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Full Length Research Paper

Sensitivity of Alternaria alternata; cause of foliar and seedling blight disease of *Cassia fistula* in Pakistan to fungicides and biological control agents

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Foliar blight disease is caused by *Alternaria alternata*, cosmopolitan saprophyte in nature, considered a virulent pathogen in different crops. Pathogens cause foliar and seedling blight of *Cassia fistula*, which is an important ornamental and timber tree plant grown on sidewalk, roads. pathogen infection resulted in timber deterioration and reduce esthetic value of tree. So, it's very important to develop a cost-effective strategy to control this disease either by chemical and biological agents to save the *C. fistula* trees. For this purpose, different fungicides viz; Mancozeb 75% WP, Carbendazim 50% WP, Thiophanate methyl 70% WP, Copper oxy 50% WP, Difenoconazole 25% EC and Propiconazole 25% EC and biological agents viz; *Aspergillus niger, Aspergillus flavus* and *Trichoderma harzianum* were used *in vitro* against *A. alternata*. Results of this experiment demonstrated that Propiconazole 25% EC and Difenoconazole 25% EC were found statistically significant in reducing mycelial growth of *A. alternata* (71.5%, 72.7%, 73.9%, 72.1%, 72.1%, and 73.0%) after 3, 6 and 9 days of incubation at various concentrations. On the other hand, *Trichoderma harzianum* was found to be most successful in inhibition (76%) of this pathogen. Therefore, we concluded that Propiconazole 25% EC, Difenoconazole 25% EC and *T. harzianum* are effective in managing this pathogen responsible for foliar and seedling blight in *Cassia fistula*.

Keywords: Seedling blight, Foliar blight, *Trichoderma harzianum,*Propiconazole, Alternaria alternata, Cassia fistula.

INTRODUCTION

Cassia fistula is the fourth largest legume genus belonging to the family Fabaceae, with about 600 species present. *Cassia fistula* is commonly known as Golden Shower or Amaltas. *C. fistula* is a deciduous tree with irregular rounded crown having yellow luminous flowers. It's a tree of tropical region, mostly found in Asia and usually scattered across India, Pakistan, Malaysia and Indochina.*C. fistula* served as shade tree and as an ornamental tree on the edges of the roads, parks and gardens due to this reason it has been introduced in tropical regions of America and Africa. Now due to the high medicinal and water purifying qualities of *C. fistula,* it is gaining admirations all over the globe (Kumar *et al.,* 1998; Gupta *et al.,* 2000; Adebayo *et al.,* 2004; Hanif *et al.,* 2007). *C. fistula* is highly nutritive, medicinally, phytochemically, pharmacologically important and having water purifying attributes which make this tree very important ornamentally and economically. It was considered

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that *C. fistula* is not an important commodity for food but now soybean meal is replaced by *C. fistula* meal due to its medicinal importance. However, medicinally it is an important tree with high potential for the tumor treatment (Gupta *et al.,* 2000), hepato-protective, anti-inflammatory (Ilavarasan *et al.,* 2005) antitussive (Bhakta *et al.,* 2001), hypo-cholesterol-laemic, hepato-protective, anti-oxidant (Ilavarasan *et al.,* 2005; Manonmani *et al.,* 2005), curative against wound (Kumar *et al.,* 2006), antifertility and antifungal (Yadav and Jain, 1999) activities of *C. fistula* extracts are currently well recognized. Being ornamentally and medicinally important the *C. fistula* is still prone to diseases, mainly diseases caused by viruses, bacteria, nematodes and fungi. But fungal diseases are more prevalent. Recently, In Pakistan foliar and seedling blight disease of *C. fistula* is more dominating among other fungal diseases of anthracnose, powdery mildews, scab, false rust, root, stem and foliage rots. The incidence of this disease is very high to all *C. fistula* growing areas of Pakistan. The disease is prevailing more drastically which will lead the trees to die back. The aesthetic sense of this tree is endangered due to this disease. Two types of characteristic symptoms are produced; (1) foliar blight**;** It firstly appears as depletion of chlorophyll which progress with the necrosis of diseased area, large irregular spots collapse together and give light brown to dark brown coloration to the leaves. High humidity favors the disease development while rain and soil debris are the sources of dissemination from one part to another part or one plant to another plant. (2) seedling blight; described as severe lesions on leaves and defoliation with small black necrotic spots appearing during the winter season on the seedlings of *C. fistula* trees. These spots further progress into black irregular necrotic area and become cankerous. With further progress of this disease, stunting or death of the whole plant has been observed.The pathogen of this disease is *A. alternata*, a saprophytic fungus in nature, requires humidity to cause disease or infection. Produces two types of spores called macro and micro spores which spread through wind and rain from one place to another and from one part of the plant to another part. Therefore, its management before rainy season is necessary to minimize the spore load of the present inoculum to cause less infection in the favorable conditions. *A. alternate* was inhibited by fungicide and by bio-controlling agents. By using poisoned food technique three different concentrations of eleven fungicides was tested against *A. alternate* (Nene and Thapliyal, 1993). In this study, management of this fungus through fungicides and biological control agents will help to manage this disease with cost effective, less hazardous and more reliable manner.

MATERIALS AND METHODS

Isolates

Previously isolated culture of *A. alternata* isolate A 27 was used in this study.

Dual Culture technique for sensitivity of A. alternata against biological agents

Dual culture technique was studied for the biological management of the pathogenic fungus *Alternaria alternata*. Three different antagonistic fungi viz; *Aspergillus flavus*, *Trichoderma harzianum* and *Aspergillus Niger* were used in this study. In this method 4-day old culture of each bio-agent was selected and disc (6mm) of agar was taken and placed in petri plates having PDA media (5cm). Another disc of *A. alternata* with the same size was placed at the fringe but on opposite end of the petri plate. On another PDA plate same disc of *Alternaria alternata* was placed referred as a control. Quadruplicate was used for incubation. Incubation was done at 28^0C. Data was recorded after 3, 6, 9th day of incubation by assessing the radius of *A. alternate* in the direction of antagonist's colony (R2) and *Alternaria alternata* colony radius in the control plate (R1). Two readings of data were converted into percentage inhibition (PIRG) by using formula. Whereas continued observations were done on duel culture plates after 3, 6, and 9 days of incubation and PIRG was calculated.

Formula is as followed,

PIRG=R1-R2/R1×100

Poison Food Technique for fungicidal sensitivity of A. alternata

Different fungicides (Mancozeb 75% WP, Carbendazim 50% WP, Thiophanate methyl 70% WP, Copper oxy 50% WP, Difenoconazole 25% EC and Propiconazole 25% EC) were tested *in vitro* for evaluation of fungicides on mycelial growth of isolated fungi by using poisoned food technique. All commodities were tested at 50, 100, 150, 200 and 250*µ*g/ml. 1gm/ml or equivalent of each fungicide were dissolved in 100ml of sterilized water to prepare stock solution required concentration were prepared (Rehman *et al.,* 2015). Before media pouring, in each (9 cm) sterilized plates 1ml of each solution was added. 5mm discs of seven days old isolated fungus culture were inoculated in solidifying media plates. Each treatment consists of three replications and without fungicide treatment served as control. Incubation of inoculated plates takes place at 22°C and after 4-5 days of incubation data was recorded on the radial colony diameter.

RESULTS

Sensitivity of *A. alternata* against biological agents

Disease data as the percent inhibition showed that *T. harzianum* (Max. value 76%) can inhibit the growth of the *A. alternata in vitro* as compare to the other two fungi

Table 1. Efficacy of biological agents against *Alternaria alternata* after 3, 6 and 9 days.

Treatments	Colony growth after 3 das	PRIG	Colony growth after 6 days	PRIG	Colony growth after 9 days	PRIG
Trichoderma harzianum	0.73 ± 0.20	29	1.80 ± 0.20	45	3.8 ± 0.20	76
	0.78 ± 0.20	31	1.60 ± 0.20	40	3.0 ± 0.20	60
	0.75 ± 0.20	30	1.52±0.20	38	3.5±0.20	70
Aspergillus flavus	0.50 ± 0.20	20	1.24 ± 0.20	31	3.2 ± 0.20	64
	0.40 ± 0.20	16	1.16 ± 0.20	29	2.6 ± 0.20	52
	0.35 ± 0.20	14	0.92 ±0.20	23	2.7±0.20	54
Aspergillus niger	0.45 ± 0.20	18	1.20 ± 0.20	30	3.0 ± 0.20	60
	0.30 ± 0.20	12	1.00 ± 0.20	25	2.3 ± 0.20	46
	0.25 ± 0.20	10	1.28 .± 0.20	32	2.8±0.20	56
Control	2.5 ± 0.50	–	4 ± 0.50	–	5 ± 0.50	–

Table 1. The sensitivity of the pathogen against antagonistic biocontrol agent represents the potential of *Trichoderma harzianum* in managing the disease pathogen (Figure 1-3).

Fungicidal sensitivity of A. alternata

Propiconazole and Difenoconazole was found excellent in reducing mycelial growth of *Alternaria alternate* (71.5%, 72.7%, 73.9% and 72.1%, 72.1%, 73.0%) after 3, 6 and 9 days of incubation at various concentrations and both are statistically at par to each other followed by Mancozeb 75% WP (55.0%, 55.1%, 58.9%) Carbendazim 50% WP (41.7%, 42.1%, 47.2%) Thiophanate methyl 70% WP (8.6%, 19.6%, 26.3%) and Copper oxy 50% WP with values of (12.2%, 20.8%, 31.0 %,) disease over control after 3, 6 and 9 days of incubation as in Table 2.

DISCUSSIONS

Cassia fistula is an ignored ornamental and timber tree and is being vigorously affected by the seedling and foliar blight which results in poor quality of wood and poor aesthetic value. *Alternaria alternata* is responsible for the foliar and seedling blight of *Cassia fistula*. Our study was limited *in vitro* and we were only able to collect sufficient data to prove that this disease can be managed by biological and chemical means without effecting the environment and thus saving this tree. Results indicate that *Trichoderma harzianum* is the best bio-controlling agent and inhibit maximum mycelial growth after 3, 6 and 9 days of incubation period with maximum percent value (31%, 45%, 76%) followed by *Aspergillus flavus* and *Aspergillus Niger* with percent values (14%, 31%, 64% and 10%, 32%, 60%) respectively. *T. harzianum* considered an effective bio-control agent against different type of soil born pathogenic fungi. Now a day it is produced commercially and used as bio-control agent against different pathogens. Various types of applications have been recommended such as secretion of chitinolytic enzymes, competition for space and nutrients, production of inhibitory compounds and mycoparasitism which are responsible for their bio-control activity (Haram *et al.,* 1996; Zimand *et al.,* 1996) and *T. harzianum* possess top position as a bio-control agent and used against various fungal plant pathogens. Fungi may eliminate other organisms from possessions possibly available to each other by mechanism of interference competition referred as antibiosis (Gomathy and Ambikapathy, 2011). It is also reported that *T.harzianum* served as best bio-control for *Alternaria alternate* (Roco and Perez, 2001; Monte, 2001; Sempere and Santamarina, 2007). Elad *et al.,*1996) described that enzymes produced by *Trichoderma* spp play a vital role in the lysis and fragmentation of mycelium of test fungus. Kumar, (2008); Gveroska and Ziberoski, (2012); Rajput *et al.,* (2013) evaluated *T. harzianum* and its antagonistic efficacy against *A. alternata* under *in vitro* condition by using dual culture technique. Akin results were made by Balai and Ahir, (2011) that *T. harzianum* was found utmost effective in impeding the mycelial growth of *A. alternata* followed by *A.niger*. Jat and Agalave, (2013) described the properties of *Trichoderma* sp. as an antagonist against *A. alternata*. Panwar *et al.,* (2013) observed inhibiting properties of *A.niger* against *Alternaria alternata* through dual culture

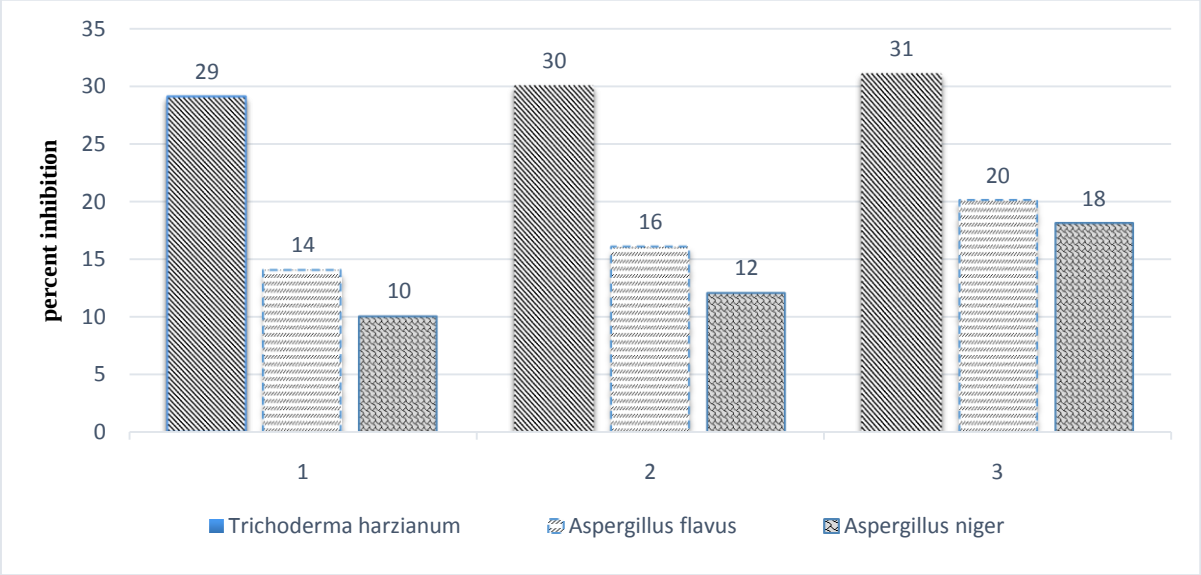

Fig 1. Graphical presentation of efficacy of different biological control agent against *Alternaria alternata* after 03 days.

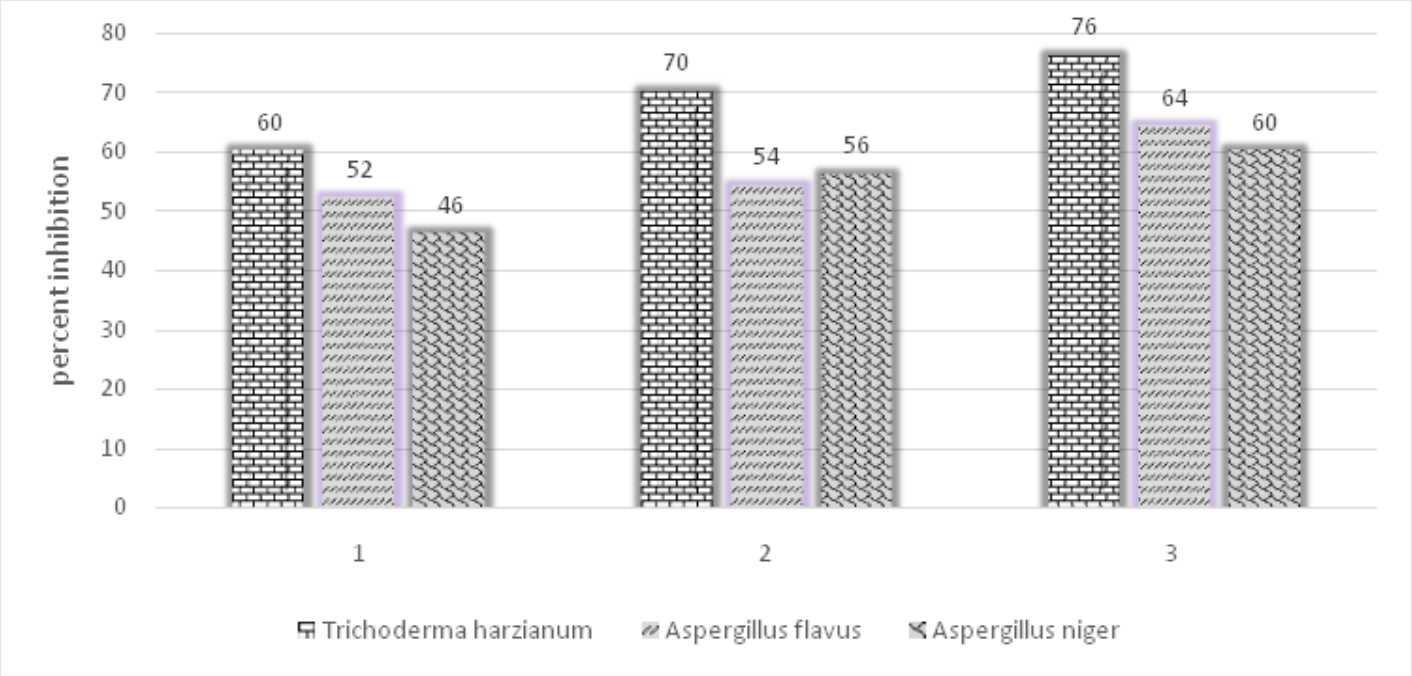

Fig 2. Graphical presentation of efficacy of different biological control agent against Alternaria alternata after 06 days.

method. Our *in vitro* results demonstrated that propiconazole 25% EC and difenoconazole % EC was best against *A. alternata* with inhibiting efficacy of (71.5%, 72.7%, 73.9% and 72.1%, 72.1%, 73.0%) after 3, 6 and 9 days of incubation at various concentrations and both are statistically at par to each other. By, using *In vitro* evaluation method, we can deliver comprehensive and useful information of fungicides against certain type of pathogens. Hence serve as chaperons for future field testing. Another experiment was conducted on evaluation of fungicides and found that propiconazole (Tilt) was the best inhibitory fungicide against *A.alternata.*

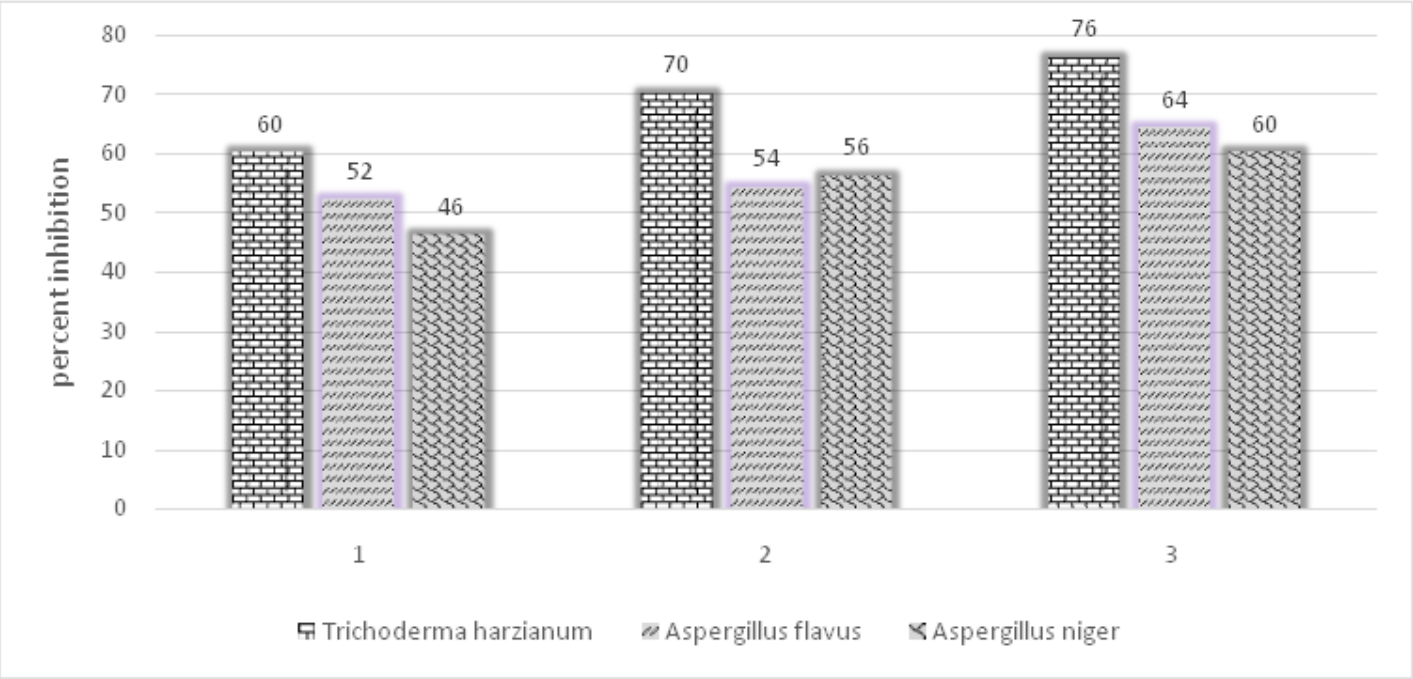

Fig 3. Graphical presentation of efficacy of different biological control agent against Alternaria alternata after 09 days.

Table 2. Effect of different fungicides treatments on mycelial growth of*Alternaria alternata* at 0, 50, 100, 150, 200 and 250ug/ml concentration after 3, 6 and 9 daysof incubation.

Fungicides	after 3 days	% decrease over control	after 5 days	% decrease over control	after 7 days	% decrease over control
Mancozeb 75% WP	0.78±0.198D	58.9	1.15±0.228D	50.0	1.48±0.328D	55.1
Carbendazim 50% WP	1.10±0.166C	42.1	1.34±0.211C	41.7	1.74±0.304C	47.2
Thiophanate methyl 70% WP	1.40±0.140A	26.3	2.10±0.146A	8.6	2.65±0.207B	19.6
Copper oxy 50% WP	1.31±0.145B	31.0	1.82±0.173B	20.8	2.90±0.184A	12.2
Difenoconazole 25% EC	0.53±0.226E	72.1	0.64±0.284E	72.1	0.89±0.393E	73.0
Propiconazole 25% EC	0.54±0.225E	71.5	0.60±0.284E	73.9	0.90±0.393E	72.7
Control	1.90		2.30		3.30	

Another scientist describes difenoconazole, propiconazole and mancozeb as persuasive in inhibiting mycelial growth of fungus even at 100 ppm concentration. Another experimental result showed that Carbendazim fungicide was not effective in term of inhibition of mycelial growth of *Alternaria solani*. These findings are in complete agreement with the results obtained in our present investigation. Similar findings were also reported and results showed that propiconazole was the most effective fungicide in controlling *A. alternata* by 100% in 8 days after inoculation.

Phapale *et al.,* (2010) reported propiconazole showed cent per cent reduction of *A.alternata* at 250, 500 and 1000 ppm concentrations. Propiconazole 0.05 per cent completely inhibited the growth of the *A. alternata* (Thaware *et al.*, 2010). Similar findings of Pairashi, (2007) supported the present finding that benomyl and propiconazole found 100 per cent inhibition of *C. nicotianae*. Therefore, Trichoderma harzianum as biological agent and propicona-difenoconazole's as fungicides proved best in the in vitro experiments. There is a need to evaluate these findings under in vivo conditions for seedling and foliar blight disease management.

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