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Metabolism of Monocrotophos and Quinalphos by Algae Isolated from Soil

M. Megharaj, K. Venkateswarlu, and A. S. Rao

Department of Botany, Nagarjuna University, Nagarjunanagar 522 510, Andhra Pradesh, India

Although microalgae have been implicated in the metabolism of certain organophosphorus insecticides such as phorate (Ahmed and Casida 1958), malathion (Christie 1969), parathion (Mackiewicz et al. 1969, Zuckerman et al. 1970), etc., information on the algal degradation of organophosphates is far from complete (Wright 1978). In a very recent study involving monocrotophos and quinalphos, a significant, but selective with concentrations,enhancement in the growth of soil algae has been reported (Megharaj et al. 1986). The capabilities of five algal species, isolated from a vertisol, to degrade monocrotophos and quinalphos, the most effective insecticides in controlling cotton bollworms, were determined in the present study.

MATERIALS AND METHODS

To determine the role of soil algae in the degradation of monocrotophos (Dimethyl (E)-1-methyl-2- methyl carbamoylvinyl phosphate) and quinalphos (O,O-diethyl-O-quinoxalin -2- yl phosphorothioate),enrichment cultures were prepared by adding 1 ml of 1000 ppm aqueous solutions from the commercial formulation of each insecticide separately to 20-g portions of the soil samples, maintained at 50% of water-holding capacity. After five additions, at 15-day intervals, of each chemical, the soil samples were withdrawn for isolation of predominant algae following the most-probable number (MPN) method as described earlier (Muralikrishna and Venkateswarlu 1984). Axenic cultures were developed from the most common and consistently occurring forms in almost all the MPN tubes. Two green algae, Chlorella vulgaris Beijerinck and Scenedesmus bijugatus (Turpin) Kuetzing, and three species of blue-green algae (cyanobacteria) -

Send reprint requests to Dr.K.Venkateswarlu, Department of Microbiology, S.K.University, Anantapur 515 003, India

Synechococcus elongatus Nageli, Nostoc linckia (Roth) B & F and Phormidium tenue (Mengh) Gom.-were obtained as pure cultures. N.linckia, a dinitrogen fixer, was grown in modified nitrogen-free Chu-10 medium and the others were maintained in Bold's basal medium (BBM) as described previously (Megharaj et al. 1986).

The ability of these soil algae to degrade monocrotophos and quinalphos was tested as follows. Aliquots from stock solutions, prepared in acetone, of the technical grade insecticides were added to 250-ml sterilized Erlenmeyer flasks to provide desired final concentrations ranging from 5 to 50ppm of both insecticides keeping in view thier toxic levels to the test algae (Megharaj et al. 1986). The carrier solvent was completely evaporated to dryness and 100-ml portions of steam-sterilized BBM or modified Chu-10 medium were introduced into each flask under aseptic conditions. The residues were then equilibrated for a day to obtain aqueous solutions of the insecticides. Equal quantities of algal inocula were added to each flask as mentioned earlier (Megharaj et al.1986). Uninoculated media with an insecticide served as controls. All the culture flasks including controls were incubated under constant fluorescent illumination at room temperature (29 $\pm$ 4° C). Triplicate samples were withdrawn after 5, 10, 20, and 30 days of incubation for solvent extraction and estimation of the parent compounds. A change in the pH of the media from 6.8 to 7.0 was noticed in inoculated samples during the 30-day incubation period.

The residues of the parent compounds from the media were extracted three 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 redissolved in 2ml methanol for further analysis by colorimetry after separation by thin-layer chromatography (TLC). The residues of monocrotophos and quinalphos, dissolved in methanol, 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), as employed for other organophosphates by Adhya et al (1981), and air-dried. The authentic compounds were located by spraying with 0.25 per cent 4-(p-nitrobenzyl)-pyridine (NBP) in redistilled acetone. After separation of residues in the samples by TLC, the silica gel areas of the samples corresponding to the authentic compounds were scraped off carefuly, transferred to test tubes and the residues were then extracted into acetone.

The parent compounds were estimated by colorimetry as developed by Getz and Watts (1964) and modified by Jain et al (1974) in a Spectronic 20 (Bausch & Lomb) at 540nm. For qualitative analysis of the metabolites, if any, formed during algal degradation of the two insecticides, the TLC plates were sprayed with NBP followed by heating at 150° C for 15 min. in a hot-air oven and then spraying lightly with 10% tetraethylene pentamine in redistilled acetone until the development of intense blue spots against a white backgrouond (Watts 1965). Analyses of significant differences ($P<0.05$) between values of each sampling were performed using Duncan's new multiple range test.

RESULTS AND DISCUSSION

The standard curves which formed the bases in calculating the amount of insecticides after their metabolism by soil algae in culture media were linear over a range of 2 to 15 ug. With the complex extraction and analytical procedures used, the initial recoveries of monocrotophos and quinalphos immediately after application to the media were 96 and 97.5 %, respectively. The composition of the media was found to have no impact on the recovery of insecticides. The concentration of monocrotophos (Table 1) and quinalphos (Table 2) in uninoculated media also decreased appreciably during the 30-day incubation period. Thus,

Table 1. Degradation of monocrotophos by soil algae

Organism	% monocrotophos recovered after incubation, in days			
	5	10	20	30
Uninoculated	95.8a	89.6a	79.2a	60.4a
Chlorella vulgaris	85.4a	77.1a	20.2e	traces
Scenedesmus bijugatus	89.6a	81.9a	29.2d	16.7b
Synechococcus elongatus	89.6a	81.3a	42.3b	15.2b
Phormidium tenue	86.9a	79.2a	39.6bc	traces
Nostoc linckia	90.2a	79.8a	33.3cd	16.0b

Monocrotophos added to 100-ml medium, 5mg
Means (n=3) in each column followed by the same letter are not significantly different ($P<0.05$) according to Duncan's new multiple range test

about 38% monocrotophos and 46% quinalphos were lost from the controls. Such a decomposition of these

insecticides even in uninoculated media with pH 6.8 could be expected since the organophosphate ester insecticides are highly susceptible to hydrolysis and are inherently unstable, decomposing slowly even at normal temperatures (Brown et al. 1986).

All the five species of soil algae started metabolizing monocrotophos only after 10 days of their incubation. At the end of 20-day incubation period, the algae effected significant degradation of monocrotophos followed by a further increase in the rate of its metabolism. After 30 days, about 60.4% monocrotophos was recovered from uninoculated samples as compared to its decrease to 16.7% down to insignificant levels in inoculated media. The parent compound was almost completely degraded during this incubation period in samples inoculated with Chlorella vulgaris or Phormidium tenue. Thus, the rate of degradation of monocrotophos by different algae followed the order: P. tenue = C. vulgaris > Synechococcus elongatus > Nostoc linckia > Scenedesmus bijugatus. Thin-layer chromatographic analysis of the residues in organic solvent extract of the media revealed that monocrotophos (Rf,0.17) during its algal metabolism yielded four unidentified metabolites with Rf values 0.38, 0.45, 0.75 and 0.91 as against to only two spots (Rf, 0.45 and 0.75) from uninoculated controls. The formation of two metabolites even in uninoculated medium thus confirmed the slow decomposition of monocrotophos probably due to its chemical hydrolysis. The rapid degradation of monocrotophos by the algal cultures was accompanied by accumulation of degradation compounds in large quantities as evidenced by thier color intensities on TLC plates. Presumably, the four metabolites are the resultant products of hdrolysis as 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).

The two filamentous cyanobacteria, P. tenue and N.linckia, degraded quinalphos significantly even by the end of 5 days of incubation (Table 2). As with monocrotophos, the degradation of quinalphos progressively increased with time. At the end of 30 days after inoculation, the insecticide was either completely degraded or decreased to negligible amounts.

Table 2. Metabolism of quinalphos by soil algae

Organism	Quinalphos concentration (ppm)	% quinalphos recovered after incubation, in days			
		5	10	20	30
Uninoculated	20	88.2a	78.5a	63.1a	52.8
C. vulgaris	5	92.3a	64.9b	37.6b	0
S. bijugatus	5	94.0a	68.4b	41.0b	0
S. elongatus	10	90.6a	44.4c	13.7c	traces
P. tenue	20	63.2b	42.7c	8.6c	0
N. linckia	10	73.5b	44.5c	12.0c	traces

Means (n=3) in each column followed by the same letter are not significantly different ($P<0.05$) according to DMR test

Thus C. vulgaris, S. bijugatus and P. tenue effected complete degradation of parent compound while only traces remained in the media inoculated with S. elongatus and N. linckia. Qualitative analysis of residues in the organic solvent fraction by TLC showed the formation of two unidentified metabolites (Rf, 0.40 and 0.59) during the degradation of quinalphos (Rf, 0.89) after five days of inoculation with all the species of algae as compared to only one spot (Rf, 0.40) from uninoculated samples. Interestingly, these two intermediate compounds of algal degradation were not observed subsequently indicating that the metabolites were further utilized completely by the algae.

The results of the present study suggest that algal metabolism of organophosphate insecticides like monocrotophos and quinalphos is highly likely in soil environments only when such chemicals are encountered by the microalgae at nontoxic levels. Further, it is also evident that both categories of soil algae, greens and blue-greens, are equally potential in detoxifying these insecticides.

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