

Research Article

Investigation of Potential Biological Control of *Fusarium Oxysporum* f.sp. *Lycopersici* by Plant Extracts, Antagonistic sp. and Chemical Elicitors *In Vitro*

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Received date: June 13, 2018; Accepted date: June 27, 2018; Published date: July 2, 2018

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Abstract

Tomato (Lycopersicon esculentum L.) is the most popular vegetable in the world. These fruits carry heavy spore loads when they leave the field. As a result of poor packaging and improper management, the fruits arrive at their destination bruised and squeezed and different types of rots set in under favourable conditions. Fusarium rot is one of the most devastating diseases of ripe tomatoes. The pathogen was isolated from the infected material and it was identified as Fusarium oxysporum f.sp. lycopersici. Diseases caused by fungal pathogens have been a major challenge to agriculture, health as well as national economy. The resistance of this pathogen to the wide variety of chemicals has stimulated the search of new alternatives for control measures. Use of plants extract is one of the most promising, effective, safer and eco-friendly method to eradicate the pathogen from ripe tomato. The present paper aims at the study on the potential for the control of Fusarium oxysporum f.sp. lycopersici by nine plant extracts (Calotropi sprocera, Curcuma domestica, Moringa oleifera, Tricosanthes dioica, Nigella sativa, Wedelia calendulacea, Andrographis paniculata, Trigonell afoenum-graceumand Momordi cacharantia), three fungicides (Sulcox, Indofil M 45 and Ridomil MZ 68) and three antagonistic fungi. It was found that almost all plant extracts at 25% concentration were effective in reducing the mycelium growth of Fusarium oxysporum f. sp. lycopersici. The highest inhibition (87%) of mycelium growth of this pathogen was observed when treated after the plant extract of Calotropis proceraon 12 DAI at 25% concentration and lowest inhibition (27%) of this pathogen was observed when treated after the plant extract of Momordi cacharantia on 12 DAI at 5% concentration. Antagonistic effect of Trichoderma sp., Sclerotium sp. and Aspergillus sp. shows that the highest percent inhibition radial growth (82%) was of Trichoderma sp. and the lowest (68.95%) was of Aspergillus sp. against Fusarium oxysporum f.sp. lycopersici. Chemical treatment with Sulcox was proved that the most effective fungicides against this pathogen.

Keyword:

Tomato; Rot disease; Pathogen; Biological Control; Antagonistic; Sulcox

Introduction

Despite of inadequate food supplies more than 800 million people in developing countries faces 10% of food lost due to plant disease [1]. Fungi, bacteria, viruses and nematodes, develop through soil-borne, above-ground infections are pivotal candidate for plant disease production in some instances it may be transmitted through insect feeding [2]. Compared to other plant parasites, fungi cause the greatest impact with regard to disease and crop production losses. Tomato (Lycopersicon esculentum L.) is economically vibrant and popular vegetables not only in Bangladesh but also throughout the world. Fruit rots caused by fungi are common pre and post-harvest diseases of tomatoes. Several fungal species including Phytophthora parasitica, Fusarium oxysporum, Colletotrichum phomoides, Penicillium sp., Alternaria solani, Phytophthora infestans, Aspergillus niger have been reported as pathogens. Fusarium rot caused by Fusarium oxysporum f.sp. lycopersici is one of the most destructive diseases on ripe tomatoes. Pathogenic fungi can attack effortlessly due to having low

pH, high moisture content and nutrient composition, eventually causing rots may also make them unfit for consumption by producing mycotoxins [3]. Several fungal species can cause fruit decay in tomatoes such as the sour rot pathogens caused by Geotrichum candidum, Rhizopus rot caused by Rhizopus stolonifer, buckeye rot caused by Phytophthora species, Black mold rot caused by Alternaria arborescens and Fusarium rots caused by Fusarium species. Harvested tomatoes are susceptible towards infections caused by Fusarium species due to its succulent epicarp which enable the fungal hyphae to penetrate deeply into the fruit [4,5]. The infected tissue is discoloured and appears pale brown whereas the rotted water-soaked tissue and becomes covered by white, yellow or pinkish mycelium externally [6]. Several evidence of root rot cause by Fusarium sp. have been reported in Carthamus roseus L., Ruta graveolens L., Salvia officinalis, Capsicum annuum, Hypericum perforatum L. etc. Leaf blight, loss of turgidity, drooping and wilting of leaves followed by brown to black discoloration are also observed in frequent as a reason of Fusarium sp. attack.

Plant extracts, antagonistic microorganisms are being used an approach of biological control and which are implemented progressively more on a commercial scale in greenhouse crops. Biological control of soil borne pathogen offers environmentally safe, durable and cost effective alternative to chemicals. Numbers of studies showed that efficacy of *Calotropis procera, Curcuma domestica, Moringa oleifera, Tricosanthes dioica, Nigella sativa, Wedelia calendulacea, Andrographis paniculata etc* [7,8]. Similarly many species of fungi and bacteria are reported to be effective bio-control agents against soil borne plant pathogens. *Trichoderma* spp. was found to be very potential bio-control agents against many pathogens including *Fusarium* spp. again enhancement of the activity of the different fungicide using different volume of plant extract are implemented to control [9].

Considering all the facts, present study have been undertaken to develop a sustainable bio control strategy using different plant extracts ,ant agnostic organisms and suitable fungicide against Fusarium rot disease and determining their relative efficiency.

Materials and Methods

The study was conducted in the Laboratory of Plant Pathology, Mycology and Microbiology Laboratory, Department of Botany, University of Rajshahi, during 2017. The objective of this study was to isolate the causal organism of Fusarium rot of tomato and its management by using nine medicinal plant extracts, three fungicides and three antagonists. A good number rot tomato of samples was collected from related localities to the laboratory for undergoing different experimental study. Survey was done in immediate localities. Symptoms of Fusarium rot on fruits were appeared as white mycelium.

Origin of isolation

Fusarium oxysporum f.sp. *Lycopersici* was obtained from the diseased fruits of tomato (*Lycopersicon esculentum Mill.*).

Sterilization of sample

Infected parts of tomato fruits were sterilized by 0.1% of mercuric chloride (HgCl2) solution and continued for 2-3 min. Then given several washes in sterilized distilled water and excess water was removed by pressing. Under this condition, materials were then incubated to support profuse conidiophores and conidia of the fungus.

Plating of fungi

Identified fungus was placed into the sterilized Petri dishes and incubated at $26 \pm 2^{\circ}$ C. Maintaining optimum condition growth rate was follow upped at two days interval up to 10 days.

Control measure

Control measures were done by using medicinal plant extracts, antagonistic fungi and fungicides.

Preparation of extract of the plant parts

Nine different water plant extract concentrations were prepared by weighting 100 gm each plant part and 100 ml of sterilized distilled water was added. Extract concentration of 100% were thus obtained. 5 ml of each extract concentration was added with 95 ml of molten PDA. Thus we obtained 5%, 10%, 15%, 20% and 25% extract concentrations.

Preparation of fungicides

Three fungicides were used against *Fusarium oxysporum* f.sp *lycopersici* and each fungicide was used for 0.1%, 0.15%, 0.2%, 2.5% and 0.3% concentrations in a petri dish plate. Inoculated plates were incubated for 7 days at 27° C.

Isolation of antagonistic fungi from soil

Soil samples were collected from rhizosphere of tomato plants Botanical Garden, Rajshahi University and different tomatoes fields of Godagari Upazila, Rajshahi. *Add* 1.0 g soil to 10 ml of sterile distilled water afterwards; suspension was serially diluted to 10-3. Then was incubated for 7 days at 27°C. The antagonistic properties of three fungi were tested for their efficacy to inhibit the growth of *F. oxysporum* f.sp. *lycopersicion* dual culture on PDA medium.

Statistical analysis

The effect of concentration of plant extract on the growth *of Fusarium oxysporum* f.sp. *lycopersicion* PDA media was evaluated by a two way analysis of variance (ANOVA). Mean difference between treatments or concentration levels of the extracts were separated by Fisher's (1932) least significant difference (LSD) at 5% significant probability level.

Result and discussion

Identification of rot disease of tomato has been done from different locations of Binodpur Bazar and Saheb Bazar of Rajshahi and Deluabari Bazar of Manda, Naogaon. The highest percentage of Fusarium rot infected tomatoes were counted at Deluabari Bazar, Manda, Naogaon and lowest percentage of Fusarium rot infected tomatoes were counted at Saheb bazar, Rajshahi (**Table 1**).

Morphology of the causal Organism (*Fusarium oxysporum* f.sp. *lycopersici*). In culture, mycelium colony was circular to slightly irregular, pale colourless or rarely light brown, aerial mycelium white in PDA media, this fungus changed its mycelium color from white to blackish brown. It is shown in cotton to floccose. The Micro conidia are fucoid broadly falcate with curved pointed tip and well developed foot cell. Chlamydo spores are intercalary in the hyphae, solitary or in chains. The mycelium of this fungus is septate (**Figure 1, Plates 1-4**).

Name of the sample collected areas	No. of rotted tomato	No. of Fusarium rotted tomato
Deluabari Bazar, Manda, Naogaon	100	21
Saheb Bazar, Rajshahi	50	13
Binodpur Bazar, Rajshahi	80	15

 Table 1: List of locations of survey and collection of rot diseased tomato.

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In our study 9 plant extracts and 3 antagonistic fungi were evaluated against the pathogen. 3 fungicides were also evaluated against this pathogen at lowest concentration. All the products tested significantly reduced the fungal growth (**Figure 2**) Result indicated that the 25% concentration of all plant extracts on 12 DAI were found to be inhibitory in most plant extracts *In vitro* (**Tables 2 & 3**).

Scientific Name	Name of used parts	Concentration	Inhibition of growth (%)	LSD(0.05)
Wedelia calendulacea	Leaf	15	53	0.259
		20	53.5	
		25	54.1	
Andrographis paniculata	Leaf	15	48.2	0.334
		20	48.8	
		25	50.9	
Trigonella foenum- graceum	seed	15	42.8	1.21
		20	43.1	
		25	44.7	
Nigella sativa	Seed	15	54.9	3.329
		20	56	
		25	58.25	
Curcuma domestica	Rhizome	15	66.34	6.706
		20	85.2	
		25	86.4	
Momordica charantia	Fruit	15	31.5	2.239
		20	37.9	
		25	39.5	
Tricosanthes dioica	Leaf	15	70.15	3.159
		20	73.5	
		25	82.25	
Moringa oleifera	Leaf	15	68.8	5.918

		20	83.2	
		25	84.9	
Calotropis procera	Leaf	15	77.4	4.884
		20	82.67	
		25	87	
Ridomil MZ 68wa	-	15	0.2	3.5
		20	0.25	
		25	0.3	
Sulcox	-	15	0.2	0
		20	0.25	
		25	0.3	
Indofil M 45	-	15	0.2	3.7
		20	0.25	
		25	0.3	
		PDA(Control)	74 ± 4.33	

Table 2: Effect of different plant extracts on the inhibition of mycelium growth (mm) of *Fusarium oxysporum* f.sp. *lycopersicion* 12 DAI.



Figure 2: Inhibition of *Fusarium oxysporum* f. sp. *Lycopersici* treated with *Curcuma domestica* extract (1) *Calotropis procera* extract (2) *Andrographis paniculata* extract (3) *Wedelia calendulacea* extract (4) *Trigonell afoenum-graceum* extract (5)

Antagonistic fungi	PIRG of mycelium after 7 days of incubation
Trichoderma sp.	82%
Sclerotium sp.	77%
Aspergillus sp.	68.95%

Table 3: Antagonistic effect of *Trichoderma* sp., *Sclerotium* sp. and *Aspergillus* sp. against *Fusarium oxysporum* f. sp. *lycopersici* on 7 days of incubation at 27°C.

Chandra and Singh [10] described that the plant extract of C. procera significantly reduced the wilt disease of chickpea caused by Fusarium oxysporum f.sp. Ciceri, here in our result shows mycelium growth of Fusarium oxysporum f.sp. Lycopersici was measured 13 mm at 25% concentration of C. procera (leaf) extract on 12 DAI it observed 87% of inhibition. Similarly extract of Curcuma domestica (Rhizome) also measured 13.6 mm so 86.4% of decrease in growth. which was reported for antifungal activity for containing tumerone and their LSD value at 5% level are found significant 4.88, 6.70 respectively. In case of Moringaoleifer, Tricosanthesdioica (leaf) extract the result is satisfactory with 84.9%, 82.25% of mycelium growth inhibition, whereas the inhibitory effect of Nigella sativa (seed) extract concentration on 12 DAI found similar with [11] and its LSD at 5% level was 3.329 which reports 58.25 % of inhibition. Minimum inhibitory effect on leaf extract were obtained on Wedelia calendulacea and Andrographis paniculata with 0.259, 0.334 at 95% CI. Against bakane disease of rice and panama diseases of banana Andrographis paniculat have inhibitory effect [12,13] reported that the plant extract of Momordica charantia is found to be inhibitory for the growth of Fusarium oxysporum [14]. Described that the antifungal activity in aqueous and organic extracts of it in our study we found 39% of mycellial growth inhibition in respect of 2.239 LSD value at 5% level.

Out of tested 3 fungicides were Indopil M 45, Sulcox and Ridomil MZ 68wa; Sulcox was the most effective against this pathogen in 0.3% concentration on 12 days of incubation it observed 0.30 % of inhibition. Theworthiness of Sulcox was reported by on controlling rhizome rot of ginger [15].

Because of jeopardized implementation of fungicides and their species particularity their popularity is down falling. Broad spectrum efficiency of antagonistic species so their demand is uprising as fungicide. Antagonistic effect of Trichoderma sp., Sclerotium sp. and Aspergillus sp. has been shown. Because of antibiotics secreted by them can work as inhibitory effector [16]. Other inhibitory substances produced by the antagonists such as geodin, terricin, terric acid, aspergillic acid, dermadin, etc. Whipps and reported that A. niger, A. terreus, G. virens, P. citrinum, T. harzianumand species of Bacillus control soil-borne diseases. Observed that A. flavus, A. niger, and T. virideamended in soil suppressed the growth of F. oxysporum f.sp. Lycociceri and exhibited strong fungi static activity against germination of conidia of tested pathogen from this study the highest (82%) PIRG was of Trichoderma and the lowest was (68.95%) of Aspergillus sp. against Fusarium oxysporum f.sp. lycopersici. Percent Inhibition Radial Growth (PIRG) of Fusarium sp. values were recorded 82%, 72% and 68.95% after 7 days of incubation at 27°C were measured by Trichoderma sp., Sclerotium sp. and Aspergillus sp. respectively when cultured on PDA plates [17-21].

Conclusion

So our study signifies that, plant extracts had equal potential as fungicides for the reduction of mycelium growth of this pathogen. The findings of this study suggest that, in view of the high cost of chemical fungicides and their hazardous consequence, the medicinal plant and antagonistic bio-agents are important source of compounds that are effective against some fungi and these could be good replacements of fungicides.

Acknowledgement

This total work was done in the Plant Pathology and toxicology Laboratory of University of Rajshahi, So grateful to all the supervisor and fellow scientist there.

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