

Screening of some plant root extracts for their antifungal activity against seed borne pathogenic fungi.

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Abstract: In the present study, root extracts of five plants were screened against two seed borne pathogenic fungi *Alternaria solani*, *Fusarium moniliforme*. Out of the five root extracts, two root extracts showed strong antifungal activity. The extract of *Hemidesmus indicus* showed maximum activity while minimum activity was observed by *Rauwolfia tetraphylla*. The purpose of investigation was to search for alternative approach to prevent biodeterioration of seeds in an ecofriendly way.

Index Terms : Antifungal activity; Biodeterioration; Carbendazim; Exploitation; Seed-borne Fungi; Root extracts; Whatmann filter paper.



INTRODUCTION

Plant extracts represent a rich source of antimicrobial agents and have been used by Indian system of medicine in preventive, promotive and curative applications. Extracts of various plant parts are found to be effective against seed borne pathogenic fungi. Fungi form the largest group among microorganisms causing seed damage at various growth stages. Seeds are treated by various means to get rid of such pathogens. Though efficient control of seed borne fungi can be achieved by using synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity [3]. Biological treatment can avoid losses due to diseases by seed borne pathogens and also provide sustainable and environmental friendly approach.

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides [14]. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails [11]. Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promising. In view of these the present investigation was undertaken to screen some root extracts against seed borne pathogenic fungi and the data has been presented in this paper.

MATERIALS AND METHODS

Collection of plants

The plants used in the present studies were collected from different regions of Marathwada particularly Nanded. After pressing and drying herbarium sheets of these plants, their identification was confirmed through consultation with Department of Botany, Yeshwant Mahavidyalaya, Nanded using the "Flora of Marathwada" [9]. The leaves, rhizomes, tubers, and roots, of the selected plants were collected separately, surface sterilized with 0.1 % HgCl₂ and washed two to three times with sterile distilled water. Plant parts like leaves, rhizomes, tubers, and roots, were separated and dried in an oven at 50-60 °C for 48 hours. Fine powders of these plant parts were prepared and preserved separately in polythene bags at room temperature (28 ± 2°C) for 48 hours.

Isolation of Phytopathogenic fungi

The test fungus namely *Alternaria solani*, was isolated from diseased leaves of tomato and *Fusarium moniliforme* was isolated from Maize seeds. For this the affected parts of the host were brought to the laboratory in polythene bags. They were cut into small pieces; surface sterilized with 0.1 per cent HgCl₂ solution and passed through three changes of sterile distilled water. The affected bits were placed aseptically on Glucose Nutrient Agar (GNA) plates. The fungal growth from the affected bits was picked up and transferred on GNA slant. The fungus identification was confirmed using manual of fungi [1] maintained on GNA slants for further investigation.

Preparation of plant powder

The processed plant part materials were used in this study. The collected plant parts were shredded and dried completely at 50-60 °C for 48 hrs in hot air oven. The dried materials were

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then ground into fine powder and stored in polythene bags at room temperature till extraction.

Preparation of plant extracts

For testing efficacy of plant extracts hot water, cold water, alcoholic (ethanolic) and ethyl acetate extracts of these plant parts were prepared. Hot water extract was prepared by heating extract in a container at 80 °C temperature for 20 minutes. 5 ml of alcoholic (ethanolic) and ethyl acetate extracts were evaporated on water bath and sterile distilled water was added to make up the volume of 5 ml. These extracts were used for further experiments

Plant Extracts

2.5 g / 5 g / 7.5 g / 10 g powder each of the plant parts were suspended / mixed separately in 100ml sterilized distilled cold water, hot water, alcohol (ethanol) and ethyl acetate in 250 ml conical flasks. They were thoroughly shaken and then the conical flasks were allowed to stand for 12 hours at room temperature. The contents were filtered through Whatman filter paper No.1. The filtrates were used as 2.5 %, 5 %, 7.5 % and 10 % plant extracts respectively.

Poisoned food method

In this method PDA media was used 1ml of the plant extract was mixed with 20 ml of the sterile medium. This was poured on to the sterile Petri plates and allowed to solidify at room temperature for thirty minutes. Spots of fungal spore suspension were aseptically inoculated. The inoculated plates were incubated at 25 °C and the test fungus was allowed to grow on poisoned plate. Petri plates inoculated with standard fungicide carbendazim. The effect of sample on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate after 72 hrs. The experiment was conducted in three replicates.

RESULTS AND DISCUSSIONS

The antifungal activity of root extracts (10 %) of five selected plants was tested against two plant pathogenic fungi *Alternaria solani* and *Fusarium moniliforme* by poison food method after 72 hours. The root extracts were extracted in cold water, hot water, ethyl acetate and alcohol (ethanol). 20 ml PDA medium in the plate was poisoned with 1ml of 10 % of each extracts separately. Carbendazim at 200 µg / ml in water was used as standard antifungal agent for comparison. The results are shown in Table 1.

Out of the four extracts of each of the five plants tested, *A. solani* showed maximum inhibition in colony diameter against ethyl acetate and alcohol extracts. These inhibitions were more than the standard. Hot water and cold water extracts of these five plants did not show much inhibition in colony diameter.

Ethyl acetate extract of *Hemidesmus indicus* showed complete inhibition of the fungus (standard 16 mm).

Four extracts of each of the five plants tested against *F. moniliforme*, showed maximum inhibition in colony diameter against ethyl acetate and alcoholic extracts. These inhibitions were more than the standard. Hot water and cold water extracts of these five plants did not show much inhibition in colony diameter. Ethyl acetate and alcoholic extract of *H. indicus* showed complete inhibition of the fungus.

Table 1 : Antifungal activity of Root extracts of selected plants in different solvents against some phytopathogens (1ml of 10 % extract in 20ml PDA) By poison food method.

Colony diameter in mm (After 72hrs)										
<i>Alternaria solani</i>						<i>Fusarium moniliforme</i>				
Name of test plants						Name of test plants				
Sol-vent	Ar	Ct	Hi	Ws	Rt	Ar	Ct	Hi	Ws	Rt
CW	19	20	16	22	28	35	37	34	36	36
HW	20	23	18	24	26	37	38	34	37	39
EA	23	16	00*	12*	18	27*	30*	00*	18*	32*
AL	6*	7*	00*	4*	11*	28*	26*	00*	11*	29*
Std	16	16	16	16	16	33	33	33	33	33

Ar - *Asparagus racemosus* (L.)

Ct - *Chlorophytum tuberosum* (Roxb.)

Hi - *Hemidesmus indicus* (R.Br.)

Ws - *Withania somnifera* (L.)

Rt - *Rauwolfia tetraphylla* (L.)

Std-Standard fungicide (Carbendazim @ 200µg/ml of water)

*-More effective than standard fungicide.

CONCLUSION

The antimicrobial activities of the five plants selected varied distinctly. Out of the four solvent extracts cold water, hot water, ethyl acetate and ethanol, the ethanol and ethyl acetate of all the plant extracts showed appreciable antimicrobial

activities against all the tested phytopathogens. The ethyl acetate and alcoholic extracts of *Asparagus racemosus*, *Chlorophytum tuberosum*, *Hemidesmus indicus*, *Withania somnifera* showed significant antimicrobial activity higher than the standard antibiotics. *Rauwolfia tetraphylla* showed relatively less activity compared to other plants. *Hemidesmus indicus* showed higher antimicrobial activity which have not been recorded earlier by any worker

These findings gave an idea that the plant extracts may be used as potential alternatives to synthetic fungicides for seed treatment to protect them against seed borne pathogens.

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