolized in comparison to control. Further, at 14 day incubation, 69-74% of methyl parathion was degraded by isolated cultures. Even though, methyl parathion can undergo chemical hydrolysis at alleviated pH, it was evident that microorganisms are responsible for accelerated degradation of the chemical in mineral salts medium, utilizing it as sole source of carbon since the pH of the medium was observed to increase only marginally from 6.5 to 6.7 after 14 days of incubation with cultures. Chromatographic analysis revealed that samples inoculated with *Bacillus* species exhibited the presence of one major unidentifed, metabolite from methyl parathion as well as chlorpyrifos, incubated for 14 days. But due to non-availability of standard intermediate metabolite for comparison, identification of the compound is not performed. Present findings indicate that *Bacillus sps*. are more predominant in biodegradation of methyl parathion than *Pseudomonas*.

Experimental evidence as shown in Fig. 1 indicates rapid disappearance of phorate parent compound in inoculated samples compared to uninoculated. Thus, about 70-76% of phorate added initially was degraded efficiently by microbial cultures at the end of 7days. The significant differences in the rate of degradation between inoculated and uninoculated samples indicate the active role of soil microbes in transformation of phorate. Present finding also reveal that phorate, an aliphatic compound was found to be completely metabolized after 2 weeks (Fig. 1).

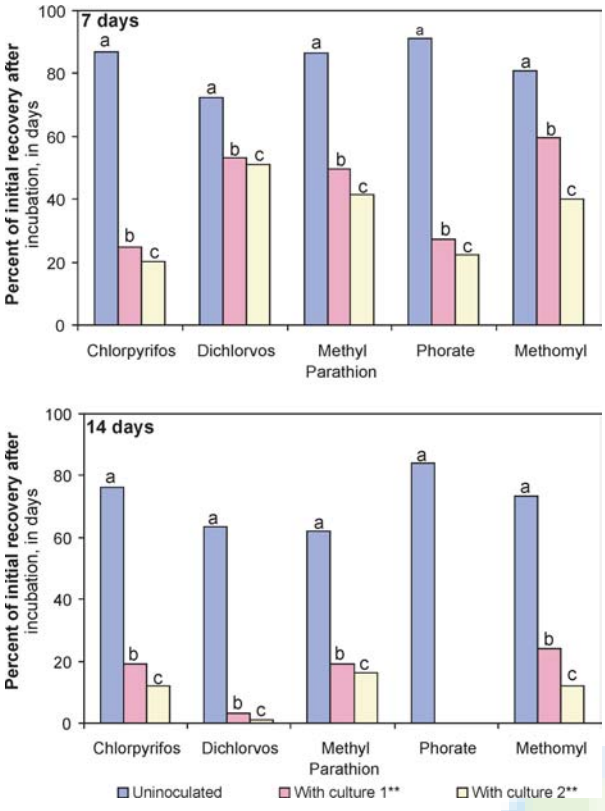
Degradation studies of methomyl, a carbamate insecticide show that about 26-58% of pesticide residue has been eliminated solely by sps of *Pseudomonas* in comparison to control, by the end of 7 days. Rate of methomyl metabolism by the two bacterial cultures was more significant (69-84%) on further incubation for 14 days.

Discussion

Degradation of the selected insecticides even in uninoculated samples is reported to be due to that organophosphates and carbamates are highly susceptible to chemical hydrolysis at high pH and decomposed even under normal temperature. Gas chromatographic analysis of presence of extra peak is may be the hydrolytic product of parent compound since it has been reported in literature that microbial transformation of chlorpyrifos yields hydrolytic products such as TCP, TMP etc. (Getzin, 1981a and b; Racke and Coats, 1988). Coinciding with the present data of microbial role in degradation, Sigleton (1994) reported rapid metabolism of chlorpyrifos by *Lactobacilllus bulgaricus* and *Streptococcus thermophiluss* than in control media Lal *et al*. (1987) observed disappearance of chlorpyrifos by *Saccharomyces cerevisiae*. But there is no data available on chlorpyrifos degradation in tropical ground nut soils of Andhra Pradesh, India. Limited information was available regarding the biodegradation of dichlorvos. However, in a study made by Leiberman and Alexander (1983), microbial enrichment culture composed of *Pseudomonas aeroginosa, Pseudomonas sps*. and *Bacillus sps.,* were found to degrade dichlorvos to dichloroethanol, dichloroacetic acid and ethyl dichloroacetate in temperate soils.

Ou *et al*. (1983) demonstrated that more than 40% of methyl parathion was mineralized after 14 days in Webster soil .But in the present experiment, rapid degradation was noticed (72%). Similar results were obtained by Mishra *et al*. (1992) in paddy soils. Formation of one unidentified metabolite in methyl parathion degradation was in contrast to the results obtained by Ou *et al*. (1983). He postulated the formation of *p*-nitrophenol, the major hydrolytic product of methyl parathion, *p*-aminophenol and also three unidentified metabolites, during methyl parathion transformations. As observed in the present finding, Sharmila *et al*. (1989a and b) also reported that *Bacillus sps*. play major role in hydrolysis of methyl parathion in mineral salts medium.

Similar to the present data where 75% of the compound is degraded, Ahmed and Casida (1958) found that phorate incubated for 8 days in cultures of two species of bacteria showed 58 and 75% breakdown of parent compound. The significant



* Bacteria were isolated by enrichment culture technique from soil treated four times with the respective insecticide.Insecticide added to 50 ml medium, 2.5 mg.

** The insecticide-degrading capacity of bacteria sps., isolated from soil treated with an insecticide was tested with only the respective insecticide.

Bars marked with the same letter are not significantly different (P≤0.05) from each other according to DMR test

Fig. 1. Degradation of selected insecticides by bacteria* isolated from soil

difference in the rate of degradation of phorate between inoculated and uninoculated samples indicate the active role of microbes in biotransformation of the chemical.

The only evidence available regarding methomyl was enzymatic degradation of the compound by *Rhizobium* cultures, *Aspergillus niger, Aspergillus fumigatus, Fusarium oxysaporum* and *Penicillium notatum* in culture media (Mostafa *et al.*, 1990). But no information was available on its degradation by *Bacillus* and *Pseudomonas sps.*

Conclusions

The present experimental data on biodegradation of selected insecticides in groundnut soils of Andhra Pradesh, INDIA indicate rapid degradation of phorate, an organophosphate, than the remaining pesticides and in methomyl, a carbamate insecticide, degradation was least. With respect to dichlorvos and methomyl, initial rate of degradation was low (26-29%). But on further incubation, the rate of degradation increased to a greater extent. Present findings reveal the order of degradation of the five insecticides was as follows; phorate > dichlorvos > methylparathion > chlorpyrifos > methomyl.

Commercial application of these cultures in bioremediation of soil samples contaminated by these insecticides is presently under field trail.

Acknowledgements

Authors are thankful to University Grants Commission, New Delhi, for providing financial support in the form of Major Research Project.

References

Ahmed, MK and Casida, JE (1958). Metabolism of some organophosphorus insecticides by microorganisms. *J. Econ. Entomol.,* **51**: 9-63.

Anonymous (2003). Agriculture production plan for Anantapur district for Kharif season. Department of Agriculture, Anantapur, Andhra Pradesh, India, pp. 1-10.

Bhandari, P, Rahman, MDH and Varshney, I (2003). Relative toxicity of chlorpyrifos 20EC and methyl chlorpyrifos 45EC against Bihar hairy caterpillar, *Pestol.,* **27**(2): 27-28.

Cement, RE, Eiceman, GA and Koester, CJ (1995). Environmental analysis. *Anal. Chem.,* **67**: 221-225.

Dharne, PK, Patil, SK and Patil, RB (2001). Field evaluation of some insecticides against tobacco caterpillar on groundnut. *J. Maharashra. Agric. Univ.,* **26**(2): 164-165.

Galgunde, LP and Kurundkar, BP (2002). Occurrence of groundnut diseases in rabi crop. *J. Maharasha, Agric. Sci.,* **115**: 15-17.

Getzin, LW (1981a). Degradation of chlorpyrifos in Soil. *J. Econ. Entomol.,* **74**: 158-162.

Getzin, LW (1981b). Dissipation of chlorpyrifos from dry soil surfaces. *J. Econ. Entomol.,* **74** : 707-713.

Graebing, P, Frank, M and Chib, JS (2002). Effects of fertilizer and soil components on pesticide photolysis. *J. Agri. Food. Chem.,* **50**: 7332-7339.

Kori, RN, Patil, SL, Salakinakop, SR, Hunshal, CS and Nagagouda, BT (2002). Economics of integrated pest

management in irrigated groundnut. *J. Oil. Seeds. Res.,* **17**(1): 61-65.

Lal, R, Lal, S, Dhanraj, PS and Saxena, DM (1987). Genetic manipulation of microorganisms for degradation of pesticides. In: pesticides, crop protection and environment. (Ed. VS. Pavmall and S. Walia), Oxford and IBH, New Delhi, pp. 264-277.

Liebermann, MT and Alexander, M (1983). Microbial and non-enzymatic steps in the decomposition of dichlorvos. *J. Agric. Food. Chem.,* **31**: 265-267.

Marois, JJ and Wright, DL (2003). Effect of phorate on tomato spotted wilt of peanut. *Agron.,* **95**: 386-389.

Megharaj, M, Singleton, I and Mc Clura, NC (1999). Effects of DDT and its metabolites on soil algae and enzymatic activity. *Biol. Fertil. Soils.,* **29**(2): 130-134.

Mishra, D, Bhuya, S, Adhya, TK and Sethunathan, N (1992). Accelerated degradation of methyl parathion by suspensions from methyl parathion treated soils. *Soil. Bio. Biochem.,* **24**: 1035-1042.

Mostafa, IY, Helal, GA and El Zawahry, YA (1990). Fate of 14labeled insecticide methomyl by some soil microorganisms. *Isotope and Radiation Res.,* **22**(1): 39-48.

Ou, LT, Rao, PSC and Davidson, JM (1983). Methyl parathion degradation in soil. *Soil. Bio. Biochem.,* **15**(2): 24-45.

Racke, KD and Coats, JR (1988). Comparative degradation of organophosphorus insecticides in soil. *J. Agr. Food. Chem.,* **36**: 193-199.

Ramesh Babu, K, Santharam, G and Chandrasekharan, S (2002). Bioefficacy of imidacloprid against Leaf miner on groundnut. *Pestol.,* **26**(6): 13-16.

Rangaswamy, V and Venkateswarlu, K (1992). Degradation of selected insecticides by bacteria isolated from soil. *Bull. Env. Contam. Toxicol.,* **49**: 797-804.

Raymond, D, Jeffrey, K and Alan, S (2003). Detection of a methyl carbamate degrading gene in agricultural soils. *Comm. Soil. Sci. Pl. Anal.,* **34** (3,4): 393-406.

Sawashe, SG, Gondkar, PP, Magaure, KK and Uhale, SS (2003). Chemical control of special diseases of groundnut. *Pestol.,* **27**(5): 34-37.

Shah, S and Thakur, IS (2003). Enzymatic dehalogenation of insecticide by *Pseudomonas fluorescens* of the microbial Community from tannery effluent. *Curr. Microbiol.,* **47**: 65-70.

Sharmila, M, Ramanand, K and Sethunathan, N (1989a). Hydrolysis methyl parathion in a flooded. *Soil. Bull. Enz. Contam. Toxicol.,* **43**: 45-51.

Sharmila, M, Ramanand, K and Sethunathan, N (1989b). Effect of yeast extract on the degradation of organophosphorus insecticides by soil enrichment bacterial cultures. *Can. J. Microbiol.,* **35**: 1105-1110.

Sigleton, L (1994). Microbial metabolism of xenobiotics. *J. Chem. Tech. Biotech.,* **59**: 9.

Weeks, JR and Wells, L (2003). Evaluation of novel insecticides for control of thrips and Lepidopterus larvae on peanuts in Alabama. *Andhra. Agr. J.,* **49** (3,4): 73.

Zabik, MJ, El Hadidi, MFA, Cash, JN, Zabik, ME and Jones, A.L. (2000). Reduction of chlorpyrifos and methomyl residues in apple cultivated soil. *J. Agr. Food. Chem.,* **48**: 4199-4203.

Received : 08.12.2008
Accepted: 27.02.2009