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UTILIZATION OF ANTAGONIST AGAINST SEED BORNE FUNGI

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ABSTRACT
Diseases are the major constrains in decreased productivity and post harvest deterioration of crop plants. Diseases caused by fungi are major in plants and ultimately to seeds. Among the different disease causing agents, fungi are major agents responsible for seed deterioration. Hence present investigation was carried out to know mycoflora associated with selected oil seeds, causes of deterioration and its control by integrated methods. The effects of bio control agents, antagonist i.e. *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were observed on dominant fungi like *Aspergillus flavus* (Link), *Aspergillus niger* (Van Tiegh). *Fusarium oxysporum* (Schlecht).and *Alternaria alternata* (Fr.) Keissler. The present investigation will be helpful in control of seed borne fungi of oils seeds. By using these treatments and methods, health of seeds can be maintained and seeds can be stored for longer time and diseases can be controlled .Hence this investigation will be useful in increasing productivity of oil seeds.

KEY WORDS:

INTRODUCTION

An antagonist is an organism which has inhibitory relationship with other organism. In nature microbes grows in various association ranging from antibiosis, symbiosis, synergism to commensalisms. These associations may exist within or between different groups of microbes.
The selected antagonists are two species of fungi and two species of bacteria.

Fungi:
1. *Trichoderma viride*
2. *Trichoderma harzianum*

Bacteria:
1. *Bacillus subtillis*
2. *Pseudomonas fluorescens*

Keeping in view the potentiality of biocontrol agents and seriousness of disease, the present investigation were undertaken to the antagonistic action of *Trichoderma*, *Bacillus* and *Pseudomonas* sp. against selected fungi. An attempt was made in the present study to control dominant fungi through application well- known bio control agents.

MATERIALS AND METHODS

Collection of oil seeds from the oil research centre, Latur, Assessment of Mycoflora.
The surface mycoflora of selected seeds were detected by agar plate and blotter paper test method as recommended by International seed testing association ISTA (1966), De Temple (1970), Neerguard (1973) and Agarwal (1967).

Identification and isolation of seed borne fungi:
The fungi occurring on seeds plated on agar plate and moist blotter paper were preliminary identified on the basis of sporulation characters. Detail examination of fungal characters was done by using compound microscope and identification was confirmed with the help of manuals.

Biocontrol agents including *Trichoderma viride*, *Trichoderma harzianum* and *Bacillus subtilis*, *Pseudomonas fluorescens* were assessed. In vitro for their antagonistic activity against selected fungi such as, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria* alternate. Microbial antagonist in the laboratory is tested by dual culture method on PDA plates.Two antagonists namely *Trichoderma viride*, *Trichoderma harzianum*, were tried in vitro against the isolated pathogens for knowing their antagonists properties.

Their testing was done by dual culture technique, Dennis and Webstar, (1971) using Potato Dextrose Agar medium. Each pathogen was tested separately by keeping three replications and the medium inoculated with the pathogen alone served as control. The petriplates were incubated in BOD incubators at 25 $\pm$ 1^{o}c.

Dual Culture Technique

Dual culture technique for fungi given by (Huang and Hoes, 1976).It consists of growing the test organism and the pathogenic organism on the same plate. This can be done by the following procedure.

Pour 20 ml of melted cooled (45- $50^{o)}$ PDA in each petri plates. Allow the medium to solidify. Place a 9mm mycelial disc cut from the margin of the actively growing colonies of pathogenic culture (*Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum*) near the periphery on one side of the PDA plate. Place another disc of 9 mm of test organism (*Trichoderma viride* and *Trichoderma harzianum*)on the other side of same plate first opposite to the first disc i.e. at an angle of 180^{o}c.

Antagonistic properties of bacterial strains were tested (*Pseudomonas fluorescens, Bacillus subtilis*) against *Aspergillus niger, A. flavus, Fusarium oxysporum and Alternaria alternata* on NA plates using a dual culture technique (Skidmore and Dickinson, 1976). Agar blocks (5 days old, 5mm diameter) containing 5 days old mycelia were placed in the centre of NA plate. A loop full 42 hrs. Old culture of bacterial strains was inoculated at 2cm just opposed to the pathogen on each plate. The fungal pathogen inoculated centrally on NA plate but uninoculated by bacteria strains served as control. The plates were incubated at 28 -+ 1 C for 5 days and colony growth inhibition (%) was calculated. Observed the development of inhibition zone. The diameter of the mycelial growth was measured when controlled plates shows the maximum growth. i.e. after 8 days of incubation. The percentage inhibition of mycelial growth was calculated as per formula given by Vincent (9147).

$$\% \text{ growth Inhibition} = \frac{\text{Growth of pathogenic organism in control plate} - \text{Growth of pathogenic organism in presence of test organism}}{\text{Growth of pathogenic organism in control plate}} \times 100$$

RESULTS AND DISCUSSION

Effect of fungal biocontrol agents i.e. *Trichoderma viride,* and *T. harzianum* was tested against *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum* and *Alternaria altternata* by dual culture technique. The bacterial species including P. *fluorescnes* and *B. subtilis* were tested by dual culture technique. The petri plates were incubated with test fungi and bio control agents. The zone of inhibition was measured in relation to its growth and results were recorded.

1. **Effect of *Trichoderma* sp. on the growth of *Aspergillus flavus* (Link).**

The growth of *Aspergillus flavus* in presence of *Trichoderma viride* it was 2.5 cm and % of growth inhibition was 67.0. While the growth of *A. flavus* on control plate was 8.5cm. from the table it is clear that *Trichoderma* viride inhibits the maximum growth of *A. flavus*, as compared to *Trichoderma harzianum.* (Table 1).

2. **Effect of *Trichoderma* sp. on the growth of *Aspergillus niger* (Van Tiegh**).

The growth of *Aspergillus niger* in presence of *Trichoderma viride* it was 2.9 cm and % of growth inhibition was 66.2. In presence of *Trichoderma harzianum* it was 3.0cm and % of growth inhibition was 65%. From the table it is clear 2.5 cm and % of growth inhibition was 61.5%.From the table it is clear that *Trichoderma viride* inhibits the maximum growth of *Fusarium oxysporum* on control plate was 6.5 cm., (Table 2).

3. **Effect of *Trichoderma* sp. on the growth of *Alternaria alternate*(Fr.) keisler.**

The growth of *Alternaria alternaria* in presence of *Trichoderma viride* was 2.2 cm and % of growth inhibition was 67.6. In presence of *Trichoderma harzianum* it was 2.9 cm and % of growth inhibition was 57. From the table it is clear that *Trichoderma viride* inhibits the maximum growth of *Alternaria alternata* as compared to *Trichoderma harzianum,* while the growth of *Alterneria alternata* on control plate was 6.8 cm., (Table 3).

4. Effect of Trichoderma sp. on the growth of *Fusarium oxysporum* (Schlecht.)

The growth of *Fusarium oxysporum* in presence of *Trichoderma viride* was 1.8 cm and % of growth inhibition was 72.3. In presence of *Trichoderma harzianum* it was 2.5cm and % of growth inhibition was 61.5% from the table 95 and Fig.49 it is clear that *Trichoderma viride* inhibits the maximum growth of *Fusarium oxysporum* as compared to *Trichoderma harzianum*, while the growth of *Fusarium* on control plate was 6.5cm., (Table 4).

5. Effect of Bacterial sp. on the growth of *Aspergillus flavus* (Link).

Table 5.The growth of ***Aspergillus flavus*** in presence of *Pseudomonas fluorescens* it was 2.2cm and % growth of inhibition was 67.6.In presence of *Bacillus subtilis* it was 2.9cm and % of growth inhibition was 57.3. While the growth of *Aspergillus flavus* on control plate was 6.8 cm. from the table it is clear that *Pseudomonas fluorescens* inhibits the maximum growth of *A. flavus* as compared to *Bacillus subtilis*.

6. Effect of Bacterial sp. on the growth of *Aspergillus niger* (Van Tiegh).

Table 6. shows that the growth of *Aspergillus niger* was 2.0cm in presence of *Pseudomonas fluorescence* , while 3.0 cm in the presence of *Bacillus subtilis*, the % of growth inhibition was 73.00 and 60.00 respectively, while the growth of *A. niger* on control plate was 7.5cm.

7. Effects of Bacterial sp. on the growth of *Fusarium oxysporum* (Schelcht.)

Table 7. reveals that the growth of *Fusarium oxysporum* in presence of *Pseudomonas fluorescens* was 2.8 and % of growth inhibition was 65, while in the presence of *Bacillus subtilis* the growth was 3.5 cm and % of growth inhibition was 56.2.The growth of *Fusarium oxysporum* on control plate was 8.0 cm. it is clear that % of growth inhibition was maximum by *Pseudomonas fluorescens.*

8. Effects of Bacterial sp. on the growth of *Alternaria alternata* (Fr.) keiller.

Table 8. reveals that the growth of *Alternaria alternata* in presence of *Pseudomonas fluorescens* was 2.5 cm and % of growth inhibitions was 66, while in the presence of *Bacillus subtilis* the growth was 3.5 cm and % of growth inhibition was 53.3.The growth of *Alternaria alternata* on control plate was 7.5 cm. It is clear that % of growth inhibition was maximum by *Pseudomonas fluorescens*.

Table 1. Effect of *Trichoderma* sp. on the growth of *Aspergillus flavus* (link.) in Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.5	70.5
2	*Trichoderma harzianum*	2.8	67.0
3	Control	8.5	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.67 16.58 7.18	10.84 107.64 46.61

Table 2. Effect of *Trichoderma* sp. on the growth of *Aspergillus niger* (Van Tiegh.) in Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.9	66.2
2	*Trichoderma harzianum*	3.0	65.1
3	Control	8.6	----
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.53 15.19 6.57	9.41 93.44 40.46

Table 3. Effect of *Trichoderma* sp. on the growth of *Fusarium oxysporum* (Schlecht.) in Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A .flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	1.8	72.3
2	*Trichoderma harzianum*	2.5	61.5
3	Control	6.5	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.19 11.81 5.11	10.82 107.44 46.52

Table 4. Effect of *Trichoderma* sp. on the growth of *Alternaria alternata*(Fr.) Keissler. in Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.2	67.6
2	*Trichoderma harzianum*	2.9	57.3
3	Control	6.8	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.16 11.51 4.98	10.12 100.49 43.51

Table 5. Effect of *Trichoderma* sp. on the growth of *Aspergillus flavus* (Link.) by Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.2	67.6
2	*Trichoderma harzianum*	2.9	57.3
3	Control	6.8	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.16 11.51 4.98	10.12 100.49 43.51

Table 6. Effect of *Trichoderma* sp. on the growth of *Aspergillus Niger* (Van Tiegh.) by Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.0	73.3
2	*Trichoderma harzianum*	3.0	60.0
3	Control	7.5	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.38 13.70 5.93	10.90 108.23 46.87

Table 7. Effect of *Trichoderma* sp. on the growth of *Aspergillus niger* (Van Tiegh.) by Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.8	65.0
2	*Trichoderma harzianum*	3.5	56.2
3	Control	8.0	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.32 13.10 5.67	9.75 96.81 41.92

Table 8. Effect of *Trichoderma* sp. on the growth of *Aspergillus niger* (Van Tiegh.) by Dual Culture

Sr. No.	Fungal Antagonists	Growth of *A. flavus* against *Trichoderma* sp. in cm	% of growth inhibition
1	*Trichoderma viride*	2.5	66.0
2	*Trichoderma harzianum*	3.5	53.3
3	Control	7.5	---
4	S.E. ± C.D at p = 0.01 C.D at P= 0.005	1.24 12.31 5.33	9.84 97.71 42.31

REFERENCES

Agrawal V.K., Mathure S. B. and Neegaard P.(1972). Some of seed health testing with respect to seed borne fungi rice, wheat, green gram and soybean growth in India. *Ind. Phytopathol*. **25**: 91-100.

Anonymous (1966).International rules for seed testing. *Proc. Int. Seed Test. Assoc.* 31: 1-150.

Bells D. K., Wells H.D and Morkhane C.R. (1982). In-vitro antagonism of Trichoderma sps. Against six fungal pathogens. *Phtytopathol.***72**: 379-382.

Bhatnagar Himani (1966). Influence of environmental conditions on antagonistic activity of *Trichoderma* sp. against *Fusarium udum*. Inidan. *J. Mycol. Plant. Path.* **26**(1):58.

Biswas K.K. (1999). Screening of isolates of *Trichoderma harzianum* for their relative biocontrol efficacy against *Fusarium oxysporum* and *Rhizoctonia solani*. *Ann. Plant Prot. Sci.* **7** (2): 125-13.

Nema S., Bandyopadhyaya S., Sharma N.D. (2001). Some studies on *Trichoderma* as biocontrol agent. *J. Mycopatho. Res.* **40**(2): 81-87.

Padmodaya B. and Reddy H. R. (1966). Screening of *Trichoderma* sp. against *Fusarium oxysporum* f.sp *Lycopersici* causing wilt of tomato. *Ind. J. Mycol. Plant. Pathol.* **26**(3): 266- 270.

Pant Rajiv and Mukhopadya P.N. (1988). Biological seed treatment with *Glicocladium virens* and *Trichoderma harzianum* for management of seed and seedling rot complex of soybean. J. Indian Phytopath .Ab. 549.

Zaki M.J. and Ghaffar A. (1988). Control of *Rhizoctonia solani* in soybean by farmyard manure culture of *Trichoderma harzianum*. *Ind. J. Agri. Sci.* **596**: 596- 598.

