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RESEARCH ARTICLE

BIOEFFICACY OF SOME PLANT EXTRACTS AGAINST *FUSARIUM* SPECIES CAUSING WILT IN TOMATO

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ABSTRACT

This study was carried out with an objective to investigate the antifungal potential of Turmeric (*Curcuma longa*) (Family Zingiberaceae), Black pepper (*Piper nigrum*) (Family Piperaceae) and Clove (*Syzygium aromaticum*) (Family Myrtaceae). The aim of the study is to evaluate the potential of antifungal activity against fungal strains *Fusarium oxysporum* f.sp. *lycopersici* causable for wilting in tomato plant and to determine the zone of inhibition. The antifungal activity of dried parts powder (250, 500, 750, 1000 mg/ml) were tested in acetone solvent against tomato wilt pathogen by paper disc diffusion essay. The results showed that the Clove exhibits highest inhibition of the fungal growth as 18.9 mm inhibition zone. So this study confirms the ethno medical use of daily used kitchen spices for treating various fungal infections.

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INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. In many developing countries, plants are the important source of medicine having potent drugs (Srivastava, Lambert and Vietmeyer, 1996). Medicinal properties of plants have been used as antibacterial, antifungal and antiviral activities from hundreds of years in different parts of the world (Ali et al., 1998; Barbour et al., 2004; Yasunaka et al., 2005). All the plants have an extreme efficiency and ability to synthesize aromatic substances, most of which are phenols or phenol's oxygen substituted derivatives (Geissman, 1963). Most of these aromatic compounds are secondary metabolites, out of which more than 12,000 have been isolated, their number estimated to be less than 10% of total (Schultes, 1978). All these active chemical compounds are the most important part of plant's defense mechanism. In nature these secondary metabolites are specific and varies from species to genera. These secondary metabolites are not used by plants in normal primary metabolic need but they enhance the overall capacity of it to survive and prepare to face challenges by giving them ability to interact with their environment (Harborne, 1993).

These substances are fatal towards various microorganisms, Insects and herbivores naturally. Like some terpenoids provides specific odours to its native one, at the same time tannins and quinons are equally responsible for pigment formation quality of plant parts. Many of them are flavoring in nature as the flavour of chili pepers is because of capsaicin a terpenoid. Some main catagories of phytochemicals extracted from medicinal plants are studied to evaluate their medicinal activity. *Fusarium* species are usually found in cereals and often produces mycotoxins in the kernels of various cereal species. *F. oxysporum* causes vascular wilts on many crops, whereas in other cases, Root and stem rot are caused especially by *F. solani*, and rots of seeds that are accompanied by the production of mycotoxins. Moreover, some *Fusarium* species can cause disease in immunocompromised human population (Agrios, 2005; Seifert, 2010). The species *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. semitectum*, *F. tricinctum* and *F. sporotrichioides* are found in cereals; *F. nygamai*, *F. verticilloides* and *F. subglutinans* in corn; *F. thapsinum* and *F. chlamydosporum* in sorghum, while *F. nygamaian* and *F. fujikuroi* are found in rice. In legumes *F. chlamydosporum* and *F. tumidum* are typically encountered. *F. solani* usually attack potatoes. The species *F. acuminatum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani* and *F. sambucinum* can attack a variety of fruits,

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vegetables and ornamental plants (Samuels, 2001). The Fusarium wilt of tomato (*Lycopersicon esculantum* Mill) caused by *Fusarium oxysporum* f. sp. *lycopersici* (Snyder and Hansen). It (Fol) is recognized as a devastating disease in tomato growing areas all over the world (Beckman, 1987; Bondad-Reantaso *et al.*, 2005) also in different regions of India from severe to moderate (50-60%) percentage (Sherf and Macnab, 1986; Jiskani *et al.*, 2007; Chakraborty and Chatterjee, 2009). Plants possess antimicrobial properties because of the presence a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc. Microbiologists have strongly two reasons to be interested in the antimicrobial properties of plant extracts and their scopes in medical science. First, it is very likely discovered that these phyto chemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians. It is reported that, approximately two or three antibiotics at an average rate derived from microorganisms are launched each year (Clark, 1996). Second reason is that, the public is becoming aware of problems with the over prescription and overdose with antibiotics and at the same time because of the alter effects of allopathic medicinal system. This study focused at evaluating the antifungal activity of locally available three extracts of turmeric, clove and black pepper against growth of *Fusarium oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Dried powder of Turmeric (*Curcuma longa*) (Family Zingiberaceae), Black pepper (*Piper nigrum*) (Family Piperaceae), Clove (*Syzygium aromaticum*) (Family Myrtaceae) were collected from local market. 2.0 gram of organic extracts were prepared by extracting sample successively with acetone in a ratio of 1:5. The resultant extract was weighed and stored in airtight sample bottles. All the extracts were oven evaporated till complete dryness for 5 - 7 days at 30 ± 2 °C. Plant extract was second time extracted with DMSO. 200 mg of each extract was weighed into a sterilized sample bottle and dissolved in DMSO (Sigma) to make a concentration of 200mg/ml. For the study 250, 500, 750, 1000 mg/ml concentrations were prepared. Microorganism used in this study *Fusarium oxysporum* f. sp. *lycopersici* was obtained from Microbial Type Culture Collection MTCC, Institute of microbial technology, Chandigarh.

Antimicrobial Activity

In Paper disc diffusion method some amount of Potato Sucrose Agar (PSA) was dispersed in petridishes and allowed to solidify. A micropipette will be used to introduce 0.1 ml. spores on agar medium and spread with glass rod spreader under sterile conditions. Sterilized discs (6 mm, Whatmann No. 1 filter paper) will be prepared by soaking in different concentrations of the extracts ie, 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 with different concentrations of the extracts will be placed on the fungal spore or mycelium with the help of sterilized forceps. The petridishes incubated at 35 °C for 48 h. Antifungal activity will be determined by measurement of the zone of inhibition around the discs after the period of incubation. Experiments were performed in the laboratory of L.S.M.G.P.G. college Pithoragarh.

Data Analysis

Data from antifungal activity screening were analyzed using simple statistics from Microsoft Excel and recorded in appropriate tables as mean \pm standard deviation of mean.

RESULT AND DISCUSSION

Table below represents the average concentration of plant material extracted from acetone solvent, prior to the disc diffusion method. Table 1 showed that the dried turmeric extract was effective against the tested pathogen *Fusarium oxysporum* f.sp. *lycopersici*. Its acetone extract showed 11.25 mm inhibition zone at 250 mg/ml concentration. 500 mg/ml concentration was moderately effective with 13.75 mm inhibition zone. At 750 mg/ml and 1000 mg/ml the zone of inhibition were observed to be as 14.75 mm and 17.95 mm (Plate 1). While acetone extract of black pepper showed 9.8 mm inhibition zone at 250 mg/ml concentration. 500 mg/ml concentration was effective with 11.7 mm inhibition zone. 13.8 mm inhibition zone was observed at 750 mg/ml concentration. And 1000 mg/ml concentration of black pepper showed 15.8 mm. zone of inhibition (Plate 2). Dried powdered clove in acetone solvent was highest effective at its 1000 mg/ml concentration as 18.9 mm inhibition zone.

Table 1. In -vitro antifungal activity of some medicinal plant extract a t various concentrations against fungal pathogen *Fusarium oxysporum* f.sp. *lycopersici*

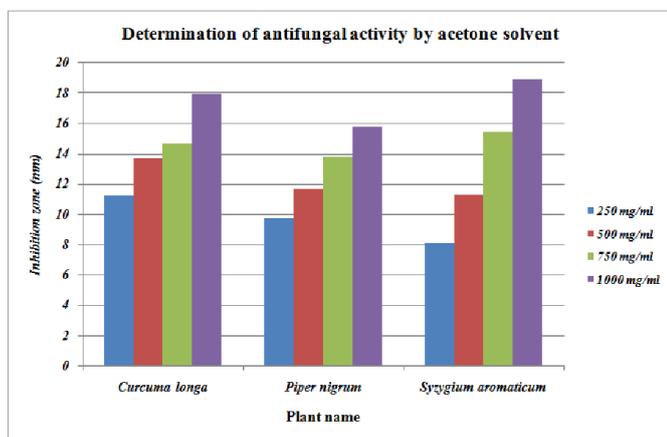
Plant Name	Solvent	Mean Inhibition Zone (mm) in various concentrations (mg/ml)			
		250 mg/ml	500 mg/ml	750 mg/ml	1000 mg/ml
<i>Curcuma longa</i>	Acetone	11.25 \pm 0.9	13.75 \pm 0.4	14.75 \pm 0.6	17.95 \pm 0.9
<i>Piper nigrum</i>		9.8 \pm 0.6	11.7 \pm 0.6	13.8 \pm 0.5	15.8 \pm 0.6
<i>Syzygium aromaticum</i>		8.1 \pm 0.4	11.3 \pm 0.6	15.5 \pm 0.5	18.9 \pm 0.6

Results are the mean of four replications \pm S.D

Fungus culture (MTCC 1755) was maintained in Potato sucrose agar medium for an optimum pH of 6.8. The fungal spore suspension was prepared by the addition of a loopful of fungal spores in a 5 ml of sterile distilled water and 1 ml Tween 20. Then 0.1 ml fungal spore suspension was mixed well in aseptic conditions and spread evenly on the petri dishes containing 20 ml of solidified potato sucrose agar.

Its other concentrations were also very effective. 8.1 mm inhibition zone was obtained at 250 mg/m concentration. While 500 and 750 mg/ml concentrations were similarly effective as 11.3 and 15.5 mm inhibition zone (Plate 3). Member of Zingiberaceae family are found to be a rich source of substances of phytochemical interest. They are rich in curcuminoids, and known for their broad spectrum of biological activities, curcuminoids vary in chemical structures, physico-chemical characteristics as well as the functional

properties (Revathy *et al.*, 2011). Antimicrobial activity of piperine increases as the piperine an alkaloid the major constituent of piperamides concentration increases against fungus which is present in the skin and seed of the black pepper and responsible for the antimicrobial activity. Cloves main component is eugenol which is responsible for antifungal properties against various pathogens. The results suggest that turmeric, clove and black pepper exhibited significant antimicrobial effects.



Graph Antifungal activity of some plants against *Fusarium oxysporum f.sp. lycopersici*



Plate 1. Antifungal activity of acetone extract of *Curcuma longa*



Plate 2. Antifungal activity of acetone extract of *Piper nigrum*



Plate 3. Antifungal activity of acetone extract of *Syzygium aromaticum*

Conclusion

Turmeric, black pepper and Cloves are aromatic spices that have many useful properties. Their extract was successfully effective in suppressing the *Fusarium* growth *in-vitro*. These plants and their extracts could be promising as a source of natural eco-friendly phytofungicidal compounds for *in-vivo* applications. Confirmations of the efficacy *in vivo* of these extracts against *Fusarium* are needed.

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