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TOLERANCE OF ALIETTE BY RHIZOSPHERE MYCOFLORA OF *CAPSICUM ANNUUM L.*

V. B. Sonawane P.G. Department of Botany, K. T. H. M. College Nashik-2

ABSTRACT The present exploration was undertaken to study the tolerance of fungicide (Aliette) by the rhizosphere and soil mycoflora of *Capsicum annuum* L. was studied by using Food Poisoning Soil Dilution (FPSD) technique. Isolation of rhizosphere mycoflora was carried out on "Czapek-Dox agar medium" by soil dilution plate count method. By this method each viable fungus was developed into a colony on the plates. Then the slides were prepared and identified. Most of the fungal species tolerated from rhizosphere and soil were: *Aspergillus niger, Aspergillus carbonarius*, *Aspergillus fumigatus* and *Penicillium funiculosum* tolerated at higher concentration. As the fungicidal concentration increases in the plates, the fungal population decreases.

KEYWORDS : Fungicide, tolerance, rhizosphere, soil, fungi

INTRODUCTION

The work done by Starkey is an excellent and pioneering in the field of rhizosphere study. He was the first to work out rhizosphere microflora in detail and unearthed several microbiological problems regarding rhizosphere. He worked out qualitative and quantitative effects of different plant species increase in the number of microorganisms in rhizosphere with the age of the plant and also the seasonal variation in the number of the rhizosphere microflora. The native home of chilli is considered being Mexico. Chilli was introduced in India by the Portugese in Goa in the middle of the 17th century and since then it had rapidly spread throughout the country. The soil borne fungus, *Phytophthora capsici,* causes *Phytophthora* root rot also called chilli wilt. This fungus is a serious pathogen on peppers worldwide. Seedling disease, commonly called 'Damping off', can be caused by a number of soil borne fungi such as *Rhizoctonia solani, Phytophthora capsici* and *Fusarium* sp. Damping off occurs when seeds or young seedlings are attacked by theses pathogens. Fungicidal compounds marketed in India were tested in vitro for their tolerance by rhizosphere and soil microfungi of chilli with the help of rapid food poisoning, soil dilution technique variability in the tolerance of fungicides with different isolates of these organisms from rhizosphere and soil was observed.

Many fungicide compounds are directly introduced into agricultural land for combating soil borne disease and pests. These chemicals reach the soil and thus influence the microbial balance of soil by Wainwright (1979) and Bollen (1981). Chemical control of these diseases is being widely practiced to achieve higher yields of *Capsicum annuum* L. all over the world. For this reason use of pesticide has become an integral and economically essential part of agriculture. They are most extensively used in countries with high agronomic technology. At present chemical fungicides viz. mancozeb, captan, carbendazim, thiram, copper oxichloride, benlate, ziram etc. are used to manage the disease. Various pathogens like *Gloeosporium amelophagum, Fusarium oxysporum, Colletotichum capsici, Pythium* sp. and *Phytophthora* sp. have been reported Gangawane (1990) to develop resistance against commonly used fungicides.

Tayal (2009) reported that the application of fungicide in agriculture soil decreases the total number of soil fungi. The interesting examples of the resistance to agricultural pesticides have been reviewed by Dekkar (1976) and Georgopoulos (1976 and 1977). Tolerance of fungicides by pathogenic fungi is reported by some workers Anderson (1978), Greaves (1979) and Saler and Gangawane (1981 and 1994).

Aktar et al., (2009) gave a detailed account of effect of contamination of air, soil and non target organism by different pesticides. Das et al., (2005) found markedly increase of the number of fungi in soil treated with insecticide phonate. Tapwal et al., (2003) studied by rhizosphere is a zone of increased microbial activity in the vicinity of plant roots. Increases in microbial community are due to the exudation of plant roots. Quantitative and qualitative rhizosphere mycoflora and their biological interaction influence the growth and development of seedlings by Maisuria and Patel (2009). Andreu and Pico (2004) was studied the present investigation was carried out to determine the rhizosphere mycoflora of chilli. Amran and Hasan (2003) investigated the effect of fungicides on mycoflora of rice field soil. The result showed that many fungi such as *Fusarium* sp., *Absidia* sp. and *Alternaria* sp. appeared less in soil before treatment with fungicides than after treatment. While common fungi in the soil declined after treatment with fungicides such as *Penicillium* sp. and *Aspergillus* sp. Lower concentration of benomyl (10 ppm to 100 ppm) gave strong inhibitory action against non rhizosphere fungi. Rhizosphere fungi and rhizoplane fungi compared with mancozeb at 500 ppm and 1000 ppm. Channabasava et al., (2015) studies by fungicide treatments affect the root colonization by *R. fasciculatus* and growth of Proso millet plants. Treatment with Benomyl, followed by Bavisin and Mancozeb, significantly decrease the root colonization, spore number, plant growth and grain yield of mycorrihizal plants compared with mycorrhizal plants without fungicide treatment.

MATERIALS AND METHODS

Seeds of the *Capsicum annuum* L. were sown in the experimental plots using garden soil in the Botanical Garden, K.T.H.M. College Nashik. They were observed for the germination after 15th days. Plants were collected to study the tolerance of fungicides by rhizosphere and soil mycoflora.of *Capsicum annuum* L. The samples were properly labeled. Isolation of rhizosphere mycoflora was carried out on "Czapek-Dox agar medium" by "soil dilution plate count method" Subba Rao (2004). By this method each viable fungus was developed into a colony on the plates. Then the slides were prepared and identified.

Food Poisoning Soil Dilution (FPSD) technique:

Tolerance of rhizosphere and soil fungi was studied by modified food poisoning soil dilution (FPSD) technique Nene (1971), Saler and Gangawane (1981) were used for quantitative and qualitative studies of soil. The media employed were equal volume of 2x medium (served as food) and 2x concentration of fungicide (served as poison) along with 1 ml of spore suspension from a dilution flask (served as soil dilution). Thus the medium had the final concentration 100, 200, 500, 1000, 1500 µg/ml concentration of fungicide (aliette). Media with single strength without fungicidal compound served as control. Control concentration was considered as 0 µg/ml. 'R' abbreviation used as rhizosphere mycoflora and 'S' used as soil mycoflora. R/S referred as Rhizosphere effect. Plates were incubated in an inverted position at room temperature until good growth of fungi was observed. The identification of fungal organism was done by referring various monographs, research papers and other literature such as a manual of soil fungi etc.

RESULT AND DISCUSSION

Tolerance of Aliette by Rhizosphere and Soil mycoflora of *Capsicum annuum* L. at 15 day growth period.

Qualitative Results:

A total of 17 fungal species was recorded; out of this 15 species were recorded in rhizosphere and soil on poisoned plates. At control µg/ml, 14 species were recorded in rhizosphere and 15 species were in the soil. 100 µg/ml Aliette was tolerated by 10 species in rhizosphere and soil, 200 µg/ml was tolerated by 10 species in rhizosphere and 8 in soil, 500 µg/ml was tolerated by 8 species in rhizosphere and 6 in soil, 1000 µg/ml was tolerated by 5 species in the rhizosphere and 4 species in soil and 1500 µg/ml was tolerated by 2 species in rhizosphere and 3 species

in soil (Table No. I).

The species tolerant to 1500 µg/ml Aliette were *Aspergillus carbonarius* (S), *Aspergillus fumigatus* (S), *Aspergillus niger* (R and S), *Penicillium funiculosum* (R); the species tolerances at 1000 µg/ml Aliette were*: Aspergillus carbonarius* (R and S), *Aspergillus fumigatus* (S), *Aspergillus niger* (R and S), *Aspergillus sclerotiorum* (R and S), *Penicillium funiculosum* (R) and *Trichoderma viride* (R). The species tolerant at 500 µg/ml aliette were: *Aspergillus carbonarius* (R and S), *Aspergillus flavus* (R), *Aspergillus fumigatus* (R and S) , *Aspergillus niger* (R and S), *Aspergillus sclerotiorum* (R and S), *Fusarium redolens* (R), *Trichoderma harzianum* (R and S) and *Trichoderma viride* (R and S).

The species tolerant at 200 µg/ml aliette were : *Aspergillus carbonarius* (R and S), *Aspergillus flavus* (R and S), *Aspergillus fumigatus* (R), *Aspergillus nidulans* (R), *Aspergillus niger* (R and S), *Aspergillus sclerotiorum* (R and S), *Cladosporium herbarum* (R), *Fusarium redolens* (R), *Fusarium oxysporum*(R), *Helminthosporium tetramera* (S), *Mucor globosus* (S) , *Penicillium funiculosum* (R), *Trichoderma harzianum* (S) and *Trichoderma viride* (S)*;* the species tolerant at100 µg/ml aliette were: *Aspergillus carbonarius* (R and S), *Aspergillus flavus* (R and S), *Aspergillus fumigatus* (R and S), *Aspergillus nidulans* (R and S), *Aspergillus niger* (R and S), *Aspergillus sclerotiorum* (R and S), *Cladosporium herbarum* (R and S), *Fusarium redolens* (S), *Fusarium oxysporum* (R and S), *Helminthosporium tetramera* (S) and *Mucor globosus* (R).

The species more or less unaffected in their counts both in rhizosphere and soil were: *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus sclerotiorum, Penicillium funiculosum, Trichoderma harzianum* and *Trichoderma viride.* The fungal species were *Alternaria tenius* and sterile white mycelium cannot survive on poisoned plates.

Quantitative Results:

At 1500 µg/ml 5.0 and 7.5 thousand populations survived as against 35.0 and 37.5 at '0' µg/ml aliette in the rhizosphere and soil respectively. The rhizosphere effect was increased at 500 µg/ml aliette. There were 5 fold reductions in the fungal population, both in rhizosphere and soil (Table No. II).

Table No. II : Number of fungal colonies (10^3 /gm oven dry soil) tolerated to different concentration of Aliette (µg/ml) in rhizosphere and soil at 15 day growth period of *Capsicum annuum* L.

	Aliette (µg/ml)					
	0	100	200	500	1000	1500
R	35.0	27.5	25.0	20.0	12.5	5.0
S	37.5	27.5	20.0	15.0	10.0	7.5
R/S	**0.93**	**1**	**1.25**	**1.33**	**1.25**	**0.66**

Table No. I: Number of fungal colonies tolerated to Aliette (µg/ml) for Rhizosphere and Soil at 15 day growth period of *Capsicum annuum* L.

Sr.No.	Fungal species	Control		100		200		500		1000		1500	
		R	S	R	S	R	S	R	S	R	S	R	S
1	*Alternaria tenius* Auet.	1	1	-	-	-	-	-	-	-	-	-	-
2	*Aspergillus carbonarius* (Bainier)	3	2	2	2	2	1	2	1	1	2	-	1
3	*Aspergillus flavus* Link (Lundae)	3	1	1	1	2	1	1	-	-	-	-	-
4	*Aspergillus fumigatus* Fresen	2	3	1	2	2	-	1	1	-	1	-	1
5	*Aspergillus nidulans* (Eidam) Wint.	2	2	2	2	1	-	-	-	-	-	-	-
6	*Aspergillus niger* Van Tiegh.	3	2	3	1	1	2	1	1	1	2	1	2
7	*Aspergillus repens* (Corda) de Bary	3	2	1	1	-	-	-	-	-	-	-	-
8	*Aspergillus sclerotiorum* Humber	3	2	2	2	1	1	1	1	1	1	-	-
9	*Cladosporium herbarum* (Pers.) Link.	2	2	1	1	1	-	-	-	-	-	-	-
10	*Fusarium redolens* Wr. Wollenw	2	-	-	2	3	-	1	-	-	-	-	-
11	*Fusarium oxysporum* Schlecht	-	1	1	3	2	-	-	-	-	-	-	-
12	*Helminthosporium tetramera* Mc.Kinney	2	-	-	1	-	1	-	-	-	-	-	-
13	*Mucor globosus* Fischer	1	1	1	-	-	1	-	-	-	-	-	-
14	*Penicillium funiculosum* Thom	2	3	-	-	1	-	-	-	1	-	2	-
15	Sterile white mycelium	2	1	-	-	-	-	-	-	-	-	-	-
16	*Trichoderma harzianum* Rifai	-	1	-	-	-	2	1	1	-	-	-	-
17	*Trichoderma viride* Pers. Ex Fr.	-	2	-	-	-	2	1	2	2	-	-	-

CONCLUSION

The effect of Aliette on rhizosphere and soil mycoflora during 15 days growth period of *Capsicum annuum* L. Statistically, highly significant difference of fungal population was observed in concentration of Aliette in both rhizosphere and soil. The present investigation concludes total Seventeen fungal species belonging to eight genera were recorded from rhizosphere and soil of *Capsicum annuum* L. Application of the Aliette to soil significantly reduces the fungal population, in the rhizopshere and soil. It has been observed that the tolerance limit of the fungal population of Aliette was 1500 µg/ml concentration. Most of the fungal species tolerated from rhizosphere and soil were: *Aspergillus niger, Aspergillus carbonarius*, *Aspergillus fumigatus* and *Penicillium funiculosum* tolerated at higher concentration. As the Aliette concentration increases in the plates, the fungal population decreases. Some of the fungal species were unaffected, stimulated or appeared and decreased or eliminated from rhizosphere and soil.

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