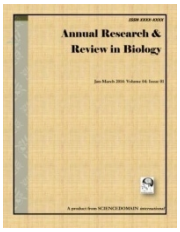

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Inhibitory Effect of Selected Medicinal Plant Extracts on Phytopathogenic Fungus *Fusarium oxysporum* (Nectriaceae) Schlecht. Emend. Snyder and Hansen

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Author's contribution

Author designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The study was aimed to screen antifungal activity of some medicinal plant extracts against *F. oxysporum* by screening fungistatic, fungicidal activities and minimum inhibitory dilution (MID).
Study Design: All the data were subjected to analysis of variance followed by mean separation through Duncan's multiple range tests using computer software.
Place and Duration of Study: Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka between June 2012 and May 2013.
Methodology: *In vitro* studies were carried out to test the antifungal activity of 6 plant extracts; *Oxalis corniculata* L. (creeping wood sorrel), *Ocimum gratissimum* L. (wild basil), *Tithonia diversifolia* (Hemsl.) A. Gray (wild sunflower), *Azadirachta indica* A. Juss. (neem) ,*Kaempferia galangal* L.(aromatic ginger) and *Zingiber officinale* Roscoe (ginger). All plant extract were screened for their fungistatic, fungicidal activities and minimum inhibitory dilution (MID) against *F. oxysporum.*
Results: Results showed that radial growth of *F. oxysporum* was significantly impaired (P = .05) by all extracts except wild sunflower and creeping wood sorrel. *F. oxysporum* differed in their reaction to the different extracts but on the whole, growth inhibition increased with the concentration of each extract. At 25 %, the most active extracts were

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aromatic ginger, wild basil and neem, which are shows inhibition values of 91%, 89% and 83% for *F. oxysporum* respectively. The minimal inhibitory dilution (MID) were 3.125 % (v/v) for aromatic ginger and wild basil and 6.25% (v/v) for ginger. Out of six plants extract screened, wild basil, aromatic ginger and neem showed more than 80% fungal inhibition after 6 hour immersion and other extracts could not exceed 60% inhibition after any exposure time.

Conclusion: The study revealed that methanol crude extract of aromatic ginger, wild basil and neem exhibit strong fungistatic and fungicidal activities against *F. oxysporum,* whereas aromatic ginger and wild basil could be used as an effective antifungal agent.

Keywords: Antifungal activity; Fusarium oxysporum; fungistatic; fungicidal; plant extracts.

1. INTRODUCTION

Fusarium oxysporum (Nectriaceae) schlecht. Emend. snyder & hansen is a plant pathogenic fungus that causes 'fusarium wilt' in more than a hundred species of plants such as tomato, potato, sugarcane, cowpea, musa, pea, ginger, etc. it colonizes the xylem of the host plant, and as a result, blockage and breakdown of the xylem leads to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant [1]. Management of *F. oxysporum* is required, as this pathogen and its many special forms affect a wide variety of hosts of economic value. Control of *Fusarium* wilt disease has been accomplished primarily by the application of chemical fungicides, long crop rotations, pasteurizing seedbeds with steam or fumigants [2]. Nevertheless, the massive use of synthetic fungicides in crop defense had severe environmental impact. The inappropriate use of agrochemicals especially fungicides were found to possess adverse effects on ecosystems and a possible carcinogenic risk than insecticides and herbicides together [3]. Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective [4]. Due to the aforementioned considerations, there may be a need to develop new management systems to reduce the dependence on the synthetic agrochemicals.

In this respect, plant extract may represent an ideal solution to the problem be easily tested in vitro. In recent years much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens [5], medicinal plants are part and parcel of human society to combat disease. The antimicrobial activity of medicinal plant oils and extracts has been recognized for many years. Aqueous extracts of 46 plants against *Fusarium* spp. revealed that 12 plants have recorded significant antifungal activity and that these plants could be exploited for eco-friendly management [6]. However, in Sri Lanka few investigations have conducted to find antimicrobial activity of oils and extracts of traditional medicinal plants against plant pathogenic microbes.

Considering the vast potentiality of medicinal plant as sources for antimicrobial agents, the present study was designed to evaluate the *in vitro* antifungal activity of some plant extracts ; *Oxalis corniculata* L. (creeping wood sorrel), *Ocimum gratissimum* L. (wild basil), *Tithonia diversifolia* (Hemsl.) A. Gray (wild sunflower), *Azadirachta indica* A.Juss. (neem) ,*Kaempferia galangal* L.(aromatic ginger) and Zingiber officinale Roscoe. (ginger) against fungal strains *F. oxysporum* by screening fungistatic, fungicidal activities and minimum inhibitory dilution (MID) .

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extracts

Fresh leaves of *Oxalis corniculata* L. (creeping wood sorrel), *Ocimum gratissimum* L. (wild basil), *Tithonia diversifolia* (Hemsl.) A. Gray (wild sunflower), *Azadirachta indica* A.Juss. (neem) ,*Kaempferia galangal* L.*(*aromatic ginger) *and Zingiber officinale* Roscoe (ginger) were collected (Table 1) from the surrounding areas of Belihuloya, Sri Lanka. The leaves and rhizome were washed in clean water and dried in room temperature. The dried plant materials were milled to a fine power using grinder and stored in the dark at room temperature in airtight containers. 1 g of finely ground plant material was used for the extraction in 5 ml of methanol. The solution was kept overnight at room temperature and filtered using filter papers (Whatman filter paper No. 1) and stored at 4^0C temperature.

Table 1. Common, scientific names, families and used parts of studied plants

Common name	Scientific name	Family	Used part
Aromatic Ginger	*Kaempferia galanga*	Zingiberacea	rhizome
Ginger	*Zingiber officinale*	Zingiberacea	rhizome
Wild basil	*Ocimum gratissimum*	Lamiaceae	leaves
creeping woodsorrel,	*Oxalis corniculata*	Oxalidaceae	leaves
Neem	*Azadirachta indica*	Meliaceae	Leaves
Wild Sunflower	*Tithonia diversifolia*	Asteraceae	leaves

2.2 Effect of Different Concentrations of Extracts on Radial Growth of Test Organisms

Plant extracts which could suppress the fungal growth in the disk diffusion method were further tested for their efficiency against the pathogen by using an agar dilution technique [7]. Different concentrations of the extracts; 25%, 12.5%, 6.25%, and 3.125% of effective plant were amended with PDA. The amended medium was dispensed into sterile Petri plates and allowed to solidify and to avoid bacterial contamination 0.5g of antibacterial streptomycin was added to 1L of PDA medium. Two perpendicular lines were drawn at the bottom of each plate to cross each other at the centre of the plate. Each plate was inoculated with *F. oxysporum*. A 4-mm diameter mycelia disc of each of the test organisms was inoculated on each amended agar plate. Inoculated plates were incubated at 25±2ºC and growth measured along the perpendicular lines. Daily radial growth of each test organism in any of the test extracts was recorded for 7 days. Each treatment was replicated four times. The media amended with methanol and recommended fungicide for respective fungal strain were consider as negative and positive control respectively.

$$\%\ of\ inhibition\ = \frac{Diameter\ of\ control\ colony\ -\ Diameter\ of\ treated\ colony}{Diameter\ of\ control\ colony} x\ 100$$

In here four replications were prepared for each treatment. Then all the culture plates were incubated at 27ºC in dark condition. The mycelia growth of fungus was measured after 24, 48, 72 and 96 hours. Calculate the percent inhibition of the mycelia growth over control by using the following formula [8].

2.3 Minimum Inhibition Dilution (MID)

The minimal inhibition concentration was taken from the results of the fungistatic activity test. The lowest bio extracts dilution with highest inhibition percentage was taken as MID.

2.4 Fungicidal Activity

Further study about fungicidal activity of each plant extract and carbendazim were done for the minimal inhibition dilution (MID). Fungicidal activities of each treatment against *F. oxysporum* done by immersing the fungal block in minimal inhibition concentration of each solution separately for 1, 3, 6, 12 and 24 hours and incubate for 7 days to get inhibition percentages. PDA media plates were prepared and treated fungal blocks were inoculated aseptically in the center of the plate. The agar blocks were washed prior to inoculate on PDA plates to remove the crude extracts. Fungal blocks which were dipped in sterilized distilled water for the above time periods were used as controller. Three replications were prepared for each treatment. All culture plates were incubated at room temperature in dark condition. The mycelia growth was measured by taking the colony diameter after 24, 48, 72 and 96 hour.

The comparison of the fungicidal activity of minimal inhibition concentrations of plant extracts and carbendazim were done by calculating the present inhibition of the mycelia growth over control by using the above formula [8].

2.5 Data Analysis

The experiment was conducted using a completely randomized design. Standard errors of means of three replicates were computed using computer software Microsoft Excel. All the data were subjected to analysis of variance followed by mean separation through Duncan's Multiple Range Test. All statistical analyses were carried out using SAS soft ware.

3. RESULTS AND DISCUSSION

The present study tested the antifungal activity of methanol crude extracts and their respective dilutions of selected medicinal plants against fungal strain *F. oxysporum* based on their traditional use as antiseptics (Table 1). All the plant extracts screened by agar diffusion method, showed different level of antifungal activity as evidenced by a zone inhibition except *Tithonia diversifolia* (Wild sunflower) and *Oxalis corniculata* (creeping woodsorrel)). Plant species, *Ocimum gratissimum* (wild basil), *Azadirachta indica* (neem), *Kaempferia galangal* L. (aromatic ginger) and *Zingiber officinale* (ginger) significantly (P = .05) suppressed the growth of *F. oxysporum* compared to control. *Ocimum gratissimum* (wild basil) and *Kaempferia galangal* L. (aromatic ginger) and *Azadirachta indica* (neem) showed exceptionally prominent activity against *F. oxysporum* (Fig. 1).

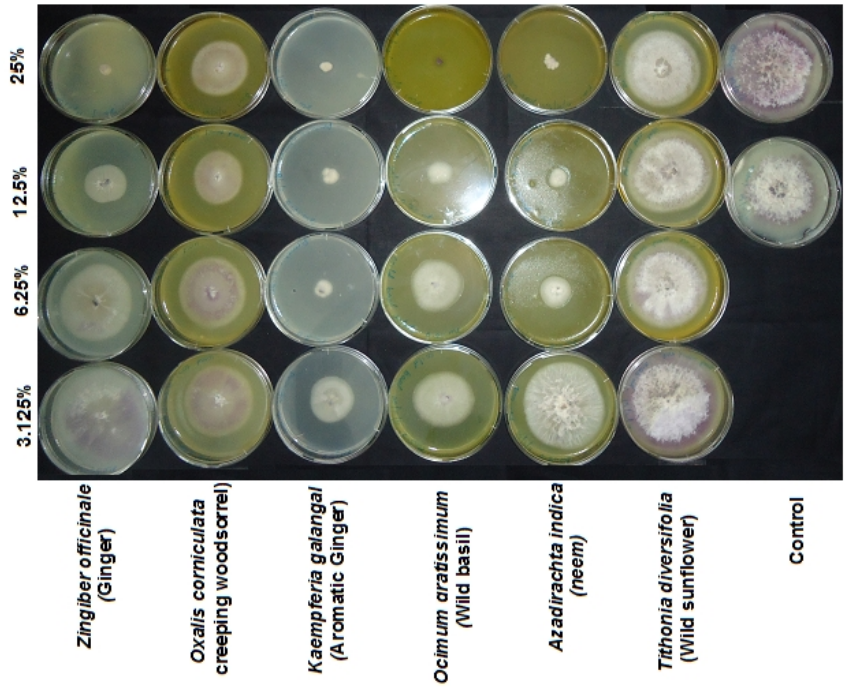
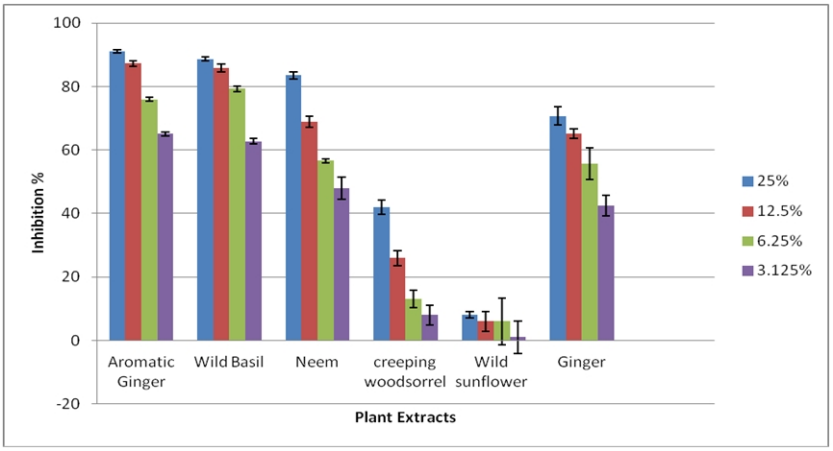


Fig. 1. Mycelium growth inhibition effect at different concentrations of leaf extract of O*. corniculata, O. gratissimum*, *T. diversifolia, A. indica* and rhizome extract of *K. galangal* and *Z. officinale* against *F. oxysporum*

3.1 Effect of Different Dilution of Extracts on Radial Growth of test Organism

The antifungal effects of the studied plant extracts, *Oxalis corniculata* (creeping), *Ocimum gratissimum* (wild basil), Wild sunflower (*Tithonia diversifolia)* and *Azadirachta indica (neem) Kaempferia galangal* (aromatic ginger) and *Zingiber officinale* (ginger) on fungi strain *F. oxysporum* was compared with the control by agar dilution method. The results showed that the growth inhibition of the tested fungi produced by plants extracted at concentration ranging from 3.125% - 25% were significantly (P = .05) different from control values. All concentrations of methnol extracts of aromatic ginger, neem and wild basil was significantly impaired (P = .05) the mycelial growth *F. oxysporum* by the addition of the plant extracts into culture medium compared with the control treatment and the effect was proportional to concentration (Fig. 2). The test fungi differed in their reaction to the different extracts but on the whole, growth inhibition increased with the concentration of each extract. Fig. 2 shows a marked effect of the 25% crude extracts from aromatic ginger, wild basil and neem with inhibition values of 91%, 86% and 83 % for *F. oxysporum* respectively. The plant extract of creeping woodsorrel and, Wild sunflower showed comparatively very low activity against *F. oxysporum.* However, ginger showed moderate level of antifungal activity (75%-42%).



Mean ± S.E.M = Mean values ± Standard error of means of four experiments

Fig. 2. Mycelium growth inhibition effect at various dilutions of *O. corniculata O. gratissimum*, *T. diversifolia*, *A. indica, K galangal* and *Z. officinale* against *F. oxysporum*

3.2 Minimum Inhibition Dilution (MID)

All plant extracts were effective reduced the redial growth the fungal strains with varying degree of efficacy over the different dilution (Fig. 3).The percentage of inhibition increased with the increase of the plant extract concentrations. In lowest concentrations 3.125% of aromatic ginger showed effectiveness in minimizing the colony growth of fungi up to 69% and followed by wild basil (65%) against *F. oxysporum.* Neem and Ginger shows more than 50% inhibition at 6.25 % dilution others showed very low effectiveness less than 25% (Fig. 3).Methanol crude extracts of Neem, wild basil and aromatic ginger were found highly effective in suppressing the growth of *F. oxysporum* even at 3.125% concentration. Minimal inhibition Dilution (MID) of aromatic ginger and wild basil was 3.125%, whereas MID for ginger and Neem was recorded as 6.25%.

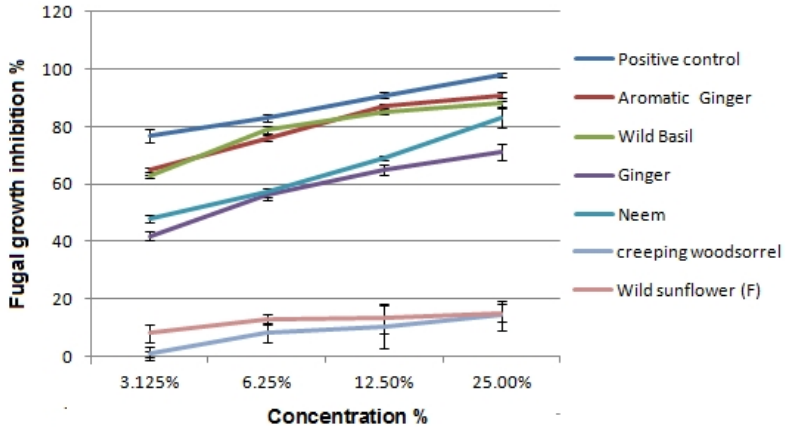


Fig. 3. Effect of different dilutions % of *O. corniculata*, *O. gratissimum*, and *A. indica, K. galangal* and *Z. Officinale* on mycelium growth of *F. oxysporum*

3.3 Fungicidal Activity

The fungicidal activities of selected plant methanol crude extracts against *F. oxysporum* are shown in Table 2. This test was done to study the ability of different plant crude extract to kill the vegetative forms of *F. oxysporum* after immersed in solutions for different time periods. Inhibition ratios were calculated after the incubation period and data was analyzed with ANOVA by using statistical analyzing software (SAS). Significant differences of two variables were determined by Duncan's Multiple Range test at (P = .05). In this study neem (6.25%), wild basil (3.125%), aromatic ginger (3.125%) and positive control (carbendazim 0.05%) was shown the significant inhibition of the vegetative growth of the fungi. In addition to that fungicidal activity was varied with exposure time. carbendazim 0.05% was shown the 76% inhibition after 1 hour immersion and neem, wild basil and aromatic ginger was more than 80% inhibition after 6 hours immersion where as ginger shows moderate level of inhibition after 24 hours immersion (Table 2). Other plant crude extracts wild sunflower, creeping and woodsorrel were shown no significant inhibition of the fungal growth even at 24 hours immersion time.

Table 2. Fungicidal activity of various concentration of crude extracts against the growth of *Fusarium oxysporum*

Time	Inhibition ratio of growth of *F. oxysporum* Crude extracts						
	K. galangal	***Z. officinale***	***O. gratissimum***	***T. diversifolia***	***O. corniculata***	***A. indica***	**Positive control (0.05%)**
1hr	53.3^{y}	44.6^{x}	55.5^{y}	18.5^{x}	12.3^{x}	48.3^{x}	76^{z}
3hr	73.3^{z}	48.3^{x}	65.5^{y}	20.3^{x}	19.0^{x}	69.0^{y}	80^{z}
6hr	95.8^{z}	56.4^{y}	85.5^{z}	23.0^{x}	24.0^{x}	80.0^{z}	92^{z}
12hr	98.8^{z}	59.8^{y}	95.3^{z}	22.4^{x}	26.4^{x}	90.4^{z}	100^{z}
24hr	100^{z}	65.2^{y}	100^{z}	29.6^{x}	29.8^{x}	96.8^{z}	100^{z}

Numbers followed by the same letters in each row were not significantly different according to Duncan's Multiple Range test at (P = .05). Minimal inhibition Dialution (MID) of aromatic ginger and wild basil was 3.125% whereas, MID for neem and ginger, was 6.25% for others as 20%.

Biological control had attained importance in modern agriculture to curtail the hazards of intensive use of chemicals for pest and disease control [9]. Accordingly, the efficacy of different plant extracts *of* Wild sunflower, creeping woodsorrel, wild basil, *neem,* aromatic ginger and ginger against *F. oxysporum* was studied *in vitro.* Wild sunflower, creeping and woodsorrel at all concentrations, showed a slightly inhibition for the *F. oxysporum* thus was excluded from further studies whereas wild basil, neem *and* aromatic ginger methanol crude extracts were found to be highly effective in controlling the growth of the *F. oxysporum*. These all types of extracts showed different levels of antimicrobial activity and the relative differences were found to vary within the tested extracts and with increasing concentration of the extracts, a gradual increase in the inhibition potential of the *F. oxysporum* was recorded.

Comparing leaf extracts of aromatic ginger and wild basil with methanol, the inhibition percentage in each increased gradually with the extract concentration, both completely suppressed the growth of *F. oxysporum* at 50%. Results of the present study are in agreement with [9] who noticed that methanol extracts of aromatic ginger and wild basil showed fungitoxic properties against 5 pathogenic fungi (*Alternaria brassicola*, *Colletotrichum capsici*, *F. oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*) when tested under laboratory conditions at 500 and 1000 µg/ml.

In this study, four concentrations of the methanol crude extract of neem leaf effectively suppressed mycelial growth of the *F. oxysporum*. These results are in agreement with [10]. who found that the growth of four pathogens (*F. oxysporum*, *R. solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*) which incite wilt and rot in *Cicer arietinum*, was inhibited in liquid medium by extracts of leaf, trunk park and oil from the neem tree. [11] Who found that the mycelia growth inhibition rate increased with plant extract concentration, also, 100% aqueous neem (*A. indica*) leaf extract caused complete inhibition of spore germination of *Fusarium* spp. And with notes of [12] who reported that active compounds of neem are distributed throughout the tree parts but are concentrated in seeds and leaves, and were extractable by water and organic solvents. Our previous study [13] shows aqueous extract of neem were less effective against *F. oxysporum* than methanol extract .These findings agree with [14] who found that both ether and methanol neem seed extracts gave the best results at 3000 µlitre/100 ml concentrations but the methanol was found effective against *A. niger*, *F. oxysporum* and *Trichoderma resii.* According to findings of [15], there are 15 plant extracts capable to inhibit completely conidial germination of *F. oxysporum*.

These three extracts exhibit significant fungicidal properties that support their traditional use as antiseptics. In the case of fungal infection, these mechanisms include synthesis of bioactive organic compounds [16] and antifungal proteins [17] and peptides [18] .Therefore further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity.

4. CONCLUSION

In the conclusion, the present study explores the possibilities of controlling *F. oxysporum*, by using extracts of neem, aromatic ginger and wild basil. The fungitoxic effects of the phyto-extracts indicate the potentials of selected plant species as a source of natural fungicidal material. Antifungal activity was confirmed by all of the selected plant species and the results revealed neem, wild basil and aromatic ginger are the most effective inhibitor for the mycelia growth of *F. oxysporum*. The finding of the present investigation could be an important step towards the possibilities of using natural plant products as biopesticides in the control of plant diseases caused by *F. oxysporum*. Further purification, extraction and photochemical analysis of the active compounds of those plants would give a strong antifungal activity comparable to synthetic fungicides.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Agrios GN. Plant Pathology. 4th ed. Academic Press, Inc, New York; 1997.
2. Spletze ME, Enyedi AJ. Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato. Phytopathol. Tomato. Phytopathology. l999;89:722-727.
3. Osman KA, Al-Rehiayam S. Risk assessment of pesticide to human and the environment. Saudi J. Biol. Sci. 2003;10:81-106.
4. Zhonghua MA, Michailides TJ. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 2005;24:853-863.
5. Nwachukwu EO, Umechuruba CI. Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. J. Appl. Sci. and Enviro. Manag. 2001;5:29-32.
6. Satish S, Raghavendra MP, Raveesha KA. Anti-fungal Potentiality of Some Plant Extracts against Fusarium spp. Archives of Phytopath and Pl. Prot. 2009;42:618-625.
7. Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Pfaller MA, Tenover FC, Baron EJ, Yolken RH, ed. Manual of clinical microbiology, 7th ed. Washington, DC: ASM Press: 1999;1526-1543.
8. Vincent JM. Distortions of fungal hyphae in the presence of certain inhibitors. Nature. 1947;6:159:850.
9. Tuber MJ, Baker, Every Every R. Other alternative biological control. Bio Science. 1998;38:660-661.
10. Shivpur A, Sharma OP, Jhamaria SL. Fungitoxic properties of plant extracts against pathogenic fungi. J. Mycol. and Pl. Pathol. 1997;27:29-31.
11. Singh UP, Singh HB, Singh RB. The fungicidal effect of neem (A. indica) extracts on some soil borne pathogens of gram (*Cicer arietinum*). Mycologia. 1980;72:1077-1093.
12. Dwivedi BP, Shukla DN. Effect of leaf extracts of some medicinal plants on spore germination of some *Fusarium* species. Karnataka J. of Agric. Sci.2000;13:153-154.
13. Banumathy B. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. Phytoparasitica. 1998;26:109-11.
14. Dissanayake MLMC, Kumari WKMT. Efficacy of various plant extracts to control *Fusarium* wilt of *Polyscias balfouriana* variety Marginata. Asian journal of Experimental. Science. 2012;3:129-135.
15. Upasana ST, Anurag AK, Upadhyay U, Tewari A. *In vitro* antimicrobial study of Neem *(A. indica*) seed and leaf extracts. J. Ind. of Vet. Med. 2002;22:109-111.
16. Domenico R, Filomena M, Elisabetta S. Inhibitory Effect of Plant Extracts on Conidial Germination of the phytopathogenic Fungus *Fusarium oxysporum.* American Journal of Plant Sciences. 2012;3:1693-1698.
17. Morrisey JP, Osbourn A. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiology and Molecular Biology Reviews. 1999;63:708-724.
18. Selitrennikoff CP. Antifungal proteins. Applied and Environmental Microbiology. 2001;67:2883-2894.

19. Broekaert WF, Cammue BPA, De Bolle MFC, Thevissen K, De Samblanx GW, Osborn RW. Antimicrobial peptides from plants. Critical Reviews in Plant Sciences.1997; 16:297-323.

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