Integrated disease management strategy of *Fusarium oxysporum* f. sp. *sesami* causing Wilt of Sesame

SELIMA KHATUN

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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SELIMA KHATUN*
Department of Botany, Government General Degree College, Singur, Jalaghata, Hooghly 712409, West Bengal

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Biological control of Wilt disease of Sesame caused by *Fusarium oxysporum* f. sp. *sesami* was attempted with the application of antagonistic agents like *Trichoderma harzianum, T.viride, T.reesei, T.lignorum* and *T.hamatum.* The effect of volatile and non-volatile antibiotics of *Trichoderma* on growth inhibition of the wilt pathogen was studied. *T.harzianum* showed maximum growth inhibition (100%) of the pathogen through mycoparasitism.The volatiles produced by the *T. harzianum* showed maximum growth inhibition (76.66%) and the non-volatiles produced by the same agent exhibited its excellent and durable antagonism to the growth of the pathogen (100%) under *in vitro* condition. The degree of inhibition of mycelial growth of the pathogen by different phytoextracts (biocides) such as *Coleus forskohlii, neem, zinger, kulmegh, Catharanthus roseus, Achyranthes aspera* and *Solanum indicum* was studied. Among all the fungicides, Bavistin, a systemic fungicide, observed to be the most efficient one providing highest growth inhibition (100%) at 0.5% concentration. Integrated application of *T. harzianum*, captan and neem extract (1:1:1) showed maximum growth inhibition (82.22%) of test pathogen. This recommends the application of eco-friendly strategy of integrated disease management (IDM) for control of Wilt disease of Sesame.

Key words : Sesame, wilt pathogen, antagonistic agents, biocides, fungicides, integrated disease management

INTRODUCTION

Sesame, (*Sesamum indicum* L. family Pedaliaceae) is cultivated for their seeds. It is one of the most ancient oil seed crop cultivated in tropical and sub-tropical countries. The sesame seeds are very important today as a source of protein- rich edible oil for human nutrition. The oil contains two constituents sesamin and sesamolin which are responsible for their synergistic action as insecticides. Irrespective of the agro-climate conditions, sesame is liable to be infected by various pathogenic fungi.

Among the fungal diseases, wilt disease of sesame caused by *Fusarium oxysporum* f. sp. *sesami* (Zap.) Cast is the most devastating disease causing losses in seed yield in India. Fusarium wilt of sesame is quite serious wherever the crop is grown. In India, it has been reported from all the sesame growing areas, such as Madhya Pradesh, Maharastra, Andra Pradesh, Rajasthan, Haryana, Punjab (Adiver and Kumari, 2010). The disease is quite serious when it starts in the early stages of crop growth. *T. harzianum* was highly effective as compared to other *Trichoderma* species in controlling fusarial wilt of crop plants (Ojha, 2008). *Azadirachta indica* which was found to be the most efficient extract (98% inhibition), could be a promising material for controlling this fungus. Significant control of different wilt and root-rot diseases caused by *Fusarium* spp. with the application of systemic fungicides was documented by a number of workers (Kopacki and Wagner, 2006). Kapil and Kapoor (2005) reported the management of white rot of pea using *Trichoderma* species and biopesticides. Management of soil borne plant pathogens shall be effective economically attained following integrated way of disease management (Zewain *et al.* 2005).Integrated disease management strategy of *Fusarium oxysporum* f. sp. *sesami* causing wilt of sesame has been attempted by using antagonistic agents like different species of *Trichoderma*, phytoextracts and fungicides.

*Email: shelly_selima_india@yahoo.com

MATERIALS AND METHODS

Pathogens and biocontrol agents

Isolation of the pathogen (*Fusarium oxysporum* f. sp. *sesame*) from the diseased plants and its identification has been made following standard protocols. Among the five species of *Trichoderma, T.viride, T. lignorum and T.reesei* were isolated from Mycology and Plant Pathology laboratory, Burdwan University, West Bengal and the remaining two antagonists viz. *T. harzianum and T. hamatum* were procured from Indian Agricultural Research Institute (IARI), New Delhi. The pathogen as well as *Trichoderma* strains were grown on potato dextrose agar (PDA) plates for a week at $28^0 \pm 1^0$ C.

Competition and mycoparasitism of the screened antagonists against the test-pathogen

To study the competition and mycoparasitism of the antagonistic fungi, 'dual culture plating method' (Royse and Ries, 1978) and the antibiosis by 'food poisoning technique' (Mondal *et al*. 1995) has been adopted. Effect of volatile and non volatile antibiotics were performed following Dennis and Webster (1971 a,b) and Whipps (1987).

Screening of phytoextracts against the growth of the pathogen

Preparation of plant extracts

Plant extracts were prepared following the method of Ansari (1995). The fresh and healthy parts of locally available plants like leafy twigs of leaves of neem (*Azadirachta indica*), leaves of *Solanum indicum*, leaves of *Achyranthes aspera*, leaves of *Andrographis paniculata,* rhizome of zinger (*Zingiber officinale*), roots of *Coleus forskohlii* and leaves of *Catharanthus roseus* were collected. About 50 grams of each of the plant materials was washed thoroughly with distilled water and crushed in 50 ml distilled water in a mortar and pestle separately. It was passed through a double layered cheese cloth and then through Whatman filter paper No. 1. The filtrate was centrifuged at 5,000 rpm for 20 minutes. The supernatant was filtered with sterilized sintered glass filter (pore size 1-2 µ). The sterilized filtrates were stored at 10^0C for future use.

In vitro efficacy of plant extracts

In order to evaluate the efficacy of the prepared phytoextracts as biocides against *in vitro* growth of the test pathogen*, F.oxysporum* f. sp. *sesami* 'food poisoning technique' (Mondal *et al.* 1995) was adopted.

In vitro effect of fungicidal chemicals on growth of the pathogen

The effect of five fungicides viz. Hexaconizole, Copper hydrochloride, Bavistin (BASF India Ltd., Mumbai), Captan and Mancozeb (Anum-45, Anu Production Ltd., Haryana) were tested by using poisoned food technique in liquid medium under laboratory condition. Different doses (0.05%, 0.1% and 0.5%) of each fungicide were prepared in distilled water. *In vitro* growth of fungicidal chemicals on growth of the pathogen was tested by using "food poisoning technique" (Mondal *et al*. 1995).

Bioagent based integrated disease management (IDM) approach towards control of the pathogen

The investigation was made to study integrated management of the pathogen with application of screened potential antagonists (*T. harzianum* and *T. viride*), the fungicide, Captan and the plant extracts (Neem and Achyranthes). Although Bavistin was recorded as the most effective fungicide against the growth of the pathogen but the antagonistic fungi, *Trichoderma* spp. were found to be very much sensitive to Bavistin. So during integrated disease management strategy Captan was tested at 0.1% concentration as *Trichoderma* spp. were noted to be insensitive to that concentration of Captan. Preparation of plant extracts, culture filtrate of *Trichoderma* species and fungicidal concentration (%) were made individually as done previously during their individual *in vitro* studies.

Different compatible combinations were made for the experiment viz. *T. harzianum* + Captan, *T.viride* + Captan, *T. harzianum* + Captan+ Neem, *T.viride*+ Captan+ Neem, *T. harzianum* + Captan+ *Achyranthes*, *T.viride* + Captan + *Achyranthes*. About 15 ml of PDA medium at pH 7.2 was sterilized and plated in Petri dishes and just before solidification of the medium, selected quantities of IDM approaches were added separately to the Petri dishes and mixed thoroughly to have a homogeneous mixture. The medium was cooled at room temperature and inoculated with a 5 mm inoculum disc cut out aseptically from the actively growing 6 days old culture of the test pathogen, *F.*

oxysporum f. sp. *sesam*i. Control plates without any treatments were also simultaneously inoculated for comparison. The inoculated plates were incubated at $28^0 \pm 1^0$C till the pathogen completely covered the control plates. The radial growth of the colony in each treatment was measured and the per cent inhibition of growth was calculated.

Statistical analysis

Data were expressed as mean±standard error. Significant differences among the means were determined by Fisher's least-significant difference test after one-way analysis of variance. Significance of between-treatment means was tested at the 0.05 level of probability using Stat Plus Version 4.8, 2007 software.

RESULTS AND DISCUSSION

Evaluation of potentiality of the screened antagonists for control of the pathogen

Antagonistic efficiency of the screened fungi against *F. oxysporum* f. sp. *sesami* was done in the following manner:

Competition and mycoparasitism of the screened antagonists against the test-pathogen

Table 1 reveals that five *Trichoderma* spp., were the most effective antagonists against the test pathogen. Highest response was performed by *T. harzianum* (100%) followed by *T.viride* (95.33%), *T.reesei* (90.88%), *T. lignorum* (84.88%) and *T. hamatum* (70.89%). Thus evident from Table 1, *T. harzianum* was proved to be most potent one.

Out of five *Trichoderma* spp., *T. harzianum* was proved to be most deterrant to *F. oxysporum* f. sp. *sesami* showing 100% growth inhibition followed by *T. viride* (95.33%) and *T.reesei* (90.88%) respectively which corroborate the findings of Rajeswari and Kannabiran (2011). Inhibition of colony growth of *F.oxysporum* was earlier reported (Brasier, 1975). Fakhrunnisa and Ghaffar (2006) confirmed that *T. harzianum* inhibited radial growth of *F. oxysporum* to the extent of 79.97%. Effectiveness of different *Trichoderma* species against *F. oxysporum* was reported by Dubey and Suresh (2006). Interaction between *Trichoderma* and plant pathogenic fungi includes competition for nutrients and space (Alabouvette *et al.,* 2009), inactivation of the enzymes produced by the pathogens (Ozbay and Newman, 2004), modifying the environmental conditions (Benitez *et al*. 2004), production of inhibitory soluble metabolites (Ghisalberti *et al.* 1993), production of inhibitory volatiles (Bruce *et al*. 1984).

Production of soluble metabolites by Trichoderma spp.

Table 2 shows the growth of inhibition of the test fungus with soluble metabolic product of *Trichoderma* spp. The antagonistic agents for biocontrol were more or less able to bring about significant reduction of radial growth of *F.oxysporum* f. sp. *sesami* and that potentiality of inhibition depended on the amount of metabolites used. Among the five *Trichoderma* spp. maximum retardation of growth of the pathogen (100%) was achieved when 10 ml. of matablites of *T.harzianum* was added to 15 ml of PDA medium while minimum was recorded with *T.hamatum* (28.28%). The other three species viz. *T. viride, T. reesei* and *T. lignorum* showed efficiency in this regard as 90.67%, 80.22% and 52.00% respectively. It is also clear from the result that with the rise in the amount of growth metabolites of the antagonists, the percentage growth biomass of the pathogen was retarded, which resulted its cent per cent inhibition when treated with 10 ml of the metabolites of the antagonists *T.harzianum* experimented. Statistically it may be concluded that all the *Trichoderma* species exhibited significant reduction in mycelial growth of the pathogen of which *T. harzianum* is significantly most efficient.

The results (Table 1 and 2) show that *Trichoderma* spp. were able to check the growth of the test pathogen through competition for nutrients and space and parasitism through the production of soluble metabolites. *Trichoderma* spp. produced antifungal substances act as mycoparasite and lyse the pathogenic superior capacity to take up and mobilize nutrients compared to other organisms (Benitez *et al.* 2004). *Trichoderma* spp. were reported to compete for space and nutrients, production of diffusible and/or volatile and non-volatile antibiotics and hydrolytic enzymes which partially degrade the pathogen cell wall and leads to its parasitisation (Rajeswari and Kannabiran, 2011). The result (Table 2) suggests that toxic metabolites, secreted in culture broth by five

Table 1: Antagonistic potentiality of *Trichoderma* spp. through mycoparasitism on growth of *F. oxysporum* f. sp. *sesami* following 'dual culture plating'. Data showing mean± standard error of five replicates indicate significant differences between the control and treated fungi

Antagonists	Radial growth of the pathogen (cm)	Radial growth of the antagonists(cm)	Growth inhibition of the pathogen (%)
Trichoderma harzianum	0	9	100±0
T.viride	0.4	8.6	95.33±0.41
T. reesei	0.8	8.2	90.88±0.42
T.lignorum	1.3	7.7	84.88±1.09
T.hamatum	2.6	6.4	70.89±0.89
Control	9.0	0	0

Table 2: Effect of soluble metabolites of *Trichoderma* spp. on growth of *F. oxysporum* f. sp. *sesami* following 'food poisoning technique'. Data showing mean± standard error of five replicates indicate significant differences between the control and treated fungi (P<0.05)

Antagonists	Dosage of the culture filtrate of the antagonists(ml)	Radial growth of the pathogen(cm)	Growth inhibition of the pathogen(%)
Trichoderma harzianum	1ml	5.6	37.55±0.65
	5ml	2.1	75.77±0.65
	10ml	0	100±0
T.viride	1ml	7.6	14.89±1.56
	5ml	3.6	60.00±0.70
	10ml	0.8	90.67±0.44
T. reesei	1ml	8.1	9.77±0.65
	5ml	5.6	37.55±0.89
	10ml	1.7	80.22±0.82
T.lignorum	1ml	9.0	0
	5ml	7.3	18.89±0.93
	10ml	4.3	52.00±1.02
T.hamatum	1ml	9.0	0
	5ml	8.4	6.44±0.54
	10ml	6.4	28.88±0.70
Control	-	9	0

Trichoderma species, brought about significant reduction in vegetative growth of *F. oxysporum* f. sp. *sesami* and maximum inhibition was achieved with *T. harzianum*. *Trichoderma* isolates have been reported to produce a wide range of soluble metabolites, which are inhibitory to other fungi (Horvath *et al.* 1995) and harzianolide is one of the important antifungal metabolites produced by *Trichoderma* (Avent *et al.* 1992). The culture filtrates of *Trichoderma* spp. contain some kinds of antibiotics or enzymes (Di Pierto *et al*. 1993) which are involved in antifungal activity of the antagonists.

Studies on the production of inhibitory volatiles and non-volatiles by Trichoderma spp.

The data presented in Table 3 indicate that all the five *Trichoderma* species were capable of producing some volatile substances which inhibit the growth of the test-pathogen. The *Trichoderma* spp. again showed inter specific variability in growth inhibition of the pathogen by volatile antibiotics of their own. The result demonstrated that the volatiles produced by *T. harzianum* were most promising to check the radial growth of the pathogen wherein *T. harzianum* exhibited 76.66% inhibition. This was followed by 71.38% growth inhibition with *T.viride* and 49.16% with *T. reesei* respectively. From the results of Table 3 it was clear that non-volatiles produced by the antagonists were inimical to the growth of the pathogen and all the *Trichoderma* spp. were almost effective to check the growth of the pathogen. So it was clear from the results (Table 3) that *Trichoderma* spp. were able to produce both volatile and non-volatile antibiotics and the non-volatiles were recorded to be more durable in their action than the volatiles.

Table 3: Antagonistic effect of volatile and non volatile antibiotics produced by *Trichoderma* spp. on growth of *F.oxysporum* f. sp. *sesami*. Data showing mean± standard error of five replicates indicate significant differences between the control and treated fungi (P<0.05)

Antagonists	Antibiotics	Radial growth of the pathogen(cm)	Growth inhibition of the pathogen(%)
Trichoderma harzianum	Volatiles	2.1	76.66±0
	Non volatiles	0	100±0
T.viride	Volatiles	2.6	71.38±0.27
	Non volatiles	0	100±0
T. reesei	Volatiles	4.6	49.16±0.28
	Non volatiles	0	100±0
T. lignorum	Volatiles	4.8	46.61±0
	Non volatiles	0	100±0
T. hamatum	Volatiles	5.5	38.87±0
	Non volatiles	0	100±0
Control	Volatiles	9	0
	Non volatiles	9	0

Table 4: Effect of phytoextracts on growth of *F. oxysporum* f. sp. *sesami* following "food poisoing technique" in solid medium. Data shows mean ± standard error of 5 replicates, indicate significant differences between the control and treated ones (P < 0.05)

Phytoextracts	Quantity of the extract (ml)	Radial growth of the pathogen(cm)	Growth inhibition of the pathogen(%)
Coleus forskohlii	1ml	6.6	73.55±1.13
	5ml	0	100±0
	10ml	0	100±0
Neem	1ml	7.5	83.33±0
	5ml	0	100±0
	10ml	0	100±0
Zingiber officinale	1ml	6.4	71.11±0.50
	5ml	0	100±0
	10ml	0	100±0
Kulmegh	1ml	6.4	71.33±0.65
	5ml	0	100±0
	10ml	0	100±0
Catharanthus roseus	1ml	5.5	60.66±0.67
	5ml	0	100±0
	10ml	0	100±0
Achyranthes aspera	1ml	7.4	82.44±0.41
	5ml	0	100±0
	10ml	0	100±0
Solanum indicum	1ml	5.4	60.22±0.42
	5ml	0	100±0
	10ml	0	100±0
Control	-	9	0

It is apparent from the results (Table 3) that production of volatile and non-volatile antibiotics was involved in the antagonistic activity of the members of the genus *Trichoderma* against the test pathogen. Inhibition of germination and mycelial growth of pathogenic fungi *in vitro* was attributed to the antifungal properties of volatile compounds (alkyl pyrones) produced by *T. harzianum* (Claydon *et al*. 1987). Role of diffusible volatile compounds produced by *T. viride* and *T. harzianum* in the inhibition of germination and mycelial growth of *Fusarium oxysporum in vitro* was reported (Khatun, 2012). It had been reported that volatile and non-volatiles produced by *Trichoderma* spp. showed inhibitory effect on soil-borne plant pathogens including *Fusarium* spp. (Wani, 2005). *Trichoderma* strains antagonizing *F. oxysporum* through the production of volatile and non-volatile compounds had been reported (Dubey and Suresh, 2006). Strains of *Trichoderma* spp. produce many types of secondary metabolite (Alabouvette *et al*. 2009) including antibiotics (Howel, 1998) and CWDEs (Lorito, 1998), the role of which has been clearly established in biocontrol

Table 5: *In vitro* effect of fungicidal chemicals on growth of *F. oxysporum* f. sp. *sesami*. Data shows mean ± standard error of 5 replicates, indicate significant differences between the control and treated ones ($P < 0.05$)

Treatments	Dose (%)	Radial growth of the pathogen(cm)	Growth inhibition of the pathogen(%)
Hexaconazole	0.05	2.5	27.99±0.74
	0.1	5.5	60.89±0.65
	0.5	6.1	68.44±0.27
Copper hydrochloride	0.05	0.5	5.55±0.93
	0.1	1.8	19.77±0.89
	0.5	2.5	27.33±067
Bavistin (Carbendazim)	0.05	4.8	52.89±1.20
	0.1	5.7	63.77±1.14
	0.5	0	100±0
Captan	0.05	1.7	19.11±1.08
	0.1	3.9	43.55±0.42
	0.5	5.8	64.88±0.90
Mancozeb	0.05	0.8	8.44±1.20
	0.1	1.9	20.66±1.03
	0.5	4.8	53.33±0.93

Table 6: Integrated management strategy towards *in vitro* growth of *F. oxysporum* f. sp. *sesami* with biocides and fungicide. Data shows mean ± standard error of 5 replicates

Treatments	Amount (ml)	Radial growth of the pathogen (cm)	Growth inhibition of the pathogen (%)
Azadirachta indica+Captan	1+1	2.8	68.88±0
(0.01%)	2+1	2.0	77.76±0
	3+1	1.2	90.01±0
	4+1	0.0	100±0.00
Achyranthes aspera +	1+1	4.2	53.30±0.02
Captan (0.01%)	2+1	2.8	68.87±0
	3+1	2.3	74.44±0
	4+1	0.0	100±0.00
Coleus forskohlii + Captan	1+1	4.8	46.66±0
(0.01%)	2+1	3.7	58.82±0.01
	3+1	2.9	67.71±0.01
	4+1	0.0	100±0.00
Control	-	9	0

activity. Woo and Lorito (2007) demonstrated that a strain of *T. harzianum* produced different secondary metabolites depending not only on the plant to which it was applied but also on the target pathogen infecting that plant.

A comparison between the inhibition by volatile and non-volatile antibiotics of *Trichoderma* isolates (Table 3) revealed that non-volatiles were able to check the growth of the test-pathogen almost completely (with *Trichoderma harzianum, T. viride, T. hamatum*) whereas the volatiles resulted in reduction of fungal growth by 76.66% with *Trichoderma harzianum*, 71.38% with *T. viride* and 49.16% with *T. reesei*. Similar superior activity of non-volatiles of *Trichoderma* over volatiles was reported by Kucuk and Kivanc (2003). Most *Trichoderma* strains produce volatile and non-volatile toxic metabolites that impede colonization by antagonizing microorganisms; among these metabolites, the production of harzianic acid, tricholin, peptaibols, 6-pentyl-α-pyrone, massoilactone, viridian, glioviridin, glisopenins, heptilidic acid have been described (Vey *et al*. 2001). In addition to these suzukacillin (Ooka *et al.* 1966), alamethicine (Meyer, 1966), demadin

Table 7: Integrated management approach towards *in vitro* growth of the pathogen with biocontrol agents, fungicide and biocides. Data shows mean ± standard error of 5 replicates, indicate significant differences between the control and treated ones (P < 0.05)

Treatments	Ratio	Radial growth of the pathogen(cm)	Growth inhibition of the pathogen(%)
T.harzianum+Captan	1:1	5.2	42.22±1.05
T.viride+ Captan	1:1	6.2	30.44±0.97
T.harzianum+Captan+Neem	1:1:1	1.6	82.22±0.22
	3:3:1	0	100±0
T.harzianum+ Captan + *Achyranthes*	1:1:1	2.2	76.88±1.24
	3:3:1	0	100±0
T.viride+ Captan +Neem	1:1:1	3.2	64.22±1.28
	3:3:1	0	100±0
T.viride+ Captan + *Achyranthes*	1:1:1	4.4	50.66±1.47
	3:3:1	0	100±0
Control	-	9	0

(Pyke and Dietz, 1966), trichodermin (Krivoshchekova and Mischenk, 1990) are some of the antibiotics extracted from culture filtrates of *Trichoderma* spp. A new antifungal compound viz. 6-substituted 2H-pyran-2-one named viridipyronone has been isolated from the culture filtrate of a strain of *T. viride* showing *in vitro* activity towards plant pathogenic fungi (Evidente *et al.* 2003).

The Table 3 confirmed that *T. harzianum*, *T. viride* and *T. reesei* are able to inhibit the growth of the pathogen efficiently and among them *T. harzianum* is found to be most potent antagonist. Vyas and Mathur (2002) also reported that *T. harzianum*, *T. viride* and *T. hamatum* effectively inhibited the growth and sporulation of *F. oxysporum* through production of voilatile and non-volatile antibiotics. When a small amount of mycelium of the pathogen from the experimental petriplate was transferred to a fresh PDA medium, it showed renewal growth. Thus the antibiotics produced by *Trichoderma* are fungistatic rather than fungicidic.

Screening of phytoextracts against the pathogen

The results (Table 4) reveal that *in vitro* growth of *F. oxysporum* f. sp. *sesami* was effectively inhibited by some plant extracts. The vegetative growth of the pathogen was drastically reduced with the extracts of *Azadirachta indica* (Neem) and *Achyranthes aspera* where Neem preparation exhibited highest significant (P<0.05) growth inhibition against the mycelia growth of the pathogen. Extracts of *Coleus forskohlii, Andrographis paniculata, Zingiber officinale,* and *Catharanthus roseus w*ere effective even at their minimum concentrations. The effectiveness of the extracts was found to increase with increase in quantity of extracts and maximum inhibition of growth of the pathogen was recorded when 1 ml of the extracts was mixed with 15 ml of PDA medium. Neem extract showed 83.33 % and100% inhibition of growth of the pathogen at a dose of 1 ml and 5 ml in 15 ml of growth medium respectively followed by *Achyranthes aspera* extract which showed 82.44% and 100 % inhibition at the same concentrations. The effectiveness of other biocides remained at 73.55% for *Coleus forskohlii*, 71.33% for *Andrographis paniculata*, 71.11% for *Zingiber officinale*, 60.66% for *Catharanthus roseus* and 60.22% for *Solanum indicum* at a dose of 1 ml. The extracts of *Catharanthus roseus* and *Solanum indicum* were least effective.

In vitro evaluation of plant extracts against the test-pathogen revealed that different extracts differed markedly in their fungitoxicity (Table 4). The vegetative growth of the pathogen was drastically reduced with the extracts of *Azadirachta indica* (Neem) and Achyranthes aspera where Neem extracts showed significantly (P < 0.05) highest for the mycelial growth inhibition of the pathogen. Leaf extracts of *Azadirachta indica* (Neem) effectively reduced the mycelial gowth of different plant pathogenic fungi as evidenced from the studies of different workers (Natarajan *et al.* 2003; Ramachandra and Kalappanavar, 2006b).

Plant extracts are being used to control the diseases since last several years. The extract of *Azadirachta indica* showed maximum activity to control of *Fusarium moniliforme* has been observed by Pawar (2011). Effective control of *Fusarium* spp. and their races with the application of Neem preparation has also been reported by earlier investigators (Haseeb and Kumar, 2007; Chakraborty *et al*. 2009). Brahmachari (2004) concluded that such an antifungal activity of Neem extract might be due to the presence of active chemicals like azadirachtin, nimbidin, nimbinin, nimbolidin, nivasin etc. contained in the extract. Obongoya *et al.* (2010) used *Azadirachta indica, Nicotiana tabacum* and *Vinca rosea* for the control of *Fusarium oxysporum* Schl. f. sp. *phaseoli*. Neem leaves inhibited the soil borne fungi *F. oxysporum* when used as a soil treatment (Ojha, 2008). The present findings complied with the views of Bansal and Gupta (2000); and Agabenin and Marley (2006) where leaf extracts of *A. indica* were most effective against *F. oxysporum*. Chloroform and methanolic root and shoot extracts of *A. aspera* showed good amount of antifungal activity against *Fusarium* spp. (Khatun, 2012). In the present studies antifungal effect of Achyranthes extract may also be due to presence of alkaloids, long chain of alcohol or essential oil or may be due to some another unidentified bioactive secondary metabolites with antagonistic activities. The phytochemical analysis of methanolic extract (alkaloids, sterols, triterpenes, flavonoids, carbohydrates) choloroform and petroleum ether extract (triterpenes, Sterol and azulene derivatives) of *Achyranthes aspera* obtained by infusion revealed no antimicrobial effects (Londonkar *et al*. 2011).

In vitro effect of fungicidal chemicals on growth of F. oxysporum f. sp. sesami

Results presented in Table 5 indicate the effect of commonly available fungicides on growth inhibition of *F. oxysporum* f. sp. *sesami* under laboratory conditionds. The fungicides showed different grades of inhibition in terms of biomass production of the pathogen. The systemic fungicide Bavistin had shown its superiority in inhibiting vegetative growth of the pathogen where at 0.5% concentration, 100% inhibition was obtained. Next to Bavistin, Hexaconizole exhibited 68.44% growth inhibition at a 0.5% concentration where Captan and Mancozeb showed 64.88% and 53.33% inhibition of mycelia growth of the test pathogen at 0.5 % concentration. Mancozeb (64.88% at 0.5 % concentration) and Copper hydrochloride (27.33 % at 0.5 % concentration) were least effective.

The systemic fungicide Bavistin (Carbendazim) had shown its superiority in reducing the growth of the pathogen. Carbendazim (Bavistin) was proved to be the most effective fungicide against many fungal pathogens as evidenced from the works by Ramachandra and Kalappanavar (2006a), and Clara *et al*. (2007). The promising effect of carbendazim (Bavistin) on systemic pathogen like *Fusarium avenacerum* (Kopacki and Wagner, 2006), *F. moniliforme* (Titone *et al*. 2004), *F. oxysporum* (Bharath *et al*. 2005) was well documented.

It is evident from the result that Captan provided 64.88% growth inhibition of the test fungus at 0.5% concentration (Table 5). Similar effective response of Captan towards growth inhibition of *Fusarium* sp. was reported by Khatun (2012). Lyr (1987) also noticed that Captan inhibits the radial mycelia growth of *F. oxysporum*. According to Arinze and Yubedee (2000) Captan inhibited the activity of cellulase of *Fusarium moniliforme*. Another report of Dutta and Chatterjee (2000) was obtained where *Fusarium dampieri* remains less susceptible to Mancozeb and Tridemorph.

Integrated disease management (IDM) strategy

Integrated management of the pathogen with the application of a combination of plant extracts, minute dose of Captan and antagonistic *Trichoderma* species was carried out.

Integrated management strategy towards in vitro growth of F. oxysporum f. sp. sesami with biocides and fungicide

It is evident from the result (Table 6) that treatment with Neem extract + Captan was most effective against *in vitro* growth of the pathogen. Combination of *Achyranthes* extract and Captan was proved to be the next best treatment. When 3 ml of plant extract was combined with 1 ml (0.01%) of fungicide, i.e. Neem + Captan, it showed 90.01 % while *Achyranthes* + Captan showed 74.44 % growth inhibition of the pathogen respectively. Cent per cent inhibition of mycelia growth of the pathogen was exhibited with a combination of 4 ml of plant extract and 1 ml of fungicide in case of Neem and *Achyranthe*s. Coleus + Captan

treatment was recorded to be less effective than the other treatments. From statistical point of view, integrated treatment with Neem + Captan is significantly superior over the others.

It may be noted from the result (Table 6) that a combined treatment with the effective biocides and a fungicide (Captan) showed significant inhibition of *in vitro* growth of the pathogen. Kapoor *et al*.(2006) reported that combined treatment with plant extract and fungicide was effective against *F. solani* and *F. oxysporum.*

Integrated management approach towards in vitro growth of the pathogen with biocontrol agents, fungicide and biocides

The integrated management of the pathogen with the application of potentially effective antagonists, plant extract and minimal dose of the fungicide (Captan) was performed. It is evident from the result (Table 7) that a combined treatment with 1 ml of cell free culture filtrate of *T. harzianum* + 1 ml 0.01% Captan showed 42.22 % inhibition of growth of the pathogen. A combination of *T.viride* with Captan in similar proportion showed 30.44% growth inhibition. Thus integration of *T. harzianum* with Captan was found to be superior over the other combination. Combinations like culture filtrate of *T. harzianum* + Neem extract + Captan, *T.harzianum* + *Achyranthes* extract + Captan, *T.viride*+Neem extract+Captan, *T.viride*+ *Achyranthes* extract+ Captan, all at a proportion of 3 ml+ 3 ml+ 1 ml offered cent per cent growth inhibition of *F.oxysporum* f. sp. *sesami*. The treatment with culture filtrate of *T. harzianum*+ Neem extract+ Captan at a proportion of 1 ml + 1 ml + 1 ml resulted inhibition at a rate of 82.22% as compared to the other treatments. Statistically it may be concluded that significant ($P<0.05$) growth inhibition of the test pathogen, *F. oxysporum* f. sp. *sesami* could be achieved with different integrated combinations. It is also apparent from statistical analysis that an integrated combination of *T. harzianum* + Neem + Captan is the most significant one.

The result indicated that successful control of the pathogen can be achieved with the application of different integrated treatment. Application of *T. harzianum* and *T. viride* in combination with different fungicides were effective against a number of fungal pathogen including *Fusarium* species (Omar *et al.* 2006). Dubey (2002) reported that combined treatment with Captan and *T. viride* resulted in significant inhibition of collar rot of French bean caused by *Macrophomina phaseolina.* Although Bavistin (carbendazim) was recorded as the most effective fungicide against the pathogen (Table 5), the potentially effective antagonists like *T. harzianum* and *T. viride* were found to be very much sensitive to Bavistin even at the lower concentrations but they were less sensitive to Captan. Similar responses of carbendazim and Captan on growth of *T. harzianum* and *T. viride* were also reported by Sharma *et al.* (2001) and Khalko *et al*. (2006). Kay and Stewart (1994) found that *Trichoderm*a spp. including *T. harzianum* were insensitive to Captan. Papavizae *et al.* (1982) recorded that Captan was tolerant for *T. harzianum* even at higher concentrations. Since biocontrol agents have to be applied in soil, it becomes imperative to ascertain its tolerance to agrochemicals used in crop production technology (Sharma and Mishra, 1995). Thus during integrated disease management strategy, the fungicide Captan was employed.

Integrated management approach, being the safest and ecofriendly pragmatic strategy for the management of wilt disease, has also been reported (Manju *et al*. 1999). So the integration of *T. harzianum* as biocontrol agent with fungicides for the effective management of soil-borne diseases is present day need (Chakraborty *et al*. 2009, Khatun, 2012).

The present study indicates that the treatments in permutation and combinations with biocontrol agents, phytoextractives and a minimal dose of fungicide reduce *in vitro* growth of wilting pathogen of sesame significantly which is suggestive of an eco-friendly alternative to existing expensive and hazardous chemical management practices.

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REFERENCES

Adiver, S.S. and Kumari. 2010. *Management of fungal plant patho-*

gens. In: Fungal disease of oilseed crops and their management, CAB International, U.K., pp. 263-274.

Agabenin, O.N. and Marley, P.S. 2006. *In vitro* assay of some plant extracts *against Fusarium oxysporum* f. sp. lycopersici causal agent of tomato wilt*. J. Plant Prot. Res*. **46**: 215-220.

Alabouvette, C., Olivain, C., Migheli, Q. and Steinberg, C. 2009. Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. *New Phytol*. **184**:529-544.

Ansari, M.M. 1995. Control of sheath blight of rice by plant extracts. *Indian Phytopathogy*. **48**: 268-270.

Arinze, A.E. and Yubedee, A.K. 2000. Effect of fungicides on Fusarium grain rot and enzyme production in maize (*Zea mays* L.). *Glob. J. Appl. Sci*. **6:**629-634.

Avent, A.G., Hanson, J.R. and Trunen, A. 1992. The biosynthesis of harzianolide by *Trichoderma harzianum. Phytochemistry*. **31**: 791-793.

Bansal, R.K. and Gupta, R.K. 2000. Evaluation of plant extract against *Fusarium oxysporum*, wilt pathogen of fenugreek. *Indian Phytopathology*. **53**: 107-108.

Benitez, T., Rincon, A.M., Limon, M.C. and Codon, A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbiol.* **7:**249-260.

Bharath, B.G., Lokesh, S., Rai, V.R., Prakash, H.S., Yashovarma, B. and Shetty, H.S. 2005. Role of foliar spray in the infection biology and management of fungal diseases of watermelon [*Citrullus lanatus* (Thumb.) Matsum and Nakai]. *World J. Agri. Sci*. **1**: 105-108.

Brahmachari, G. 2004. Neem-an omnipotent plant: a retrospection. *Chem. Biochem*. **5**: 408-421.

Brasier, C.M. 1975. Stimulation of sex organ formation of *Phytophthora* by antagonistic species of *Trichoderma*. *New Phytology*. **74**:183-194

Bruce, A., Austin, W.J. and King, B. 1984. Control of growth of Neolentinus lepidus by volatiles from *Trichoderma. Trans. Br. Mycol. Soc*. **82**: 423-428.

Chakraborty, M.R., Chatterjee, N.C. and Quimio, T.H. 2009. Integrated management of fusarial wilt of eggplant (*Solanum melongena*) with soil solarization. *Micol. Aplicad. Int*. **21**:25-36.

Clara, M.L., Kadvani, D.L. and Dangaria, C.J. 2007. Efficacy of fungicides in controlling blast disease of pearl millet. *Indian Phytopathology*. **60**:68-71.

Claydon, N., Allan, M., Hanson, J.R. and Avent, A.G. 1987. Antifungal alkyl pyrenes of *Trichoderma harzianum*. *Trans. British Mycol. Soc*. **88**:503-513

Dennis, C. and Webster, J. 1971a. Antagonism properties of species groups of *Trichoderma* I. Production of non-volatile antibiotics. *Trans. Brit. Mycol. Soc*. **57**: 25-39.

Dennis, C. and Webster, J. 1971b. Antagonism properties of species groups of *Trichoderma II*. Production of volatile antibiotics. *Trans. Brit. Mycol. Soc*. **57**: 41-48.

Di Pierto, A., Lorito, M., Hayes, C.K., Broadway, R.M. and Harman, G.E. 1993. Endochitinase from *Glicladium virens*: Isolation, characterization and synergistic antifungal activity in combination with gliotoxin. *Phytopathology*. **83**:308-313.

Dubey, S.C. 2002. Bio-agent based integrated management of collar rot of French bean. *Indian Phytopathol*. **55**: 230-231.

Dubey, S.C. and Suresh, M. 2006. Randomly amplified polymorphic DNA markers of Trichoderma species and antagonism against *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *J. Phytopathology*. **154**:663-669.

Dutta, S. and Chatterjee, N.C. 2000. Induction of resistance against fusarial wilt of glory pea (*Clianthus dampier*). *Indian Biology*. **32**:97-102.

Evidente, A., Cabras, A., Maddau, L., Serra, S., Andolfi, A. and Motta, A. 2003. Viridepyronone, a new fungal 6-substituited 2H-pyran-2-one produced by *Trichoderma viride*. J. *Agric. Food Chem*. **51**: 6957-6960.

Fakhrunnisa, H. M. H. and Ghaffar, A. 2006. *In vitro* interaction of *Fusarium* spp., with their fungi. *Pak. J. Bot*. **38**:1317-1322

Ghisalberti, E.L., Rowland, C.Y., and Sivasitamparam, K. 1993. Metabolites of *Trichoderma harzianum:* 6th ICPP Montreal, *Canada Abstract*. p. 292.

Haseeb, A. and Kumar, V. 2007. Efficacy of bioagents and organic amendment materials against *Fusarium oxysporum* causing brinzal wilt. *Indian Phytopathology*. **60**:108-111.

Horvath, E.M., Burgel, J.L. and Messner, K. 1995. The production of soluble antifungal metabolites by the biocontrol fungus *Trichoderma harzianum* in connection with the formation of conidiospores. *Mat. Org*. **29**: 1-14.

Howel, C.R. 1998. *The role of antibiosis in biocontrol*. In: *Trichoderma and Gliocladium*, Volume 2, Enzymes, biological control and commercial application. London, UK: Taylor and Francis Ltd, pp.173–180.

Kapil, R. and Kapoor, A.S. 2005. Management of white rot of pea incited by *Sclerotinia sclerotiorum* using *Trichoderma* spp. and biopesticides. *Indian Phytopathology*. **58**: 10-16.

Kapoor A.S., Paul Y.S. and Singh A. 2006. Integrated management of white rot and root rot-wilt disease complex of pea. *Indian Phytopath*. **59:** 467-476.

Kay, S.J. and Stewart, A. The effect of fungicide on fungal antagonists of onion white rot and selection of dicarboximide-resistant biotypes. *Plant Pathol*. **43**: 863-871.

Khalko, S., Jash, S., Bose, S., Roy, M. and Pan, S. 2006. Evaluation of tolerance in *Macrophomina phaseolina, Trichoderma harzianum, Trichoderma viride* and *Gliocladium virens* to fungicides. *J. Mycopathol. Res*. **44** : 109-111.

Khatun, S. 2012. *Management of fusarial wilt of the medicinal plant*, *Coleus forskohlii* (Willd.) Briq. Ph.D. Thesis. The University of Burdwan, West Bengal, India.

Kopacki, M. and Wagner, A. 2006. Effect of some fungicides on mycelium growth of *Fusarium avenaceum* (Fr.) Sacc. pathogenic to chrysanthemum (*Dendranthema grandiflora* Tzvelev). *Agronomy Res*. **4**:237-240.

Krivoshchekova, T.G. and Mischenk, V.S. 1990. The effectiveness of trichodermin. *Zasch. Ras. (Moskovo)*. **11**:22.

Kucuk, C. and Kivanc, M. 2003. Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. *Turk. J. Biol.* **27**: 247-253.

Londonkar, R., Reddy, C.V. and Kumar, A.K. 2011. Potential antibacterial and antifungal activity of *Achyranthes aspera* L. *Recent Res. Sci. Tech*. **3**:53-57.

Lorito, M. 1998. *Chitinolytic enzymes and their genes*. In: *Trichoderma* and *Gliocladium*: enzymes, biological control and commercial applications, Vol. 2. Taylor and Francis. Ltd. London, pp. 153- 172.

Lyr, H. 1987. *Selectivity in modern fungicides and its basis*. In: Modern selective fungicides-properties, applications, mechanisms of action, Longman Group UK Ltd., London. pp. 31-33.

Manju, Sinha, P.P. and Patralkesh, L.N. 1999. Integrated management of bacterial wilt of brinjal. *Indian Phytopathology*. **52**:325.

Meyer, C.E. 1966. U21963, a new antibiotic II: Isolation and characterization. *Appl. Microbil*. **14**: 511-512.

Mondal, G., Srivastava, K.D. and Aggarwal, R. 1995. Antagonistic effect of *Trichoderma* spp. on Ustilago segetum var. tritici and their compatibility with fungicides and biocides. *Indian Phytopathol*. **48**: 466-470.

Natarajan, V., Venugopal, P.V., and Menon, T. 2003. Effect of *Azadirachta indica* (neem) on the growth of dermatophytes. *Indian J. Med. Microbiol*. **21**: 98-101.

Obongoya, B.O., Wagai, S.O. and Odhiambo, G. 2010. Phytotoxic effect of selected crude plant extracts on soil-borne fungi of common bean. *Afr. Crop Sci. J*. **18**: 15-22.

Ojha, S. 2008. *Studies in wilt of tomato (Lycopersicon esculentum) caused by Fusarium oxysporum and its integrated control.* Ph.D. Thesis. The University of Burdwan, West Bengal, India.

Omar, I., O'Neill, T.M. and Rossall, S. 2006. Biological control of *Fusarium* crown and root rot of tomato with antagonistic bacteria and integrated control when combined fungicide carbendazim. *Plant Pathol*. **55**: 92-99.

Ooka, T., Akimoto, T. and Abe, J. 1966. A new antibacterial peptide. Suzukacillin. *Agric. Bio. Chem.* **30**: 700-702.

Ozbay, N. and Newman, S.E. 2004. Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. *Pak. J. Biol. Sci.,* **7**: 478-484.

Pawar, B. T. 2011. Antifungal activity of some leaf extracts against seed-borne pathogenic fungi. *Int. Multidis. Res. J.* **1:**11-13

Papavizas, G.C., Lewis, J.A. and Abd-El Moity, T.H. 1982. Evaluation of new biotypes of Trichoderma harzianum for tolerance to benomyl and enhanced biocontrol capabilities. *Phytopathology.***72:** 126-132.

Pyke, T.R.and Dietz, A. 1966. U21963, a new antibiotic, discovery and biological activity. *Appl. Microbiol.* **14**: 506-510.

Rajeswari, P.and Kannabiran, B. 2011. In vitro effects of antagonistic microorganisms on *Fusarium oxysporum* [Schlecht. Emend. Synd & Hans] *infecting Arachis hypogaea L. J. Phytopathol.* **3**: 83-85.

Ramachandra, C.G. and Kalappanavar, I.K. 2006a. Evaluation of fungicides against leaf blight of dicoccum wheat both laboratory and field conditions. *Int. J. Agric. Sci*. **2**: 405-407.

Ramachandra, C.G. and Kalappanavar, I.K. 2006b. Non-chemical management strategy for leaf blight of dicoccum wheat. *Int. Agric. Sci*. **2:** 503-504.

Royse, D.L. and Ries, S.M. 1978. The influence of fungi isolated from peach twigs on the pathogenecity of Cytospora cincta. *Phytochemistry*. **68**: 603-607.

Sharma, S.D. and Mishra, A. 1995. Tolerance of *Trichoderma harzianum* to agrochemicals. *Indian J. Mycol. Pl. Pathol*. **25**: 129-130.

Sharma, S.D., Mishra, A., Pandey, R.N. and Patel, S.J. 2001. Sensitivity of *Trichoderma harzianum* to fungicides. *J. Mycol. Pl. Pathol*. **31**: 251-253.

Titone, P., Polenghi, L., Tamborini, L. and Garibaldi, A. 2004. Chemicals and chemotherapy for rice seed dressing. In: Proceeding of the conference, challenges and opportunities for sustainable rice based production systems, Torino, Italy.

Vey, A., Hoagland, R.E. and Butt, T.M. 2001.*Toxic metabolites of fungal biocontrol agents*. In: *Fungi as biocontrol agents* (Eds. Butt, T.M., Jackson, C., Magan, N.): Progress, problems and potential. CAB International, Bristol. pp. 311-346

Vyas, R.K. and Mathur, K. 2002. Distribution of *Trichoderma* spp. in cumin rhizosphere and their potential I suppression of wilt. *Indian Phytopathology*. **55**: 451-457.

Wani, A.H. 2005. Biological control of wilt of brinjal caused by *Fusarium oxysporum* with some fungal antagonists. *Indian Phytopathology*. **58**: 228-231.

Whipps, J.M. 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. *New Phytology*. **107:** 127-142.

Woo, S.L.and Lorito, M. 2007. Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. In: Novel biotechnologies for biocontrol agent enhancement and management, Dordrecht, the Netherlands: *Springer*, pp.107–130.

Zewain, Q.K., Bahadur, P. and Sharma, P. 2005. Integrated disease management of stalk rot of cauliflower. *Indian Phytopathology*. **58**: 167-173.