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EFFICACY OF BIOLOGICAL ANTAGONISTS AND PHYTO EXTRACTS AGAINST *FUSARIUM OXYSPORUM* IN OKRA SEEDS

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ABSTRACT

In present study, selected seed samples of okra (Abelmoschusesculentus L. Monech) carrying 35-45 % natural infection of Fusariumoxysporum were used for the treatment. Pure suspension culture of antagonists Trichodermaharzianum and Gliocladiumvirens were tried as seed treatments against the pathogen. T.harzianumwas highly antagonistic to F. oxysporumand showed significant percent disease control (75% and 63%) in asymptomatic and symptomatic treated seeds respectively. T.harzianumand G. virens enhanced seed germination in treated asymptomatic (85%, 70%) and symptomatic (65%, 55%) seeds as compared to their control (60%, 42%). Leaf extracts of 11 plants viz. Azadirachtaindica, Catharanthusroseus, Calotropisprocera, Cassia tora, Daturainnoxia, Eucalyptus rudis, Lantana camera, Lawsoniarosea, Neriumindicum, Ocimum sanctum, and Ricinuscommunis were used for the control of seed–borne infection of F.oxysporum. Among the leaf extracts used, Eucalyptus rudis (pure,100% conc.) and Azadirachtaindica (30% dil.) were found most efficacious and showed maximum percent control of pathogen incidence and seedling infection (81.81% , 80%) & (81.81%, 75%) respectively. Treatments with Eucalyptus rudis and Lawsoniarosea enhanced significant seed germination (90%, 78.33%) with respect to their control (50%). Pure extracts of rhizome/ bulb of ginger, turmeric, onion and garlic were more effective to promote seed germination than 30% dilution. However, the extract (both pure and diluted) of turmeric was found significantly superior over all other extracts in promoting seed germination and percent control of pathogen incidence. Application of bio-agents and plant extracts against seed-borne diseases are cost effective and eco-friendly alternative.

Keywords: *Plant Leaf /Rhizome /Bulb Extracts, Trichodermaharzianum, Gliocladiumvirens, Fusariumoxysporum and Okra.*

Introduction

In India, Okra is an important vegetable crop known to suffer from many fungal diseases. Root rot and wilt disease of okra caused by *Fusariumoxysporum*f.sp. *vasinfectum* .Grover and Singh (1970) reported a severe wilt disease caused 25 -35 % significant loss of okra crop due to *Fusariumoxysporum*f.sp. *vasinfectum* in the region of Haryana and Punjab. In okra, the seed–borne nature of fungus was reported by Gangopadhyay and Kapoor (1977). In present study efforts were made to reduce the incidence of pathogen and enhancement of seed germination by using fungal antagonists and various plant leaf/rhizome/bulb extracts as seed treatment against *F. oxysporum* in okra.

Materials and Methods

Naturally infected seed samples of okra by*F. oxysporum* were selected to test the efficacy ofplant leaf / rhizome/ bulb extracts and biological antagonists for the control of pathogen. For extract preparation, 10 g fresh leaves/ bulb/ rhizome of each plant were taken, washed and crushedin sterilized

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distilled water at the rate of 1g tissue in 1ml of water (1:1/w/v) usingpastel and mortar and filtered with double layered cheesecloth, formed stock aqueous extracts. Seeds were categorizedby dry seed inspection (Neergaard, 1977) as asymptomatic (normal bold healthy looking) and symptomatic seeds (seeds with brown/black surface discoloration or with white crust) for the treatments. Seeds were treated separately with aqueous extracts in two different concentrations, pure (w/v) and diluted (1:3/w/v) for 4 h. Seeds soaked in distilled water were used as control.

Pure culture suspension of *Trichoderma harzianum* and *Gliocladiumvirens* were used as seed treatment (figure-1). 20 ml of sterilized distilled water was added to each 12 day old culture plates of *T. harzianum* and *G.virens* and suspensions were obtained in flask. Naturally infected asymptomatic and symptomatic seeds (60 seeds per treatment) were surface sterilized with 2% NaOCl solution and soaked in spore suspension of *T. harzianum* and *G.virens*for 4 h. Three replicates of 20 seeds for each treatment were sown on moistened blotters by standard blotters method (SBM) [ISTA 1966].Observations on percent seed germination, percent control of seedling infection and percent incidence of pathogen were recorded on 8th day of the incubation and the data of treated and untreated seeds were analyzed statistically by Completely Randomized Design (CRD) method. Disease control was calculated by the following formula:

$$\text{Percent disease control} = \frac{\text{Incidence in control} - \text{Incidence in treatment}}{\text{Incidence in control}} \times 100$$

Plant Leaf Extracts

The maximum percent control of pathogen incidence and seedling infection were observed in leaf extract of *Eucalyptus rudis* (81. 81%; 80, 80 %) and *Lantana camera* (72.72%; 70, 75%)) in pure (100%) and 30% diluted extracts respectively. In 30% dilution, *Azadirachtaindica* and *Eucalyptus rudis* showed 81.81% and 72.72% significant control of pathogen followed by *Lantana camera, Oscimum sanctum* and *Calotropis procera* ((Table-1).Sharma (1999) observed good control of *F. oxysporum* by seed treatment with extractof *Cassia indica, Ricinuscommunis*and *Oscimum sanctum* in green gram. *Eucalyptusrudis, Cassia occidentalis & Catharanthusroseus* were found also effective against *Fusarium oxysporum*in pigeon pea (Kumar,2000). Datar, Hayat, Anis and Zaki (2008) reported control of root rot fungi *viz*., *Macrophominaphaseolina, Fusarium* spp.& *Rhizoctoniasolani* by the seed treatment with microbial antagonists *Bacillus thurigiensis, Rhizobium meliloti, Aspergillusniger* and *Trichodermaharzianum*on okra and sunflower plants.Choudhary, Ashraf and Musheer (2017) recorded effective reduction in mycelial growth of *F.oxysporum*f.sp.*vignio*f mung bean by the use of Neem and Amlaextracts in *in vitro*.

Rhizome /Bulbs Extracts

In seed treatment with rhizome/ bulb extracts, the significant reduction of pathogen incidence was observed inturmeric (68.75% in 30% dilution) and onion extract (56.25% in 100% concentration).For the control of seedling infection, turmeric extract(77.77 % in 30% dilution) was found most superior to all other extracts followed by onion (55.55 %) and ginger (44.44 %) in their pure concentration(Table-2). Kapadiya, Akbari, Talaviya and Siddhapara (2014) obtained maximum inhibition of *Fusariumsolani* in turmeric rhizome extract followed by jatropha and neem leaves extracts. Choudhary, Ashraf and Musheer (2017) recorded effective reduction in mycelial growth of *F.oxysporum*f.sp.*vignio*f mungbean by the use of garlic extract at 30% concentration. Datar (1999) reported efficacy of garlic followed by onion, turmeric and ginger in reduction of mycelial growth of *M.phaseolina.* The variable toxicity of the extracts may be due to different activity of antifungal compounds in different plants such as phenolic substances, gummy resinous and non-volatile substances of unknown nature (Sindhan, Hooda and Parashar, 1999). Seed treatments with plant leaf /rhizome /bulb extracts are eco-friendly, non-toxic and promising alternative to control the fungal diseases of crops.

Biological Antagonists

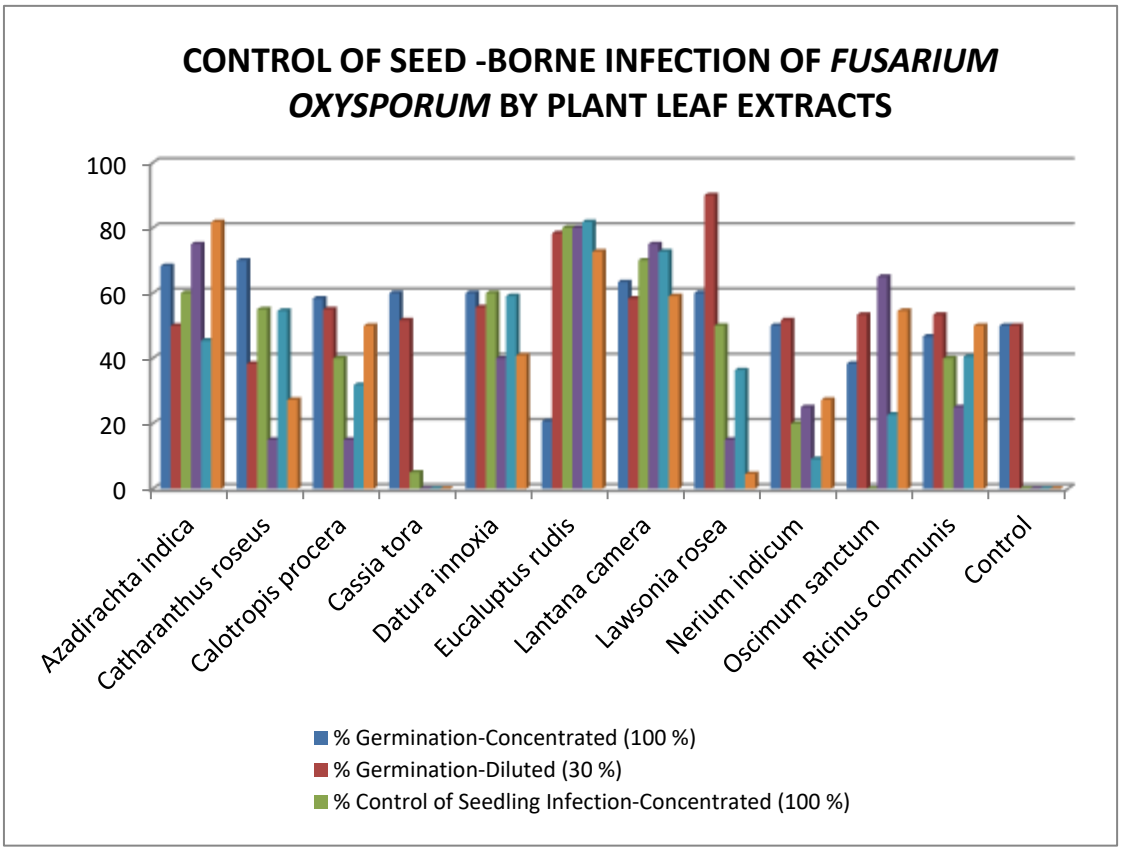
In presentstudy**,** *Trichodermaharzianum* gave effective percent disease control in treated seeds of asymptomatic (68%) and symptomatic (59%) categories. The germination was also enhanced in treated seeds 85, 65% as compared to their control (60, 40%) in asymptomatic and symptomatic categories respectively(Table-3;Figure-1).Goel and Mehrotra (1974d) were used *T.harzianum*, *Paecilomyceslilacinus, Bacillus subtilis, Streptomyces* sp.and *G. virens* as seed treatments for the control of *Fusarium* spp*., M.phaseolina, R.solani* in okra, soybean, moong and sunflower . Sharma (1999) also proved the significant effect of *Gliocladiumvirens* as seed treatment against the *F.*oxysporum in mung bean. Increased seed germination index and seedling growth by the use of culture filtrate of

*Trichoderma*sp. in chilli seeds were observed by Ahsanur, Sultana, Ferdousi Begum and Firoz Alam (2012).Sultana and Ghaffar (2013) reported significant effect of *T.harzianum* and *G. virens* on seed germination, reduction in seedling mortality against the *F.oxysporum* causing seed rot and root infection in bottle gourd and cucumber .Shah Syed, Khanzada, Rajput and Lodhi (2015) proved the efficacy of plant extracts(neem and garlic) and biological antagonists i.e. *G.virens Paecilomyceslilacinus* & *T.harzianum* in control of *F. oxysporum* caused wilt disease in okra .They reported percent inhibition colony growth of pathogen in dual essay test and seed germination enhancement and low plant mortality of okra in pot experiments respectively. Seed treatments with plant leaf /rhizome /bulb extracts and biological antagonists are eco-friendly, non-toxic and promising alternative to control the fungal diseases of crops.

Table 1: Control of seed -borne infection of *Fusarium Oxysporum* by Plant Leaf Extracts

	Plant Leaf Extract	% Germination		% Control of Seedling Infection		% Control of Pathogen	
		Concentrated (100 %)	Diluted (30 %)	Concentrated (100 %)	Diluted (30 %)	Concentrated (100 %)	Diluted (30 %)
1	*Azadirachta indica*	68.33(13.66)	50 (10)	60 (2.66)*	75 (1.66)*	45.45 (4)*	81.81 (1.33)*
2	*Catharanthus roseus*	70 (14)	38.33 (7.66)	55 (3) *	15 (5.66)	54.54 (3.33)*	27.27 (5.33)
3	*Calotropis procera*	58.33(11.66)	55 (11)	40(4)	15 (5.66)	31.81 (5)	50 (3.66)*
4	*Cassia tora*	60 (12)	51.66 (10.33)	5(6.33)	0 (6.66)	0 (7.33)	0 (7.33)
5	*Datura innoxia*	60 (12)	56.66 (11.33)	60(2.66)*	40 (4)	59.09 (3)*	40.90 (4.33)
6	*Eucaluptusrudis*	20.66 (5.33)	78.33 (15.66)	80(1.33)*	80 (1.33)*	81.81 (1.33)*	72.72 (2) *
7	*Lantana camera*	63.33 (12.66)	58.33 (11.66)	70 (2)*	75 (1.66)*	72.72 (2)*	59.09 (3)*
8	*Lawsonia rosea*	60 (12)	90 (18)	50(3.33)	15 (5.66)	36 .36 (4.66)	4.54 (7)
9	*Nerium indicum*	50 (10)	51.66 (10.33)	20(5.33)	25 (5)	9.09 (6.66)	27.27 (5.33)
10	*Oscimum sanctum*	38.33 (7.66)	53.33 (10.66)	0(6.66)	65 (2.33)*	22.72 (5.66)	54.54 (3.33)*
11	*Ricinus communis*	46.66 (9.33)	53.33 (10.66)	40(4)	25 (5)	40.49 (4.33)	50 (3.66)
12	Control	50(10)	50 (10)	0(6.66)	0 (6.66)	0 (7.33)	0 (7.33)
13	CD at 5%	3.07	4.3	1.97	2.68	2.74	3.27

The figures in parentheses are in mean value of three replications

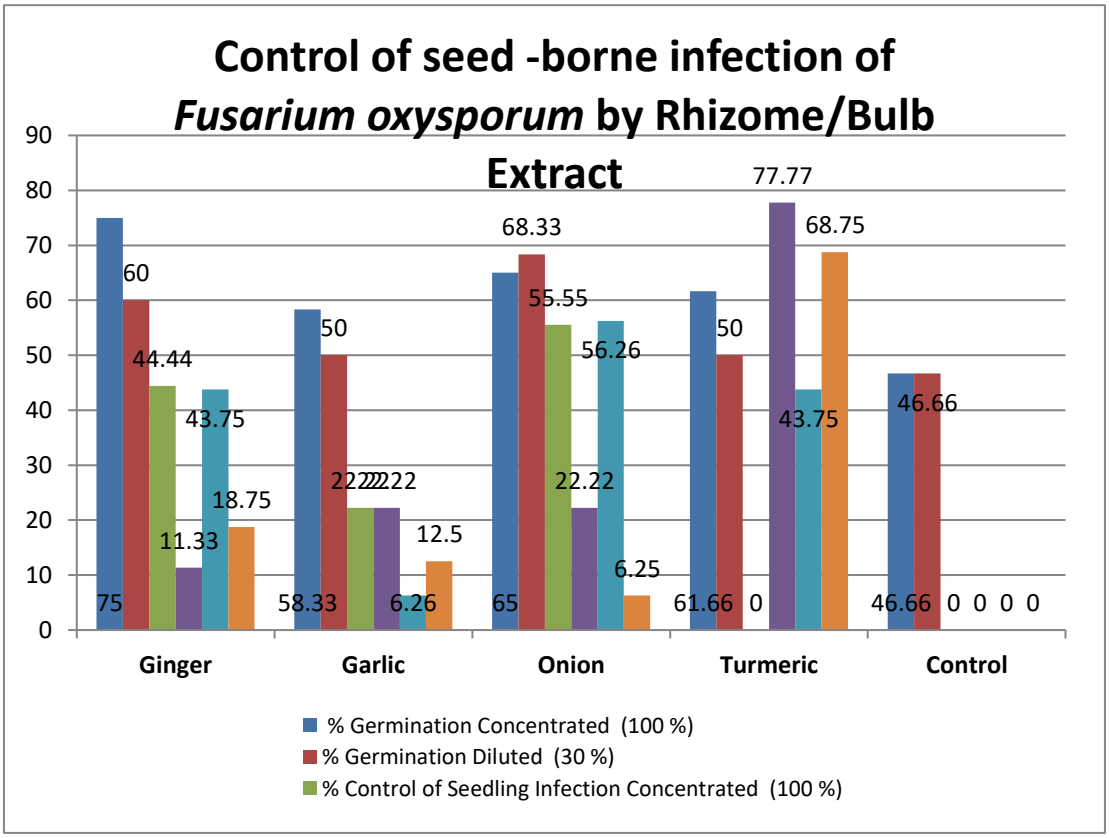


* Significant value at 5% level

Table 2: Control of Seed -Borne Infection of *Fusarium Oxysporum* by Rhizome/Bulb Extract

	Rhizome/ Bulb Extract	% Germination				% Control of Seedling Infection				% Control of Pathogen			
		Concentrated (100 %)		Diluted (30 %)		Concentrated (100 %)		Diluted (30 %)		Concentrated (100 %)		Diluted (30 %)	
1	*Ginger*	75	(15)	60	(12)	44.44	(1.66)	11.33	(2.66)	43.75	(3)*	18.75	(4.33)
2	*Garlic*	58.33	(11.66)	50	(10)	22.22	(2.33)	22.22	(2.33)	6.26	(5)	12.5	(4.66)
3	*Onion*	65	(13)	68.33	(13.66)	55.55	(1.33)	22.22	(2.33)	56.26	(2.33)*	6.25	(5)
4	*Turmeric*	61.66	(12.33)	50	(10)	0	(3)	77.77	(0.66)*	43.75	(3)*	68.75	(1.66)*
5	*Control*	46.66	(9.33)	46.66	(9.33)	0	(3)	0	(3)	0	(5.33)	0	(5.33)
6	*CD at 5%*	3.37		3.34		1.92		1.45		2.14		1.78	

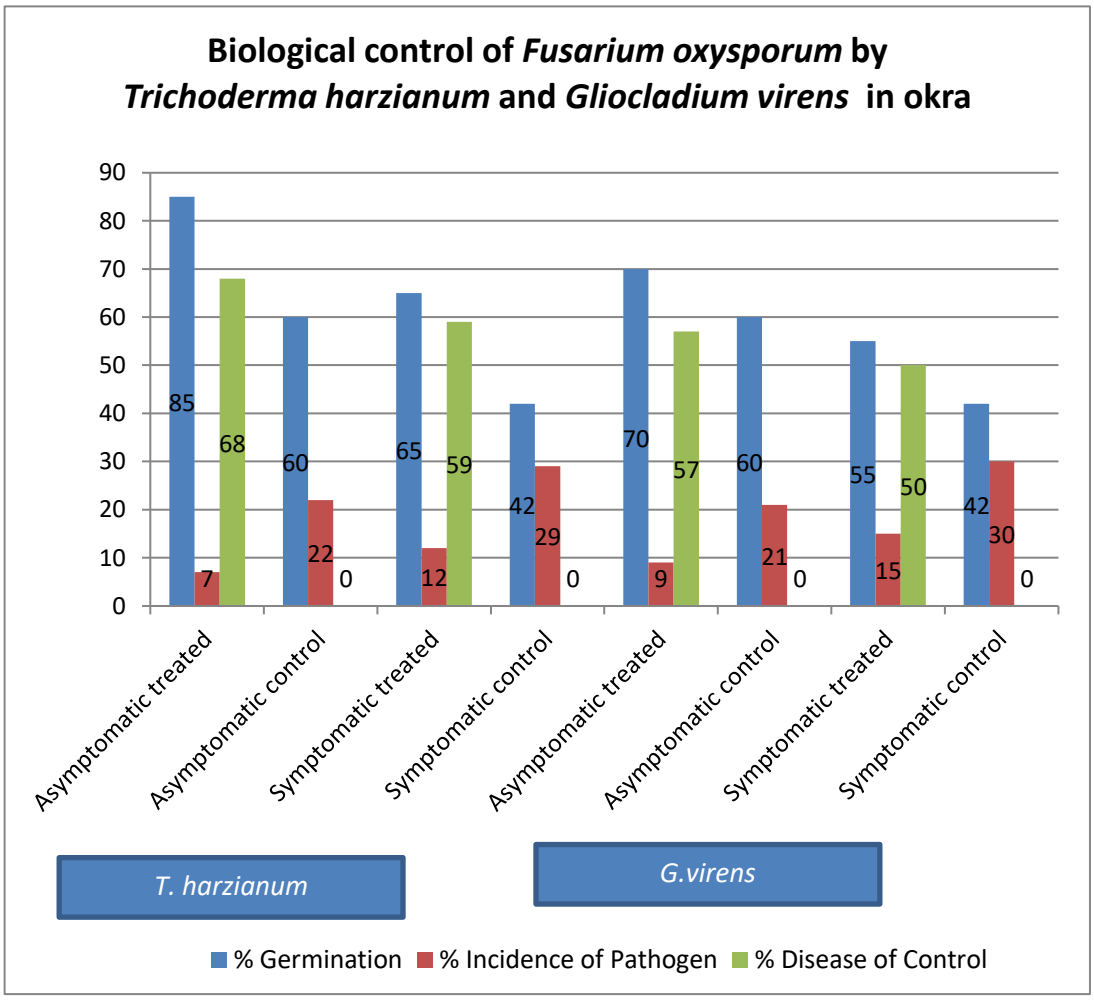
The figures in parentheses are in mean value of three replications

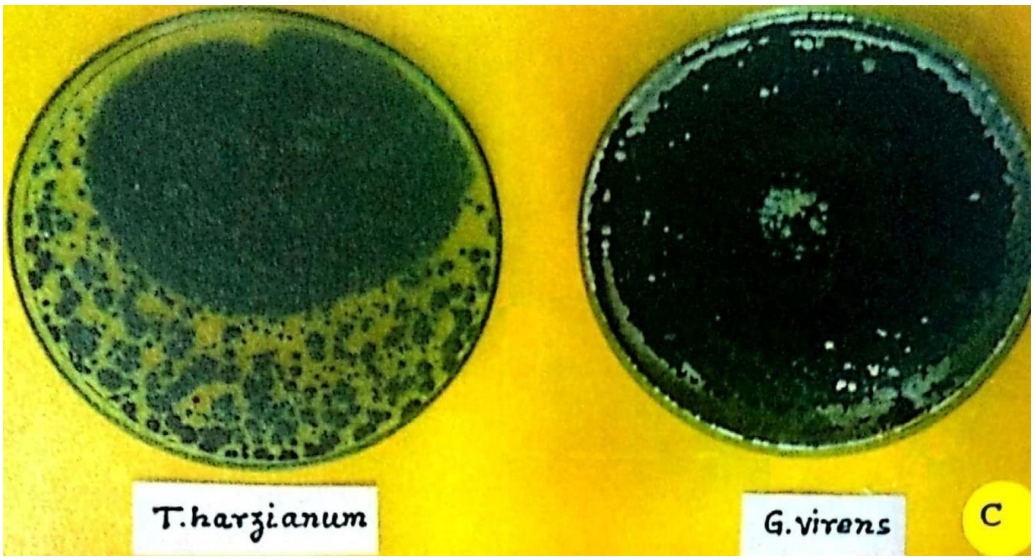


* Significant value at 5% level

Table 3: Biological control of *Fusarium Oxysporum*by *Trichoderma Harzianum* and *Gliocladiumvirens* in okra

Antagonists / Categories	% Germination	%Incidence of Pathogen	% Disease Control
Trichoderma harzianum			
Asymptomatic treated	85	7	68
Asymptomatic control	60	22	0
Symptomatic treated	65	12	59
Symptomatic control	42	29	0
Gliocladium virens			
Asymptomatic treated	70	9	57
Asymptomatic control	60	21	0
Symptomatic treated	55	15	50
Symptomatic control	42	30	0


Biological control of Fusarium oxysporum by Trichoderma harzianum and Gliocladium virens in okra
90
80
70
60
50
40
30
20
10
0
85
7
68
60
22
0
65
12
59
42
29
0
70
9
57
60
21
0
55
15
50
42
30
0
Asymptomatic treated
Asymptomatic control
Symptomatic treated
Symptomatic control
Asymptomatic treated
Asymptomatic control
Symptomatic treated
Symptomatic control
T. harzianum
G.virens
% Germination
% Incidence of Pathogen
% Disease of Control


T.harzianum
G.virens
C

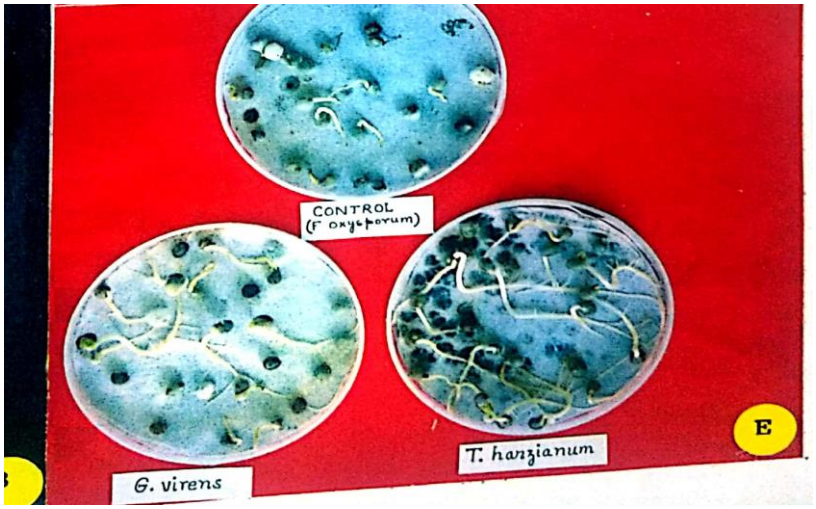


Figure 1: Control of *Fusariumoxysporum* by seed treatment with *Trichodermaharzianum* and *Gliocladiumvirens* . Petri plate (C) Pure growth of both bio- agents (E) Better germination and low infection on naturally infected seeds by *F. oxysporum*.

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