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Effect of Pesticides on the Diazotrophic Growth and Nitrogenase Activity of Purple Nonsulfur Bacteria

A. V. Chalam, C. Sasikala, C. V. Ramana, N. R. Uma, P. R. Rao

Microbial Biotechnology Laboratory, Department of Botany, Osmania University, Hyderabad 500 007, India

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Application of pesticides for improving crop productivity has become necessary in the present day agricultural practices. This has resulted in either stimulatory or inhibitory effect on the soil microflora including nitrogen fixing organisms. Most of the pesticides, being xenobiotic, are degraded by very few microorganisms, which developed the tendency to degrade them while they may be toxic for others. Extensive studies on the effect of some commonly used pesticides on the soil nitrogen fixing microorganisms has been well worked out on chemotrophic bacteria and cyanobacteria. Anoxygenic phototropic bacteria (APB) are one of the major groups of diazotrophic microorganisms existing in high numbers in paddy soils and contributing significantly to soil fertility (Habte and Alexander 1980). The effects of some of the xenobiotic nitroaromatic compounds (2,4-dinitrophenol, 4- nitrophenol, 2-amino-4-nitrophenol, 4-aminophenol), haloaromatic compounds (4-chlorophenol) (Roldan et al. 1994), antibiotics (Keshav and Subha, 1992, Nogales et al. 1994) and s-trazine herbicides (atrazine) (Sutton et al. 1984; deVirty and Diner 1984) on APB have been studied. In the present communication the effect of a herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D); fungicides- captan (N-trichloro-methyl mercapto-4-cyclohexene-1,2,dicarboximide), carbendazim (2-benzimidazole carbamic acid methyl ester), and insecticides - quinolphos (0,0-diethyl-0-quinoxy-alinyl-2-thio oxophosphate) and monocrotophos (E-phosphoric acid dimethyl l-methyl-3-(methylamino)-3-oxo-l-propenyl ester) on the diazotrophic growth and nitrogenase activity of two purple nonsulfur bacteria, *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* isolated from paddy fields is discussed.

MATERIALS AND METHODS

Rhodobacter sphaeroides 1B and *Rhodopsuedomonas palustris* 3A were isolated as a dominating species of purple non-sulfur APB existing in two different paddy soils of Andhra Pradesh, India. Auxenic cultures of these organisms were grown photoheterotrophically in Biebl and Pfennig's (1981) medium with malate (0.2% w/v) and ammonium chloride (0.04% w/v) as carbon and nitrogen source, respectively, at 30 ± 2°C and 2400 lux. For diazotrophic growth, ammonium chloride and yeast extract were omitted in the medium. Seven mL of medium with malate (0.2% w/v) as carbon source/ electron donor and a vitamin cocktail (Biotin-15µg;

Correspondence to: P. R. Rao

Niacin-500µg; Thiamine-500µg; Paraaminobenzoic acid-300µg; Pysidoxin- 15µg per litre of medium) was taken in 15x125 mm rimless test tubes and sealed with suba seals. The gas phase in the tubes was replaced with ultra pure nitrogen and growth was measured turbidometrically using Systronics colorimeter at 660 nm. All the pesticides used were of commercial grade (quinolphos (trade name-Ekalux, 25%), monocrotophos (Hycrophos, 36% SL), captan (captoff-50% wp) were obtained from Rallis India ltd., India. Carbendazim (Derosol 50% wp) was from Hoechest limited, India. 2,4-D (Fernoxone with 2,4-D Sodium salt 80%) was obtained from Imcomos limited, India) and were added into the medium used for diazotrophic growth before autoclaving. Biomass yield was calculated in terms of dry weight and nitrogenase activity of resting cell suspension at growth inhibitory concentration was assayed in terms of acetylene reduction (Sasikala et al. 1990).

RESULTS AND DISCUSSION

The ability to fix nitrogen is well known among members of purple nonsulfur bacteria (Madigan et al 1984). *Rb.sphaeroides* and *Rps.palustris* could grow by fixing nitrogen photosynthetically with malate as carbon source/electron donor. Since most of the compounds chosen are aromatic compounds, two purple bacteria were chosen for the present study, *Rps.palustris* with the capability to degrade aromatic compounds and *Rb.sphaeroides* without such a capability. Growth (Fig, 1) of the two organisms was studied at various concentrations of the pesticides, i.e, from 0-900 mg/L and nitrogenase activity (Tables 1,2) was determined in resting cell suspensions of the organisms grown in the absence of the above compounds. The concentration of the pesticides used during nitrogenase assay was more than that required to inhibit growth but less than lethal. Though the biomass yield of diazotrophically grown *Rps.palustris* (Table 2) was more compared to that of *Rb.sphaeroides* (Table 1), its nitrogenase activity (acetylene reduction) was only half of that of *Rb.sphaeroides.*

None of the aromatic pesticides used, viz., 2,4-D, quinolphos, captan and carbendazim were photobiodegraded even by *Rps.palustris* (data not shown) under the experimental conditions tested, though this organism is known to assimilate both heterocyclic (Sasikala et al 1994a) and homocyclic (Sasikala et al 1994b) aromatic compounds. However, in an earlier report carbendazim was shown to be photoassimilated by *Rps.palustris* strain as sole carbon and nitrogen source (Rajkumar and Lalithakumari 1992).

Except for captan at 200 mg/L (Fig. 1) for *Rps.palustris,* all the pesticides had an inhibitory effect on diazotrophic growth of both the strains. In general, *Rps.palustris* was more resistant to the compounds used than *Rb.sphaeroides* showing species variation in the growth inhibition, though the patterns were same, with monocrotophos being most inhibitory for both strains followed by quinolphos, captan, 2,4-D and carbendazim. Carbendazim could not stop growth of *Rps.palustris* even at a concentration of 900 mg/L. Such a species variation in susceptibility was also noted by Brown et al (1990) who observed for the herbicide atrazine that *Rps.palustris* and *Rhodocyclus gelatinous* were less resistant than was *Rps.acidophila* but still 5 times more resistant than was *Rb.sphaeroides.*

Relatively higher resistance of the strains tested for the pesticides studied may be

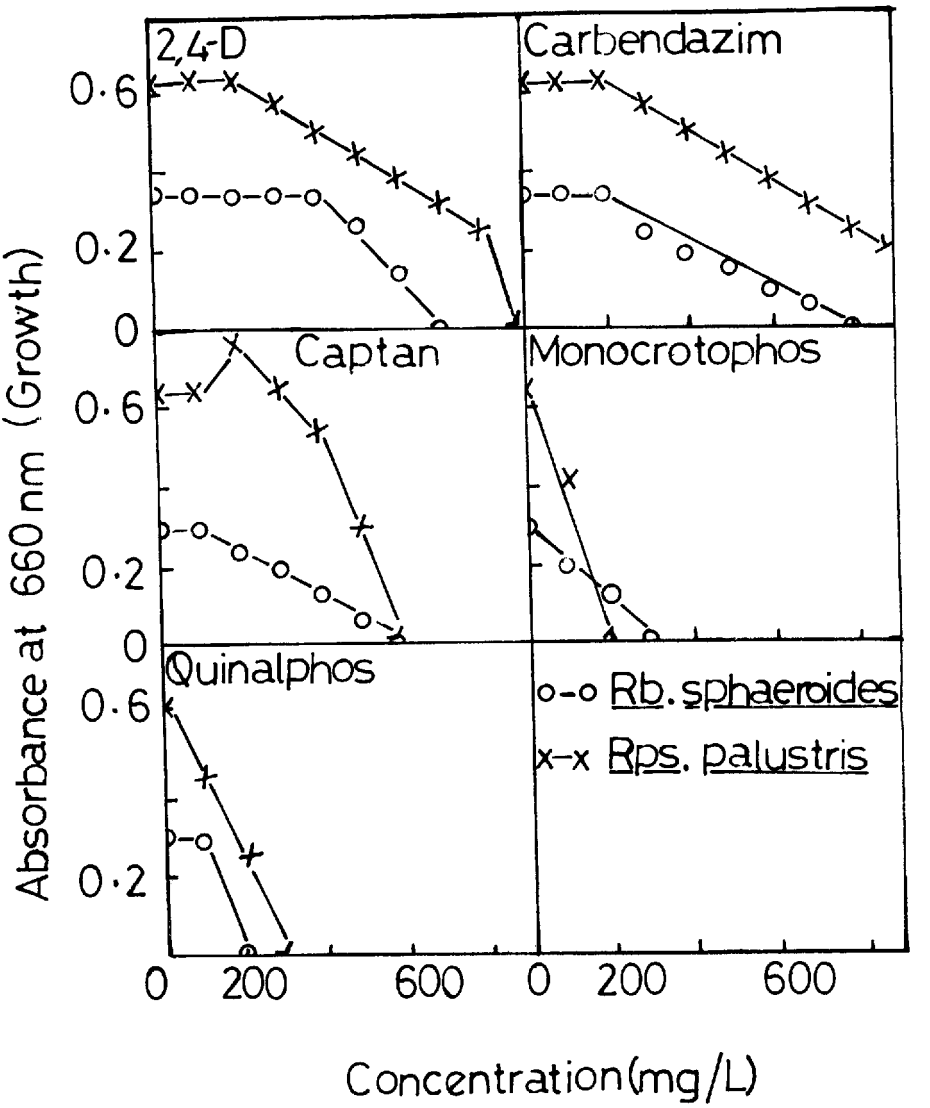

Figure 1. Effect of pesticides on the diazotrophic growth of *Rb.sphaeroides* and *Rps.palustris.* Results expressed are after 7 days of light (2400 lux) anaerobic growth at 30 ± 2°C.

because of the habitat of the organisms from which they were isolated, i.e, paddy soils which receive the above compounds during pesticide application to the crop where the compounds accumulate since they are recalcitrant being xenobiotic. This is supported by the fact that *Rb.sphaeroides* showed 50% growth inhibition at 35 µM (approximately 7.56 mg/L) atrazine and while resistant strains selected under laboratory conditions (Sutton et al. 1984) or under naturally occurring conditions (Brown et al. 1990) showed no susceptibility even at a concentration of 100 µM (approximately 21.6 mg/L) atrazine. There are a few reports of stimulation of growth and nitrogenase activity in oxygenic phototrophs by the herbicide 2,4-D (Subramaniam and Shanmugasundaram 1986); however no such stimulatory effect was observed on APB in the present investigation. 2,4-D inhibited growth above 400 and 200 mg/L, respectively, for *Rb.sphaeroides* and *Rps.palustris* (Fig 1); lethal concentrations were at 700 and 900 mg/L, respectively. Nitrogenase activity as observed at the inhibitory concentration selected suggests that 2,4-D has more inhibitory effect on *Rb.sphaeroides* than on *Rps.palustris.*

The pattern of inhibition of nitrogenase activity (Table 1,2) was also similar for both the strains at the selected concentrations with quinolphos being the most potent. Though monocrotophos inhibited growth at very low concentration (lethal doses being 150 and 200 mg/L for *Rb.sphaeroides* and *Rps.palustris* respectively), nitrogenase activity was least affected at these concentrations. Carbendazim was the least inhibitory to nitrogenase activity with only 18 and 25% inhibition (compared with control) at 400 and 300 mg/L of the compound for *Rb.sphaeroides* and *Rps.palustris* respectively. Even with quinolphos which was most potent inhibitor of nitrogenase activity, at a concentration of 100 mg/L also about 8% of activity was still retained for *Rps.palustris.* In contrast, quinolphos and monocrotophos totally inhibited nitrogenase activity in cyanobacteria even at very low concentration (5 mg/L) (Meghraj et al. 1988). The fact that APB are more resistant to pesticides than oxygenic phototrophic bacteria (cyanobacteria), makes them more suitable for application as biofertilizer under field conditions where considerable amounts of these compounds are found accumulated.

Table 1. Effect of pesticides on the diazotrophic growth (biomass yield) and nitrogenase activity (acetylene reduction) of *Rb.sphaeroides 1B.*

Pesticides mg/L	Biomass yield (mg dry wt./mL)	% growth inhibition	Nitrogenase activity *	% inhibition
2,4-D (500)	0.30	25	50.3	88
Quinolphos (150)	0.20	50	00.0	100
Monocrotophos(100)	0.25	37	243.6	44
Captan (400)	0.40	00	189.6	56
Carbendazim (300)	0.30	25	325.0	25
(-) pesticide medium control	0.40	00	433.4	00

* n mol ethylene formed/mg dry wt./hr.

Table 2. Effect of pesticides on the diazotrophic growth (biomass yield) and nitrogenase activity (acetylene reduction) of *Rps.palustris* 3A.

Pesticides mg/L	Biomass yield (mg dry wt./mL)	% growth inhibition	Nitrogenase activity *	% inhibition
2,4-D (500)	0.61	22	66.4	69
Quinolphos (100)	0.60	23	17.6	92
Monocrotophos(50)	0.53	32	115.0	46
Captan (400)	0.66	15	56.0	74
Carbendazim (400)	0.62	21	176.1	18
(-) pesticide medium control	0.78	00	214.5	00

* n mol ethylene formed/mg dry wt./hr.

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