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Research Article

Antimycotic Properties of Potentials Botanical Crude Extracts and Bio-Control Agent against Fusarium Wilt of Chili caused by *Fusarium oxysporum* f.sp. *capsici*

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Abstract

Fusarium wilt is a devastating disease of Chili in Pakistan and its management is very difficult. Therefore the studies were conducted in *in-vitro* conditions. The results showed that the disease is present in high frequency throughout the districts surveyed. The maximum disease incidence was recorded in Hyderabad (55%) whereas; the incidence of the disease from the Tando Allahyar district was recorded up to (35%). The used botanical extracts showed high variation in reducing the colony growth of the fungus. All the botanical extracts significantly reduced the mycelial growth of *Fusarium oxysporum.* Eucalyptus leaves was found most effective in reducing the colony growth of the fungus at their highest dose (6.33 mm), Aloe suppress the fungal mycelial growth (21.00 mm). Whereas the Mari gold reduce the fungal growth (25.33 mm) and Mint leaves (26.33 mm). All the botanical extracts at their respective doses significantly retarded the growth of fungus as compared to control (82.00 mm). *Trichoderma* spp. as bio control agent was tested *in-vitro* condition against *Fusarium oxysporum* and significantly reduced the mycelial growth of *Fusarium oxysporum*. *Trichoderma* spp. reduces the activity of the *Fusarium oxysporum* (10.00 mm) as compared to control (82.00 mm). Based on the *in-vitro* result Eucalyptus leaves extract can be effectively used for field experiment. These studies will be helpful for researchers and growers.

Keywords: Antimycotic; Botanical Extracts; Bio-Control Agent; Potentials

Introduction

Chili (*Capsicum annum L.*) is one of the world's significant vegetable and flavor crop. Universally, Chili crop is grown on 1776 thousand ha which produce 7182 thousand tons. Major Chili producing countries are china, Pakistan, Ethiopia, Myanmar, Mexico, Vietnam, Peru, Ghana and Bangladesh. Pakistan contributes around 6% to the world's aggregate Chili generation (Quresh *et al.*, 2021). Chili is known as financially critical and significant cash crop of Pakistan. There are two species are generally grown in Pakistan

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which are *C. annum* and *C. frutescens*. Area under Chili diminished from 103,213 hectares in 2010-11 to 100,106 hectares in 2013-4, thus showing decline of 3%. Sindh produces around 88% of Chili in Pakistan to around 130,350 tons. Areas in Sindh province including Kunri, Umerkot, Mirpurkhas, New Koat and some different towns are major hub of Chili production. The normal production of 1.7 tons for each hectare contributes 1.5% of the country's GDP Memon (2019). Chili is to a great extent produced in Sindh and southern Punjab in the country. In sindh, Chilies produced large scale and contributes 85% of the production followed by Punjab with 11% (GOP, 2019).

Kunri, which is otherwise called "Stew Capital of Asia produces Chili of around 55%. Alternate urban communities of Sindh region in which Chilies are produced on large scale included Mirpurkhas, Hyderabad and Ghotki (Chowdhury *et al.*, 2015). *Capsicums* are a fantastic source of vitamins A, B, C, E and P. Fresh green Chili peppers contain more vitamin C than citrus foods and fresh red Chili has more vitamin A than carrots (Marín *et al.*, 2004). *Capsicum* spp. contains extent of basic supplements and bioactive mixes which are known to show cancer preventive agent, antimicrobial, antiviral and anticancer properties (Khan *et al.*, 2014). A part of the known chemicals contained in *Capsicum* regular item fuse steam-unstable oils, greasy oils, capsaicinoids, carotenoids, vitamins, proteins, fiber and mineral segments (Bosland *et al.*, 2012). Chili is influenced by number of pests attack on Chilies crop during the developing season. For example aphids, thrips, leaf hoppers, ear wigs, crickets, mites, root grubs, pod borers, cut worms, flea Beatles, etc harm or destroy the crop. Normally fungal diseases cause more harm than diseases cause by different pathogens. Several fungi cause different diseases in Chili plants. Sometime these fungi can cause similar symptoms and confused to one another. There are some pre-collecting fungal diseases such as anthracnose, leaf spot, charcoal rot, damping-off and root rot, downy mildew, Fusarium stem rot, leaf spot, gray mold, Powdery mildew, Verticillium wilt White mold and Fusarium wilt are affecting in Chili crop. *Fusarium* is a soil-borne fungus and can survive for several years it is normally developed in wet soil and high temperature. It causes wilting of the plant and upward and downward rolling of the leaves as result leaves turn yellow and stop living. It is also discolored the vascular arrangement of plant, especially in the lower stem and roots (Hussain and Abid, 2011).

Wilt disease caused by *Fusarium oxysporum* f. sp. *capsici* is an important problem of Chili pepper generation worldwide that requires a superior comprehension of protective instruments in the Chili plant. Various proteins have been suspected to facilitate *Fusarium oxysporum* infection (Wongpia and Lomthaisong, 2010). The Fusarium wilt disease, caused by the soil-borne fungus, *Fusarium oxysporum* is a staid disease that lessens development, fruit production, and quality, threatening Chili production. The fungus enters the vascular systems by means of the root tissues and therefore utilizes the xylem vessels as roads to quickly spread and colonize the plant, leading to the characteristic wilt symptoms *F. oxysporum* is inflexible to manage as it can survive in turf soil for long period time (Dubey *et al.*, 2007). Alternatively application of BCA is helpful strategies for best controlling harmful or pathogenic organisms. Mostly fungi and bacteria serve such Trichoderma is significant potential agent for controlling soil borne pathogens (Muhammad *et al.*, 2023; Bashir *et al.*, 2020). In any case, such studies have

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mainly been conducted under controlled conditions (Bibi *et al.*, 2017; Shahzaman *et al.*, 2016; Shakoor *et al.*, 2015). An attempt is made to isolate *Trichoderma* obtained from various sources are screened for efficacy in the control of wilt chili caused by *Fusarium* ssp under both lab and Field conditions. (Najar *et al.*, 2016). Isolation of *Fusarium solani* by dual culture method. Five mm discs from seven-day-old cultures of *Fusarium solani* were placed at one end of the petri dishes and exactly after 86 cm, five mm discs of *Trichoderma* (quickly developing) was placed at the other end of petri dish to compensate for their growth behavior. In all, three replications were maintained for each treatment with suitable control for *Trichoderma* and *Fusarium* (Bhat *et al.*, 2016).

Furthermore plant extract are being used for managing pathogenic fungi. Tested various extract such as Eucalyptus citrosodora natural product, bark extracts and leaves methanol extracts for controlling of Fusarium wilt of chili. Neem is significant plant sources having medically valuable compound and has antimicrobial activities Agbenin and dry neem seed extract totally inhibited the mycelial development of *Fusarium oxysporum* at all concentration. Agbenin *et al.* (2004) reported that using neem seed powder also controlled *Fusarium* spp. Concentrates of safeda and different plants in various extracts diminished the development of *Fusarium solani.* The marigold (*Calendula officinalis*) is cultivated in different countries, and a few reviews have been produced utilizing this plant extract to control some species of nematode and *Fusarium* (Niaz *et al.*, 2008; Joseph *et al.*, 2016).

Therefore, present study was planned to investigate the bioassay of botanical extracts and bio-control agent against *Fusarium oxysporum* f. sp. *capsici* the causal agent of fusarium wilt of Chili , so that better crop yield could be obtained to meet the domestic and export requirements.

Methodology

Survey and sampling

Sampling of Chili crop showing wilt symptoms was carried out from different localities of districts Tando Allahyar and Hyderabad. The collected specimens were brought to Mycological laboratory for isolation and identification of the causal agent.

Isolation and identification

Isolation of pathogenic fungus was done by different isolation techniques. The samples were washed thoroughly, than cut into small pieces along with some healthy and infected portion after surface sterilized with 5% sodium hypochlorite (NaClO). The samples were then put on PDA medium and incubated at 25°C + 1 for 7 days. The observations for the fungal growth were monitored on daily basis. The fungi which colonize on these pieces were purified and identified with help of key Frank (2005).

Pathogenicity test

Pathogenicity test was performed on Chili plants with culture plate, soil drenching, and dipping methods. There were three replication for each method. Uninoculated plants served as control. Mortality, Severity and re-isolation was performed from inoculated and controlled plant after 30 days inoculated.

The efficacy of selected botanical extracts

The effect of selected plant extract was studied under *in-vitro* condition against Fusarium

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wilt of Chili. Four botanical extracts Marigold flower, Mint leaves, Aloe vera, Eucalyptus leaves were used with the doses of 2%, 4% and 8% as described by Shafique *et al.* (2015).

The efficacy of selected bio-control agent

In *in-vitro* study assessing the efficacy of *Trichoderma* sp. against *Fusarium oxysporum* f.sp. *capsici*, the selected *Trichoderma* strain was cultured on suitable media to attain a high biomass. Simultaneously, *Fusarium oxysporum* f.sp. *capsici* was cultured separately. A confrontation assay was conducted by inoculating the pathogen and biocontrol agent in close proximity on a compatible medium, allowing for direct interaction through dual culture method as described by Sahi and Khalid (2007). The experiment was replicated thrice to ensure reliability.

Results and Discussion

Disease incidence of Fusarium wilt of chili fields in two districts of Sindh

Survey of some Chili producing areas of Sindh i.e. Hyderabad and Tando Allahyar. The observation recorded maximum disease incidence in Hyderabad (55%) and Tando Allahyar (35%) (Figure 1).

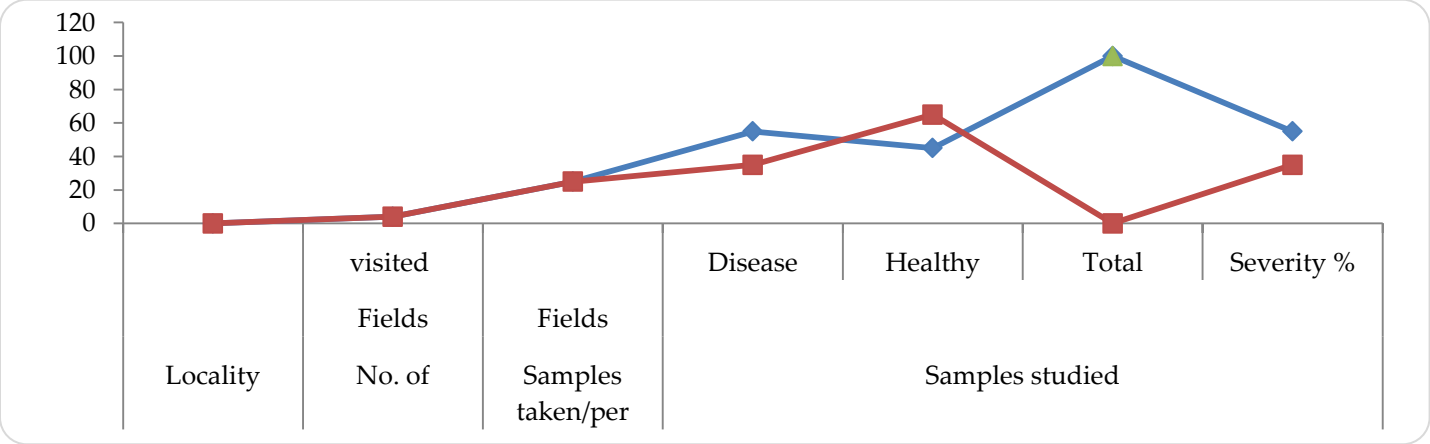

Figure 1. Disease incidence of Fusarium wilt of chili fields in two districts of Sindh.

Isolation and identification of fungi

Fusarium oxysporum f.sp. *capsici* was isolated and identified from healthy and infected plant of Chili with highest frequency (81%), and other *Macrophomina* (14.33%), *Alternaria* sp (4.6%), Among them *Fusarium oxysporum* was found most pre-dominant from two districts surveyed,100 percent sample were infected with *Fusarium oxysporum* (Table 1).

Table 1. Isolation frequencies % of fungi from chili tissue.

Isolated Fungus	Roots	Branches	Stem	Overall%
Fusarium oxysporum	80	78	85	81%
Macrophomina phaseolina	15	16	12	14.33%
Rhizoctonia solani	5	6	3	4.6%

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Pathogenicity test of *Fusarium oxysporum* f.sp. *capsici*.

Pathogenicity test performed in disinfected earthen pots. All pots were filled with steam-purified sandy soil (1 kg/pot), which was completely blended with a crisp culture of *Fusarium oxysporum* in three strategies i.e. Culture plate technique, Soil Drenching strategy, Drippings strategy. The untreated soil (without parasite) was used as control. Ten plants for each pot were sown and the pots were kept for 30 days under open sky. The plants were watered on different days and re-isolation was done from roots of plants showing wilting symptoms (Table 2, Figure 2)

Table 2. Disease mortality in pathogenicity test of *Fusarium oxysporum* on Chili plant.

Method of Inoculation	Total No. of Plants	Disease plants	Healthy plants	%age of Mortality
Culture plate method	10	7	3	30%
Soil Drenching method	10	8	2	20%
Dipping method	10	3	7	70%
Total	30	18	12	40%

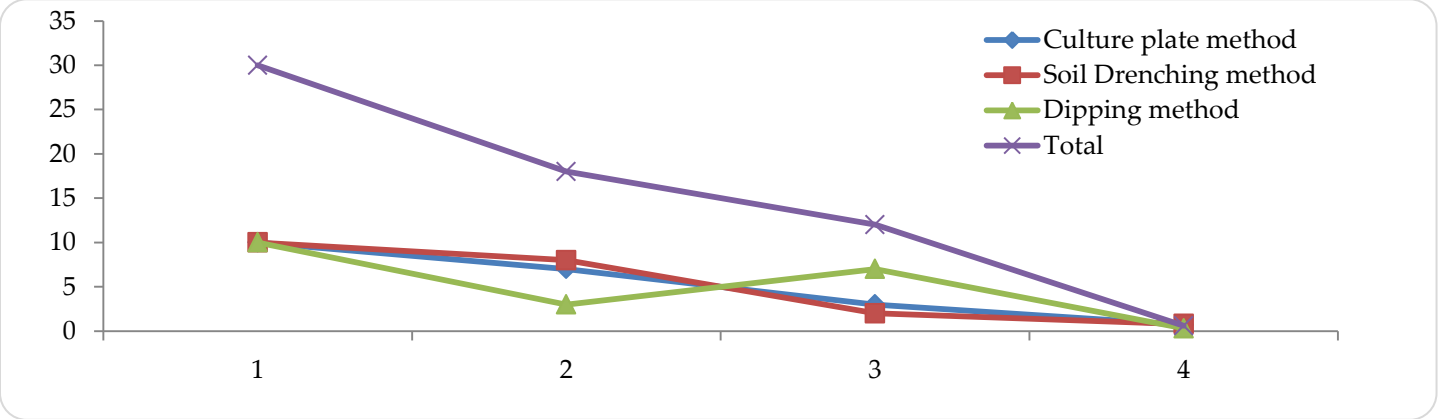

Figure 2. Disease incidence in pathogenicity test of *Fusarium oxysporum* on chili plant.

Effect of different botanical extracts and *Trichoderma* sp on the mycelial growth of the fungus (*Fusarium oxysporum* f.sp. *capsici*).

Four different botanical extracts and *Trichoderma* spp. were tested *in-vitro* conditions for their efficacy against *Fusarium oxysporum* at different doses. The botanical extracts which were used are marigold flower, Mint leaves, Aloe Vera, Eucalyptus leaves. All the botanical extracts significantly reduced the mycelial growth of *Fusarium oxysporum* ($p<0.0000$) Among all the used botanical extract the marigold flower was create powerful in reducing the mycelial development of the fungus at their highest dose (25.33mm) and lowest dose (56.33mm) respectively, followed by Mint leaves, which reduce the fungal growth at highest dose (26.33mm) and lowest dose (59.33mm) whereas the Aloe Vera reduce the mycelial growth of the fungus its highest dose (21.00mm) and lowest dose (54.66mm) respectively. Eucalyptuses were found most effective as compared to other three botanical extracts. Eucalyptus reduce the colony growth of the fungus at highest dose (16.66mm) and at the lowest dose (6.33mm) and *Trichoderma* spp. also controlled

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(16.67). All the botanical extracts at their respective doses and *Trichoderma* spp. significantly retarded the growth of fungus as compared to control (82.00mm) (Table 3).

Fusarium wilt is most serious and devastating disease of Chili field throughout the world including Pakistan especially in Sindh province district Hyderabad, which causes serious losses to plants and may lead to their death. So, therefore keeping in view the incidence and the losses caused by the disease in Chili field, the survey of two, districts of Sindh, e.g. Hyderabad and Tando Allahyar was carried out to record the incidence of the disease in different Chili fields of these two districts. During the survey it was observed that the Fusarium wilt of Chili was found frequently in all the Chili fields of the two districts with more or less incidence. The maximum incidence was recorded from Hyderabad district (55.0%) whereas, the incidence of the disease from the Tando Allahyar district was recorded up to (35.0%). The results were parallel with the previous findings of Hussain *et al.* (2013); Saleem *et al.* (1996) and Mushtaq and Hashmi (1997).

Table 6. Effect of selected botanical extracts and *Trichoderma* on the mycelial growth of *Fusarium oxysporum* f.sp. *capsici.*

Botanical extracts tested	Dose(%)/100ml.medium	Radial colony growth (mm)
	i 2	56.333 B
Mari Gold	ii 4	35.000 C
	iii 8	25.333 CDE
	i 2	59.333 B
Mint Leaves	ii 4	37.667 C
	iii 8	26.33 CDE
	i 2	54.667 B
Aloe Vera	ii 4	34.000 CD
	iii 8	21.000 DEF
	i 2	16.667 EFG
Eucalyptus Leaves	ii 4	8.667 FG
	iii 8	6.333 G
Trichoderma	5mm disc	16.667 EFG
Control	---------	82.000 A
LSD(P<0.05)	---------	0.0000

In our studies, isolation and identification of the collected diseased specimens showed the association of three fungi i-e., *Fusarium oxysporum, Macrophomina phaseolina, Rhizoctonia solani* from roots, stem and branches tissues. Among them *Fusarium oxysporum* was found in highest frequency from all the associated fungi. The isolated fungi were then identified on the basis of morphological characteristics and color of the colonies of fungi. The identification was also done with help of taxonomical keys described by Nelson *et al.* (1983); Seifert (1996) and with help of a hand book "isolation and identification of fungi" written by Frank (2005). Isolated 3 different species of fungal microbes from roots, stems and branches of diseased Chili plant and found that the *Fusarium oxysporum* were frequently associated with fields of Chili.

Keeping in view Chili has a great economic importance , its export value losses caused

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by the Fusarium wilt, different management practices like chemical, botanical, and biological were carried out under *in-vitro* conditions against the *Fusarium oxysporum* to find out the most effective eco-friendly cost effective and easily available control measure to manage the Fusarium wilt disease of Chili. For this, reason our studies showed that, among all the tested plant crude extracts was found most efficient in arresting the mycelial colony growth of the fungus and reducing the mycelial colony growth of the fungus as compared to control. These studies are in connection with the previous studies of Khan *et al.* (2016).

Conclusion

Fusarium wilt disease is one of the major serious threats to Chili crops throughout the Chili growing areas of Sindh, Pakistan. The present studies were conducted to report the severity and losses occurs due to attack of Fusarium wilt disease of Chili in Hyderabad and Tando Allahyar Districts of Sindh, Pakistan. During the studies regarding the management it was found that the disease can be managed through different management strategies such as the use of botanical extracts and bio-control agent. The fungicides were most effective as compared botanical extract, found to be best followed by *Trichoderma*. Eucalyptus leaves was most effective as compared to Marigold flower, Mint leaves and Aloe Vera respectively. As for as concern the use of bio agent *Trichoderma* spp. was also found effective *in-vitro* against the mycelial growth of *Fusarium oxysporum* f.sp. *capsici* causing Fusarium wilt disease in Chili.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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