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Antagonistic properties of *Trichoderma viride* and *Trichoderma harzianum* against storage fungi

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ABSTRACT

Different varieties of groundnut, soybean, sesame, safflower and sunflower were collected from different parts of store houses and market places of Marathwada region of Maharashtra state. By using different agar media mycoflora associated with these oilseed varieties was isolated. It was found that, Ghungru variety of groundnut, KSL-441 variety of soybean, Se1B variety of sesame, Bhima variety of safflower and Kargil variety of sunflower showed maximum incidence of fungi. *Rhizopus stolonifer*, *Fusarium oxysporum* and *Macrophomina phaseolina* showed minimum growth in presence of *Trichoderma harzianum.* Growth of *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Curvularia lunata* was retarded due to *Trichoderma viride*.

Introduction

Agriculture is the backbone of the Indian economy and villages are the life lines of growth of India. It is a very important sector for the sustained growth of the Indian economy. About 70% of the rural households and 8% of urban households are still principally dependent on agriculture for employment. India has undergone a series of successful agricultural revolutions-starting with the 'green' revolution in wheat and rice in the 1960's and 1970's, the 'white' revolution in milk to the 'yellow' revolution in oilseeds in 1980's. As a result, India has achieved self-sufficiency in agriculture. India is one of the largest producers of oilseeds in the world and this sector occupies an important position in the agricultural economy covering an area of 38 million hectares, and accounting for the production of about 32 million tonnes of oilseeds annually. India contributes about eight per cent of the world oilseeds production after China, EU-15 and USA (Kakde, 2011). India has a wide range of oilseeds crops grown due to the different agro-climatic zones. In Maharashtra state seed for sowing is available at seed industries and Government Agriculture institute. Whereas, poor farmer keep the reserved seeds with him for his consumption and sowing purpose in next season. High nutritive value makes oilseeds prime target of attack of various microorganisms like bacteria, viruses, fungi and insects. Among these, fungi play a dominant role in decrease in quality and longitivity of oilseeds (Christensen and Kaufman,1969). Therefore, such seeds are not fit for human consumption and are also rejected at industrial level. Hence, emphasis has been given on to investigate fungi associated with different varieties of oilseeds and ten fungi were selected on the basis of their quantitative occurrence of dominance. Importance of biocontrol has been enhanced for the sustainable agricultural practice and environmental protection. The adaptation of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining worldwide recognition. One of the key elements of such sustainable agriculture is the application of biocontrol agents. *Trichoderma* spp. is antagonistic by nature with rich resource and a broad action scope. In this regard experiments were carried out to screen the antagonistic nature of *Trichoderma harzianum* and *Trichoderma viride* against storage fungi.

Materials and Methods

Isolation of storage fungi of oilseeds

Collection of oilseed samples

Oilseeds samples of groundnut, soybean, sesame, safflower and sunflower of different varieties were collected from market places and store houses from different parts of Marathwada region of Maharashtra state. These seeds were then packed in pre-sterilized polythene bags and kept in laboratory conditions until use.

Isolation of oilseed mycoflora

For detection of seed mycoflora associated with seed samples, the method recommended by ISTA (1966), Neergaard (1973) and Agarwal (1976) were adopted. 10 seeds per pre-sterilized petriplates were equispaced asceptically on autoclaved Potato Dextrose Agar (PDA) and Glucose Nitrate Agar (GNA) media. Plates were then allowed to incubate at room temperature for seven days.

Antagonistic activity of *Trichoderma viride* and *Trichoderma harzianum* against storage fungi

Trichoderma harzianum and *Trichoderma viride* were isolated from the sesame on PDA. A mycelial disc (1.2 cm diam), obtained from the peripheral region of 5-7-day-old cultures of *Alternaria dianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum* was placed on a fresh PDA plate (3 cm from the center) and incubated at 28^0C for 48 h to initiate growth. Then a 1cm diameter mycelial disc, obtained from the periphery of a 5-7day old culture of *Trichoderma harziaum* and *Trichoderma viride* was placed 3 cm away from the inoculum of the pathogen, the plates were incubated at 28^0C and measurements were taken after 7 days. In the control

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experiment a sterile agar disc (l.2 cm diam) was placed in the dish. At the end of the incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control (Edington et al., 1971) as follows:

$$\frac{C-T}{C} \times 100 = \%\ \text{Inhibition of radial mycelial growth}$$

Where, C = radial growth measurement of the pathogen in control

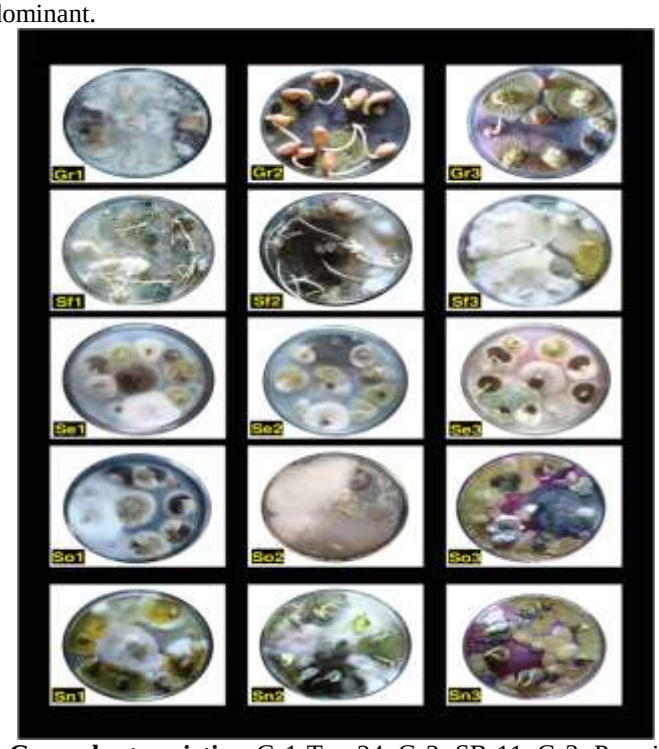
T =radial growth of the pathogen in the presence of *Trichoderma*

Results and Discussion

Percent incidence of fungi on different oilseed varieties

Groundnut

Incidence of fungi on five different varieties of groundnut viz., Tag-24, SB-11, Ghungru, Pasari and TSL-45 was studied and results are summarized in table 1. Ghungru and Tag-24 yielded eleven and ten fungi respectively. On the other hand, six fungi each on SB-11 and TSL-45 while, five fungi on Pasari variety were found to be associated. *Aspergillus flavus* and *Rhizopus stolonifer* grown on all the varieties of groundnut while, *Trichoderma viride* and *Aspergillus fumigatus* only grown on Tag-24 and Ghungru variety respectively. *Alternaria alternata* and *Aspergillus niger* were found to be associated with Tag-24 and SB-11, Ghungru variety of groundnut. *Rhizoctonia solani* associated with Ghungru, Pasari and TSL-45 variety while, *Curvularia lunata*, *Fusarium chlamydosporum*, *Fusarium oxysporum*, *Penicillium digitatum* and *Penicillium chrysogenum*, occurred on Ghungru variety. On TSL-45 and Pasari variety, *Aspergillus flavus* and *Rhizoctonia solani* were found to be dominant.



Groundnut varieties: Gr1-Tag-24; Gr2- SB-11; Gr3- Pasari,
Safflower varieties: Sf1- Bhima; Sf2- PBNS-12; Sf3- Parbhani-40
Sesame varieties: Se1- Phule no. 1; Se2- Se1B; Se3- Se2P
Soybean varieties: So1- KSL-44; So2- Yashoda; So3- JS-335
Sunflower varieties: Sn1- SuH; Sn2- Suraj; Sn3- Kargil

Fig 1: Isolation of storage fungi from different oilseed varieties

Soybean

JS-335, Eagle, KSL-441, Yashoda and Nirmal varieties of soybean were screened for the incidence of the mycoflora and results are summerized in table 2. All variety showed occurrence of *Macrophomina phaseolina* and *Rhizopus stolonifer*. Eleven and thirteen fungi were found to be associated with JS-335 and KSL-441 while, Eagle yielded six fungi, Yashoda and Nirmal showed occurrence of five fungi. *Aspergillus niger*, *Cercospora kikuchii*, *Colletotrichum* sp., *Curvularia lunata*, *Fusarium oxysporum*, *F. verticillioides*, *F. solani*, and *Penicillium chrysogenum* were found to be associated with JS-335 and KSL-441 varieties. *Alternaria alternata* were grown only on Eagle and Nirmala while, *Helminthosporium* sp. restricted its growth on KSL-441.

Sesame

Five varieties of sesame viz. Phule no.1, Se_1B, Se_2P, Se_3N and Se_4H were selected to study the occurrence of fungi and results are given in table 3. Phule no.1 and Se_1B yielded seven and nine fungi respectively. On the other hand Se_2P and Se_3N showed growth of four fungi and six fungi were grown on Se_4H. Phule Til no.1 and Se_1B showed occurrence of *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata* and *Penicillium chrysogenum*. All varieties except Se_3N and Se_4H showed incidence of *Penicillium chrysogenum*. *Aspergillus niger* and *Fusarium oxysporum* grown on all the varieties except Se_2P. *Alternaria dianthicola* was grown on Phule no.1, Se_2P and Se_4H varieties. Phule no.1 and Se_3N yielded *Trichoderma viride* while Se_2P and Se_4H yielded *Penicillium digitatum*.

Safflower

Bhima, PBNS-12, Sharda, Parbhani-40 and Sf-J varieties of safflower were selected to screen the mycoflora associated with them and results are summerized in table 4. *Aspergillus niger* and *Rhizopus stolonifer* were occurred on Bhima, PBNS-12, Sharda and Parbhani-40. Bhima, Sharda and Sf-J showed occurrence of *Alternaria carthami* and *Curvularia lunata*. *Aspergillus ustus*, *Curvularia pellescens*, *Fusarium oxysporum*, *Mucor* sp., *Penicillium digitatum* and *Rhizopus stolonifer* yielded on PBNS-12 and Parbhani-40. *Fusarium equiseti* and *Macrophomina phaseolina* were found to be associated only with Bhima and Sharda varieties.

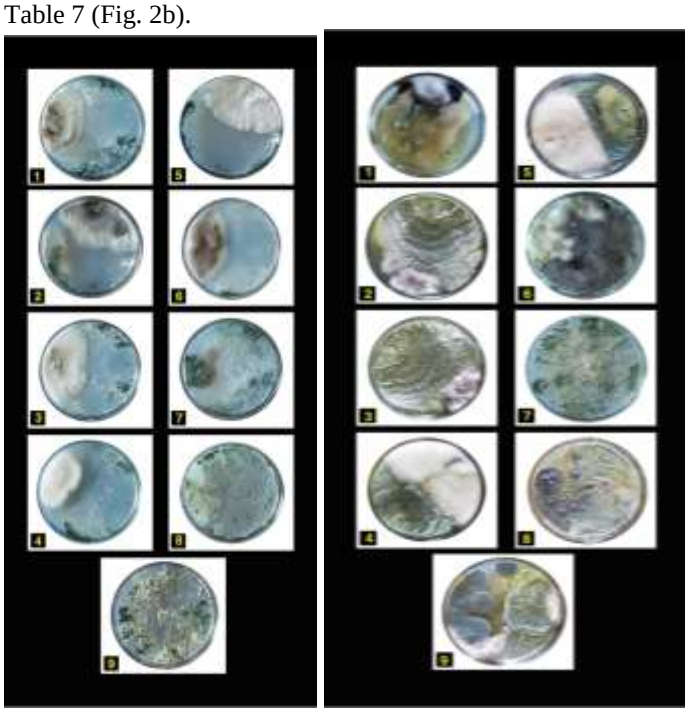
Sunflower

Su-H, Suraj, Kargil, LSF-8 and Ravikiran-2 varieties of sunflower were screened for the incidence of fungi on them and results are summarized in table 5. *Aspergillus terreus* and *Mucor* sp. were found to be associated with Su-H, Suraj, Kargil and LSF-8. *Aspergillus niger*, *Fusarium equiseti* and *Rhizopus stolonifer* occurred on Su-H and Kargil varieties. Kargil and Suraj varieties showed occurrence of *Alternaria dianthicola* and *Fusarium oxysporum*. *Trichoderma viride* was grown on Suraj and Ravikaran-2 varieties. It is interesting to note that only Su-H yielded *Trichoderma harzianum*. Such types of variations in incidence of storage fungi in different oilseed crops have also been reported by various workers as in case of groundnut (Reddy et al., 1991), soybean (Murthy and Raveesha, 1996), sunflower (Agarwal and Singh, 1974), safflower (Singh et al., 1987) and in sesame (Vyas et al., 1984).

Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* against storage fungi

Antagonistic activity of *Trichoderma harzianum* against storage fungi was studied and the results are summarised in the table 6 (Fig. 2a). Growth of *Rhizopus stolonifer* was greatly hampered due to *Trichoderma harzianum*. *Fusarium oxysporum* and *Macrophomina phaseolina* showed minimum growth in

presence of *Trichoderma harzianum*. On the other hand *Trichoderma harzianum* not showed any considerable effect on the growth of *Penicillium digitatum* and *Penicillium chrysogenum*. Antagonistic activity of *Trichoderma viride* against storage fungi was studied and results are given in the Table 7 (Fig. 2b).



(a) **(b)**

Fig. 2: a) Antagonistic activity of *Trichoderma harzianum* against storage fungi
b) Antagonistic activity of *Trichoderma viride* against storage fungi

Trichoderma viride showed its maximum antagonistic activity against *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Curvularia lunata*. Growth of *Macrophomina phaseolina* and *Fusarium equiseti* was not considerably affected due to *Trichoderma viride*. From these results it can be concluded that *Trichoderma* spp. is antagonistic by nature with rich resource and a broad action scope. Similar results were reported by Bandopadhyay et al. (2008) investigated the antagonistic efficacy of *Trichoderma viride* against *Macrophomina phaseolina* while Hussain et al. (2009) found that *Trichoderma harzianum* reduced the growth of *Alternaria alternata* by 67.07%. On the other hand, Howell (2003) reported the interaction between *Trichoderma viride* and *Rhizoctonia solani*, *Macrophomina phaseolina* and *Rhizopus oryzae* by different mechanism. Sempere and Santamarina (2007) analyzed *Trichoderma harzianum* as possible biocontrol agent of *Alternaria alternata* under different environmental conditions.

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Table 1: Percent incidence of fungi on different groundnut varieties

Fungi	Varieties				
	Tag-24	SB-11	Ghungru	Pasari	TSL-45
Alternaria alternata	10	20	10	--	--
Aspergillus niger	50	30	20	--	30
Aspergillus flavus	80	70	80	60	70
Aspergillus fumigatus	--	--	30	--	--
Curvularia lunata	20	--	30	20	--
Fusarium chlamydosporum	--	--	20	--	20
Fusarium oxysporum	20	--	10	--	10
Fusarium equiseti	10	10	--	--	--
Macrophomina phaseolina	30	--	--	20	--
Rhizoctonia solani	--	--	10	50	60
Rhizopus stolonifer	30	40	50	10	30
enicillium digitatum	20	--	20	--	--
Penicillium chrysogenum	--	20	20	--	--
Trichoderma viride	20	--	--	--	--

Table 2: Percent incidence of fungi on different soybean varieties

Fungi	Varieties				
	JS-335	Eagle	KSL-441	Yashoda	Nirmala
Alternaria alternata	--	10	--	--	10
Aspergillus niger	30	20	30	--	40
Aspergillus flavus	30	20	--	20	--
Aspergillus fumigatus	--	--	20	10	20
Cercospora kikuchii	20	--	20	--	--
Colletotrichum gloeosporioides	20	--	20	--	--
Curvularia lunata	20	--	20	--	--
Fusarium culmorum	--	20	--	20	--
Fusarium solani	10	--	20	--	--
Fusarium oxysporum	40	--	40	--	--
Fusarium verticillioides	10	--	10	--	--
Macrophomina phaseolina	50	50	70	50	10
Rhizoctonia solani	--	--	20	--	--
Rhizopus stolonifer	30	10	60	20	20
Penicillium chrysogenum	30	--	30	--	--
Helminthosporium papulosum	--	--	20	--	--

Table 3: Percent incidence of fungi on different sesame varieties

Fungi	Varieties				
	Phule Til no.1	Se_1B	Se_2P	Se_3N	Se_4H
Aspergillus flavus	70	90	80	--	60
Aspergillus ustus	--	10	--	10	--
Aspergillus niger	50	60	--	50	20
Alternaria dianthicola	20	--	40	--	20
Curvularia lunata	20	20	--	--	--
Curvularia pellescens	--	--	20	--	--
Fusarium equiseti	--	40	--	--	40
Fusarium oxysporum	30	40	---	40	20
Penicillium chrysogenum	20	20	--	--	--
Penicillium digitatum	--	--	30	--	20
Trichoderma harzianum	--	30	--	--	--
Trichoderma viride	20	--	--	40	--
Verticillium tenerum	--	20	--	--	--

Table 4: Percent incidence of fungi on different safflower varieties

Fungi	Varities				
	Bhima	PBNS -12	Sharda	Parbhani -40	Sf-J
Aspergillus terreus	10	--	--	10	--
Aspergillus ustus	--	10	10	10	--
Aspergillus niger	30	20	20	20	--
Alternaria carthami	20	--	40	--	10
Curvularia lunata	20	--	30	--	20
Curvularia pellescens	--	10	--	10	--
Fusarium chlamydosporum	30	--	--	30	--
Fusarium equiseti	20	--	20	--	--
Fusarium oxysporum	40	20	--	30	10
Fusarium roseum	--	--	10	--	--
Macrophomina phaseolina	20	--	20	--	--
Mucor indicus	10	10	--	10	--
Penicillium digitatum	--	20	--	30	--
Rhizopus stolonifer	10	30	20	10	--

Table 5: Percent incidence of fungi on different sunflower varieties

Fungi	Varieties				
	Su-H	Suraj	Kargil	LSF-8	Ravikiran-2
Aspergillus niger	20	--	20	--	--
Aspergillus terreus	30	10	30	--	--
Alternaria dianthicola	--	10	10	--	--
Fusarium equiseti	30	--	30	20	--
Fusarium oxysporum	--	40	10	--	20
Mucor indicus	20	20	20	20	30
Trichoderma harzianum	30	--	--	--	--
Trichoderma viride	--	20	--	--	20
Rhizopus stolonifer	50	--	60	70	40

Table 6: Antagonistic activity of *Trichoderma harzianum* against storage fungi

Fungi	(C)	(T)	% Inhibition
Alternaria dianthicola	5.8	3.1	46.55
Curvularia lunata	5.6	3.0	46.42
Curvularia pellescens	6.0	3.8	36.66
Fusarium oxysporum	7.0	3.6	48.57
Fusarium equiseti	7.8	4.2	46.15
Macrophomina phaseolina	8.4	4.3	48.80
Rhizopus stolonifer	7.8	2.0	74.35
Penicillium digitatum	7.0	5.5	21.42
Penicillium chrysogenum	6.9	5.2	24.63

Radial growth (mm) in control set (C)
Radial growth (mm) in treated set (T)

Table 7: Antagonistic activity of *Trichoderma viride* against storage fungi

Fungi	(C)	(T)	% Inhibition
Alternaria dianthicola	5.4	2.2	59.25
Curvularia lunata	5.2	2.0	61.53
Curvularia pellescens	6.2	2.4	61.29
Fusarium oxysporum	7.4	4.0	45.94
Fusarium equiseti	7.2	4.4	38.88
Macrophomina phaseolina	7.8	5.2	33.33
Rhizopus stolonifer	7.9	2.2	72.15
Penicillium digitatum	7.0	2.3	67.14
Penicillium chrysogenum	7.5	2.1	72.00

Radial growth (mm) in control set (C)
Radial growth (mm) in treated set (T)