



## ANTAGONISTIC ACTIVITY OF TRICHODERMA SPECIES AGAINST FUNGI ASSOCIATED WITH OIL SEEDS

Yadav S.G

Department of Botany Shivaji Mahavidyalaya Renapur District Latur. (413527) Maharashtra

### ABSTRACT

The species of *Trichoderma* are effective biocontrol agent used for management of various crop plant diseases. The effectiveness of *Trichoderma* species is due to their extracellular secretion of metabolites. The oil seeds, Ground nut (*Arachis hypogea* L.), Soyabean (*Glycine max* L.), Sunflower (*Helianthus annuus* L.), Safflower (*Carthamus tinctorius* L) are the major oil seed crops grown in the Marathwada region of Maharashtra. The seed mycoflora was detected by using Blotter paper method and Agar plate method and 19 fungal species i.e. *Alternaria alternata*, *Alternaria tenuis*, *Aspergillus tamari*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus candidus*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliformae*, *Fusarium semitectum*, *Penicillium notatum*, *Penicillium digitatum*, *Rhizopus nigricans*, *Rhizopus stolonifer*, *Rhizoctonia solani*, *Rhizoctonia bataticola*, *Sclerotium* sp. were obtained and these fungal species screened by the three species of *Trichoderma* i.e. *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma virens*, of these *Trichoderma harzianum* proved to be superior for inhibition of the above mentioned pathogens as compared to *Trichoderma viride* and *Trichoderma virens*.

**KEYWORDS :** Oil seeds, biocontrol, mycoflora, trichoderma species.

### Introduction:

The Ground nut (*Arachis hypogea* L.), Soyabean (*Glycine max* L.), Sunflower (*Helianthus annuus* L.), Safflower (*Carthamus tinctorius* L) are the major oil seed crops grown in the Marathwada region of Maharashtra. About 32% yield loss in crops due to plant diseases. The diseases are classified as seed borne, air borne and soil borne diseases caused by fungal pathogens causes qualitative and quantitative loss to crops. The term seed mycoflora is used for both qualitative and quantitative analysis of fungi occurring on or in the seeds. (Niggard and Paul, 1973). The fungi associated with the seeds at the stage of harvest and under storage bring about several undesirable changes making them unfit for consumption and sowing. (Bhikane, 1998).

In advanced technology of agriculture, use of trichoderma species as biocontrol agents for the management of crop plant diseases, now it becomes a modern trend. Apart from enzymes, the species of trichoderma are also producers of various metabolites with toxic antibiotic properties (Bruckner et al, 1990., Dennis and Webster, 1971., Stepanovic and Howell, 1982., Tomoda et al. 1992., Huang et al. 1995). Number of secondary metabolites are produced by their metabolic pathways. (Sivasithamparan and Ghisalberti, 2002). They are frequently associated with both biocontrol activity and promotion of plant and root growth. (Chet et al. 2006, Howell, 1998). Aspite et al. (1996) extracted trichodermin from *Trichoderma viridae* and *Trichoderma harzianum*. Trichonitrin was obtained from the several strains of *Trichoderma harzianum* (Kulisler, 1997). Shanmugam (2001) was made purification and characterization of phytotoxin from *Trichoderma viridae*. The species of *Trichoderma* are known to inactivate the phytotoxins produced by many plant pathogenic fungi. (Sriram et al. 2000). Culter and Le Flies (1978) reported that Trichodermin is a potent inhibitor of plant growth and produces other phytotoxic effects. It inhibits wheat coleoptile growth; phytotoxic to tobacco at high concentration and inhibits growth at lower concentrations.

Recently several techniques were used for the control of seed borne fungi. The fungi associated with seeds, successful chemical treatment has been developed over the years. The chemicals have played a significant role in maximizing crop productivity they are causing harmful and undesirable effects not only on man and wild life, but also on the whole ecosystem. The biological control of root diseases of crop plants by introduction of antagonistic microorganisms has been suggested as environmentally safer alternative to use of

fungitoxic chemicals. (Baker and Cook, 1979), protection of mung bean seedling from *Rhizoctonia solani* by using trichoderma species (Patale, et al. 2009), control of fungi associated with green gram seeds, by using trichoderma species (Patil, et al. 2012). In the present investigation the biological control of trichoderma species against some oil seed pathogenic fungi under the laboratory conditions.

### Materials and Methods:

#### a) Collection of seed samples:

For the present investigation the oil seeds like groundnut, soyabean, sunflower and safflower were collected from the local market of Renapur tehsil.

#### b) Detection of seed mycoflora:

The seed mycoflora of selected oil seeds were detected by blotter paper method and by agar plate method recommended by International seed testing association, ISTA, (1966) and Agarwal, (1976).

#### c) Blotter paper method (Doyer, 1938 and De Temp, 1953):

A pair of white blotter paper of 8.5cm diameter, 10 seeds of groundnut, soyabean, sunflower and safflower per petriplate placed at equal distance on moist blotter paper aseptically. These petriplates were incubated at room temperature (28°C) for seven days. The seeds were observed under the microscope for determination of various fungal growths. Identifications and confirmations of different fungal species were made by preparing slides.

#### d) Agar plate method (Musket, 1948):

In Northern Netherland, Musket (1948) very firstly used this method for the seed health management. In this method sterilized petriplates were poured with 15ml of autoclaved Potato dextrose agar (PDA). 10 seeds of each selected oil seeds were placed at equal distances, the petriplates were incubated at room temperature for seven days. Identifications and confirmations of different fungal species were made by preparing slides. The pure culture of these fungi was prepared and maintained on PDA slants for further studies.

#### e) Biological control:

The antagonistic properties of trichoderma species were studied through dual plate method (Biswas and Sen, 2000). The mycelial disc of 6mm diameter cut from the margin of three days old cultures of both test pathogen and antagonists were placed opposite to each other on PDA in petriplates. Inoculated plates were incubated at 28°C in BOD chamber. These replicates were kept for each treatment and

observations on colony diameter (mm), overgrowth and formation of inhibition zone were recorded to select highly effective nature of trichoderma species. On the basis of inhibition of mycelial growth of pathogen over control the percent inhibition was calculated by Vincent, (1972) method.

**Table 1: Isolated fungi from Oil seeds by Blotter and Agar plate method:**

SR.NO.	Name of Fungi	Blotter paper method	Agar plate method
1	Alternaria alternate	12	19
2	Alternariatenuis	21	28
3	Aspergillus tamari,	10	16
4	Aspergillusflavus	38	47
5	Aspergillusniger,	41	52
6	Aspergillusfumigatous	28	34
7	Aspergillusterrus	18	20
8	Aspergilluscandidus,	34	46
9	Fusariumoxysporum	20	23
10	Fusariumsolani	12	16
11	Fusariummoniliformae	06	08
12	Fusariumsemitectum,	08	10
13	Penicilliumnotatum	14	17
14	Penicilliumdigitatum	04	07
15	Rhizopusnigricans	03	04
16	Rhizopusstolonifer	02	02
17	Rhizoctiniasolani	13	18
18	Rhizoctoniabataticola,	16	23
19	Sclerotiumsp	02	04

**Table 2: Antagonistic activity of Trichoderma species against fungi associated with Oil seeds :**

Sr. No.	Plant pathogenic fungi	Control (growth of fungus in mm )	inhibition of fungal growth due to Trichoderma harzianum	inhibition of fungal growth due to Trichoderma viride	inhibition of fungal growth due to Trichoderma virens
1	Alternaria alternate	88.10	80.20	76.20	72.33
2	Alternariasolani	92.16	85.60	75.70	70.10
3	Alternariatenuis	80.00	84.41	78.22	72.22
4	Aspergillusflavus	88.20	83.22	74.14	69.44
5	Aspergillus tamari,	83.00	70.66	78.12	75.44
6	Aspergillusniger,	83.22	72.41	80.13	73.78
7	Aspergillusfumigatous	80.0	75.14	81.10	76.17
8	Aspergillusterrus	85.00	70.15	75.18	72.87
9	Aspergilluscandidus,	90.00	82.13	78.22	75.32
10	Fusariumoxysporum	90.00	80.15	75.45	70.16
11	Fusariumsolani	92.10	86.14	78.12	80.90
12	Fusariummoniliformae	82.00	78.88	71.55	70.48
13	Fusariumsemitectum,	80.00	75.64	70.29	69.22
14	Penicilliumnotatum	62.00	90.46	88.56	85.22
15	Penicilliumdigitatum	60.00	89.62	85.11	86.45

16	Rhizopusnigricans	85.00	82.10	79.49	80.22
17	Rhizopusstolonifer	80.00	75.96	72.88	70.44
18	Rhizoctiniasolani	72.23	90.46	86.41	82.63
19	Rhizoctoniabataticola,	70.00	88.44	80.10	80.92
20	Sclerotium sp.	60.00	89.92	82.77	78.90

**Results and Discussion:**

The seed borne mycoflora was detected by moist blotter paper method and by agar plate method and a total of 19 different fungal species under 07 genera were isolated from the oil seeds. (Table 1).The percent incidence of agar plate method was highest than blotter paper method. The species of Aspergillus flavus and Aspergillus niger shows dominance and followed by species of Fusarium. It is also clear that agar plate method is more suitable for the fungal growth due to nutrients from the PDA medium than that of blotter paper method. Karreppa, (1998), Baig, (2005), AliyuKhutama, (2007).

In the present investigation three trichoderma species were used against the isolated plant pathogenic fungi. The fungi associated with oil seeds were found to be significant in inhibition of fungal growth in the presence of trichoderma species and among these antagonists, Trichoderma harzianum proved to be more stronger than Trichoderma viride and Trichoderma virens. (Table 2). The biological control of three trichoderma species against the fungi associated with oil seeds supports the earlier investigations Biswas and Sen (2000), Singh and Thapliyal, (1998).

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