Research Article

Control of tomato crop pathogen using *Ficus carica* extracts

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Abstract

Objective: The present work was carried out to evaluate the effectiveness of plant extract to control disease of tomato (*Lycopersicon esculentum* L.) caused by phytopathogenic fungi (*Fusarium* sp) in world over. **Material and methods** *Ficus carica* was extracted with different solvents. Antifungal activity of the extract against phytopathogenic fungi *Fusarium* sp., was tested by using disc diffusion technique in different concentration (250, 500, 750, 1000mg/ml) on mycelial growth. **Results and Conclusion:** Ethanolic extracts of *Ficus carica* (1000mg/ml) showed effective inhibition of *Fusarium solani*. This indicates that the medicinal extracts could be alternative for the management of *Fusarium sp. Fusarium* sp. causing severe losses in crop field of tomato. This *Ficus carica* showed antifungal activity against *Fusarium s*p. So crop loss due to fungal disease can be controlled.

Keywords: *Ficus carica* ,*Fusarium* sp., disc diffusion technique, ethanol

Introduction

Agriculture is the main backbone of India as it has to support its huge population .Tomato plant (*Lycopersicon esculentum* L) is the main backbone of India as it has to support its huge population. Tomato plant is affected by various disease causing phytopathogenic fungi like *Fusarium oxysporum, Alternaria solani, Aspergillus niger, Phytophthora capsici*, produce extensive damage to crop plants and adversely affect agricultural economy.Pesticide is an essential input for preventing pre and post harvest crop losses. Synthetic pesticides are commonly used in order to control phytopathogenic microorganism. Incessant and extensive use of these synthetic pesticide are posing serious problem to the life supporting systems due to their residual toxicity (Andrea et al., 2000; Harris et al.2001; Campos et al., 2005). It is estimated that hardly 0.l% of the agro-chemicals used in crop protection reaches the target pest, leaving the remaining 99.9% to the environment to cause hazards to non target organisms including humans. The large numbers of synthetic pesticides have been banned in the western world because of their undesirable attributes such as high and acute toxicity, long degradation periods accumulation in the food chain and an extension of their power to destroy both useful and harmful pests.

Many phytopathogenic bacteria have acquired resistance to synthetic pesticides. Considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms. Green plants represent a reservoir of effective chemotherapeutics and can provide valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effective substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant by-products, which possess antimicrobial properties, on several pathogenic bacteria and fungi (Kilani, 2006).

Medicinal higher plants have been used extensively as a source for numerous active constituents for treating disease and they as well, have high contain of therapeutic value *Ficus* is a gens of about 800 species and 200 varieties of *Ficus* of woody shrubs and vines in the family Moraceae occurring in most tropical and subtropical regions with throughout the

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world (Hamed, 2011). *Ficus carica* is commonly referred as `Fig'varios parts of the plant like bark, leaves, tender shoots, fruits, seeds and latex are medicinally important.

Its fruit, root and leaves used in the native system of medicine because of high content of alkaloids, flavonoids, coumarins, saponins, terpenes and phenolic compounds. These compounds cause antimicrobial activity.

Oliveira et al. (2009) reported Phenolic acids such as 3-O- and 5-O-caffeoylquinic acids, ferulic acid, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, psoralen, bergapten, and organic acids (oxalic, citric, malic, quinic, shikimic, and fumaric acids) have been isolated from the water extract of the leaves of *Ficus carica* L.

Saeed and Sabir (2002) investigated on four triterpenoids, bauerenol, lupeol acetate, methyl maslinate and oleanolic acid,have been isolated from the leaves *of Ficus carica*. The leaves of *Ficus carica* consist of various volatile compounds which are identified distinct chemical classes such as methyl-butanol, 2-methylbutanol, (E)-2-hexanal,alcohols:1-penten-3-iol, 3-methyl-1-butanol, 2-methylbutanol, heptanol, benzyl alcohol, (E)-2-nonen-1-ol,andpheylethyl alcohol, ketone:3-pentanone, esters:methyl butanoate, methyl hexanoate, hexylacetate, ethyl benzoate and methyl salicylate, monoterpenes:limonene and menyhol, sesquiterpenes:α-cubenene, α-guaiene, and miscellaneous compunds:psoralen (Oliveira et al., 2010).

The present study report antibacterial activity *Ficus carica* against plant pathogenic bacteria. This work investigates the antibacterial activity of ethanolic and petroleum ether extract of *Ficus carica* leaves by agar well diffusion.

Materials and methods

Collection of diseased parts of the tomato plant

Diseased parts of tomato plant were collected from the field and put into sterile polythene bags and brought into the laboratory.

Isolation and Identification of the pathogens

Infected parts of the tomato were collected, washed then surface sterilization was made with 70% ethanol and rinsed in sterile distilled water, transferred to potato dextrose agar (PDA) medium in petri dishes and incubated in the dark at 28°C for 7 days. Pure cultures were maintained on PDA slants and Petriplates at 4°C. Identification was made by using lactophenol cotton blue mounting technique.

Antifungal activity

Antifungal activity was determined by Disc diffusion technique. In Paper disc method, 20ml of Potato Sucrose Agar (PSA) was dispersed in petridishes and allowed to solidify. After solidification of the media, introduce 0.5 ml. spores on agar medium and spread with glass rod spreader under sterile conditions. Sterilized discs (6 mm, Whatman no. 1 filter paper) will be prepared by soaking in different concentrations of the ethanolic and petroleum ether extracts of *Ficus carica* i. e, 250, 500, 750, 1000 mg/ml for 6 hour. The discs will be then removed and allowed to dry. To assay for antifungal activity various discs impregnated different concentrations of the extracts was placed on the fungal spore or mycelium with the help of sterilized forceps. The petri dishes incubated at 35 °C for 48 h. Antifungal activity was determined by measuring the zone of inhibition, around the discs after the period of incubation.

Determination of Minimum Inhibitory Concentration (MIC) of *Ficus carica*

The broth dilution method was used to determine the minimum inhibitory concentration of the *Ficus carica* leaves extract. The minimum inhibitory concentration test of the leaf extract was screened against pathogenic fungi. The minimum inhibitory concentration was determined using the tube dilution method. To all test tubes 5ml of sterile nutrient broth for pathogenic bacteria and Potato dextrose broth for pathogenic fungi was prepared and sterilized at 121^0C for 15 minutes. Different concentration (250, 500, 750, 1000mg/ml) of leaf was added to all tubes except control. The tubes were shaken well till extract is completely dissolved. Then appropriate amount of microorganisms was inoculated at 37^0C over night for bacteria and room temperature for fungi (Muschietti et al., 2005).

Phytochemical study- GC-MS analysis

Phytochemical compound of ethanolic extracts of *Ficus carica* was determined by GC-MS analysis.

Equipment:	Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II
Column:	ZB 5 -MS Capillary Standard Non-Polarcolumn
Dimension:	30 Mts, ID: 0.25mm, Film: 0.25 µM
Carrier Gas:	He, Flow: 1.0 ML/Min
Temp Prog:	Oven Temp 70 °C raised to 26 °C at 6 c/min
Injection Volume:	1 Microliter

If there is inhibition of the phytopathogenic fungi *Fusarium* it is due to the compound present in the ethanolic extracts of *Ficus carica.*

Results

Morphological and cultural characteristics of isolated phytopathogenic fungi

After incubation, isolated colonies were observed in Potato

Dextrose Agar by plating method. On Potato dextrose agar, the colonies were found to be flat, raised whooly, whitish profuse growth with compact mycelium. Using lactophenol cotton blue mounting technique irregular branched mycelium, conidial spores were also observed, it was given in figure 1.

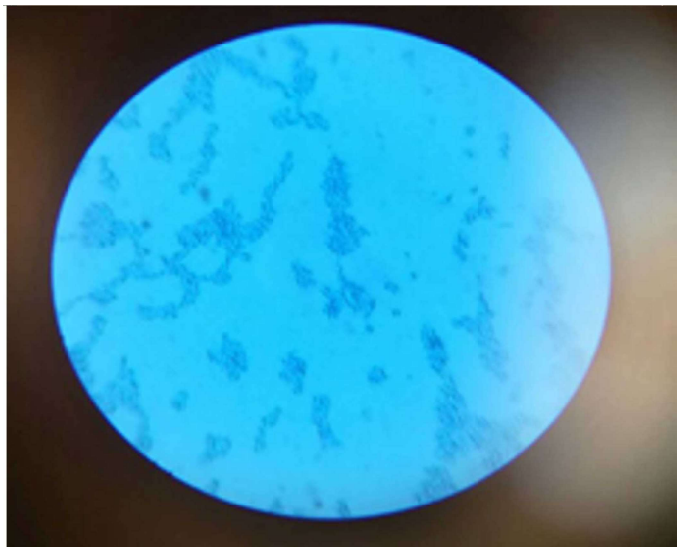

Figure 1. Phytopathogenic fungi *Fusarium* was identified by Lactophenol cotton blue staining

Effect of *Ficus carica* extract against *Fusarium sp* using disc diffusion technique

After incubation zone of inhibition was found to be high 12.9mm in 750mg/ml and 13.8mg/ml in 1000mg/ml in the ethanolic extract of *Ficus carica.* In petroleum ether extract zone of inhibition was found to be 13.0mm in 1000mg/ml and results was given in table 1.

Table 1. Antifungal activity of *Ficus carica* against *Fusarium sp*.

S. No.	*Ficus carica*	Concentration (mg/ml)	Inhibition zone (mm) against *Fusarium* sp
1.	Ethanol	250	6.5
		500	8.9
		750	12.9
		1000	13.8
2.	Petroleum ether	250	4.1
		500	7.4
		750	10.0
		1000	13.0
3.	Control	0	0

-, indicates negative; +, indicates positive

Determination of minimum fungicidal concentration (MIC) of ethanol and petroleum ether extracts of *Ficus carica*

Ethanolic extracts of *Ficus carica* showed to possess an inhibitory activity against *Fusarium solani* 0.54 in 1000mg/ml than Petroleum ether 0.69 in 1000 mg/ml OD at 360nm. The result was tabulated in table 2

Phytoconstituents of *Ficus carica*

Phytochemical screening of Ethanol and petroleum ether extracts of *Ficus carica* showed the presence Flavanoid, phytosterol, phenolic compound and absence of alkaloids tabulated in table 3. The compound which are determined by GC-MS Analysis are 3-Methyl-4-isopropylphenol, 2, 6-

Table 2. Determination of Minimum fungicidal Concentration (MIC) of ethanol and petroleum ether extracts of *Ficus carica*

Fungal pathogen	Optical density at 360nm								
	(ethanol extract) dilution mg/ml					(petroleum ether extract) dilution mg/ml			
	Control	200	500	750	1000	200	500	750	1000
Fusarium solani	0.92	0.82	0.74	0.61	0.54	0.96	0.88	0.76	0.69

Table 3. Phytochemical screening of various extracts of *Ficus carica*

S. No.	Phytochemical tests	Ethanol extract	Petroleum ether extract
1.	Alkaloid	-	-
2.	Flavanoids	+	+
3.	Phytosterol	+	+
4.	Phenolic compound & tannin	+	+

Bis[(5-ethoxycarbonyl-2-hydroxyphenyl)methyl]-4-nitrophenol which has the area of 1.31 % 29.58 % which possess the antifungal activity inhibition of *Fusarium solani*. Sharma and Sharma (2010) reported the methanolic extract of *Ficus carica* possessed the antifungal activity in the same way ethanolic extract was more effecient than petroleum ether, ethyl ether, chloroform extract.

Discussion

Halima and Kafah (2016) reported morphological characters of *Fusarium*, as white to grey, Microconidia and Macroconidia in large numbers and are oval-shaped, Chlamydia spores with rough a wall. In my present study the colonies was found to be flat, raised whooly, whitish profuse growth with compact mycelium of *Fusarium* on potato dextrose agar.

Pratibha et al. (2015) reported 16.5mm inhibition zone was obtained at 750 mg/ml and with leading inhibition zone of ethanol extract 19.6mm zone was observed at 1000 mg/ml concentration using *Acorus calamus* plant extract against *Fusarium oxysporum*. In my present study found 12.9mm in 750mg/ml and 13.8mg/ml in 1000mg/ml, using ethanolic extract of *Ficus carica* against *Fusarium* sp. In my present study, petroleum ether extract of *Ficus carica,* zone of inhibition was found to be 10.00mmin 750mg/ml, 13.0mm in 1000mg/ml .In the same way Pratibha and Rajendran (2016) reported 15.00mm inhibition zone at 250mg/ml concentration, 500mg/ml concentration was effective with 16.87mm inhibition zone. 20.50mm inhibition zone was observed in 750mg/ml concentration using *Zingiber officinale* plant extract.

Aref et al. (2010) reported minimal inhibition concentration (MIC) of the methanol fraction showed a total inhibition against *Candida albicans* (100%) at a concentration of 500 µg/mL and showed negative effect against *Cryptococcus neoforman*; methanolic extract (75%) strongly inhibited *Microsporum canis* and ethyl acetate extract at a concentration of 750 µg/ml. In my present study minimal inhibition concentration (MIC) of the ethanol extracts of *Ficus carica* showed effective inhibition of fungi *Fusarium solani* in 1000mg/ml than petroleum ether. Giliani et al. (2008) have reported the presence of alkaloids, flavonoids, coumarins, saponins and terpenes in aqueous extracts of fruit *Ficus carica*. In present studies also showed the presence of flavonoids, phytosterol and phenolic compound. Dilek Keskin et al. (2012) reported the compound identified by GC-MS Analysis were benzothienoquinoline (0.94%), 6-methylthiol (0.84%). Quinolone derivatives useful as an Antimicrobial agent.

Conclusion

Fusarium sp. causing severe losses in crop field of tomato. This *Ficus carica* showed antifungal activity against *Fusarium s*p. So crop loss due to fungal disease can be controlled.

Future aspect

It was concluded from the present research that plant extracts *Ficus carica* are the cheap source and be effective fungicide and it do not have human and environmental health implication. So in the future aspects the work deals with the control of bacterial plant pathogen using the same extracts for the protection of the tomato crop.

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Conflicts of interest

Authors declare no conflict of interest.

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