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Effect of *Annona muricata*, *Abutilon indicum* and *Evolvulus alsinoides* Extract on Spore Germination of Sorghum Grain Mold Fungi

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Abstract

Sorghum grain mold fungi like *Alternaria solani*, *Curvularia lunata*, *Fusarium moniliforme* and *Helminthosporium* sp, were tested with hexane fraction (AMIE-HF), chloroform fraction (AMIE-CF), ethyl acetate fraction (AMIE-EAF), crude extract (AMIE) of *Annona muricata,* petroleum ether extract of *Abutilon indicum* (AIPE) and alkaloid enriched fraction of *Evolvulus alsinoides* (EAMAA I). AMIE HF at 1000, 2500 and 5000 ppm and AMIE EAF at 2500 and 5000 ppm concentration showed significant effect on *F. moniliforme* while AMIE CF and AMIE showed no effect. AIPE remarkably inhibited *A. solani* at all concentrations and *C. lunata* at 2500 and 5000 ppm concentration. EAMAA I inhibited spore germination of *A. solani* at 1000, 2500 and 5000 ppm whereas *C. lunata* at 5000 ppm concentration.

1. Introduction

The fungal diseases of plants are one of the major constrains in crop production causing severe losses every year. Indiscriminate use of various synthetic fungicides for the control of diseases of crop plants has posed a serious threat to human health and environment leading to disturbed biodiversity, outbreaks of secondary pests, and development of resistance in the pathogens and contamination of food chain in the ecosystem (Lyon et al., 1995). However, the researchers are on the way to find out alternatives of synthetic fungicides. Eco-friendly systems involve biodegradable plant products from medicinal plants and biological agents which act directly or indirectly by inducing resistance in plants (Mishra & Raja 1999). Many workers have used crude plant extracts *in vitro*, in glasshouse and field conditions against several plant pathogens (Vollekova et al., 2001; Prithiviraj et al., 1996). Various active principles isolated from the plants were proved effective against several plant pathogenic fungi *in vitro* (Singh et al., 1995; Singh et al., 2007).

"Grain mold" is an important disease of sorghum (*Sorghum bicolor* (L.) Moench), during *kharif* (rainy) season. This disease is more often caused by *F. moniliforme* and *C. lunata*, although many other fungal species are also associated with the mold complex (Zehnder et al., 2001; Janisiewicz et al., 1988). It causes economic losses by reducing the nutritional value and producing mycotoxins. Molded sorghum grain fetches lower market price and therefore affects the income of sorghum farmers (Cartwright et al., 1995).

Three medicinal plants belonging to different families were selected for the study based on their amtimicrobial property. *Annona muricata* L. (Annonaceae) is indigenous to most of the warmest tropical areas in South and North America, including the Amazon. All parts of the tree are used in natural medicine. Fruit and fruit juice are used for killing worms and parasites and as an astringent for diarrhoea and dysentery. *A. muricata* produces annonaceous acetogenins which showed selective cytotoxic activity (without harming healthy cells) at very low dosages as little as 1 ppm against various cancer cell lines when tested by National Cancer Institute, Bethesda, Maryland (USA) as a part of plant screening programme (Kim et al., 1998a, Kim et al., 1998b). The antibacterial effect of The *A.*

muricata leaf extract was screened for various gram positive and gram negative strains like *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumonia, Bacillus subtilis* and found to show good inhibition (Pathak et al., 2010).

Evolvulus alsinoides L., belonging to Convolvulaceae family is a perennial herb with a small woody and branched rootstock from tropical and subtropical regions of the world mainly of East Asia. In Ayurveda it is used as a brain tonic to treat neurodegenerative diseases, asthma and amnesia (Goyal and Singh, 2005). It was found to be potent fungicide when tested against three pathogens, viz., *Alternaria brassicae, A. brassicola* and *Fusarium oxysporu*m (Aulakh et al., 1988). Also, antibacterial reports on *E. alsinoides* against various strains like *Microocus pyogenes* var. *aureus, Sarcinia lutea, Escherischia coli, Salmonella typhosa* and *Shigella dysenteriaes* var. *shiga* have been reported.

Abutilon indicum L. is belonging to family Malvaceae, commonly known as Country mallow in English and Atibala in Sanskrit (Kuo et al, 2008; Mohite et al., 2012). In traditional medicine the roots are used to cure uterine haemorrhagic discharges and as diuretics. The bark is used as febrifuge, anthelmentic, astringent and diuretic (Mohite et al., 2012; Shivhare et al., 2010). The extract of fruits, root, and leaf of *A. indicum* was found to be inhibitory against various microorganisms like *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* (Kumar et al., 2006). The seed extract of *A. indicum* showed mycelial inhibition (%) against *Absidia ramos* and *Aspergillus niger* by 6.97 and 37.25 respectively (Pandey et al., 1983).

In view of the reported antimicrobial activities of the selected plants, the efficacy of methanolic extract of leaves of *Evolvulus alsinoides* and *Abutilon indicum,* and *A. indicum* seeds were tested against spore germination and mycelial growth of some fungi.

2. Materials and Methods

2.1. Plant material

The aerial parts of *Evolvulus alsinoides* and *Abutilon indicum* were collected during the month of October 2010 from Viruthunagar district, Tamilnadu, India and seeds of *Annona muricata* were collected from Trivandrum, Kerala, India during September 2011. The plants were identified and authenticated by Dr. Chelladurai, Research Officer, Botany, C.C.R.A.S, Government of India, Tirunelveli dist, Tamilnadu, India. A specimen sample (EVL, ABI, ANM /2011/02, 03 & 04) of the raw materials used for the investigation has been preserved in the Department of Pharmacy, BITS-Pilani Hyderabad Campus, Andhra Pradesh, India.

2.2. Extraction of plant material

The aerial parts of *Evolvulus alsinoides* and *Abutilon indicum* were dried in shade and pulverized by a mechanical grinder. Then 500 g of powder was extracted with methanol (1 L × 2 times) using a Soxhlet extractor, for 48 h. The obtained organic extract was concentrated under reduced pressure using Buchi Rotavapor (Model: R-210, Switzerland). The extract obtained (11.4% w/w) from *Evolvulus alsinoide* was suspended in water and extracted using petroleum ether, in a separating funnel to remove pigments and fatty matter. The aqueous layer was made acidic (pH 3) by adding 10% HCl solution, extracted with diethyl ether and the solvent was evaporated under reduced pressure to afford acidic alkaloid enriched fraction (EAMAA 1; 0.69% w/w). *Abutilon indicum* extracts (10.79% w/w) using petroleum ether (AIPE) was also used for spore germination. The fraction was tested for alkaloids using Dragendorff's reagent [Sigma-Aldrich-44578]. Accurately weighed 500 g of the powdered seed material of *Annona muricata* was also extracted with methanol (1 L × 2 times) in round bottom flask by heat reflux extraction method. The extraction was performed for 24 h at 60 °C and then it was filtered and evaporated under reduced pressure (6.76% w/w). The extract was further fractionated with chloroform (AMIE CF), ethyl acetate (AMIE EAF), hexane fraction (AMIE HF) and crude (AMIE). Each fraction was evaporated under reduced pressure. All the fractions and the crude extract was screened for their antifungal activity against various micro organisms.

2.3. The test fungi

The test fungi, viz., *Alternaria solani*, *Curvularia lunata*, *Fusarium moniliforme* and *Helminthosporium* species were isolated from infected grains of sorghum plants. Infected grains were collected and incubated in moist Petri dishes for 24 h and the fungal growth developed after 24 h were transferred on PDA medium in Petri plates. The fungal growth developed after 48-72 h was picked up by an inoculating sterile needle from the margin of the colonies and purified by single spore isolation technique (Singh et al., 1990). Purified cultures were maintained on PDA slants for further use.

2.4. Spore germination test

Solutions of AMIE HF, AMIE CF, AIPE, AMIE EAF, AMIE and EAMAA I of 100, 500, 1000, 2500 and 5000 ppm were prepared by dissolving required amount in distilled water. A drop (30-40 µl) of different concentrations of the various test samples were placed separately on grease- free glass slides for studying spore germination. Approximately 200-300 spores of each fungus were picked up from fresh sporulating cultures by an inoculating needle aseptically and mixed in a drop of test samples kept on glass slides. Five slides of each fungus were prepared. All the slides were kept in Petri dishes which were humidified by fixing moist filter paper on the lower and upper surfaces of the base and lid of the Petri dishes. All the Petri

dishes were incubated at 25±2 °C for 24 h. After incubation, a drop of cotton blue prepared in lactophenol, was placed on the drop of test samples containing spores and finally covered with a cover slip. The spore germination was observed under Nikon binocular research microscope and finally the per cent spore germination was calculated. The data was subjected to statistical analysis. The experiments were conducted in five replicates.

3. Results and Discussion

In the present study, various solvent extracts of *Annona muricata* (AMIE HF, AMIE CF, AMIE, AMIE EAF), *Abutilon indicum* (AIPE) and *Evolvulus alsinoides* (EAMAA I) were screened for their antimicrobial activity against four sorghum grain mold fungi *viz Alternaria solani, Curvularia lunata, Fusarium moniliforme and Helminthosporium* sp.

Significant inhibition of spore germination of *A. solani* was observed at 1000, 2500 and 5000 ppm concentration with AMIE CF, EAMAA I**.** AIPE showed its inhibition effect at all concentrations on *A. solani* while no effect was observed with AMIE HF, AMIE EAF and AMIE even at high concentration.

AIPE at 2500 and 5000 ppm concentration and AMIE CF and EAMAA I at 5000 ppm concentration showed remarkable effect on spore germination of *C. lunata*. Spore germination of *F. moniliforme* was profoundly inhibited by AMIE HF at 1000, 2500 and 5000 ppm and AMIE EAF at 2500 and 5000 ppm concentration. Significant inhibition of *Helminthosporium* sp. was seen in AMIE at 5000 ppm concentration whereas no remarkable effect was observed on other cases (Table 1).

During the course of studies conducted by several workers to find out alternatives to synthetic chemical fungicides, a number of chemical compounds isolated from plants were found to be antifungal (Singh et al., 1995; Atta-ur Rehman et al., 1997; Sarma et al., 1999; Maurya et al., 2002; Basha et al., 2007; Singh et al., 2007; Singh et al., 2009; Mondal et al., 2012). Raghavendra et al. (2012) and Begum et al. (2011) reported the antifungal effect of safflower petal extracts and leaf extracts

Table 1: Spore germination of sorghum grain mold fungi with various plant extracts

Extract	Fungus	Concentrations (ppm)					
		Control	100	500	1000	2500	5000
		% germination					
AMIE-HF	*Alternaria solani*	81.3	77.6	74.0	69.6	65.3	64.3
	Curvularia lunata	91.3	87.3	82.0	81.6	81.6	77.0
	Fusarium moniliforme	84.6	81.0	72.6	66.0*	66.0*	56.0*
	Helminthosporium sp	86.0	84.3	82.6	82.6	80.0	77.0
AMIE-CF	*Alternaria solani*	81.3	70.3	65.6	62.0*	56.3*	51.6*
	Curvularia lunata	91.3	84.3	83.6	83.0	83.0	70.6*
	Fusarium moniliforme	84.6	80.6	75.6	74.3	73.3	68.6
	Helminthosporium sp	86.0	74.6	74.0	74.0	73.3	73.0
AIPE	*Alternaria solani*	81.3	54.0*	54.0*	52.0*	41.6*	39.0*
	Curvularia lunata	91.3	82.0	76.0	75.3	73.3*	68.3*
	Fusarium moniliforme	84.6	78.3	74.0	73.0	72.3	71.6
	Helminthosporium sp	86.0	75.6	75.3	73.3	71.6	71.6
AMIE EAF	*Alternaria solani*	81.3	79.6	78.3	76.0	74.3	74.0
	Curvularia lunata	91.3	81.0	78.6	76.6	74.6	74.0
	Fusarium moniliforme	84.6	80.6	76.6	67.3	65.0*	60.3*
	Helminthosporium sp	86.0	80.6	77.0	74.3	73.0	69.0
AMIE	*Alternaria solani*	81.3	78.3	77.3	76.3	71.6	71.6
	Curvularia lunata	91.3	78.6	78.3	78.0	76.6	76.0
	Fusarium moniliforme	84.6	85.0	84.3	82.6	82.0	79.6
	Helminthosporium sp	86.0	78.6	76.0	74.3	73.6	67.0*
EAMAA 1	*Alternaria solani*	81.3	71.0	65.6	63.0*	60.3*	59.3*
	Curvularia lunata	91.3	81.3	80.3	80.0	79.3	71.6*
	Fusarium moniliforme	84.6	81.6	80.6	77.3	76.6	75.0
	Helminthosporium sp	86.0	76.6	74.6	73.6	73.0	72.3

Values suffixed with asterisks are significantly different from corresponding control values at $p \leq 0.01$ based on student t-test.

of *Cestrum diurnum* L. on spore germination of some fungi. Some plants are known to produce antifungal compounds like phytoterpenoid (Singh et al., 2006).

4. Conclusion

The above study showed that plant extracts with medicinal value, inhibited spore germination of sorghum grain mold fungi. Testing of these extracts under field conditions against some fungal plant diseases may be interesting as their antifungal activity is being reported for the first time.

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