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Evaluation Of Antifungal Activity Of Plant Latex Extracts Against Resistant Isolates Of Pathogens Associated On Rumex Acetosa L.

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The in vitro antifungal potency of four plant latex extracts were evaluated for their botanical fungi toxicants on pathogenic fungi of Rumex acetosa L.The antifungal effect of aqueous extracts of latex namely Jatropa curcus, Calotropis gigentea,Ficus bengalensis and Ficus glomerata were selected. The inhibitory effect was tested by food poisoning technique and determined minimum inhibitory concentration (MIC). Due to the presence of bioactive molecules the latex extracts showed significant inhibition in different concentrations. Jatropa curcus latex extract showed 75% reduction of radial growth of Alternaria tenussima , Fusarium proliferatum at 50% conc and pytium 75% conc. In some extent, F. bengalens is also showed significant reduction of A.tenussima at 100% conc. The inhibitory effect of Callotropis gigantean was also shown in case of Pythium sp. at 100% conc.

Key word ,*Rumex acetosa,* Pathogens, Medicinal plants latex, Antifungal activity.

INTRODUCTION

Vegetables are the most important component of a balance diet and we can now, grow varieties of different round the year. India is the world's second largest producer of vegetables next to China. Vegetables are more susceptible to insects pests and diseases due to their tenderness and softness as compared to other crops and virtual absence of resistance characters because of intensive hybrid cultivation (Chiranjeevi et.al.,2002).The native value of healthy vegetables are altered because of fungal attack and sometimes fungi produce certain mycotoxin in them and make them unsuitable for human consumption.

Sorrel (*Rumex acetosa* l.) is one of the vegetable crop belongs to family polygonaceae. It is an indigenous English plant, common, too in the greater part of Europe, in almost all soils and situation. The medicinal action of sorrel is refrigerant and diuretic, febrile disorders and in scurvy. Both the root and the seed were formerly esteemed for their astringent properties, and were employed to stem haemorrhage (Cook, 1967).

Latex is a stable dispersion of naturally occurring polymer micro particles in an aqueous medium. It is found in 10% of all angiosperms. This complex emulsion consisting of alkaloids, starch, sugars, oils, tannins, resins and gums that coagulates on exposure to air. It is also rich in enzymes like proteases, glucosidases, chitinases and lipases. It has been demonstrated that this substance is a source of natural fungicides (Barkai-Golan, 2001) which is regarded as both safe and effective against various diseases of banana, papaya and other fruits. The water-soluble fraction of papaya latex can completely digest the conidia of many fungi, including important postharvest pathogens (Indrakeerthi & Adikaram, 1996).Other latex extracted from several plants showed a strong antifungal activity against *Botryti cinerea*, *Fusarium* sp. and *Trichoderma* sp. (Barkai-Golan, 2001).

Fungicides belong to a group of pesticides which inhibited fungal growth either causing damage to the cells or preventing the fungal development. As pesticides, they offer great economic and social benefits through the protection and preservation of materials, food and the prevention of diseases. Since pesticides are designed specifically to fight harmful or even dangerous life forms and therefore are toxic to them, they may present hazards to the environment by their potential effect upon non-target organisms, including humans, particularly when misused. The need to balance these benefits against the risks presents a challenge to the EPA (Environmental Protection Agency) unlike other chemicals. The aim of this study was to evaluate the

antifungal activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by pathogens.

MATERIALS AND METHODS

Plant material and latex collection: The fresh latex of *J. curcus, C. gigentea, F.bengalensis* and *F.glomerata* were aseptically collected from the aerial parts of the healthy plants as described by Aworh et al. (1994) in clean glass tubes containing distilled water to yield a dilution rate of 5:5 (v/v). The latex mixture was gently handled to maintain homogeneity during transport to the laboratory where it was stored at (4°C) until further use.

Fungal Pathogens

The three fungicide resistant pathogens such as *Alternaria tenussima* caused by leaf spot disease, *Fusarium proliferatum* caused by wilt disease and *Pythium* sp caused by damping off disease were used.

Preparation of latex extract:

The fresh latex was selectively decanted and centrifuged at 5000 rpm for 5 min. The precipitated material showing rubber aspect (poly-isoprene) was pooled apart and the supernatant was decanted carefully. Finally the samples were centrifuged as previously described and the clear soluble supernatant was collected and lyophilized The stock solutions of latex extract was diluted suitably as required from stock solution (Juncker et al.,2009).

Determination of antifungal activity

Plant latex aqueous extracts of each prepared with distilled water and condensed to serve as stock extract was determined by food poisoning technique (Mishra & Tiwari, 1992) against tested pathogens in five different concentrations. Petriplates containing Czapek Dox Agar (CZA) medium, supplemented with different plant latex extracts at five concentrations (25, 50, 75 and 100%) with three replications were inoculated with fresh 7 days old culture of test fungi in 8 mm discs and kept upside down. The plates were incubated in BOD incubator at 28 ± 2 $^{o}C_{.,}$ Plates without plant latex extracts served as control. Starting two days after inoculation (DAI), radial growth was recorded daily for 8 days or until the plates were overgrown. The growth inhibition was calculated by using the formula: 100 X C - T / C, Where C = growth in control and T = growth in treatment (Vincent, 1947). The lowest concentration of the extracts that inhibited the growth of the test pathogens was recorded as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Plant latex used in this study was tested against two pathogenic fungi to determine their antifungal activity. Different concentrations of plant latex (25, 50, 75 and 100%) were tested against pathogenic fungi. Minimum Inhibitory Concentration (MIC) was measured to determine the antifungal activity. The inhibition effects of the medicinal plant on pathogenic fungi were represented in Table.

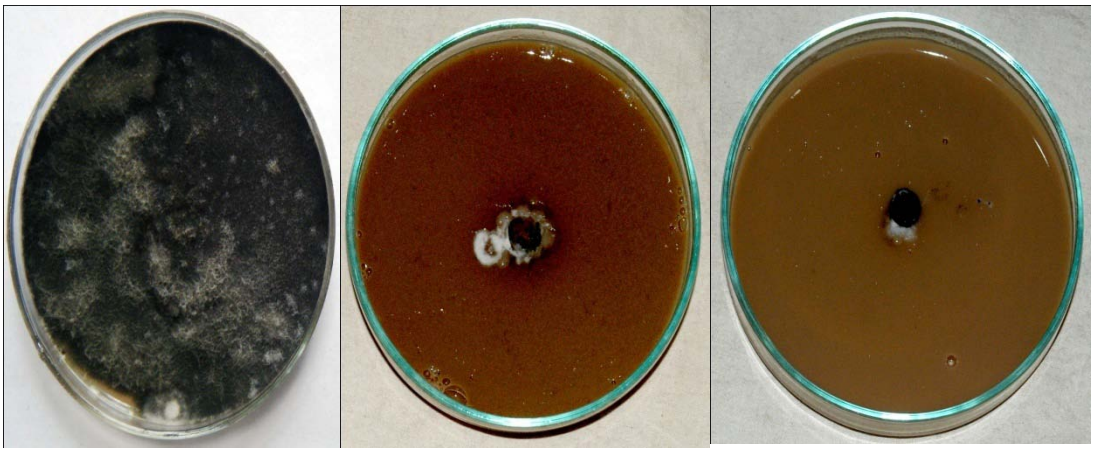
Table 1. Antifungal activity of Plant latex extracts against pathogenic fungi of fenugreek.

Plant species	**Family**	**Conc (%)**	**Radial growth of *A.tenussima* (mm)**	**Inhibition (%)**	**Radial growth of *F.proliferatm* (mm)**	**Inhibition (%)**
Jatropa curcas	Euphorbiaceae	25	16	82.02	14	84.44
		50	15	83.14	06	93.33
		75	12	86.51	00	100.00
		100	00	100.00	00	100.00
Calotropis gigantea	Asclepiadaceae	25	39	56.17	59	34.44
		50	28	68.53	40	55.55
		75	24	73.03	35	61.11
		100	22	75.28	30	66.66

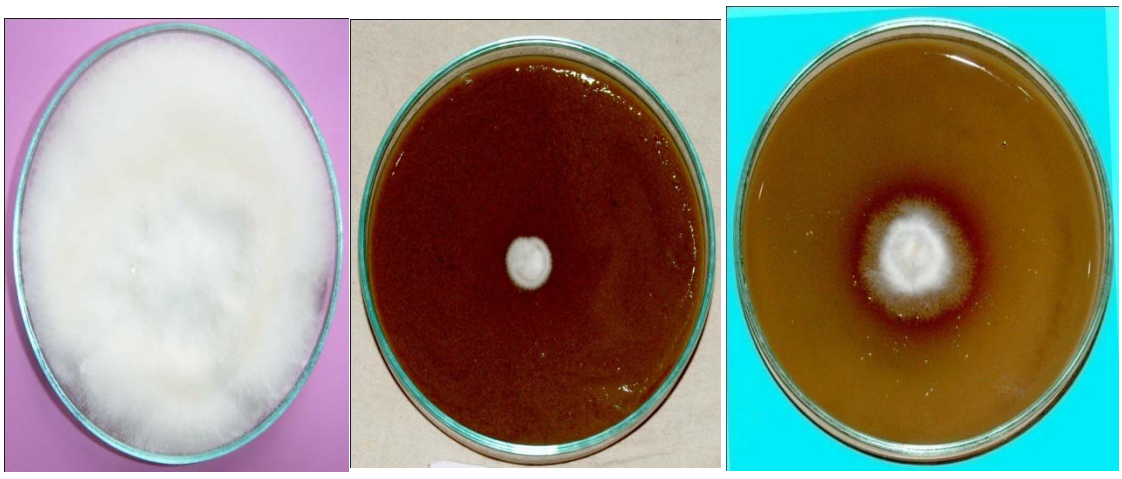
Ficus bengalensis	Moraceae	25	18	79.77	30	66.66
		50	17	80.89	28	68.88
		75	15	83.14	24	73.33
		100	13	85.39	20	77.77
Ficus glomerata	Moraceae	25	36	59.55	31	65.55
		50	30	66.29	29	67.77
		75	26	70.78	22	75.55
		100	22	75.28	20	77.77
Control			89.11	--	90.00	--
CD (P=0.05)			--		--	

*Significantly reduced mycelial growth.

Jatropa curcus latex extract showed 75% reduction of radial growth of *Alternaria tenussima,Fusarium proliferatum* at 50% and *Pythium* sp.75% conc. respectively. In some extent, *F. bengalensis* also showed significant reduction of *A.tenussima* at 100% conc. The inhibitory effect of *Callotropis gigantean* was also shown in case of *Pythium* sp. at 100% conc. However, there was no significant reduction of radial growth in case of *C. gigantea, F. bengalensis* and *F.glomerata* (Fig.).



***Alternaria alternata* (Control)** ***Jatropa curcas* (50%)** ***Ficus bengalensis* (100%)**



***Fusarium oxysporum* (Control)** ***Jatropa curcas* (75%)** ***Ficus glomerata* (100%)**

Figure1.:Antifungal activity of plant latex extracts against pathogenic fungi of fenugreek.

The result agrees with Takazawa *et al.*, (1982) that there is a need to employ broad range of extractive solvents in the extractions of possible photochemical from medicinal plants. The growth of four test fungi were inhibited by ethanol and chloroform extracts while the aqueous extract was the least effective on the test fungi. The best antifungal activity was recorded in ethanol extract of *C.procera* latex against *Candida albicans* **(**Kareem et al., 2008). Leaf extracts, chopped leaves and latex of *C. procera* have shown great promise as a nematicide *in vitro* and *in vivo* (Khirstova and Tissot, 1995). The mycelia growth, percentage spores germination and germ- tube extension in *Fusarium oxysporum* and *Aspergillus carbonaris* decreased when *Calotropis procera* extract concentration increases, where as growth of *Humicola brevis* and *Penicillium lanosum* were not affected (Rizk,2008). The minimum inhibitory concentrations (MIC) were also determined Methanolic fraction had a total inhibition against *Candida albicans* (100%) at a concentration of 500µg/ml and a negative effect against *Cryptococcus neoformans*. *Microsporum canis* was strongly inhibited with methanolic extract (75%) and totally with ethyl acetate extract at a concentration of 750µg/ml (Houda, 2010). The antifungal potency of *C. gigantea* latex extract on the *C. albicans* showed a larger diameter of clearance than that of other fungal strains (Venkatesan and Subramanian, 2010). Raghavendra (2011) reported the latex extract were screened *in vitro* against human pathogenic strains such as Gram positive; *Staphylococcus aureus, Bacillus subtilis,* Gram negative**;** *Salmonella typhi, Klebsiella phenonemia* and two fungal strains; *Aspergillus niger and Candida albicans*. The inhibitory effect was assessed by agar well diffusion method.

Table 2. Antifungal activity of Plant latex extracts against pathogenic fungi of *Rumex acetosa.*

Plant species	**Family**	**Conc (%)**	**Radial growth of *Pythium* sp. (mm)**	**Inhibition (%)**
Jatropa curcas	Euphorbiaceae	25	18	80.00
		50	16	82.22
		75	14	84.44
		100	00	100.00
Calotropis gigantea	Asclepiadaceae	25	34	62.22
		50	30	66.66
		75	26	71.11
		100	19	78.88
Ficus bengalensis	Moraceae	25	34	62.22
		50	30	66.66
		75	28	68.88
		100	25	72.22
Ficus glomerata	Moraceae	25	39	56.66
		50	33	63.33
		75	30	66.66
		100	28	68.88
Control			9o.oo	--
CD (P=0.05)			--	

Significantly reduced mycelial growth.

CONCLUSION:

The remarkable bio-fungicidal effects of *J. curcas* latex extract suggest that the latex may be a useful source for the development of novel antifungal agent against pathogenic fungi. Due to the presence of bioactive molecules the latex extracts showed significant inhibition.

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