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Research Article

Use of biopotentials of plant extracts of medicinal importance against pathogenic fungi *Fusarium oxysporum.*

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Abstract

Stem, leaf, seed and root extract of ten Indian plants (*Accacia catechu* Willd, *Accacia arabicae* Willd, *Carissa congesta* Wight, *Cassia fistula* (L.),*Cuscuta reflexa* Roxb., *Jatropha curcas* L.,*Moringa oleifera* Lam., *Syzygium cumini*, *Terminalia arjuna* Roxb., *Vitex negundo* L.) of medicinal importance from India significantly manage the *Fusarium* wilt of Tomato (*Lycopercicon esculantum*) caused by *Fusarium oxysporum sp. Lycopercicon.* Root extract of *Accacia catechu* control the growth of test fungi at 25% concentration (23.66), followed by leaf extract of *Jatropha curcas* at 25%, showed 33.33 cm growth of test fungi in treated plates, remaining extracts of plant parts inhibited the growth at 25% with less than 45cm growth. All extracts showed 100% PCE in 50% concentration against test fungi, while in 25% concentration it showed more than 99.8% PCE in several plant extracts.

INTRODUCTION

The use of medicinal plant materials for the inhibition of fungal diseases is an old practice in many countries and is still offers an enormous potential source of antifungal agent (Usharani and Chitra, 2014). Many phytochemical pesticides exhibiting broad spectrum of activity against pest and diseases have long been considered as attractive alternative to synthetic chemical pesticides as they are biodegradable, target specific and pose no or less hazard to the environment or to human health. (Walia *et al.*, 2014). Plants have been a rich source of medicines because the produce a bioactive molecules, most of which probably evolved as chemical defenses against predation or infection (Anitha *et al.*, 2016). Irrational use of chemicals pesticides pose dangerous to overall ecosystem these chemicals combat various phytopathogens which are inhibited various crop diseases, but on the other hand they have their adverse effect on human health and environment. Pesticides of chemical origin pollutes water, air soil (Manuel *et al*., 2008). Medicinal plants have played an essential role in the development of human culture. Medicinal plants are the resources of various medicinally important constituents, chemical ingredients (Saleh, 2015).

The testing of the efficacy of such potential plant based sources for antifungal activity could an important step towards the assessment of the degree of variability among the diverse natural flora (Manoorkar and Gachande, 2014). As plants contains various chemicals which showed antifungal, antibacterial activities, Therefore the present study was undertaken to manage fungal pathogen *Fusarium oxysporum* causing disease to a Tomato (*Lycoprsicon esculentum)* through root, seed and leaf extracts of ten Indian plants of medicinal important (*Accacia catechu* Willd, *Accacia arabicae* Willd, *Carissa congesta* Wight, *Cassia fistula* (L.), *Cuscuta reflexa* Roxb., *Jatropha curcas* L., *Moringao leifera* Lam., *Syzygium cumini*, *Terminalia arjuna* Roxb., *Vitex negundo* L.).

MATERIALS AND METHODS

Fresh plant materials viz. leaves, stem, seeds and roots were collected from difference parts of Maharashtra, India. These all ten plants (*Accacia catechu* Willd, *Accacia arabicae* Willd, *Carissa congesta* Wight, *Cassia fistula*(L.), *Cuscuta reflexa* Roxb., *Jatropha curcas* L., *Moringa oleifera* Lam., *Syzygium cumini, Terminalia arjuna* Roxb., *Vitex negundo* L.) have their own traditionally medicinal importance (Table No.1) collected plant materials were thoroughly washed with tap water and then followed by distilled water and kept in dark in filter papers at room temperature till completely dry. Each plant material was individually pulverized to obtained dry powder. For each plant 25 gm powder was taken. Extract of each plant material of every plant was prepared in ethanol and distilled water and serve as stock extract, the toxicity of extract was determined against pathogenic fungi, *Fusarium oxysporum* following food poisoned technique (Mishra and Tiwari, 1992)

Different concentrations of extracts were prepared (10%, 25%, 50% and 100%) supplemented with Czepox Dox agar medium, four concentrations of each plant material of every plant were made each concentration with three replications and control were kept. These petriplates then inoculated with six mm disc of fungal mycelium, which is obtained from seven day old culture of fungal pathogen. These inoculated plates kept upside down and inoculated in BOD incubator at 28°C ± 2°C. Radial growths of fungal colonies were measured at different intervals. Antifungal activities of extracts of different plant parts of all ten plants was studies, sporulation of test fungi on different extracts was calculated by following equation.

$$Spore\ per\ ml = \frac{No.of\ spores\ observed\ per\ microscopic\ field}{12.5} X \frac{40{,}000}{1}$$

The percentage control efficacy is also studied, which was calculated by following formula

$$PCE = 100\left(1 - \frac{X}{Y}\right)$$

Where,
X = The diameter of the mycelia growth on extract treated host.
Y = The diameter of the mycelia growth on untreated host (Control)

RESULTS AND DISCUSSION

Leaves, stem, seed and root extracts of different ten plants tested against test fungi *viz. Fusarium oxysporum* significantly inhibited radial growth of fungal pathogen at various concentrations, as shown in Table 2. At 50% concentration extracts of different plant parts of ten plants significantly inhibited the test fungi *Fusarium oxysporum*, root extract of *Accacia catechu* control the growth of test fungi at 25% concentration (23.66), followed by leaf extract of *Jatropha curcas* at 25%, showed 33.33 cm growth of test fungi in treated plates, remaining extracts of plant parts inhibited the growth at 25% with less than 45cm growth (table 2).

In vivo percentage control efficacy (PCE) of all plant part extracts of ten plants was studied against test fungi. All extracts showed 100% PCE in 50% concentration against test fungi, while in 25% concentration it showed more than 99.8% PCE in several plant extracts (table 4) like *Accacia catechu,Crissa congesta, Jatropha curcas, Moringa oleifera and Vitex negundo.* Aqueous and ethanolic leaf extracts of *Calotropis procera* showed antifungal activities against dominant storage seed born fungi *Curularia lunata, Alternaria alternate, Rhizoctonia solani Fusarium solani, Penicillium chrysogenum, Aspergillus niger, A. flavus, A. terrus, A. fumigates*, and *Rhizopus sp*.(Manoorkar *et al*, 2015).

Table No. 1: Medicinally importance Indian plants.

Sr. No.	Botanical Name of plant	Family	Traditional use
1	*Accacia catechu* Willd	Mimosaceae	Used in the treatment of diarrhea and throat infections.
2	*Accacia arabicae* Willd	Mimosaceae	Skin wounds, such as burns, cuts or leprosy, digestive, gonorrhea, cough, dysentery, colds etc.
3	*Carissa congesta* Wight	Apocynaceae	Stomchic, antidiarrhoel, vermifuge, antianthelmentic, astringent and antiascorbutic
4	*Cassia fistula* (L.)	Caesalpiniaceae	Anti-inflammatory, antioxidant, wound healing, laxative, purgative, useful on leucoderma, diabetes and antibacterial.
5	*Cuscuta reflexa* Roxb.	Convolvulaceae	Seeds are carminative and anthelmatic , plant used externally against itch, internally in protected fevers.
6	*Jatropha curcas* L.	Ephorbiaceae	Human and veterinary ailment, latex is disinfectant, anti cancerous, used in skin disease, piles, malaria, rheumatic and muscular pain.
7	*Moringa oleifera* Lam	Moringaceae	Antioxidant, antispasmodic, antiulcer anti-inflammatory, diuretic wound healing.
8	*Syzygium cumini*	Myrtaceae	Fruit used in diabetes, bark is anti-inflammatory, digestive ailments, leaves are antioxidants, used in blood pressure.
9	*Terminalia arjuna* Roxb.	Combretaceae	Antioxidant, anti mutagenic, cardiac tonic, anti cancerous.
10	*Vitex negundo* L.	Verbinaceae	Muscle relaxant, pain relieving, anti mosquito, anti anxiety, anti asthma

Table: 2 Antifungal activities plant extracts against *Fusarium oxysporum*

Sr. No.	Botanical extracts	Plant part used	Conc. of extract in%	Growth of *Fusarium oxysporum* in mm
1	*Accacia catechu* Willd	Root	10 25 50	55.33* 23.66 00.00
2	*Accacia arabicae* Willd	Seed	10 25 50	64.33 43.66 00.00
3	*Carissa congesta* Wight	Leaf	10 25 50	58.66 39.33 00.00
4	*Cassia fistula* (L.)	Seed	10 25 50	59.66 41.66 00.00
5	*Cuscuta reflexa* Roxb.	Stem	10 25 50	61.33 45.33 00.00
6	*Jatropha curcas* L.	Leaf	10 25 50	52.00 33.33 00.00
7	*Moringa oleifera* Lam	Leaf	10 25 50	59.00 42.33 00.00
8	*Syzygium cumini*	Seed	10 25 50	67.33 45.66 00.00
9	*Terminalia arjuna* Roxb.	Stem	10 25 50	62.33 41.66 00.00
10	*Vitex negundo* L.	Leaf	10 25 50	59.33 36.33 00.00
			Control	90.00

*Radial growth is the mean of triplicat

Table No 3: Sporulation of *Fusarium oxysporum* against different extracts.

Sr. No.	Botanical extracts	Plant part used	Conc. of extract in%	Sporulation of *Fusarium oxysporum* in mm	
				No. of spores per microscopic field*	No. of spores per ml suspension
1	*Accacia catechu* Willd	Root	10 25 50	4.0 1.0 0.0	12,800 3,200 00.00
2	*Accacia arabicae* Willd	Seed	10 25 50	5.0 0.0 0.0	16,00 00.00 00.00
3	*Carissa congesta* Wight	Leaf	10 25 50	7.0 2.0 0.0	22,400 6,400 00.00
4	*Cassia fistula* (L.)	Seed	10 25 50	5.0 1.0 0.0	16000 3,200 00.00
5	*Cuscuta reflexa* Roxb.	Stem	10 25 50	6.0 3.0 0.0	19,200 9,600 00.00
6	*Jatropha curcas* L.	Leaf	10 25 50	3.0 1.0 0.0	9,600 3,200 00.00
7	*Moringa oleifera* Lam	Leaf	10 25 50	7.0 4.0 0.0	22,400 12,800 00.00
8	*Syzygium cumini*	Seed	10 25 50	8.0 4.0 0.0	25,600 12,800 00.00
9	*Terminalia arjuna* Roxb.	Stem	10 25 50	6.0 4.0 0.0	19,200 12,800 00.00
10	*Vitex negundo* L.	Leaf	10 25 50	5.0 3.0 0.0	16,00 9,600 00.00
			Control	14.6	46,720

*No. of spores per microscopic field in 10X

According to Farzana *et al*., (2014) The antifungal activity of ethanol and acetone extracts of leaves of nine medicinal plants i.e. *Piper betel, Lowsoniainermis, Psidiumuajava, Carissa papaya, Mimosa pudica, Catheranthusroseus, Moringa oleifera, Adhatoda vasica,* and *Andrographic paniculata* against *Fusarium oxysporum*, the causal organism of *Fusarium* wilt of Tomato was assessed 100%. Antifungal activity of different concentrations (2, 4, 6, and 8% w/v) of leaf extracts of wheat (*Triticum aestivum* L) Maize (Zea mays L) Sunflower (*Helianthus annus* L) Chilies (*Capsicum annum*), Onion (*Allium cepa*), and Marogold (*Tagatus erectus* L) successfully inhibit corn rot disease of *Gladiolus* caused by *Fusarium oxysporum* (Tariq, 2008). Bhalodia *et al.*, 2011, tested *Cassia fistula* against two gram positive bacteria and three fungal pathogens i.e. *Aspergillus niger, Aspergillus clavatus* and *Candida albicans,* the result shows remarkable inhibition of the fungal growth. Methanol leaf extract of *Leucas zylanica and L. aspera.* Antifungal susceptibilities of certain clinically isolated dermatophytes to methanol extracts of *Leucas aspera and Leucas zeylanica* leaves were performed using agar well diffusion method.

Table No. 4: In vivo Percentage Control Efficacy (PCE) of the different extracts against test fungi

Sr. No.	Botanical extracts	Plant part used	Conc. of extract in%	*Fusarium oxysporum*	
				Growth in mm	PCE
1	*Accacia catechu* Willd	Root	10 25 50	17.0 8.0 0.0	99.6 99.8 100
2	*Accacia arabicae* Willd	Seed	10 25 50	19.0 11.0 0.0	99.5 99.7 100
3	*Carissa congesta* Wight	Leaf	10 25 50	18.0 9.0 0.0	99.6 99.8 100
4	*Cassia fistula* (L.)	Seed	10 25 50	20.0 12.0 0.0	99.5 99.7 100
5	*Cuscuta reflexa* Roxb.	Stem	10 25 50	21.0 12.0 0.0	99.5 99.7 100
6	*Jatropha curcas* L.	Leaf	10 25 50	15.0 9.0 0.0	99.6 99.8 100
7	*Moringa oleifera* Lam	Leaf	10 25 50	20.0 9.0 0.0	99.5 99.8 100
8	*Syzygium cumini*	Seed	10 25 50	22.0 12.0 0.0	99.5 99.7 100
9	*Terminalia arjuna* Roxb.	Stem	10 25 50	21.0 12.0 0.0	99.5 99.7 100
10	*Vitex negundo* L.	Leaf	10 25 50	20.0 10.0 0.0	99.5 99.8 100
			Control	42.0	00

The result obtained shows that all the extracts expressed remarkable antifungal activity with zone of inhibition ranging from 5 to 10 mm (Babu *et al.*, 2016). According to Gavande *et al.*, (2014) The antifungal potential seeds of *Aegle marmelos* tested against antraconose causing plant fungal pathogen *Colletotrichum acutatum,* fungal strain of *Metarhiziumani sopliae*, *Trichoderm aharzianum* and *Penicillium* sp., both ethanolic and petroleum ether extracts showed antifungal activities. Rathod *et al.,* (2015) used water and acetone extracts against fungus *Candida albicans* showed fungicidal properties, the presence of phytocompounds may be responsible for these activity, they found that acetone extracts are more efficient than that of aqueous extracts. Leaf extracts of ten plants (*Annona squamosa, Azadirachta indica, Adhatoda vasica, Ocimum sanctum , Polyalthia longifolia, Tridax procumbens ,Curcuma longa, Zingiber officinale, Allium cepa and Allium sativum)* found inhibitory against *Curvularia lunata, Phytophthora sp., Alternaria alternate, Fusarium oxysporum Asperillus niger and Rhizoctonia solani* (Swami and Alane, 2013).

According to Mogle and Maske, (2012) The effects of leaf extracts of *Argemone mexicana* L., *Semecarpus anacardium* L., *Cassia fistula* L., *Tephrosia purpurea* (L.) Pers., found useful for the control of *Collectotrichum destructivum* on seeds of cowpea. The seeds were soaked in sterile distilled water extract (10, 20 and 30%, w/v) of the leaves for 5, 10 and 15 h. All these plant extracts had significant inhibitory growth effect on the fungal pathogen.

Argemone mexicana extract was more effective followed by *Semecarpus anacardium, Cassia fistula* and *Tephrosia purpurea* plant extracts. Petroleum ether, hexane, chloroform, acetone and methanol extracts of leaf, stem and tuber of *Corallocarpus epigaeus* were investigated for antifungal activity against *Candida albicans, C. tropicalis, Aspergillus niger, A. flavus and A. versicolor* by disc diffusion method. Methanol extract of *C. epigaeus* tuber exhibited maximum activity against most of the tested fungi. The petroleum ether and hexane extracts obtained from *Corallocarpus epigaeus* stem was found to be active only against *A. niger, A. flavus and A. versicolor*. All the crude extracts exhibited activity against *A. niger and A. flavus* (Priyavardhini *et al.*, 2012).

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