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Impact of Pesticides on Selected Soil Mycoflora

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Abstract

The aim of the present investigation was to study the effect of nine pesticides and two biocides tested on fungal isolates from agricultural soil samples (Coffee,Turmeric and Vegetable) of Lammasinghi village, Chintapalli mandal in Visakhapatnam district. Nine different pesticides namely Fungicides, Insecticides and Herbicides and biocides were taken into study. During the investigation period *Pencillium chrysogenum, Fusarium, Aspergillus niger, Alternaria, Aspergillus oryzae, Pencillium* and *Cladosporium* fungal colonies were isolated. The maximum fungal counts were observed in agriculture field compared with hill area. The maximum fungal colony count (54 colonies) observed in Turmeric field and minimum count (20 colonies) observed in Vegetable field. In the hilly area the more colony count were seen in Vegetable field compared with Coffee and Turmeric. The short term inhibitory effect on the total fungal population was observed with the application of Fungicides, Insecticides and Herbicides. The study proved to be destructive on Aspergillus species by the use of Fungicides and Herbicides compared with Insecticides. The biological control did not inhibit their growth or merely in a lower degree.

Keywords: Biocides, Fungicides, Herbicides, Insecticides, Pesticides, Visakhapatnam.

Introduction

Soil microorganisms appear to be very suitable and sensitive early-warning indicators or predictive tools in soil health monitoring (M. E. Pampulha and A. Oliveira,2006). Modern agriculture is readily associated with the use of different chemical inputs. Variant classes of pesticides are used in managing different groups of pests to maximize crop production and meet the demands for higher supplies of food of the fast-growing human population. Soil is a sink of pesticide residues as well as microorganisms. Fungi are well known for solubilization of inorganic phosphates and this activity of fungal isolates may be affected by the presence of pesticide residues in the soil (Jain et al., 2015). Pesticides are extensively used in agriculture as a part of pest control strategies. Owing to their xenobiotics characteristics, pesticides may adversely affect the proliferation of beneficial soil microorganisms and their associated biotransformation in the soil (Hussain et al.,2009). Inactivation of nitrogen fixing and phosphorus solubilizing microorganisms is observed in pesticide contaminated soils. Recent studies show that some pesticides disturb molecular interactions between plants and nitrogen fixing rhizobacteria and consequently inhibit the vital process of biological nitrogen fixation.

Similarly, pesticides also influences soil biochemical processes driven by microbial and enzymatic reactions. The microbial mineralization of organic compounds and associated biotransformations such as nutrient dynamics and their bioavailability are also more or less adversely affected by the pesticides (Demanou et al., 2004; Kinney et al., 2005; Mahia et al., 2008; Niewiadomska, 2004). The applied

pesticides also reduce soil enzymatic activities that act as a ‘‘biological index’’ of soil fertility and biological processes in the soil environment (Antonious, 2003; Monkiedje et al., 2002). There are also reports documenting the ability of soil microorganisms to degrade pesticides in the soil environment (Hussain et al., 2007; Kumar and Philip, 2006). The degradation products of these pesticides are assimilated by soil microorganisms (Tyess et al., 2006) resulting in increased population sizes and activities of microorganisms (Das and Mukherjee, 2000). Recently, molecular techniques have been used to elucidate the impact of pesticides on microbial community structure and functioning (Widenfalk et al., 2008). In this study, we attempt to analyze the impacts of nine pesticides and two biocides on fungal isolates from agricultural (Turmeric, Coffee, Vegetable) soil samples of Lammasinghi village, Chintapalli mandal in Visakhapatnam district.

Materials and Methods

The study area:

The soil samples were collected from the area Lammasingi in Chintapalli mandal. The Chintapalli mandal is located on the north eastern part of Visakhapatnam district in Andhra Pradesh State of India. It lies between $17^{o}\ 44^{l}\ 22^{ll}$ north latitude to $18^{o}\ 04^{l}\ 29^{ll}$ east to $82^{o}\ 38^{l}\ 04^{ll}$ east. The climate conditions are very cool in the area on account of green vegetation and thick forest. The temperature gets down with the onset of south west monsoon and tumbles with a mean minimum of 4^{o}C by January after which there is reversal trend till the temperature reaches means maximum of 34^{0}C by end of may that is April to June are the warmest months. This tribal area with rain season account for 90% rainfall an average rainfall of 1178.mm. The in this area were dependant on agriculture.

Soil Sample Collection:

Soil samples were collected based on different crop fields, during the month of May 2015 to December 2015 at Lammasingi Panchayat Chintapalli Mandal in Visakhapatnam District. From each selected hectare,the the soil was collected (between 10:30am and 4:30pm each day) under sterile conditions with the help of 15cm iron cores from four symmetrically situated locations near the corners of a square as well as from the centre of the square. Soil samples used in this investigation were collected from both agricultural fields and hill areas. Soil samples were collected from the depth of approximately 10-15cm in sterilized polythene bags and stored at 4^{o}c in the laboratory until the examination. The collected samples along with locations were shown in the table no.1.

Table no: 1. List of collected soil samples

S.No	Crop Field	Soil Colour
T1	Turmeric (Cultivation)	Black
T2	Turmeric (Hill Area)	Brown
V1	Vegetable (Cultivation)	Black
V2	Vegetable (Hill Area)	Red
C1	Coffee (Cultivation)	Black
C2	Coffee (Hill Area)	Brown

Analytical Procedure:

Microbiological analysis of the soil samples were made as per the following procedure:

For enumeration of fungal population serial dilution method is used. This technique is commonly used to isolate distinct colonies from mixed culture. The primary suspension of the soil was prepared from 1gram of soil which was diluted up to 10^{-9} times using sterile water as diluting fluid. For fungal population, 1ml from 10^{-4} diluted suspension was transferred to potato dextrose agar supplemented with 1% streptomycin. The pH was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi. Nine different pesticides namely Fungicides (Carbendizm, Copper Oxy Chloride, Commodity Mancozeb) 1ppm each, Insecticides (Tafgor, Profenofos Chlorpyrifos) 0.5ml each, Herbicides (Ammonium Salt Glyphosate -01, 2,4-D Sodium Salt solution, All Quit Paraquat Dichloride) 1ppm each and biocides (*Trichoderma viridae, Trichoderma harzianum*) 0.5ml each were taken into study. F1 F2 and F3 are fungicides, I1, I2 and I3 are insecticides, H1, H2 and H3 are herbicides commonly used in management of pests in agriculture and plantation sectors.

Effect of pesticides on Fungus:

The Potato dextrose agar medium is amended with different (isolated 7 species) actively grown 4 days old fungal species in different conical flasks containing PDA medium. The agar blocks (5 mm diameter) from the PDA are taken using sterile cork borer. The different pesticides (Fungicides, Insecticides and herbicides) and biological controlling agent with equal concentration (0.5 ml) are placed in the wells. All the plates were incubated at room temperature at 28 $\pm$ 2^{o}C for 7 days. The growth patterns of the respective fungus were observed in the plates and zone of inhibition was noted.

Results and Discussion

During the investigation period 194 fungal colonies were isolated. The maximum fungal counts were observed in Agriculture field compared with Hill area. The maximum fungal colony count (54 colonies) observed in Turmeric field and minimum count (20 colonies) observed in Vegetable field. In the hilly area the more colony count were seen in Vegetable field compared with Coffee and Turmeric. The details were shown below in the table no:4. Three replica plates for every soil samples were done and mean values are represented in Table no:2.

Table no:2. Total Fungal count (TFC) in PDA:

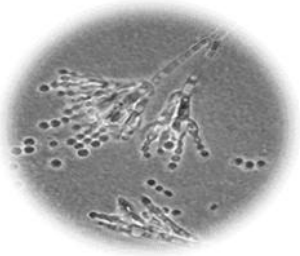
Soil Sample	Total Fungal colonies in 10^{-5} dilution CFU/100ml
Turmeric – T1	$5.40x10^2$
Turmeric – T2	$1.50x10^2$
Vegetable – V1	$2.0x10^2$
Vegetable – V2	$5.30x10^2$
Coffee – C1	$3.30x10^2$
Coffee – C2	$1.9x10^2$

In the observed soil sample the maximum TFC were present in T1 soil ($5.40x10^2$) and minimum TFC were present in T2 soil ($1.50x10^2$).

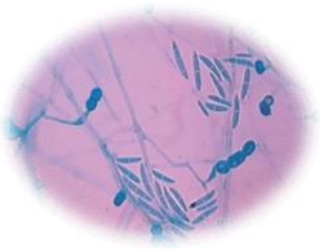
Isolation and Identification of organisms:

From the isolated samples, 7 different colonies were commonly observed in all types of soil samples. These isolated colonies were sub cultured on potato dextrose agar medium. The isolated organisms were identified macroscopically by observing the cultural characteristics and microscopically by staining with Lactophenol cotton blue (Fig 1).

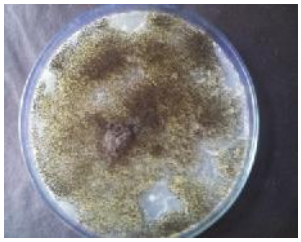
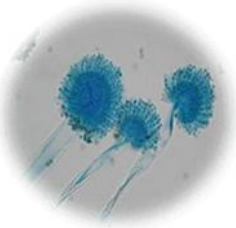
Fig.1. Isolated fungal colonies.

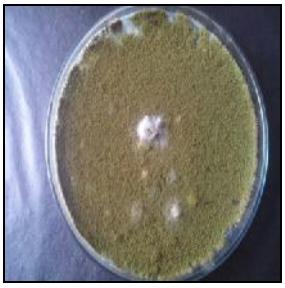
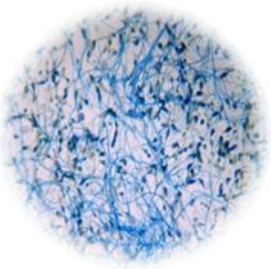
Penicillium chrysogenum

Fusarium

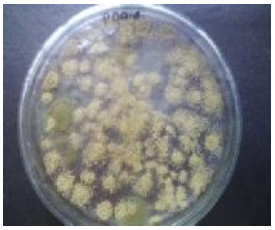
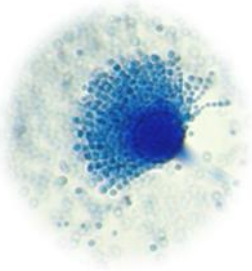



Aspergillus niger

Alternaria


***Penicillium* sp.**

Aspergillus oryzae


Cladosporium

Effect of Pesticides on the Fungal species:

Table no:3. Effect of Fungicides on different fungal species

Organisms	Zone Of Inhibition in (mm)		
	F_1	F_2	F_3
Penicillium chrysogenum	5	10	5
Fusarium	10	15	7
Aspergillus niger	20	10	Nil
Alternaria	35	2	7
Penicillium	5	15	10
Aspergillus orizae	40	5	4
Chladosporium	25	15	10

In this study the concentration of fungicides inhibiting the growth of respective fungus after 7 days of incubation and also less sporulation were observed on PDA. Compared with the three fungicides the maximum zone of inhibition were seen in *Aspergillus* species (40 mm) that is F1 pesticide and the minimum zone of inhibition were observed in F3 pesticide (Tab.3; Fig.2&4) Maribel Yanez and Andres France (2010) find similar reports on the growth of the fungus *Metarhizium anisopliae var. anisopliae.*

Table no: 4. Effect of Insecticides on different fungal species

Organisms	Zone Of Inhibition (mm)		
	I_1	I_2	I_3
Penicillium chrysogenum	10	6	2
Fusarium	5	10	7
Aspergillus niger	10	7	4
Alternaria	6	3	5
Penicillium	5	15	Nil
Aspergillus oryzae	4	8	3
Chladosporium	10	5	Nil

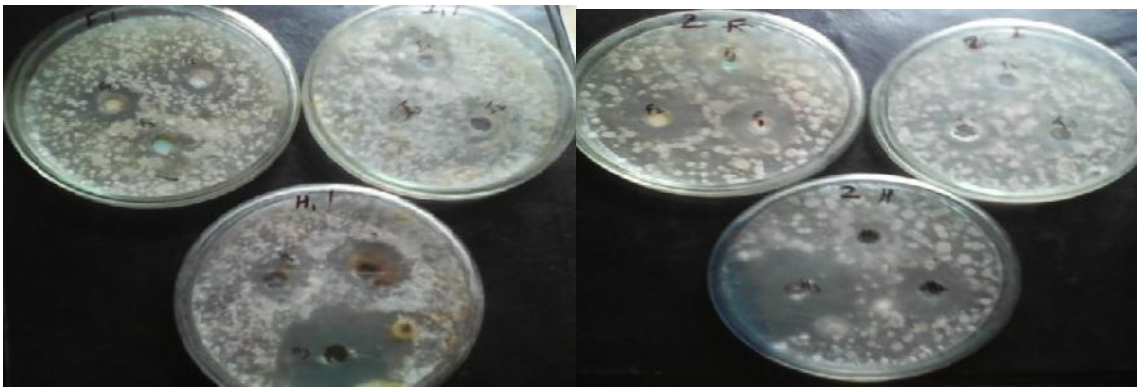
The zone of inhibition in insecticide treated petriplates in the laboratory are given in table no.1. Addition of insecticides in the plates brought a reduction in fungal population were observed (Tab.4; Fig.2&3). Maximum zone of inhibition were observed in Pencillium species (15 mm) that is I2 and minimum zone of inhibition was seen in *Pencillium chrysogenum* (2 mm) that is I3. Amutha et al., (2010) studied the effect of commonly used insecticides on the growth of the fungus *Beauveria bassiana* and stated that profenophos, indoxacarb and methyldemeton were highly toxic.

Table no:5 Effect of Herbicides on different fungal species

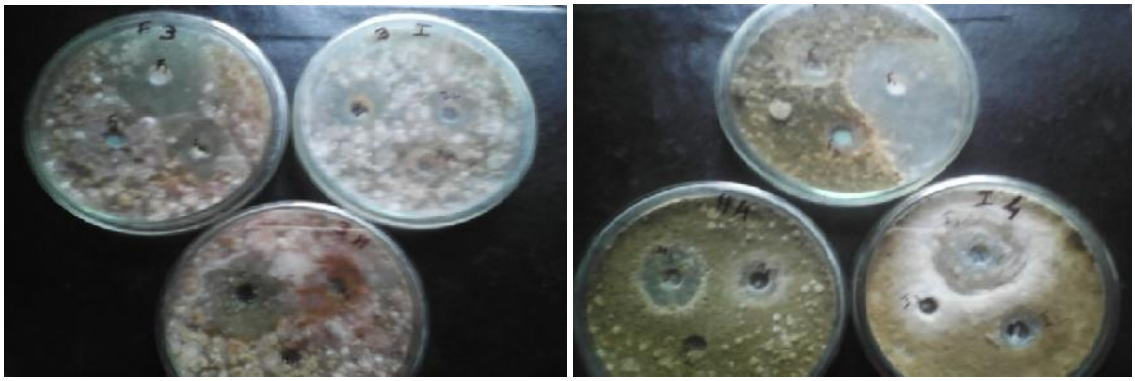
Organisms	Zone of inhibition (mm)		
	H_1	H_2	H_3
Penicillium chrysogenum	3	10	18
Fusarium	3	10	20
Aspergillus niger	40	Nil	16
Alternaria	2	1	10
Penicillium	5	2	25
Aspergillus oryzae	9	10	15
Chladosporium	Nil	Nil	19

The Herbicide effect range between 1 to 40 mm were observed in different fungal species. The highest zone of inhibition were observed in (H1 herbicide) ***Aspergillus niger*** and less inhibition were observed in ***Alternaria*** (Tab.5; Fig. 2&3). Similarly Pampulha and Oliveira, 2006 studied the impact of herbicide combination (Bromoxynil and Prosulfuron) on Soil Microorganisms. It was observed that the higher the concentration of the herbicides, the more the fungal population decreased, with the lowest values after 30 days (96% of the control at the highest concentration of the herbicides applied, and 43% for 1 p.p.m). Since the Herbicides are also rich in chemicals we need to switch towards Bio pesticides which enhances the soil fertility and enriches the soil micro organisms. The increasing use of herbicides has created concern about their persistence in soil, their toxicity to non-target organisms, and the selection of resistant species. Information of biochemical and microbial indices of soil exposed to herbicides provides valuable insight into the extent of soil disturbance and perturbation.

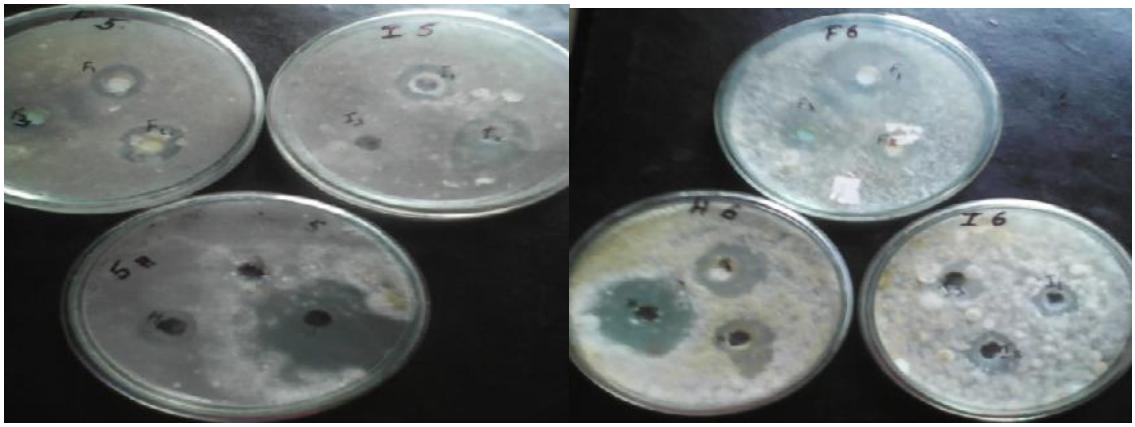
Fig:2. Plates showing the effects of Fungicides, Insecticides and Herbicides on the isolated fungi



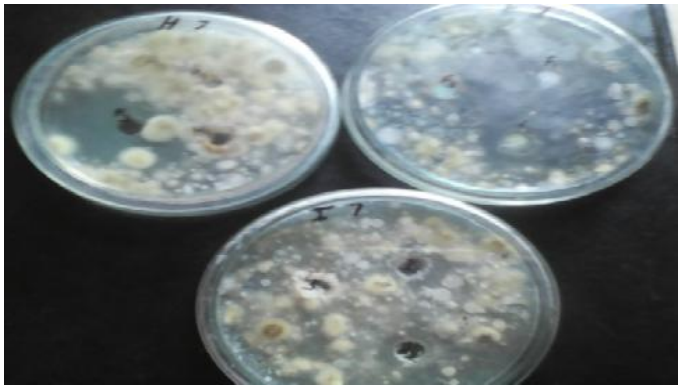
Penicillium ***Fusarium***



Aspergillus niger ***Alternaria***



Penicillium ***Aspergillus species***



Cladosporium

Effect of Biological controlling Agent:

Trichoderma is used as biological control in this study clearly showed the potential of using a biological control. The lowest inhibitory effect on the growth of the fungal species was found with the Bio fungicides – *Trichoderma viridae* and *Trichoderma harzianum* at tested concentration 1 and 2 mm, which either did not inhibit their growth or merely in a lower degree (Tab.6; Fig.3).

Table no:6 Effect of Biological control on different fungal species

S.No	Zone of inhibition (mm)	
	Trichoderma viride	*Trichoderma harzianum*
Penicillium chrysogenum	1	1
Fusarium	2	2
Aspergillus niger	1	2
Alternaria	Nil	Nil
Penicillium	Nil	1
Aspergillus oryzae	2	Nil
Chladosporium	Nil	Nil

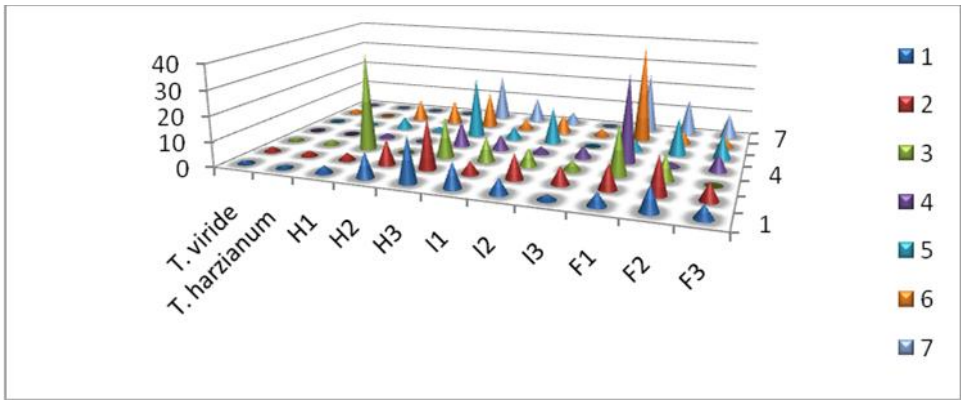


Fig.3. Effect of Pesticides on Fungal organisms

The result of present study revealed that the nine pesticides tested on the seven isolated soil fungal species. The short term inhibitory effect on the total fungal population was observed with the application of Fungicides, Insecticides and Herbicides. The study proved to be destructive on *Aspergillus* species by the use of Fungicides and Herbicides compared with Insecticides. The biological control did not inhibit their growth or merely in a lower degree. The effect of a pesticide on soil microorganisms is controlled by numerous environmental factors in addition to the persistence, concentration, toxicity of the applied pesticide, and its bioavailability (Abdel-Mallek et al., 1994). One of the major factors contributing to the net impact of applied pesticides on soil microbes is its bioavailability in soil environment.

Adsorption and desorption processes regulate concentration of a contaminant in soil solution (Bonczek and Nkedi-Kizza, 2007; Katagi, 2008) and hence its bioavailability, bioactivity, and degradability in soil environment.

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