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EFFECT OF ECO-FRIENDLY COMPONENT OF BIONEEM ON RHIZOSPHERE AND SOIL MYCOFLORA OF *CAPSICUM ANNUUM* L.

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ABSTRACT
The cultivated *Capsicum annuum* L. is grown throughout the world. The present study the effect of "Bioneem" on the rhizosphere and soil mycoflora of *Capsicum annuum* L. Isolation of rhizosphere mycoflora was carried out on "Czapek-Dox agar medium" by soil dilution plate count method. Altogether18 fungal species were recorded from rhizosphere and soil. *Aspergillus flavus* (R and S), *Aspergillus nidulans* (R and S) and *Fusarium moniliforme* (R and S) tolerate the Bioneem at higher concentration. Dominant fungal species was observed in class Deuteromycotina.

KEYWORDS: Eco-friendly component, tolerance, rhizosphere, soil fungi.

INTRODUCTION

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. The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. Soil which is not part of the rhizosphere is known as bulk soil. The rhizosphere contains many bacteria that feed on sloughed-off plant cells, termed *rhizodeposition*, and the proteins and sugars released by roots. Protozoa and nematodes that graze on bacteria are also more abundant in the rhizosphere. Thus, much of the nutrient cycling and disease suppression needed by plants occurs immediately adjacent to roots. Antimicrobial activity of *Nigella sativa* against *C. capsici* was reported Rathee et al., (1982). Shivapuri et al., (1997) investigations proved that *Azadirachta indica*, *Datura stramonium*, *Ocimum sanctum*, *Polyalthial longifolia* and *Vinca rosea* were fungi toxic against *Colletotrichum capsici*.

Jayalakshmi et al., (1998) and Korpraditskul et al., (1999) it was reported that crude extracts from different parts of Sweet flag, Palmorosa oil and Neem oil confined the growth of anthracnose fungus. 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 of chilli was observed. Some isolates showed tolerance towards the fungicides while the other was non- tolerant.

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. More et al., (2011) was studying the various antifungal properties of ten medicinal plant extracts against anthracnose of chilli caused by *Colletotrichum capsici*, the antifungal properties of plant species viz. *Cassia fistula*, *Callistemon lanceolatus*, *Duranta* sp., *Delonix regia*, *Lantana camera*, *Pongamia pinnata*, *Nerium indicum*, *Tagetus erecta*, *Eucalyptus citriodora* and *Azadirachta indica* was tested by poisoned food technique.

Park (1989) and Saleem et al., (2000) was studied by use of environmentally ecofriendly biological control agents can more effectively control the soil borne phytopathogens. From several studies, it has been confirmed that *Trichoderma* sp. have antagonistic and biologically control potential against a diversity of soil borne pathogens by (Mohiddin et al., 2010; Bajwa et al., 2004. Madhavi et al., 2006 and Rani et al., 2009) was studied *Fusarium* wilt is the most important soil borne

disease caused by *Fusarium* sp. in chilli plant. Leaf extracts of *Solanum torvum*, *Datura metel* and *Prospopis juviflora* are effective in inhibiting conidial germination by Gomathi et al., (2000).

Today, many neem Bio-pesticides are now in market world over by Khanna (1992). Commercially available neem formulation like Achook (0.15% E.C.), Bioneem (0.03% E.C.), Nimbicidine (0.03% E.C. and Neemark (0.03% E.C.) showed antifungal activity against pathogenic fungi like *Fusarium oxysporum*, *Alternaria solani*, *Curvularia lunata*. *Helminthosporium* sp. and *Sclerotium rolfsii* by Bhonde et al., (1999). Neem tops the list of 2,400 plant species that are reported to have antimicrobial properties and is regarded as the most reliable source of eco- friendly agrochemical property. Neem is also used as a bio- control agent to control many plant diseases by Kak (2000).

MATERIALS AND METHODS

Seeds were sown in earthen pots using garden soil, K.T.H.M. College Nashik. They were observed for the germination after 15th days. Plants were collected to study the rhizosphere mycoflora. The soil samples were collected from 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 bioneem based on Azadirachtin isolated from the kernel of neem seeds, this broad spectrum biocide provides the most effective, economic and lasting control of major pests of agricultural and plantation crops. It is the most environment friendly pesticide, highly biodegradable and leaves no residues on the food stuff (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, 1000, 1500 µg/ml of bioneem. Media with single strength without ecofriendly 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 by Gilman (1957) and Hand book of Soil fungi Nagmani et al., (2006) etc.

RESULT AND DISCUSSION

Qualitative results

A total of 18 fungal species was recorded during the growth period. Out of these, 15 were from the rhizosphere and also in soil on poisoned plates. At control 15 species were recorded in rhizosphere and 11 species were in the soil. There was a significant decrease in the population as the concentration of the ecofriendly compound Bioneem increased in the plate (Table No. I).

The species tolerated to 1500 µg/ml were *Aspergillus flavus* (R and S), *Aspergillus nidulans* (R and S) and *Fusarium moniliforme* (R and S)*;* the species tolerated to 1000 µg/ml were; *Aspergillus carbonarius* (R), *A. flavus* (R and S), *A. nidulans* (R and S), *Fusarium moniliforme* (R and S), *Penicillium funiculosum* (R), *Rhizoctonia bataticola* (R) and *Trichoderma viride*; the species tolerant to 500 µg/ml were: *Chaetomium globosum* (S), *Aspergillus carbonarius* (R and S), *A. flavus* (R and S), *A. nidulans* (R and S), *A. niger* (R), *Cladosporium oxysporum* (R and S), *Fusarium moniliforme* (R and S), *Penicillium verrucosum* (R) and *Trichoderma viride* (R and S); the species tolerant to 100 µg/ml were; *Rhizopus stolonifer* (R), *Chaetomium globosum* (S), *Aspergillus carbonarius* (R and S), *A. flavus* (R and S), *A. nidulans* (R and S), *A. niger* (R and S), *A. petrakii* (R), *Cladosporium oxysporum* (S), *Fusarium moniliforme* (R and S), *F. oxysporum* (R), *Helminthosporium tetramera* (R), *Penicillium brefeldianum* (R), *P. funiculosum* (S), *P. verrucosum* (R and S) and *Rhizoctonia bataticola* (R).

The species tolerant to '0'µg/ml were *Rhizopus stolonifer* (R and S), *Aspergillus carbonarius* (R and S), *A. flavus* (R and S), *A. nidulans* (R and S), *A.niger* (R and S), *A. petrakii* (R and S), *A. sclerotiorum* (R and S), *Fusarium moniliforme* (R and S), *F. oxysporum* (R), *Helminthosporium tetramera* (R), *Penicillium brefeldianum* (R), *P. verrucosum* (R and S), *Rhizoctonia bataticola* (R), *R. solani* (R and S) and *Trichoderma viride* (R and S).

Quantitative results

About 5 fold reductions was noted in rhizosphere and 4 fold reduction in the soil mycoflora of *Capsicum annuum* L. Tolerance limit of fungal population was 1500 µg/ml. It is observed that, 1500 µg/ml concentration show very less effect of rhizosphere. (Table No. II). Statically significant difference between average number of fungal colonies of concentration wise (Table No. III).

Table No I: Number of fungal colonies tolerated to Bioneem (ug/ml) for rhizosphere and soil of *Capsicum annuum* Linn.

	Concentration of Bioneem				
	Control	**100**	**500**	**1000**	**1500**
Rhizosphere	37.5	30.0	20.0	15.0	7.5
Soil	27.5	22.5	17.5	10.0	7.5
R/S	1.36	1.33	1.14	1.5	1

Table No II: Number of fungal colonies (10×3/g oven dry soil) tolerated to different concentration of Bioneem in the rhizosphere and soil of *Capsicum annuum* L.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	816.25	4	204.0625	10.88333	0.011046	5.192168
Within Groups	93.75	5	18.75			
Total	910	9				

From the above table: we observe that p value is 0.011046. Since, value < 0.05, therefore we reject H_0, i.e. there is significant difference between average number of fungal colonies of concentration wise.

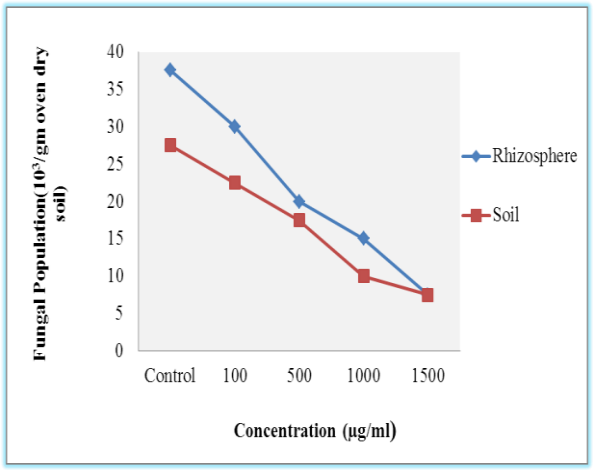

Fig. No. I: Number of fungal colonies tolerant to different concentration.

Table No III:One way ANOVA testing variation in various concentration of Bioneem for rhizosphere and soil mycoflora of *Capsicum annuum* L.

Sr. No.	**Fungal species**	**Control**		**100**		**500**		**1000**		**1500**	
		R	S	R	S	R	S	R	S	R	S
	Zygomycotina										
1	*Rhizopus stolonifer* Ehrenb	3	2	1	-	-	-	-	-	-	-
	Ascomycotina										
2	*Chaetomium globosum* Kunze	-	-	-	3	-	2	-	-	-	-
	Deuteromycotina										
3	*Aspergillus carbonarius* (Bainier)	2	2	3	2	1	2	2	-	-	-
4	*Aspergillus flavus* Link (Lundae)	4	2	3	1	2	1	2	2	2	1
5	*Aspergillus nidulans* (Eidam) Wint.	4	3	3	2	1	2	1	2	2	2
6	*Aspergillus niger* Van Tiegh.	2	3	2	2	1	-	-	-	-	-
7	*Aspergillus petrakii*	3	2	3	-	-	-	-	-	-	-
8	*Aspergillus sclerotiorum* Humber	2	3	-	-	-	-	-	-	-	-
9	*Cladosporium oxysporum*	-	-	-	3	2	3	-	-	-	-
10	*Fusarium moniliforme* J. Sheldon	3	2	2	2	1	2	1	1	2	2
11	*Fusarium oxysporum* Schlecht	3	-	2	-	-	-	-	-	-	-
12	*Helminthosporium tetramera* Mc.Kinn.	2	-	1	-	-	-	-	-	-	-
13	*Penicillium brefeldianum* (B.O. Dodge)	2	-	1	-	-	-	-	-	-	-
14	*Penicillium funiculosum* Thom	-	-	-	1	-	-	1	-	-	-
15	*Penicillium verrucosum* Peyronel	3	2	2	1	1	-	-	-	-	-
16	*Rhizoctonia bataticola* (Taub.) Butler	2	-	2	-	-	-	1	-	-	-
17	*Rhizoctonia solani* Kuehn	3	2	-	-	-	-	-	-	-	-
18	*Trichoderma viride* Pers. Ex Fr.	2	2	-	-	1	1	-	1	-	-

CONCLUSION

The present investigation concludes total 18 fungal species belonging to nine genera were recorded from rhizosphere and soil of *Capsicum annuum* L. Among Deuteromycotina dominant fungal species were recorded seven genera and sixteen species while class Zygomycotina and Ascomycotina only one species belonging to respective genera. Application of the Bioneem to soil significantly reduces the fungal population, in the rhizopshere and soil of *Capsicum annuum* L. It has been observed that the tolerance limit of the fungal population of Bioneem was 1500 µg/ml concentration. Some of the fungal species were unaffected, stimulated or appeared and decreased or eliminated from rhizosphere or soil. The species eliminated from poisoned plates were *Rhizopus stolonifer, Aspergillus petrakii , Aspergillus sclerotiorum, Fusarium oxysporum, Helminthosporium tetramera, Penicillium brefeldianum* and *Rhizoctonia solani.* As the Bioneem concentration increases in the plates, the fungal population decreases.

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