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Studies on the effect of pesticides on the mycoflora diversity in the agricultural environments

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Plant leaves are the natural habitat for growth of many microorganisms like fungi, bacteria, actinomycetes etc. Various chemicals are sprayed time to time over the leaves of economically important crops to manage the insect pests, pathogenic fungi, nematodes, pathogenic bacteria etc. Three chemicals were tested in laboratory condition over the fungal strains isolated from phylloplane. Cildon (85% Phosphamidon) showed various degrees of inhibition on *Aspergillus ochraceus, Curvularia geniculata, Alternaria solani*, *A. brassicae, A. tenuissima*, *A. tenuis*, *Nigrospora sphaerica* and on other fungi tested by cup assay method. BHC caused total inhibition over *Curvularia lunata, C. geniculata, Penicillium, Stachybotrys atra*, *T. lignorum*, *Cladosporium herbarum*, *Rhizopus nigricans* and also over pathogenic types like *Drechslera, Alternaria solani*, *A. brassicae, A. tenuissima*, *A. tenuis* etc. Although a number of fungi were inhibited totally with Bavistin, a fungicide, 12 fungi were found to grow normally including plant pathogenic *Alternaria* spp. except *A. tenuis, Drechslera*, *Helminthosporium sativum* and the saprobic *Curvularia lunata*, *C. pallescens, S. atra*, *E. purpurascens*, *Brachysporium* and *Nigrospora sphaerica*. Similar results were also obtained in dry weight method with Cildon and Bavistin. BHC caused toxicity to the whole culture medium showing nil growth in almost all fungi under experiment.

Key words: Crop fields, fungi, fungicides, growth inhibition, pesticides, phytopathogens

INTRODUCTION

The airspora studies in the crop fields revealed that pathogenic and non-pathogenic fungal spores are present at different growth stages (Uddin 2004, 2005, 2007). 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 usually use pesticides and fungicides to cope up with the problem of diseases. The chemicals are mostly sprayed on the foliar parts. Hence, studies were made to find out the effect of the chemicals on the phylloplane mycoflora which in turn affects the local airspora to some extent. The effects of pesticides were studied by Abd-Alla *et al.* (2000) on VAM fungi; on fungal and bacterial population by Pandey and Singh (2004), and on the pathogenic fungi by Olajire and Fawole (2009).

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The impact of pesticides were also studied by Johnsen *et al*. (2001) on bacterial diversity, on microbial community by Lo (2010), on phytopathogenic bacteria by Patyka *et al*. (2016). Flores *et al*. (2014) studied the effect of imazalil (fungicide) and Diazinon (insecticide) on the stream fungi associated with litter breakdown; on soil mycoflora (Rajbonshi *et al*. 2014; Saramanda and Kaparapu, 2017); on *Aspergillus niger* from agricultural soil with three fungicides, three insecticides, three herbicides and two biopesticides (Geetha *et al*. 2016); on fungal phytopathogens (Mohan *et al*. 2017); on bacterial and fungal populations in Ecuadorian tomato cultivated soils (Srinivasulu and Ortiz, 2017); on soil microorganisms (Mehjin *et al*. 2019; Singh *et al*. 2019; Meena *et al*. 2020; and Kremer, 2021). Fiedler and Sosnowska (2017) showed the side effects of fungicides and insecticides on entomopathogenic fungi while Koladar *et al*. (2018) studied the *in vitro* evaluation of non-systemic fungicides, at different concentrations on Turmeric Anthracnose caused by *Colletotricum capsici.* Very

recently the effects of pesticides on the diversity of endophytic fungi in tea plants caused by *Colletotrichum camelliae* were studied by Win *et al*. 2021.

MATERIALS AND METHODS

Cildon (85% Phosphamidon) and Benzene hexachloride *i.e*. BHC (50%) as pesticides and Bavistin (Carbendazim compound) as fungicide are frequently applied by the farmers to protect the existing crops from the severe damages due to insect pests and fungal pathogens respectively. 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 each of 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 using sterile 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. Similar agar diffusion method was also followed by Geetha *et al*. (2016), Saramanda and Kaparapu (2017) and the inhibition zone was measured.

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 were 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 at 60°C for 72 hrs. for complete drying. The dry weight of mats were taken and mean was calculated. The dry weight method was also followed by Olajire and Fawole (2009).

RESULTS AND DISCUSSION

The mean readings of four replicates of inhibition measured by the cup assay method are represented in Table 1. Cildon caused inhibition in variable degrees on *Aspergillus ochraceus, 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. ochraceus, 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. ochraceus, 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 diameter) inhibition was recorded for *A. niger*, *A. ochraceus*, *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 the fungi*, A. fumigatus, Fusarium*, *C. geniculata*, *H. oryza*e and *A. tenuis.* Surprisingly, twelve fungi were found to grow normally in presence of Bavistin which included different species of plant pathogenic *Alternaria* (excepting

A. tenuis), *Drechslera*, *H. sativum* and saprobic *C. lunata, C. pallescens*, *S. atra*, *E. purpurascens, Brachysporium* and *Nigrospora sphaerica.*

In dry weight method (Table 2), no growth was recorded in broth containing Cildon for fungi like *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. ochraceus, 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 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.

Pesticides include insecticides, herbicides, fungicides and rhodenticides. 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 to eradicate the disease. Fungal population generally decline due to fungicide treatment which are gradually recovered with time (Rajbonshi *et al.* 2014). Indiscriminate use of insecticides and fungicides may cause severe threat in the growth of some beneficial fungi, and also on other microbes (Srinivasulu and Ortiz, 2017); and even may have harmful effect on human beings and endangered species. Singh *et al.* (2019) explained that pesticides which are applied continuously in different growth phases of crop, vegetables and fruits may get deposited to some extent in the fruits, crops etc. as chemical residues and consumption of these pesticide residues showed mutagenic, carcinogenic, cytotoxic, genotoxic effects and also a range of health related issues in the human beings.

Cildon *i.e.* 85% Phosphamidon caused the death of non-pathogenic fungi (*Aspergillus ochraceus, Curvularia geniculata*) as well as pathogenic *Altemaria solani*, *A. brassicae*, *A. tenuissima, A. tenuis*; partially affected the growth in *Trichoderma lignorum*, *Fusarium, Drechslera, Helminthosporium oryzae*, *H. sativum* and *Altemaria humicola*. Thus, during the application of Cildon in the field, there would be significant reduction in the fungal level in the agricultural environment. However, some fungi were left unaffected. Similarly, Lo (2010) observed that some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms. Application of Karate (Pyrethroids compound), an insecticide significantly reduced mycelial growth in the three fungi, *viz*. *Aspergillus flavus*, *Fusarium moniliforme* and *Fusarium oxysporum* when compared with control (Olajire and Fawole, 2009).

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. Abd-Alla *et al*. (2000) reported that application of various pesticides inhibits the growth of cowpea, bean and lupin due to inhibition of arbuscular mycorrhizal root colonization. Geetha *et al*. (2016) reported from their results that fungicides, insecticides, herbicides and biopesticides caused drastic reduction in

Table 1: Effect of different pesticides on radial growth of microorganisms

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)	Nature of Inhibition	Inhibition Zone(mm)	Nature of Inhibition	Inhibition Zone(mm)
Aspergillus niger	Nil	0	Nil	0	Nil	0	Total	45
A. parasiticus	Nil	0	Nil	0	Partial	16	Total	42
A. fumigatus	Nil	0	Nil	0	Partial	12	Total	35
A. terreus	Nil	0	Nil	0	Nil	0	Total	40
A. ochraceus	Nil	0	Total	14	Partial	18	Total	45
Curvularia lunata	Nil	0	Nil	0	Total	25	Nil	0
C. geniculata	Nil	0	Total	30	Total	33	Total	30
C.pallescens	Nil	0	Nil	0	Partial	20	Nil	0
Penicillium sp.	Nil	0	Nil	0	Total	16	Total	45
P. funiculosum	Nil	0	Nil	0	Nil	0	Total	43
Stachybotrys atra	Nil	0	Nil	0	Total	30	Nil	0
Epicoccum purpurascens	Nil	0	Nil	0	Nil	0	Nil	0
Trichoderma lignorum	Nil	0	Partial	22	Total	45	Total	45
Cladosporium herbarum	Nil	0	Nil	0	Total	30	Total	45
Rhizopus nigricans	Nil	0	Nil	0	Total	20	Total	42
Fusarium sp.	Nil	0	Partial	30	Partial	27	Total	32
Drechslera sp.	Nil	0	Partial	19	Total	25	Nil	0
Helminthosporium oryzae	Nil	0	Partial	24	Partial	18	Total	30
Alternaria humicola	Nil	0	Partial	20	Partial	16	Nil	0
A. solani	Nil	0	Total	28	Total	24	Nil	0
A. brassicae	Nil	0	Total	40	Total	23	Nil	0
A. tenuissima	Nil	0	Total	30	Total	38	Nil	0
A. tenuis	Nil	0	Total	32	Total	27	Total	28
Brachysporium sp.	Nil	0	Nil	0	Partial	18	Nil	0
Cercospora sp.	Nil	0	Nil	0	Nil	0	Total	40
Chaetomium homopilatum	Nil	0	Nil	0	Total	45	Total	45
Nigrospora sphaerica	Nil	0	Total	45	Total	45	Nil	0
Helminthosporium sativum	Nil	0	Partial	18	Total	24	Nil	0

Aspergillus niger population in the soil, which agree with the present investigation. Fungicides and herbicides were proved to be more destructive on *Aspergillus* sp. as compared with insecticides, where maximum zone of inhibition was seen in *Aspergillus* spp. with three fungicides (Saramanda and Kaparapu, 2017) which is coordinated with the present findings.

The fungicide, Bavistin 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 cases of some fungi including phytopathogens. In the present investigation, total inhibition was not recorded in all fungi with Bavistin; whereas thiocarbamate fungicides demonstrated significant inhibitory action on phytopathogens (Patyka *et al*. 2016). Koladar *et. al.* (2018) reported that highest percentage of inhibition was obtained by thiram to control *Colletotrichum capsici* followed by ziram and other fungicides. Among the five fungicides,

Table 2: Effect of different pesticides on mycelial dry wt. of the different microorganisms

Fungal organism	Control	Treatment		
	(mg)	Cildon (0.06%) (mg)	50% BHC (mg)	Bavistin (1.0%) (mg)
Aspergillus niger	262	306.5	0.0	0.0
A. parasiticus	252	234.0	0.0	0.0
A. fumigatus	268	232.0	0.0	0.0
A. terreus	105	98.0	0.0	0.0
A. ochraceus	201	90.0	0.0	0.0
Curvularia lunata	244	260.0	0.0	222.5
C. geniculata	213	0.0	0.0	0.0
C. pallescens	178	89.5	4.5	148.5
Penicillium sp.	211	188.5	0.0	0.0
P. funiculosum	132	129.5	0.0	0.0
Stachybotrys atra	218	189.0	0.0	192.5
Epicoccum purpurascens	188	181.5	0.0	162.5
Trichoderma lignorum	152	99.0	0.0	0.0
Cladosporium herbarum	239	187.0	0.0	31.5
Rhizopus nigricans	156	113.0	0.0	66.5
Fusarium sp.	112	77.5	0.0	0.0
Drechslera sp.	105	16.0	0.0	74.5
Helminthosporium oryzae	223	54.0	0.0	45.0
Alternaria humicola	103	80.0	0.0	154.5
A. solani	168	0.0	0.0	138.5
A. brassicae	121	12.5	0.0	136.5
A. tenuissima	204	0.0	0.0	150.0
A. tenuis	71	0.0	0.0	0.0
Brachysporium sp.	366	241.0	0.0	313.0
Cercospora sp.	214	262.0	0.0	0.0
Chaetomium homopilatum	154	151.5	0.0	0.0
Nigrospora sphaerica	209	0.0	0.0	177.5
Helminthosporium sativum	81	28.5	0.0	71.0

Hexaconazole had highest inhibitory effect on the growth and population of fungi whereas Bavistin showed least inhibitory effect over the dominant genera, *viz*. *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp. *Alternaria* sp. and *Trichoderma* sp. (Rajbonshi *et al*. 2014). Insecticides and fungicides showed variation in reduction of growth in three fungi namely *Metarhizium anisopliae*, *Beauveria basiana* and *Acremonium* sp. as observed by Fiedler and Sosnowska (2018). Use of various agrochemicals lowered the infection rate of fungal endophytic community especially *Colletotrichum camelliae* in the leaf tissues of tea plants (Win *et al*. 2021).

Different species of *Alternaria* (excepting *A. tenuis*) were found to be unaffected with Bavistin and even Bavistin caused acceleration of growth in *A. humicola* and *A. brassicae*. The abundance, diversity and function of soil microbiota were disrupted with the use of agricultural pesticides (Meena *et al*. 2020; Kremer 2021). The *Fusarium* sp., in the present study was inhibited totally by Bavistin and BHC; while slight inhibition was also observed with the Cildon. Similarly, growth of two *Fusarium* spp., *i.e. F. oxysporum* and *F. moniliforme* was completely inhibited by Benomyl at 500 mg/L concentration (Olajire and Fawole, 2009). Herbicides and insecticides caused decrease in the microbial activities and counts of soil bacteria, fungi and actinomycetes and the effects were inversely proportional to the concentration of the pesticides (Mehjin *et al*. 2019). In the present investigation, Cildon caused growth acceleration in *A. niger*, *C. lunata* and *Cercospora* sp. as measured in dry weight, while Bavistin showed acceleration in *Alternaria humicola* and *A. brassicae*, as also observed by Lo (2010) where some pesticides stimulated the growth of microorganisms, although the fungicide tebuconazole affected greatly the growth of *Cladosporium tenuissimum*.

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