SCREENING OF SOME MEDICINAL PLANT EXTRACTS AGAINST Thielaviopsis spp-A DISEASE CAUSING PLANT FUNGUS

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ABSTRACT

Medicinal plants have been used in the prevention, treatment and cure disorders and diseases in ancient times and they provide abundant resources of antimicrobial compounds, has been used for centuries to inhibit the microbial growth. Diseases of medicinal plants were caused by the pathogenic microbes: especially fungi are common throughout the world. Microbial diseases of plants cause malfunctions such as disturb normal functions by degrading enzymes, toxin and growth substances, reduce the yield, survival capacity and resulting to death. In recent times, the medicinal plants were affected by exogenous, endogenous and microbial infections. The medicinal plant plant *Argemone mexicana L*. (Mexican poppy) is an annual exotic weed flora used for medicine in several countries throut the world and chosen for this study.This plant was frequently affected by many fungal pathogens. The present investigation focuses the isolation of frequent disease causing fungal pathogen*(*Thielaviopsis spp*)*from infected leaves and their control measures by using herbal extracts*(**Acalypha indica ,Catharanthus roseus*** and ***Murraya koenigii*** *) via. Invitro approach.*

Key word: *Argemone Mexicana;* exotic wee;, exogenous; endogenous; pathogen; toxin

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INTRODUCTION

Microbes are organisms invisible to naked eye and are visible under microscope. They are found in everywhere. Fungi may be using several modes of actions to overcome mechanical, chemical and physiological barriers to penetrate into plant tissues. The passive defense lines such as cell wall, wax layer and chemical confer broad resistance to a wide variety of pathogens (**Lebeda *et al* 2001**).During the last 20years, several valuable review studies on *in vitro* selection of resistance to pathogens (Helgeson and Deverall, 1983; wenzel, 1985; Daub, 1986) and toxins in plant pathogens and plant diseases (Durbin, 1981; Hensel and Holden, 1996; Huang, 2001; Walton, 1996; Hamer and Holden, 1997). ***A. mexcicana L. (Papavaraceae*),** commonly known as prickly poppy, is used as a medicinal plant in several countries.. In India, the smokes of the seeds are used to relieve toothache. The fresh yellow, milky seed extract contains protein-dissolving substances, effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches, dropsy and jaundice (Chopra *et al.,* 1986). Thielaviopsis spp is a filamentous, cosmopolitan and ubiquitous fungus commonly isolated from soil, plant debris, and indoor air environment. Since Thielaviopsis spp. are found in nature, they are also common laboratory contaminants. It is a member of the genus *Fusarium* species**.** In the present investigation three antifungal plant extract used to control fungal pathogens. They are *Acalypha indica ,Catharanthus roseus* and *Murraya koenigii* is an annual plant with characteristic white – purple or spotted purple flowers that flourishes in South – East Asia, China and India. It has been valued for centuries by herbalists as a treatment for upper respiratory infections, fever, sore throat and herpes. Thielaviopsis spp is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. Some species producemycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by these Thielaviopsis spp are fumonisins and trichothecenes. ***Lippia nodiflora (Phyla canescens***) was introduced as an ornamental plant to Australia. It is a toxic weed, perennial broadleaf herb with tiny serrated leaves, subsessile, Inflorescences cylindric to ovate capitula, Corolla pinkish purple or white, glabrous. Decoction of the entire plant safe and speedy laxative, constipation in children as suppository, fresh leaf, emetic and in croup, leaf paste with common salt and lime, parasiticide, useful in ringworm, applied on burns;,fresh leaf-juice, in rheumatism, arthritis and skin infections; powdered leaf: for bed sores and maggot-infected wounds. ***Acalypha indica* Linn. (Euphorbiaceae**) An annual erect herb, Leaves 2.5 - 7.5 cm long and 2-2.5 cm broad, ovate or rhomboid- ovate, It occurs as a weed in gardens, waste places and along the roadsides throughout the hotter parts of India from Bihar eastwards to Assam and southwards to Kerala.

MATERIALS AND METHODS

Collection of Plant Materials: The infected plants were collected from follow lands in and around Thanjavur and brought in to the laboratory for to carryout further processes. The leaves were surfaced sterilized by standard procedure.

Isolation and Identification of fungal pathogen (Aneja, 2003):

Composition of the Medium

Potato	:	200 grams
Agar	:	15 grams
Dextrose	:	20 grams
Distilled water	:	1000 ml

Media Preparation:

To suspend all the ingredients weighed through physical balance in 1000 ml of distilled water. The suspension was filter through Whatman filter paper for remove the impurities and then sterilized by autoclave (15 lbs pressure/121^{o}C). After autoclaving add chloramphenicol was added in sterile condition at 40^{o}-50^{o}C of medium. The medium is stored in the refrigerator for further use.The infected leaves are cut across lesions of 5-10 mm square, containing both the diseased and healthy-looking tissue. The surface sterilize the cut portions by dipping in a surface sterilant for solution for different times, varying from 12 to 120 seconds. Then, wash the treated pieces in three changes of sterile water and blot dry on clean, sterile paper towels to remove the sterilant. The plant pieces were aseptically transferred on a potato dextrose agar medium, usually 3-5 pieces per plate. Then, the plates were in an inverted position at 25ºC for 5-7 days. After the periods, the fungal growth was observed in each plate. The identification of fungus was performing standard straining method**.**

Lacto phenol cotton blue mount:

- Place a drop of lactophenol cotton blue on a clean slide.
- Transfer a small tuft of the fungus, preferably with spores and spore bearing structures, into the drop, using a flamed, cooled needle.
- Gently tease the fungal using the two mounted needles.
- Mix gently the stain with the mold structures.
- Place a cover-glass over the preparation taking care to avoid trapping air bubbles in the stain.
- Sealing lactophenol mounts: To keep the slides for many years, cover slip is sealed with nail polish as follows.

Maintenance of Fungal Inoculum: The fungal cultures were isolated and transfer to medium containing petri plates or tubes. The isolated cultures (pure form) are stored in refrigerator for the screening purposes. The periodical changes/transfer of the fungus, helped to viable condition.

Preparation of Extract: The fresh and healthy plant leaf of ***Acalypha indica ,Catharanthus roseus*** and ***Murraya koenigii***, (each 2 grams) was weighed and washed in running water for several times and then it was treated with 0.1% mercuric chloride as process of surface sterilization. The materials were thoroughly washed with sterilized distilled water for three times and they were grinded in mortar and pestle with aqueous,benzene,acetone,diethyl ether and dimethyl formamide.The extract was filtered through whatman filter paper and centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and it was made up to known volume by using distilled water.

Screening for Antifungal Activity: The *in vitro* screening of antifungal activity was performance by the pour plate technique. About 15 ml of sterilized PDA medium with 1 ml of extract of each plant was poured into each sterilized petridish. The plates were gently shacked for the thorough mixing of the medium and extract. After solidification of the medium, the test fungi maintained in PDA plates/tubes were aseptically inoculated in the middle of the plate using inoculation needle or loop. Then the plates were incubated at room temperature at 27ºC for 3-5 days. The controls were maintained throughout the study. The fungal growth was observed and the results were tabulated.

RESULTS AND DISCUSSION

The maximum antifungal activities observed in the extract prepared using dimethyl formamide and minimum in aqueous, acetone solution. It indicates that the extract prepared by using different solvent having various strength of plant extracts. ***Acalypha indica*** extract prepared by using different solvent showed significant result against the antifungal activity of fungal pathogen Thielaviopsis spp.The strong antifungal activity observed dimethy formamide acetone and benzene and mild antifungal activities aqeoues and moderate in dimethy ether extracts. From the result,among the three plants, the ***Acalypha indica*** showed strong antifungal activity in most of the extracts from the result it clearly indicates that there was a significant variation observed among the different organic solvents. ***Catharanthus roseus*** extracts showed variation against *Fusarium* species. It was found to be minimum inhibition in extract prepared by using water, acetone and diethyl ether. Maximum inhibition observed in the extract prepared by using dimethyl formamide, moderate incubation observed in benzene extracts. In control petriplate with out plant extract the inoculation showed significant growth during the inhibition period.In ***Murraya koenigii*** extract also showed significant variation in the antifungal activities .

The systematic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic fungi. The present investigation deals with the antifungal properties of three medicinal plant extracts were tested against Thielaviopsis spp, causal agent of root rot diseases in plants. Our findings showed the organic solvents extracts contribute significant activity than aqueous. Among the plant tested, the leaf extracts from ***Acalypha indica*** expressed excellent activity followed by ***Catharanthus roseus*** and ***Murraya koenigii***. This study was comparable to earlier reports showed the effect of leaf powder extracts from ***Azadirachta indica*** (a potent antimicrobial agent) expressed significantly reduced the root rot diseases in plants, caused by Thielaviopsis spp and ***A. niger*** (Sinha and Saxena, 1987). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini *et al.,* 2004). Aqueous extracts of fruits and leaves *of* ***Capsicum fruitescens***, ***Capsicum annum (Solanaceae***) and ***Nerium oleander (Apocynaceae***) were found to inhibit the germination of ***Alternaria solani*** spores (Khallil, 2001). Singh and Sudarshana (2003) tested the aqueous and ethanolic extract of ***Baliospermum axillare*** leaf and callus against some bacteria and the fungi namely ***Fusarium solani*** and ***F. oxysporum***. Antifungal activities of some extracts of ***Launaea nudicauls*** have been determined by standard methods and the activity was determined by measuring the linear growth (4^{th} day incubation) of Thielaviopsis spp (Rashid *et al*., 2000).The ethanol and water extracts of the leaves of ***Melia azadirach, Vitex negundo***, ***Broussontia papyrifera*** and fruits of ***Datura anoxia*** were evaluated against the growth of many fungi including Thielaviopsis spp (Zafar *et al*., 2002).

REFERENCES

1. Aneja K.R., 2003. Experiments in microbiology, plant pathology and biotechnology. Fourth edition. New Age International (P) Limited, Publishers, New Delhi, 437-39.
2. Chopra R.N., Nayar S.L., and Chopra., 1986. I.C. Glossary of Indian medicinal plants (Including the Supplement), Council of Scientific and Industrial Research, New Delhi.
3. Daub M.E., 1986. Tissue culture and the selection of resistance to pathogens. Annu Rev Phytopathol 24: 159-186.
4. Hammer J.E., and Holden D.W., 1997. Linking approaches in the study of fungal pathogenesis: a commentary. Fungal Genet Biol 21: 11-16.
5. Helgeson J.P., and Deverall B.J., (eds)., 1983. Use of Tissue Culture and Protoplasts in plant pathology. Academic Press, Sydney.
6. Huang J.S., 2001. Plant Pathogenesis and Resistance. Biochemistry and Physiology of Plant-Microbe Interactions. Dordrecht, Kluwer Academic Publishers, Netherland.
7. Khallil M.A., 2001. Phytofungitoxic properties in the aqueous extracts of some plants. Pak J. of Bio. Sci. 3: 177-180.

8. Lebeda A., Luhova L., Sedlarova M., and Jancova D., 2001. The role of enzymes in plant-fungal pathogens interactions. J plant Dis Protect 108: 89-111.

9. Rashid S., Ashraf M., Bibi S., and Anjum R., 2000. Antibacterial and antifungal activites of *Launaea nudicaulis (Roxb.)* and *L. resedifolia (Linn.).* Pakistan J Bio. Sci. 3(4): 630-632.

10. Singh K., and Sudarshana M.S., 2003. Antimicrobial activity of *Bailospermum axillare* plant and callus extract. Asian J. Micxrobiol. Biotechnol. and Environ. Sci 5: 571-574.

11. Sinha P., and Saxena S.K., (1987). Effect of neem leaf powder and extract on the development of fruit rot caused by *Aspergillus niger*. Neem Newsl.4:45-47.

12. Suffredini J.B., Sadar H.S., Goncalves A.G., Reis A.O., Gales A.C., Varella A.D., and Younes R.N., 2004. Screening of antimicrobial extracts from plants native to the Brazilian Amazon rain forest and Atlantic forest. Brazil. J. Med. Biol. Res. 37: 379-384.

13. Walton J.D., 1996. Host-selective toxins agents of compatibility. Plant cell 8: 1723-1733.

14. Wenzel G., 1985. Strategies in unconventional breeding for disease resistance. Annu Rev Phytopathol 23: 149-172.

15. Zafar I., Mussarat S., Farrakh h., Sheraz B., Mohammad I., Shahida Z., and Bashir A., 2002. Antifungal properties of some Indigenous plants from Peshawar Valley. Asian J Plant Sci. 1(6): 708-709.

Table 1: Antifungal activity of three plant extract prepared by using different solvent.

Plant Name	Solvents Used					
	Aqueous	**Benzene**	**Acetone**	**Diethyl ether**	**Dimethyl formamide**	**Control**
Catharanthus roseus	+	++	+	+	+++	-
Murraya koenigii.	+	++	+	+	+++	-
Acalypha indica	+	+++	+++	++	+++	-

- = control

+ = 25% growth Inhibition

++ = 50% growth Inhibition

+++ = 100% growth Inhibition