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RESEARCH ARTICLE

Antifungal activity of plant latex extracts against resistant isolates of pathogens associated on (*Rumex acetosa* L.)

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SUMMARY

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 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 100 per cent reduction of radial growth of *Alternaria alternata* and *Fusarium oxysporum* at75 per cent conc. In some extent, *F. bengalensis* also showed significant reduction of *A.alternata* at 100 per cent conc. The inhibitory effect of *F. glomerata* was also shown in case of *F.oxysporum* at 100 per cent conc.

Key Words : Fenugreek, Pathogens, Medicinal plants latex, Antifungal activity

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Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest medicinal plants, originating in India and northern Africa. In India, fenugreek is commonly consumed as a condiment, used medicinally as a lactation stimulant and used for numerous indications, including labor induction, aiding digestion, and as a general tonic to improve metabolism and health (Yoshikawa *et al.,* 1997; Ethan Basch *et al.,* 2003).

Latex is a stable dispersion of naturally occurring polymer micro particles in an aqueous medium. It is found in 10 per cent of all angiosperms. This complex emulsion consisting of alkaloids, starches, 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 and 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.

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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 two fungicide resistant pathogens such as *Alternaria alternata* caused leaf spot disease and *Fusarium oxysporum* caused wilt 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 and 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 °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 are represented in Table 1. *Jatropa curcus* latex extract showed 100 per cent reduction of radial growth of *Alternaria alternata* and *Fusarium oxysporum* at 50 and75 per cent conc., respectively. In some extent, *F. bengalensis* also showed significant reduction of *A.alternata* at 100 per cent conc. The inhibitory effect of *F. glomerata* was also shown

Table 1: Antifungal activity of plant latex extracts against pathogenic fungi of fenugreek

Plant species	Family	Conc (%)	Radial growth of *A.alternata* (mm)	Inhibition (%)	Radial growth of *F.oxysprum* (mm)	Inhibition (%)
Jatropa curcas	Euphorbiaceae	25	06	93.33*	08	91.11*
		50	04	95.55*	06	93.33*
		75	00	100.00	04	95.55*
		100	00	100.00	00	100.00
Calotropis gigantea	Asclepiadaceae	25	48	46.66	42	53.33
		50	38	57.77	30	66.66
		75	29	67.77	28	68.88
		100	22	75.55	24	73.33
Ficus bengalensis	Moraceae	25	22	75.55	39	56.66
		50	16	82.22	34	62.22
		75	15	83.33	29	67.77
		100	14	84.44*	26	71.11
Ficus glomerata	Moraceae	25	40	55.55	29	67.77
		50	34	62.22	24	73.33
		75	28	68.88	20	77.77
		100	24	74.33	18	80.00*
Control			89.11	--	90.00	--
CD (P=0.05)			--	13.04	--	14.26

*Significantly reduced mycelial growth

in case of *F.oxysporum* at 100 per cent conc. However, there was no significant reduction of radial growth in case of *C. gigantea, F. bengalensis* and *F.glomerata* (Fig.1).

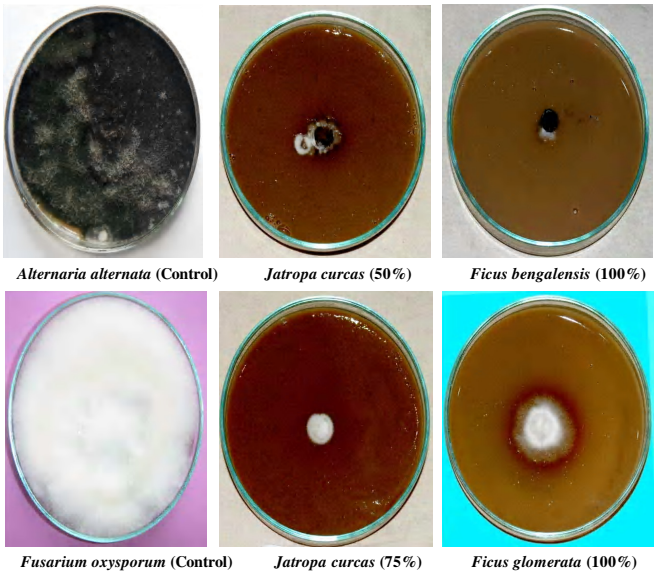

Fig.1: Antifungal activity of plant latex extracts against pathogenic fungi of fenugreek.

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 *et al.,* 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 *et al.* (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.

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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