Chapter IV

EFFECTS OF PESTICIDES ON THE FUNGI ISOLATED FROM THE PHYLLOPLANE OF DIFFERENT CROPS

INTRODUCTION

The airspora studies in the crop fields revealed that pathogenic and non-pathogenic fungal spores are present at different growth stages. These are coming out mainly from the crop plants itself acting as the immediate source. Besides fungi and bacteria, crop plants mostly suffer from the attack of insect pests damaging the plants. Consequently the farmers are to use pesticides and fungicides to face the problem. The foliar parts are mostly sprayed with the chemicals. Hence, studies were made to find out the effects of the chemicals on the phylloplane mycoflora which in turn affects the local airspora to some extent. The effects of pesticides (excluding fungicides) were studied by Kataria and Dodan (1981) on *Pythium butleri*, Singh and Dwivedi (1987) on *Sclerotium rolfsii*, Singh and Pal (1992) on *Ascochyta rabiei*, Banerjee and Dey (1992) on rhizosphere fungi, Dik and Van Pelt (1992) on the synergistic effect of fungicide and insecticide to pathogens, Vijayalakshmi and Rao (1993) on VAM fungi, and Ashour *et al.* (1993) on the pathogenic as well as the non-pathogenic fungi in recent years.

MATERIALS AND METHODS

Cildon (85% phosphamidon) and BHC (50%) as pesticides and Bavistin (Carbendazim) are frequently applied by the farmers to protect the existing crops from the severe damage due to insect pests and fungal pathogens. These three compounds were tested against 28 phylloplane fungi at field doses using cup assay and dry weight methods.

CUP ASSAY METHOD

Spore suspension was prepared from a fresh 14 days old pure culture of the test fungi. One ml of spore suspension was distributed in the sterile petridishes (four replicates for each concentration against each fungus), to which sterile but cold nutrient medium was poured thickly and rotated several times to mix the spores evenly with the medium. After solidification, two cups of 6 mm diameter were made with a cork borer in each plate. Solutions at field doses were prepared from Cildon (i.e. 0.06% concentration), BHC (i.e. 0.5% concentration) and Bavistin (i.e. 1% concentration) with sterile distilled water.

Requisite amount of solutions were poured very carefully to the cups with a micropipette. Control set i.e. distilled water without chemicals was also maintained. The plates were incubated at 28°C for 24 hrs and examined every 24 hours. The inhibition zone (in diameter) if any, was measured and recorded.

DRY WEIGHT METHOD

For this method 25 ml of nutrient liquid medium with the requisite amount of pesticides or fungicide were taken in 100 ml conical flask. To these one ml of spore suspension of each fungus was added. Four replicates for each test was maintained. Control sets (4 flasks) containing only 25 ml broth and one ml of fungal suspension were always maintained. After 14 days of growth, the mycelial mats were harvested on Whatman's filter paper No. 42 (weighed previously) and were kept in hot air oven (60°C) for 72 hrs for complete drying. The dry weight of mats were taken and mean was calculated.

RESULTS

The mean reading of four replicates of inhibition measured by the cup assay method are represented in **Table 26**. Cildon caused inhibition in variable degrees on *Aspergillus ochraceous, Curvularia geniculata, Trichoderma lignorum, Fusarium* sp., *Drechslera* sp., *Helminthosporium oryzae, Nigrospora sphaerica, Alternaria humicola, A. solani, A. brassicae, A. tenuissima, A. tenuis* and *Helminthosporium sativum.* No inhibition was recorded on the rest (15 in number) fungi tested which included different species of *Aspergillus* excepting *A. ochraceous, Curvularia lunata, C. pallescens, Penicillium* spp., *Stachybotyrs atra, Epicoccum purpurascens, Cladosporium herbarum, Rhizopus nigricans, Brachysporium* sp., *Cercospora* sp. and *Chaetomium homopilatum.*

With BHC, 15 fungi (namely *Curvularia lunata, C. geniculata, Penicillium* sp., *Stachybotrys atra, T. lignorum, C. herbarum, Rhizopus nigricans, Drechslera, Alternaria solani, A. brassicae, A. tenuissima, A. tenuis, Chaetomium homopilatum, N. sphaerica* and *H. sativum*) were totally inhibited and 8 fungi (*viz. Aspergillus parasiticus, A. fumigatus, A.*

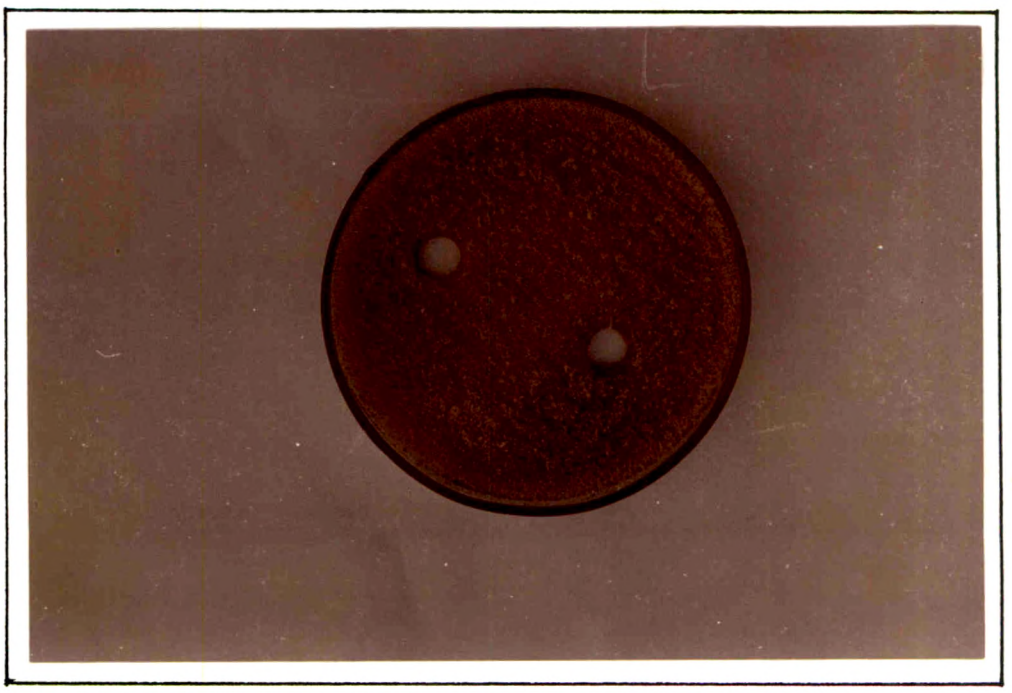

Plate A. Control (Normal growth of *Aspergillus ochraceous*)

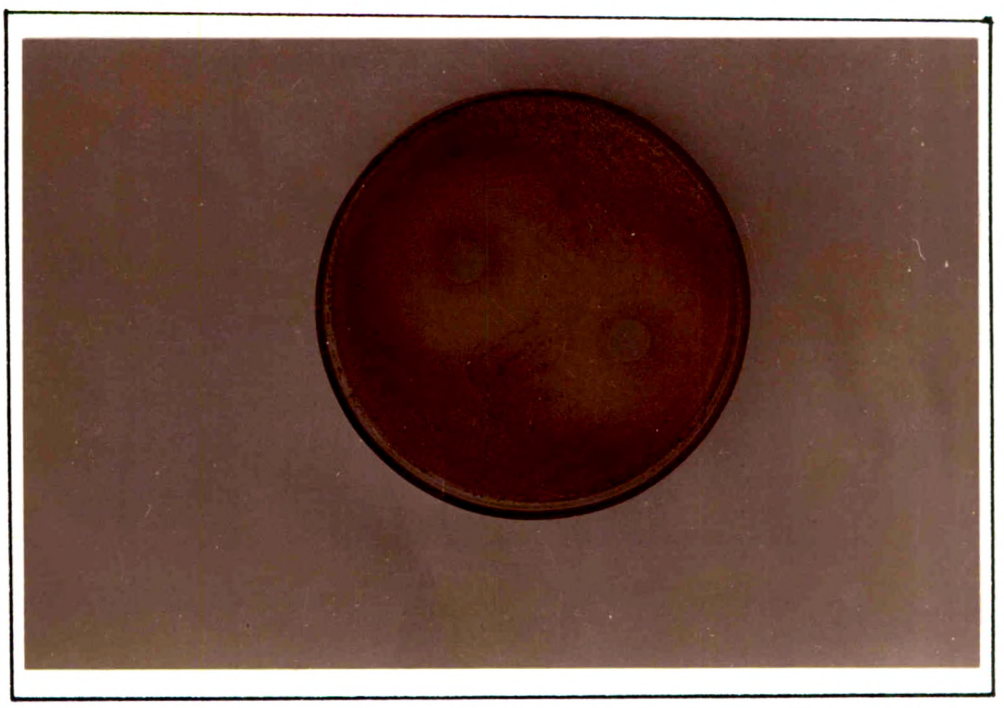

Plate B. *Aspergillus ochraceous* (showing inhibition)

Table 26. Mean growth inhibition (in diameter) of fungal types with the pesticides and fungicide by cup assay method.

Sl. No.	Fungal organism	Control (without chemical)		Cildon (0.06%)		50% BHC (0.5%)		Bavistin (1.0%)	
		Nature of Inhibition	Inhibition Zone (mm)	Nature of Inhibition	Inhibition Zone (mm diam)	Nature of Inhibition	Inhibition zone (mm diam)	Nature of Inhibition	Inhibition zone (mm diam)
1.	*Aspergillus niger*	Nil	0	Nil	0	Nil	0	Total	45
2.	*A. parasiticus*	Nil	0	Nil	0	Partial	16	Total	42
3.	*A. fumigatus*	Nil	0	Nil	0	Partial	12	Total	35
4.	*A. terreus*	Nil	0	Nil	0	Nil	0	Total	40
5.	*A. ochraceous*	Nil	0	Total	14	Partial	18	Total	45
6.	*Curvularia lunata*	Nil	0	Nil	0	Total	25	Nil	0
7.	*C. geniculata*	Nil	0	Total	30	Total	33	Total	30
8.	*C. pallescens*	Nil	0	Nil	0	Partial	20	Nil	0
9.	*Penicillium* sp.	Nil	0	Nil	0	Total	16	Total	45
10.	*P. funiculosum*	Nil	0	Nil	0	Nil	0	Total	43
11.	*Stachybotrys atra*	Nil	0	Nil	0	Total	30	Nil	0
12.	*Epicoccum purpurascens*	Nil	0	Nil	0	Nil	0	Nil	0
13.	*Trichoderma lignorum*	Nil	0	Partial	22	Total	45	Total	45
14.	*Cladosporium herbarum*	Nil	0	Nil	0	Total	30	Total	45
15.	*Rhizopus nigricans*	Nil	0	Nil	0	Total	20	Total	42
16.	*Fusarium* sp.	Nil	0	Partial	30	Partial	27	Total	32
17.	*Drechslera* sp.	Nil	0	Partial	19	Total	25	Nil	0
18.	*Helminthosporium oryzae*	Nil	0	Partial	24	Partial	18	Total	30
19.	*Alternaria humicola*	Nil	0	Partial	20	Partial	16	Nil	0
20.	*A. solani*	Nil	0	Total	28	Total	24	Nil	0
21.	*A. brassicae*	Nil	0	Total	40	Total	23	Nil	0
22.	*A. tenuissima*	Nil	0	Total	30	Total	38	Nil	0
23.	*A. tenuis*	Nil	0	Total	32	Total	27	Total	28
24.	*Brachysporium* sp.	Nil	0	Nil	0	Partial	18	Nil	0
25.	*Cercospora* sp.	Nil	0	Nil	0	Nil	0	Total	40
26.	*Chaetomium homopilatum*	Nil	0	Nil	0	Total	45	Total	45
27.	*Nigrospora sphaerica*	Nil	0	Total	45	Total	45	Nil	0
28.	*Helminthosporium sativum*	Nil	0	Partial	18	Total	24	Nil	0

ochraceous, C. pallescens, Fusarium, H. oryzae, Alternaria humicola and *Brachysporium*) were inhibited partially. No inhibitory effect was obtained in fungi like *A. niger, A. terreus, P. funiculosum, E. purpurascens* and *Cercospora* sp. adopting the cup assay method.

With the fungicide, Bavistin a number of fungi were found to be totally inhibited. Remarkable (45 mm diam) inhibition was recorded for *A. niger, A. ochraceous, Penicillium* sp., *T. lignorum, C. herbarum* and *Chaetomium homopilatum. Penicillium funiculosum* (43 mm), *A. parasiticus* (42 mm), *Rhizopus nigricans* (42 mm), *A. terreus* (40 mm) and *Cercospora* sp. (40 mm) showed a good inhibition while moderate (28-35 mm) inhibition was recorded for *A. fumigatus, Fusarium, C. geniculata, H. oryzae* and *A. tenuis*. Surprisingly, twelve fungi were found to grow normally in presence of Bavistin which included different species of plant pathogenic *Alternaria* (except *A. tenuis*), *Drechslera, H. sativum* and saprobic *C. lunata, C. pallescens, S. atra, E. purpurascens, Brachysporium* and *Nigrospora sphaerica.*

In dry weight method (**Table 27**) no growth was recorded in broth containing Cildon for *C. geniculata, A. solani, A. tenuissima, A. tenuis* and *N. sphaerica*; negligible growth was recorded in *Drechslera* and *A. brassicae*, and reduced growth was obtained in *H. oryzae, A. ochraceous, C. pallescens, T. lignorum, Fusarium, A. humicola, C. herbarum, R. nigricans* and in *H. sativum.* However, growth similar to control was measured in *A. parasiticus, A. fumigatus, A. terreus, Penicillium* sp., *P. funiculosum, S. atra, E. purpurascens, Brachysporium* and *Chaetomium homopilatum.* Hyphal growth was found to be accelerated in presence of Cildon in cases of fungi like *A. niger, C. lunata* and *Cercospora* sp.

The broth with BHC at 0.5% concentration was found to be most toxic where no growth was recorded for all fungi tested except the very negligible growth of *C. pallescens.*

A total of 13 fungi (*viz.* 5 species of *Aspergillus*, 2 species of *Penicillium, C. geniculata, T. lignorum, Fusarium* sp., *A. tenuis, C. homopilatum* and *Cercospora* sp.) were

Table 27. Mean dry weight obtained in the fungi after treating with pesticides against control.

Sl. No.	Fungal organism	Control (mg)	Treatment		
			Cildon (mg)	50% BHC (mg)	Bavistin (mg)
1.	*Aspergillus niger*	262	306.5	0.0	0.0
2.	*A. parasiticus*	252	234.0	0.0	0.0
3.	*A. fumigatus*	268	232.0	0.0	0.0
4.	*A. terreus*	105	98.0	0.0	0.0
5.	*A. ochraceous*	201	90.0	0.0	0.0
6.	*Curvularia lunata*	244	260.0	0.0	222.5
7.	*C. geniculata*	213	0.0	0.0	0.0
8.	*C. pallescens*	178	89.5	4.5	148.5
9.	*Penicillium* sp.	211	188.5	0.0	0.0
10.	*P. funiculosum*	132	129.5	0.0	0.0
11.	*Stachybotrys atra*	218	189.0	0.0	192.5
12.	*Epicoccum purpurascens*	188	181.5	0.0	162.5
13.	*Trichoderma lignorum*	152	99.0	0.0	0.0
14.	*Cladosporium herbarum*	239	187.0	0.0	31.5
15.	*Rhizopus nigricans*	156	113.0	0.0	66.5
16.	*Fusarium* sp.	112	77.5	0.0	0.0
17.	*Drechslera* sp.	105	16.0	0.0	74.5
18.	*Helminthosporium oryzae*	223	54.0	0.0	45.0
19.	*Alternaria humicola*	103	80.0	0.0	154.5
20.	*A. solani*	168	0.0	0.0	138.5
21.	*A. brassicae*	121	12.5	0.0	136.5
22.	*A. tenuissima*	204	0.0	0.0	150.0
23.	*A. tenuis*	71	0.0	0.0	0.0
24.	*Brachysporium* sp.	366	241.0	0.0	313.0
25.	*Cercospora* sp.	214	262.0	0.0	0.0
26.	*Chaetomium homopilatum*	154	151.5	0.0	0.0
27.	*Nigrospora sphaerica*	209	0.0	0.0	177.5
28.	*Helminthosporium sativum*	81	28.5	0.0	71.0

inhibited totally with Bavistin. Significant inhibition was found in *C. herbarum* and *H. oryzae* where very less amount of dry mycelia was obtained in comparison to control. *Rhizopus nigricans* and *Drechslera* were fairly inhibited. Almost similar amount of dry mycelia as control was recorded in *C. lunata, C. pallescens, S. atra, E. purpurascens, Brachysporium, N. sphaerica* and even pathogenic *A. solani, A. tenuissima* and *H. sativum.* Mycelial growth of *A. humicola* and *A. brassicae* was found to be accelerated with the presence of fungicide Bavistin.

DISCUSSION

Pesticides include insecticides, herbicides, fungicides and rodenticides (Adams and Wong 1991, Abou-Donia 1992, Ali 1992). In the field, pesticides especially insecticides are applied to crops time to time to control insect pests which may affect the non-target mycoflora of the crop foliage. Fungicides are applied during severe attack of fungal pathogens. These compounds may inhibit the growth of some beneficial fungi (Papavizas 1985, Bora *et al.* 1992) and even may have harmful effect on human beings and endangered species (Abou-Donia 1992).

Cildon i.e. 85% phosphamidon caused the death of non-pathogenic fungi (*Aspergillus ochraceous, Curvularia geniculata*) as well as pathogenic *Alternaria solani, A. brassicae, A tenuissima, A. tenuis;* partially affected the growth in *Trichoderma lignorum, Fusarium, Drechslera, Helminthosporium oryzae, H. sativum* and *Alternaria humicola*. Thus, during the application of Cildon in the field, there would be significant reduction in the fungal level of the airspora. However, some fungi were left unaffected. Kataria and Dodan (1981) observed that two herbicides caused inhibitory action on the mycelial growth of *Pythium butleri*. Adams and Wong (1991) reported that out of 46 pesticides, maximum number of these had toxic effect on the test pathogen, *Sclerotinia minor.* According to Dik and Van Pelt (1992) the effect of fungicides can significantly be enhanced with the addition of insecticide to control pathogens. Different levels of reduction with pesticides were recorded in rhizosphere fungi by Banerjee and Dey (1992), in VAM fungi by Vijayalakshmi and Rao

(1993) and in *Ascochyta rabiei* (Singh and Pal 1992). Ashour *et al.* (1993) recorded that *A. niger, P. notatum, A. candidus, F. sporotrichioides* and *A. humicola* could be controlled with the application of the insecticide "Nuvacron" apart from control of insects.

BHC, an effective pesticide caused growth inhibition or killing of a number of fungi tested in the laboratory. *Aspergillus niger, A. terreus, Penicillium funiculosum, Epicoccum purpurascens* and *Cercospora* sp. showed nil inhibition (i.e. normal growth as control) and a number of fungi showed partial inhibition with BHC in cup assay method; on the contrary showed total inhibition with dry weight method. The reason may be due to the non-permeability of BHC from the suspension placed in the cups towards the semisolid growth media; as BHC is partially soluble in water. The dry weight method showed the toxic efficacy of BHC on the fungi tested. Iyengar and Rao (1974) reported that BHC caused the inhibition in enzyme activity in *A. niger*, while colonies of *Sclerotium rolfsii* were sparsely growing in 0.1% concentration of BHC and at high doses, growth was totally retarded (Singh and Dwivedi 1987).

The fungicide, Bavistin (carbendazim compound) showed total inhibition in majority of the fungal isolates. There were some types including pathogens showing no inhibition at all, even the growth was found to be accelerated in some of the cases. Ponnayya (1978) claimed that brown spot of rice (caused by *H. oryzae*) can be controlled by treating the sprouted seeds with carbendazim for 60 minutes. This is contradictory to the present investigation where total inhibition was not recorded with Bavistin and is similar with the findings of Krishnamurthy and Lalitha Kumari (1981), and Rao and Lalitha Kumari (1987). Sen and Maity (1971) showed that metal ions and various organic substances inhibited spore germination of *H. oryzae.*

Different species of *Alternaria* (except *A. tenuis*) were found to be unaffected with Bavistin and even caused acceleration of growth in *A. humicola* and *A. brassicae.* According to Raj (1981) along with the other fungicides, Bavistin caused inhibition to spore

germination of *A. solani*, not in agreement with the present findings. Tebuconazole, a systemic fungicide was recently found to suppress *A. solani* (Shtienberg *et al.* 1990) and *A. macrospora* (Shtienberg and Dreishpoun 1991). Bavistin was found to cause growth inhibition in *Alternaria alternata* (Raut and Somani 1990) and *A. brassicae* (Biswal and Narain 1991). Babadoost *et al.* (1993) recorded Iprodione and anilazine fungicides significantly reduced incidence of *Alternaria brassicae* and *A. brassicicola* in the brassica crop; while *A. alternata* was found to be very sensitive with thiram (Appaiah *et al.* 1993).

Kannaiyan and Prasad (1973) recorded that the survival period of *Fusarium oxysporum* f. *melonis* causing wilt of muskmelon was much reduced by Benlate [methyl-1-(Butyl carbamyl)-2 Benzimidazole carmic acid] followed by Brassicol while Demonsan and Brestan were ineffective. The *Fusarium* sp., in the present study was inhibited totally by Bavistin and BHC; while slight inhibition was also observed with the Cildon. Similarly, Bavistin was reported to be most toxic to *Fusarium solani* (Kapoor and Kumar 1991) and *F. oxysporum* (Biswal and Narain 1991). Effective reduction in growth of *F. graminearum* was noticed by Troshina *et al.* (1992) with the fungicide, Baytan and of *F. moniliforme* by Appaiah *et al.* (1993) with thiram.

Cildon caused growth acceleration in *A. niger, C. lunata* and *Cercospora* sp., while Bavistin showed acceleration in *Alternaria humicola* and *A. brassicae*. Earlier, Sreenivasulu and Rangaswami (1973) reported that three organophosphorus insecticides (*viz.* thimet, disyston and dasanit) caused a significant increase in fungal, bacterial and actinomycete populations; in which *Aspergillus* was highly stimulated.