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EFFECT OF COMMON PESTICIDES ON NITROGEN FIXING BACTERIA OF MULBERRY (*MORUS ALBA* L.)

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ABSTRACT

Three fungicides and three insecticides were tested against three nitrogen fixing bacteria namely *Azotobacter chroococcum, Azospirillum brasilense* and *Beijerinckia indica* isolated from mulberry rhizosphere. There were variable effects of pesticides on the growth of three nitrogen fixing bacteria. *In vivo* study showed that amongst fungicides, Carbendazim reduced the bacterial population at all concentrations but Mancozeb and Wettable sulphur stimulated the same at 1g/l and reduced it at higher concentration. Amongst insecticides, Methylparathion followed by Dimethoate were comparatively more toxic than Endosulfan and they completely killed the nitrogen fixing bacteria at 2 and 4 ml/l concentrations respectively. *In vivo* studies indicated stimulatory effect of 0.1% carbendazim and Dimethoate on their population both in rhizosphere and on phylloplane. The other pestioides reduced the population of nitrogen fixing bacteria. There was a trend of regaining equilibrium of nitrogen fixing bacteria particularly in rhizosphere after a gap of 3 weeks.

INTRODUCTION

The use of pesticides in agriculture has become an unavoidable reality due to growing menace of diseases and pests and mulberry plantation is no exception to it. Pesticides are known to have effects on non-target microbial population of both rhizosphere (Purushothaman *et al.*, 1976; Van Schreven *et al.*, 1970) and phylloplane (Hislop, 1976; Warren, 1974). Infact, there is no organism in nature which can live like a hermit (Zehner, 1965) and man by continuous use of pesticides has further added to this natural interference. Systemic pesticides have greater inhibitory effects than contact ones on phylloplane microflora (Jayachandran and Chandramohan, 1977) and this has been explained to the adverse effects of pesticides on metabolism of plant which in turn contaminates plant leachates. Pati *et al.* (1977) have studied *in vitro* effect of pesticides on azotrophic bacteria isolated from phylloplane of wheat, potato, tomato, rice, mustard and jute and Merilyn and Thomas (1991) have tested three fungicides on *Beijerinckia* isolated from coconut rhizosphere. Rodell *et al.* (1977) have tested nine organophosphate and carbamate insecticides against *Azotobacter vinelandii* using acetylene reduction as criteria. Effect of pesticides on microbial population of phylloplane not only depend on the nature of pesticide but also on the host plant as reported by Andrews and Kenerley (1978) which also support the opinion of Jayachandran and Chandramohan (1977). The use of nitrogen fixing bacteria as inoculant in mulberry crop production is of recent origin (Das *et al.*, 1990; Dayakar Yadav and Nagendrakumar, 1992; Gangwar and Thangavelu, 1992) and needs attention.

Carbendazim (Bavistin 50WP), Mancozeb (Indofil M-45 75WP) and Wettable sulphur (Sulfex 80WP) are commonly recommended for mulberry disease control (Biswas *et al.*,1995; Philip *et al.*, 1994). Similarly, for insect pest control in mulberry, commonly recommended insecticides are Endosulfan (Thiodan 35EC), Dimethoate (Roger 30EC) and Methyl parathion (Metacid 50EC) (Gangwar and Thangavelu, 1991; Rangaswami *et al.*, 1987). As these bacteria are expected to be adversely affected by pesticides, the above fungicides and insecticides were tested both *in vitro* and *in vivo* on the population of nitrogen fixing bacteria. *In vivo* study comprised of both rhizosphere and phylloplane total population of the nitrogen fixing bacteria as influenced by foliar spray of these pesticides. While *in vitro* study was taken

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up *Azotobacter*, *Beijerinckia* and *Azospirillum* separately.

MATERIAL AND METHODS

Procurement of nitrogen fixing bacteria : Three nitrogen fixing bacteria viz: *Azotobacter chroococcum* (AZB) *Beijarinckia indica* (BJK) and *Azospirillum brasilense* (AZS) were isolated from the rhizosphere (AZB & BJK) and endorhizosphere (AZS) of mulberry plants using selective media namely Waksman 77 medium for AZB & BJK and sodium malate medium for AZS (Rupel, 1989), and maintained in pure culture. The authenticity of bacteria was confirmed by standard method (Krig and Holt, 1984). The bacteria were multiplied in specific broths to get about 10^8 cells/ml.

***In vitro* studies :** Modified food poisoned method (Gangwar and Dasgupta, 1989) was used to study the effects of pesticides on the growth of nitrogen fixing bacteria. The pesticides (Table 1) in desired concentrations (1, 2, 3, 4, 5 g or ml/l) were mixed in broths of specific media taken in 250 ml Ehrelenmeyer flasks and autoclaved at 15 psi for 20 min. Thus there were 6 treatments including check (sterile distilled water) and each treatment was replicated four times. The flasks were seeded with 1 ml of test bacterial culture as mentioned earlier and incubated at 28 ±2°C on rotary shaker (1000 rpm) for 6 days. The population count was made by dilution plate technique in respective agar media plated in 90 mm petridishes. Usually calorimetric method is followed for estimation of bacterial population in broth cultures. But, in the present experiment, dark blue colour of sodium malate broth for AZS created a problem for uniform calorimetric reading for all bacteria. Hence the dilution plate method was preferred.

***In vivo* studies :** Mulberry plant of S_1 variety were raised in 40 l earthen pots filled with farm soil and farmyard manure (10:1 ratio). The plants were maintained in green house with 90 days pruning schedule. After one year, plants were used for experiment. The plants were sprayed with recommended dose of pesticides (0.2% for Mancozeb and Wettable sulphur and 0.1% for others) at 30th day after pruning. Natural population of nitrogen fixing bacteria was studied both in rhizosphere and phylloplane. For this purpose rhizosphere soil and leaf samples were collected before spraying of pesticides and at weekly

Table 1 : Particulars of pesticides used.

Technical	Trade Name	Chemical name and Chemical formulae	AI*	Nature of pesticide	LD50 (mg/kg) (Mammals)	Source
Carbendazim	Bavistin	2, (methoxy-carbomoyl) benzymidazole $C_9H_9N_3O_2$	50 WP	Systematic fungicide	15000	BASF India Ltd., Rhone- Poulence House S.K. Ahire Marg Bombay-400 025.
Mancozeb	Dithane M-45	Maganese Ethylene bis dithiocarbamate+2%Zn ions(-SCS $NHCH_2$ CH_2 NHCS.S.Mn-) x (Zn) Y	75 WP	Cantact fungicide	5000	Indofil Chemical Company Ltd. Nirlon House, Dr. A.B. Road, Worli, Bombay-400 025.
Wettable-Sulphur	Sulfex	Sulphur (CA) Sx	80 WP	Contact fungicide	Non-toxic	Excel Industries Ltd., 184-187, Swami Vivekanand Road, Jogeswari, Bombay-400 102.
Endosulfan	Thiodan	1, 4, 5, 6, 7, 7- hexachloro 8, 9, 10-trinorborn-5-en-2, 3- ylenodimethyl sulphite ($C_9H_6Cl_6O_3S$)	35 EC	Contact insecticide	40-50 to 110	Hoechst Schering AgrEvo Ltd., GIDC, Ankleshwar-393 002, Gujarat.
Dimethoate	Rogor	Dimethyle S(N-Methyl Carbomoylmethyl) phosphorothiolothioate ($C_5H_{12}NO_3PS_2$)	30 EC	Systemic insecticide	250	Rallis India Ltd., Agrochemical Division, Ralli House, 21 D, Sukhadwala- Marg, Bombay-400 001.
Methyl-Parathion	Metacid	0, 0-dimethyle 0-P-nitro phenyl phosphorothioate ($C_8H_{10}NO_5PS$)	50 EC	Contact insecticide	12-42	Bayer India Ltd., J-2 Unti SIDCO Industrial Estate, Salem-636 004

* Active Ingradient, WP = Wettable powder, EC = Emulsifiable concentrate.

interval after spray upto 3rd week. Population of NFBs was estimated by standard dilution plate technique. LD 50 of the pesticides was calculated by log-probit analysis method (Gangwar and Dasgupta, 1989).

RESULTS AND DISCUSSION

***In vitro* results** : Available data (Fig. 1) indicated insecticides were found inhibitory to growth of the tested nitrogen fixing bacteria and their comparative effects were in the order of Methyl parathion>Diamethoate>Endosulfan. Methyl parathion at 2 ml/l and Dimethoate at 4 ml/l culture broth completely killed *Azospirillum* and *Beijerinckia* but *Azotobacter* survived mildly upto 4 ml/l concentration of Dimethoate. Amongst fungicides Carbendazim appeared to have maximum toxic effect and it reduced population of nitrogen fixing bactera even at 1 g/l concentration. Wettable sulphur and Mancozeb, on the other hand, did not exhibit any adverse effect at this concentration and, on the contrary, there was increase in their population as compared to check.

Tolerance of three bacteria varied with the nature of different pesticides. While *Azotobacter* appeared to be more tolerant to fungicides, it was inferior to *Azospirillum* and *Beijerinckia* in tolerance against the insecticides. BJK had an edge over AZB in tolerance against most of the pesticides except Wettable sulphur.

In vitro inhibitory effect of pesticides on nitrogen fixing bacteria has been studied in detail (Jana and Mishra, 1984; Omar and Abd-Alla, 1992; Rivorola *et al.*, 1992). Pati *et al.* (1984) have reported wide variation in response of pesticides on nitrogen fixing bacteria and found that certain pesticides at low concentration stimulated growth of some bacteria. In the present study, Wettable sulphur and Mancozeb stimulated the growth of nitrogen fixing bacteria at 1 g/l concentration which might be due to stimulating effect of sulphur, zinc and manganese being major components of these pesticides, Merylin and Thomas (1991) reported stimulatory effect of Carbedazim at 100 ppm on growth of *Beijerinckia* isolated from coconut rhizosphere. But this concentration was very low in comparison to the concentration taken in the present experiment. The subsequent concentrations (250 and 500 ppm) in their experiment had, however, reduced *Beijerinckia* growth.

***In vivo* results:** Data presented in Table 2 indicate that pesticides had differential effects on the population both in rhizosphere and on phylloplane. Most of the pesticides had immediate adverse effect on the population of nitrogen fixing bacteria in rhizosphere but with the passage of time the bacteria were re-established to near initial levels. Wettable sulphur, however had reverse impact. There was initial increase in the population but the same started declining 2nd week onwards after spray of the pesticides. Methyl parathion had adverse effect from the very beginning and the population continued to decline upto 3 weeks.

The population of the nitrogen fixing bacteria on phylloplane was not necessarily similar to

Table 2 : Population of nitrogen fixing bacteria in mulberry plantation under treatment with different pesticides.

Treatment	Phylloplane population (cells/sq. cm. leaf)					Rhizosphere population (x 10^4 cells/g soil)				
	Initial	1st WAS	2nd WAS	3rd WAS	Mean	Initial	1st WAS	2nd WAS	3rd WAS	Mean
Check	64.7	61.6	62.4	71.3	65.0	168.0	173.7	174.3	182.3	174.6
Carbendazim	79.3	132.6	170.7	189.6	143.1	171.7	120.3	341.3	353.3	246.7
Mancozeb	77.3	47.2	31.1	23.2	44.7	174.0	152.7	166.7	192.0	171.3
Wettable sulphur	79.3	94.8	19.8	27.5	54.0	183.3	141.0	73.0	40.7	109.5
Endosulfan	77.3	95.3	70.7	43.0	63.2	184.0	47.0	47.3	152.0	107.6
Dimethoate	74.3	28.6	53.7	43.0	55.3	178.3	42.7	43.3	47.0	70.3
Methyl parathion	73.0	59.7	56.6	40.2	57.4	190.3	57.8	52.5	41.3	85.5
Mean	87.8	74.3	66.4	62.5		180.1	120.4	132.4	144.1	

Source	CD at 5%	SE±	CD at 5%	SE±
Treatment	8.5	2.9	8.2	2.9
Period	6.4	2.3	6.21	2.2
Treat. x Period	16.9	5.9	16.4	5.8

WAS = Week after spray

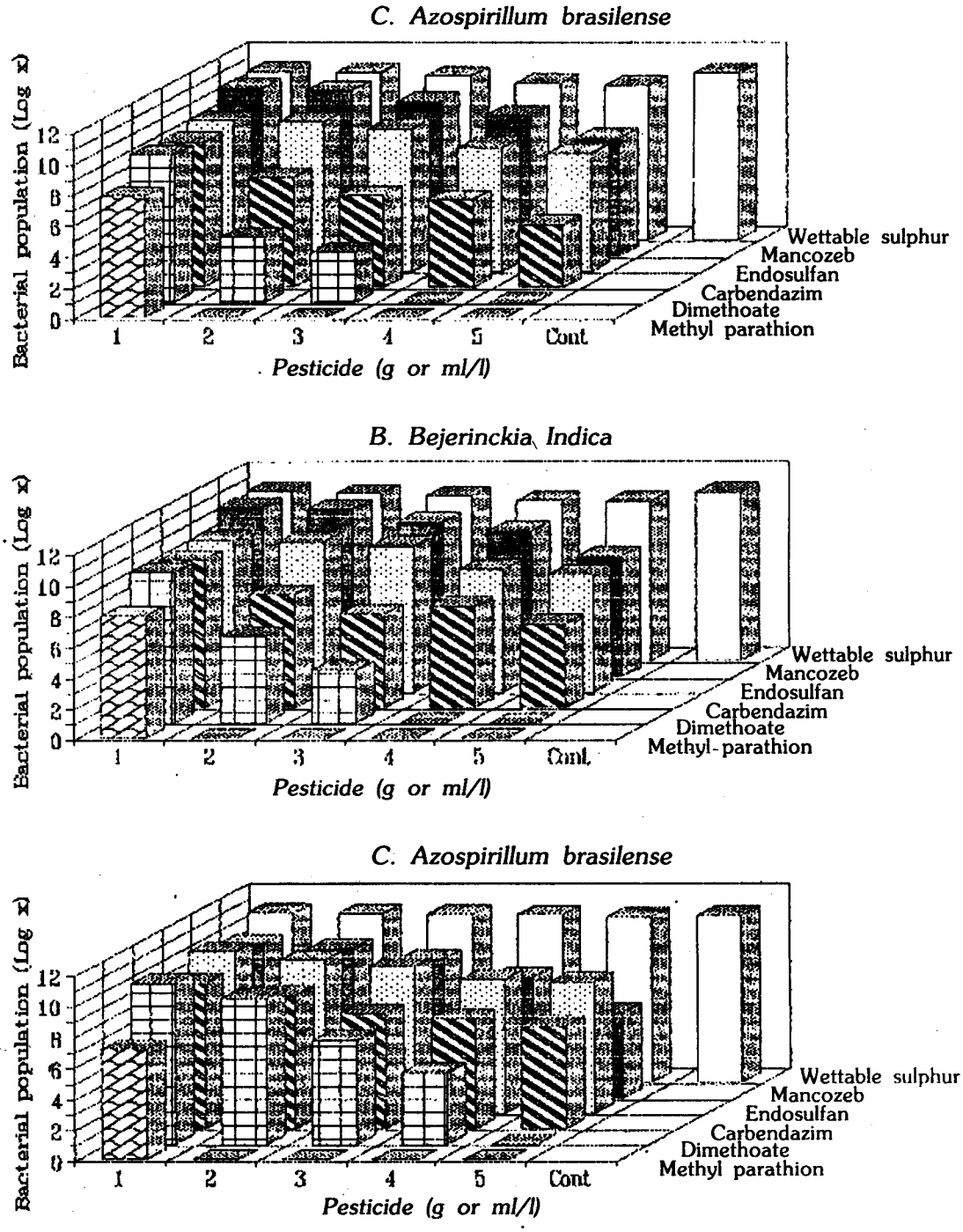

Figure 1. In Vitro effect of pesticides on NFB population.

that in rhizosphere. Carbendazim had stimulating effect on phylloplane population and so did Dimethoate. Most of the other pesticides reduced bacterial population on phylloplane and this reduction in the population continued upto 3rd week after spray of pesticides.

Effect of pesticides on the population of nitrogen fixing bacteria in the field was not similar to that in cultures and this could be explained due to their indirect effects. Pesticides, particularly systemic ones, have access to the plant metabolism and thus change the nature and composition of plant leachates (Jayachandran and Chandramohan, 1977). This, in turn, affects the microbial population both in rhizosphere and on phylloplane. Both Mancozeb and Wettable sulphur being contact fungicides had direct effect on the population of nitrogen fixing bacteria in rhizosphere and phylloplane and reduced the same immediately after the spray. Whereas, Carbendazim being systemic fungicide might have indirect effect on their population through plant leachates and thus had stimulated the same and so did Dimethoate. Another possible reason might be the attendance change in the population of competing microorganisms particularly fungi. Carbendazim being broad spectrum fungicide might have reduced competition of other fungi with nitrogen fixing bacteria and thus created indirect situation for their enhanced population. There was an indication of returning the population of nitrogen fixing bacteria to near equilibrium after 3 weeks of pesticide spray and this was in conformity with earlier report (Lal and Bhardwaj, 1981).

It is concluded from the present study that the pesticides have differential effect on the growth of nitrogen fixing bacteria and their action vary at different sites. Indication has been observed that the systemic pesticides which are moderately suppressing under *in vitro* studies turned beneficial under field condition possibly due to their low direct effect on nitrogen fixing bacteria and high indirect effect due to reduction of competing microorganisms. Carbendazim and Dimethoate, therefore, might prove effective in supporting the growth of nitrogen fixing bacteria both in rhizosphere and phylloplane while other pesticides did not do so. Parathion due to its high toxic nature also reduced the population of these bacteria under field condition.

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