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Effect of Aldicarb on a Non-Target Soil Microorganism

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ABSTRACT

The effect of aldicarb, a systemic, soil applied pesticide, on the growth, carbon and phosphate metabolism of the soil-dwelling tomato wilt pathogen, ***Pesudomonas solanacearum*** Smith, was studied. The *in vitro* growth of the organism was significantly affected at 5 and 10 ppm concentrations of the chemical while at 1 ppm the insecticide did not have much effect on the growth. In presence of ^{14}C-glucose in the medium, the insecticide treated cells incorporated more of ^{14}C activity although the cell yields were less than the untreatad cells. More ^{14}C lable was found to have been assimilated in the cold-TCA soluble fraction while the ^{14}C incorporation was less in the insoluble protein fraction of the aldicarb treated cells. However, the incorporation of ^{14}C-sodium acetate by these cells was enhanced in the lipid fraction with a reduction in ^{14}C lable in alcohol soluble, hot-TCA soluble and insoluble fractions. The enhancement in the incorporation of ^{32}P-labelled disodium hydrogen phosphate by the aldicarb treated cells indicated that the oxidative phosphorylation or electron transport chain of the bacterium was not affected.

INTRODUCTION

A considerable portion of the various pesticides applied to soil and on crop plants for controlling pests and diseases remain in soil for an appreciable length of time. Several pesticides applied to soil have been known to affect the number and activities of soil microorganisms (Robson and Gunner, 1970; Tu, 1970; Balasubramanian and Siddaramappa, 1974). However,the effect of such pesticide residues on the metebolic activities of specific, non-target soil microorganisms especially of agricultural importance, is little understood. Garretson and San Clemente (1968) observed inhibition of the nitrifying chemolithotrophic bacteria by several insecticides, while Lin *et al.* (1972) found no inhibition of nitrification by six organophosphate and three carbomate insecticides at the approximate field rate (5 ppm), but observed that *Rhizobium japonicum*, *R. meliloti* and *R. trifolii* were most sensitive. Kuseske *et al.* (1974) reported toxicity to pure cultures of the autotrophic nitrifying bacteria, *Nitrobacter agilis* and *Nitrosomonas enropea*, by Temik (Aldicarb) and Baygon (Propoxur). The effect of aldicarb (2 methyl-2 methyl thio poropional dehyde – O -methyl carbamoyl oxime), on the growth and carbon and phosphate metabolism of the soil dwelling phyto-

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pathogen, *Pseudomonas solanacearum* is presented in this paper.

One ml of uniformly suspended *P. solanacearum* cells, experiencing stationery phase of growth, was inoculated into a side - armed 250 ml Erlenmeyer flask containing 99 ml of nutrient broth (glucose 5 g, peptone 5 g, beef extract 3 g, distilled water 1000 ml with pH adjusted to 7.0). Calculated quantities of sterile aldicarb (crystalline technical grade material) solution were injected into the medium just before inoculating the bacterial cells to obtain final concentrations of 1 ppm, 5 ppm, and 10 ppm (a.i.). The flasks were then inoculated at 28° to 30°C on a gyratory shaker. At periodical intervals the growth was measured with a Klett Summerson colorimeter with blue filter (430um), and the optical density (O.D) was computed. Two replications were maintained for each treatment with appropriate controls.

For studying the influence of the insecticide on the carbon and phosphate metabolism of the organism, *P. solanacearum* cells were grown in nutrient broth medium with half the quantity of glucose for 24 hr in presence of the insecticide. Then appropriate quantities of radio - active ^{14}C - glucose or ^{14}C - sodium acetate (uniformly labelled and carrier-free) or ^{32}P - disodium hydrogen phosphate, as the case may be, was injected into the medium such that the final radio-activity in the medium was 1 microcurie per ml. Cells were harvested after varying intervals and the incorporation of radio activity in the whole cells as well as different cellular fractions were monitored following the procedure already described(Balasubramanian *et al.*,1975).

RESULTS AND DISCUSSION

Maximum growth of cells was observed in cultures treated with 10 ppm aldicarb as compared to the other treatments(Fig. 1). It was also observed that the 5 ppm aldicarb treatment showed some inhibitory effect on the growth of the organism as compared to the untreated cells. Spurr and Souza (1974) have reported that even at 100 ppm of aldicarb there was no marked inhibition in the growth of the test bacteria they used, which included *P. aeruginosa* along with several others. The present observations contradicted their findings in that, there was an inhibitory effect on the growth of *P. solanacearum* at 5 ppm but stimulation at 10 ppm concentration, with no significant effect at 1 ppm

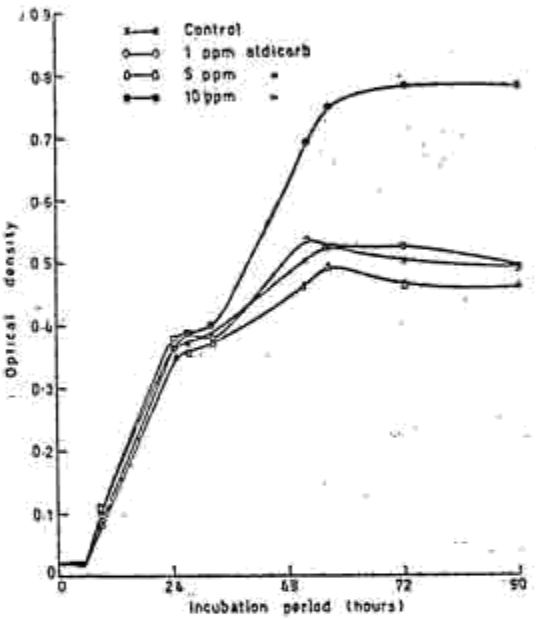

Fig. 1. Effect of Aldicarb on the growth of *Pseudomonas solanacearum in vitro*

TABLE I. Effect of aldicarb on the incorporation of ^{14}C-glucose by *Pseudomonas solanacearum*

Treatment	3 hour		6 hour		15 hour	
	Dry weight of cells (mg)	Specific activity (cpm/100 mg)	Dry Weight of cells (mg)	Specific activity (cpm/100 mg)	Dry weight of cells (mg)	Specific activity (cpm/100 mg)
0 ppm (control)	99.4	5218 ± 77.2	93.5	5169 ± 106.8	141.7	2845 ± 56.0
1 ppm	98.9	5429 ± 33.5	81.6	6524 ± 89.3	87.5	4677 ± 32.0
5 ppm	91.2	4725 ± 45.8	82.6	5589 ± 80.7	86.7	4238 ± 71.5
10 ppm	90.3	5454 ± 105.3	83.4	5784 ± 40.0	90.3	4589 ± 53.2

cpm : Counts per minute

concentration of the chemical. Such a differential effect is uncommon in the light of the reports with other insecticides, *viz.*, chlordane on *Bacillus subtilis* (Trudgill *et al.*, 1971) and carbofuran, endrin and disulfoton on *Rhizobium* sp. (Oblisami *et al.*, 1973) wherein decrease in the inhibition has been reported with decrease in the concentrations of the insecticide. Present observations, therefore, indicate the possibility that the effect of aldicarb on the metabolism of *P. solanacearum* differed at 5 ppm and 10 ppm levels, while the 1 ppm concentration did not have any significant effect.

The results on the incorporation of ^{14}C - glucose by *P. solanacearum* cells as influenced by various concentrations of aldicarb (Table I) indicated that the chemical generally enhanced the incorporation of the ^{14}C label in the cells but reduced the yield (dry weight) of the treated cells. It is, therefore, possible that, although the uptake of glucose by the bacteria was not hindered by the insecticide, the subsequent assimilation of glucose into the cellular constituents essential for cell growth, might be affected.

Incorporation of the ^{14}C label into the different cellular fractions clearly indicated that in the case of cells treated with 5 ppm aldicarb the percentage incorporation of ^{14}C label was maximum in the cold - TCA extractable fraction of the cells, which is presumably composed of simple sugars and soluble carbohydrates, as compared to the untreated cells (Table II). However, a significant reduction in ^{14}C label in the insoluble fraction and the increase in the activity in both the ether soluble as well as hot - TCA soluble fractions of the treated cells, suggest that the assimilation of ^{14}C - glucose into these cellular constituents was also altered by the insecticide treatment.

The increased incorporation of ^{14}C acetate in the ether - soluble fraction of 5 ppm aldicarb treated cells with a

TABLE II. Effect of aldicarb on the incorporation of ^{14}C-glucose in different fractions of the cells*

Treatment	Whole cells		Percentage activity in fractions				
	Weight (mg)	Specific activity (cpm/100 mg)	Cold-TCA soluble	Alcohol soluble	Ether soluble	Hot-TCA soluble	Insoluble
0 ppm (control)	117.4	1103 ± 30.5	38.16	7.83	11.45	15.26	27.48
1 ppm	75.2	2291 ± 44.0	33.33	22.22	13.33	15.55	15.55
5 ppm	78.7	2105 ± 33.0	42.04	8.52	14.20	18.88	15.34

TCA : Trichloro acetic acid

* 24 hr old cells exposed to ^{14}C-glucose for 15 hr were used for fractionation.

consequent decrease in ^{14}C activity in alcohol soluble, hot - TCA soluble and insoluble fractions of the cells indicated that the insecticide enhanced the synthesis of lipids by the cells with concomitant reduction of the other cellular constituents such as amino acids, nucleic acids and insoluble proteins (Table III). Aldrin treatment has been reported to alter the ^{14}C assimilation into the different cellular constituents of two *Rhizobium* spp. and such an effect was also found to vary with the concentration of the insecticide (Balasubramanian *et al.*, 1975).

Aldicarb treated cells incorporated more of ^{32}P-phosphate than the untreated cells (Table IV). The enhanced incorporation of ^{32}P by *P. solanacearum* cells in presence of aldicarb, as well as the increase in ^{14}C-glucose incorporation by the cells ruled out the possibility of inhibition of the oxidative phosphorylation or the electron trans-

TABLE III. Effect of aldicarb on the incorporation of ^{14}C labelled sodium acetate in the cells and in the cellular fractions*

Treatments	Whole cells		Percentage of the activity in fractions				
	Weight (mg)	Specific activity (cpm/100 mg)	Cold-TCA soluble	Alcohol soluble	Ether soluble	Hot-TCA soluble	Insoluble
0 ppm (control)	89.9	7409 ± 186.0	2.97	27.97	17.80	16.11	35.14
1 ppm	83.7	7324 ± 79.0	1.88	27.59	24.92	8.15	37.44
5 ppm	64.7	8063 ± 73.0	5.14	21.30	31.65	11.75	29.86

*24 hr old cells exposed to ^{14}C-sodium acetate for 6 hr were used for fractionation.

TABLE IV. Effect of aldicarb on the incorporation of ^{32}P labelled disodium hydrogen phosphate in the cells

Treatments	1 hr	3 hr	6 hr
	Specific activity (cpm/mg)	Specific activity (cpm/mg)	Specific activity (cpm/mg)
0 ppm (control)	684.66 ± 0.75	475.58 ± 8.10	574.57 ± 0.79
1 ppm	361.14 ± 0.42	497.51 ± 3.90	608.50 ± 0.40
5 ppm	568.38 ± 0.83	717.47 ± 6.60	543.84 ± 1.05
10 ppm	697.20 ± 0.23	725.32 ± 2.30	661.10 ± 0.87

cpm — counts per minute

port chain in the pathogen unlike the case of several other insecticides reported earlier (Nelson and Williams, 1971). In the absence of any earlier report on the effect of aldicarb on cell metabolism, it is difficult to suggest, with the available information, the specific action of the chemical on the carbon and phosphate assimilation processes of *P. solanacearum*. However, the probable machanism of action of aldicarb on the metabolism of this organism has been demonstrated by the authors and is being published elsewhere.

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