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EVALUATION OF ANTIFUNGAL ACTIVITY IN PLANT EXTRACT AGAINST *FUSARIUM MONILIFORME*

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Abstract: The present study was aimed to investigate the antifungal activity of some plant extract against *Fusarium moniliforme*. Pure culture of *Fusarium moniliforme* MTCC (156) was used. The *in vitro* studies have been performed by using sub cultured fungus and 1ml of extract was used for the screening. The agar with the sample was mixed well and then poured into petriplates. In these plates 0.2 ml of diluted spore suspension ($50X10^3$ CFU/ml) of *Fusarium moniliforme* was added and kept at room temperature for three days. These extracts were screened for their antifungal activity by the spread plate method. In out of twenty nine plants extract was screened against *Fusarium moniliforme.* Out of twenty nine plant extract, twenty seven plant extracts showed antifungal activity, while maximum activity was observed in rose mary leaves (91%) and curry leaves (83.6%), minimum activity was observed in allspice leaves (3.6%) but geranium (-3.2%) and basumati leaves (- 47.78%) were observed negative antifungal activity

Keywords: *Fusarium moniliforme,* MTCC (Microbial Type Culture Collection), CFU (Colony Forming Unit).

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INTRODUCTION

Medicinal plants were used as excellent antimicrobial agents because it possess a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as source of medical agents. Synthetic fungicides are currently used as primary means for the control of plant disease. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicide among fungal pathogens and high development cost of new chemicals. Generally the fungal infections are the most common cause of many shin diseases in developing countries. Fungal infections mainly resulting from the species of *Candida* and *Aspergillus* are life threatening in immune compromised patients[1-2].

The uses of plant derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance. Crude extracts of some well known medicinal plants are used to control some of the plants pathogens. Natural antimicrobials can be derived from plants, animal tissues or microorganism. The drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants[3-4] .Cereal grains and animal feed are colonized by moulds there is a significant risk of contamination with toxic effects towards animals and human beings and are accorded the collective term mycotoxins[5-6].

Mycotoxins from *Fusarium* species have traditionally been associated with temperate cereals, since these fungi require somewhat lower temperatures for growth and mycotoxin production than the A*flatoxigenic Aspergillus* species. Mycotoxins are the toxic products of fungal metabolism occur in a wide variety of commodities like animal feeds and human food products. Mycotoxins on ingestion can cause health hazards both in livestock and human beings. Mycotoxin contamination of feed affects dairy cattle, poultry, sheep and swine reduces growth efficiency, impairs resistance to infection and reproduction. The mycotoxins that are significance in animal feed are aflatoxin, ochratoxin and *Fusarium* toxins. *Fusarium* toxins produced by several species of *Fusarium* fungal species in feed stuffs[7]. *Fusarium moniliforme* and *Fusarium* Proliferate have been recently linked with the natural co-contamination of maize and two relatively novel mycotoxins, fusaproliferin and beauvericin. *Moniliformin* is also synthesized by *Fusarium oxysporum* which, in addition, is a recognized source of the mycotoxins wortmannin and fusaric acid.

Presence of mycotoxins in animal feeds causes health risk and effect productivity. Addition of certain antifungal compounds will be useful in preventing the growth of fungi in feed. Organic

acids are known of suppress the growth of fungi[8].Certain herbal products like spice oils and medicinal plants also possess antifungal activity[9].There are reports that plant compounds derived from garlic, onion, turmeric either prevent fungal growth or block biosynthesis of toxins[10-11]. *Fusarium moniliforme* may be transferred into milk, egg and meat. In Japan, several cases of mycotoxicosis in animals and human beings have been attributed to consumption of cereals. The present study was carried out to investigate the anti fungal activity of some plant extract against *Fusarium moniliforme*.

MATERIALS AND METHODS

Pure culture of *Fusarium moniliforme,* MTCC (156) was used.

Sub culturing of *Fusarium moniliforme*

4.1 gm of potatoes Dextrose agar was dissolved in 100ml of distilled water and heated until the agar was completely dissolved. About 15-20 ml of the clear solution was poured in a series of tubes. The tubes were sealed with the cotton plug, foiled and autoclaved at 121ºC for 20 minutes. The autoclaved tubes were kept in slanting position until the media solidified.

The slants thus prepared were inoculated with pure culture of *Fusarium moniliforme* MTCC (156) in a laminar flow under total aseptic conditions. The inoculated slants were kept in dark at room temperature for 7 days so that the fungus sporulates. The subcultures were used immediately or stored at 4ºC until used.

Spread Plate Technique

In 0.2 ml of diluted spore suspension ($50X10^3$ CFU/ml) of *Fusarium moniliforme* was added into the center of the surface of an agar plate. Dipped the L-shaped glass spreader into alcohol. Glass spreader was flamed (hockey stick) over a bunsen burner. The sample was evenly spread over the surface of agar using the sterile glass spreader, carefully rotating the petridish underneath at the same time. Plate was incubated at 37°C for 60 hours. CFU value was calculated.

Plant collection and processing

The plant leaves powder was collected from health innovation medicinal plant nursery at tirchy. 0.5 gram of leaves powder was dissolved in 100 ml of distilled water, mixed for 5 minutes and left for one hour. Then filtered through muslin cloth and finally what man filter paper no.1 to obtain a solution. These extracts were screened for their antifungal activity by spread plate method[12].The agar with the 1ml of extract was mixed well and poured to petriplates in triplicates. To these plates 0.2ml diluted spore suspension ($50X10^3$ CFU/ml) of *Fusarium*

moniliforme was added, spread well and incubated at room temperature for 5 days. On the third day, colony counting was done to judge the antifungal activity by the spread plate method.

$$\text{Antifungal activity (\%)} = \frac{\text{Colony Counts in Control} - \text{Colony Counts in Test}}{\text{Colony Counts in Control}} \times 100$$

STATISTICAL ANALYSIS

The mean value of Colony Forming Units (CFU) and their standard error (±) for each plant extracts were calculated.

RESULTS

Exploitation of the evaluation of antifungal activity of the present study revealed that the most of the plant extract possess potential antifungal activity against *Fusarium moniliforme* Table 1 presents the data on antifungal activity of some plant extract against on *Fusarium moniliforme*. rose mary leaves (91%) and curry leaves (83.6%) exhibited maximum antifungal property was preventing the growth of *Fusarium moniliforme*. Thumbe leaves (72.7%), sweet worm wood leaves (71.6%), aswagandha leaves (71.6%) and cinnamon leaves (67.3%) showed moderate anti fungal activity. This is followed by pachouli leaves (52.2%), greater galangal leaves (41.2%), clocimum leaves (31.9%), camphor basil leaves (33%), sage leaves (35%) and lavender leaves (34%) that exhibited an antifungal activity. The other plants like, mehandi leaves (27.3%), neeligida leaves (27.3%), melisa leaves (27%) and sarpaganda leaves (25.8%) showed mild antifungal activity. The remaining plants evaluated like chamomile leaves (17%), lemon verbena leaves (16.4%), origanum leaves (15.7%), worm wood leaves (12.3%) adhathoda leaves (10.7%), bergamot mint leaves (11.4%) and fennel leaves (10%) showed a lowest antifungal activity when compare to the other plant extract.

Table1: Antifungal activity of some selected plants against *Fusarium moniliforme*

Experiments were carried in triplicate and each data point is mean value ± SD.

S. No	Test Material	Scientific Name of the Plant	Colony Counts (CFU)	% Antifungal Activity	Remarks
1	Clocimum leaves	*Ocimum gratissimum*	62 ± 2.46 (91 ± 7.31)	31.9	-
2	Camphor basil leaves	*Ocimum kilimandscharium*	61 ± 2.02 (91 ± 7.31)	33.0	-
3	Melissa leaves	*Melissa officinalis*	46 ± 6.83 (63 ± 2.62)	27.0	-
4	Geranium leaves	*Pelargonium graviolens*	65 ± 3.49 (63 ± 2.62)	-3.2	-
5	Pachouli leaves	*Pogostemon cablin*	11 ± 1.65 (23 ± 1.41)	52.2	Small colonies
6	Thyme leaves	*Thymus vulgaris*	22 ± 2.01 (23 ± 1.41)	4.3	Tiny colonies
7	Sage leaves	*Salvia officinalis*	15 ± 0.75 (23 ± 1.41)	35.0	-
8	Basumati leaves	*Pandanus amerylifolius*	34 ± 1.47 (23 ± 1.41)	-47.78	-
9	Lavender leaves	*Lavendula stochyas*	46 ± 0.95 (70 ± 4.02)	34.0	-
10	Chamomile leaves	*Metricaria chamomilla*	58 ± 5.00 (70 ± 4.02)	17.0	-
11	Fennel leaves	*Foeniculum vulgare*	63 ± 4.27 (70 ± 4.02)	10.0	-
12	Bergamot mint leaves	*Mentha citrata*	62 ± 1.31 (70 ± 4.02)	11.4	-
13	Origanum leaves	*Origanum vulgare*	59 ± 2.34 (70 ± 4.02)	15.7	-
14	Curry leaves	*Murraya koenigii*	9 ± 0.47 (55 ± 0.91)	83.6	-
15	Mehandi leaves	*Lawsonia inermis*	40 ± 0.25 (55 ± 0.91)	27.3	-
16	Rosemary leaves	*Rosmarinus officinalis*	5 ± 1.10 (55 ± 0.91)	91.0	-
17	Cinnamon leaves	*Cinnamomum verum*	18 ±1.44 (55 ±0.91)	67.3	-
18	Allspice leaves	*Pimenta officinalls*	53 ± 1.08 (55 ± 0.91)	3.6	Small colonies
19	Lemon verbena	*Lippia citriodora*	46 ± 0.64 (55 ± 0.91)	16.4	-
20	Sarpaganda leaves	*Rauvolfa serpentine*	138 ± 5.76 (55 ± 0.91)	25.8	

21	Garden rue leaves	*Ruta graveolens*	173 ±17.22 (187 ± 3.10)	7.5	Tiny colonies
22	Greater galangal leaves	*Alpina galangal*	110 ± 6.37 (187 ± 3.10)	41.2	-
23	Adhathoda leaves	*Adhtoda zeylanica*	167 ± 0.64 (187 ± 3.10)	10.7	-
24	Worm wood leaves	*Artemisia absinthum*	164 ± 1.93 (187 ± 3.10)	12.3	-
25	Kalmegh leaves	*Andrographis paniculata*	180 ± 6.51 (187 ± 3.10)	3.7	-
26	Neeligida leaves	*Indigofera tinctoria*	136 ± 6.27 (187 ± 3.10)	27.3	-
27	Thumbe leaves	*Leucas aspera*	51 ± 0.48 (187 ± 3.10)	72.7	-
28	Sweet worm wood leaves	*Artemisa annua*	53 ± 0.86 (187 ± 3.10)	71.6	-
29	Aswagandha leaves	*Withania somnifera*	53 ± 0.63 (187 ± 3.10)	71.6	-

(The numbers in parenthesis indicates the CFU of control)

Garden rue leaves (7.5%), thyme leaves (4.3%), kalmegh leaves (3.7%) and allspice leaves (3.6%), exhibited minimum activity. Basumati leaves (-47.78%) and geranium leaves (-3.2%) showed a negative antifungal effect but supported the growth of *Fusarium moniliforme.*

The present study concludes that rose mary leaves and curry leaves exhibited more than 80% antifungal activity. Pachouli leaves exhibiting more than 50% antifungal activity also produced small colonies. Other plants that produced tiny colonies included thyme leaves, allspice leaves and garden rue leaves. Basumati leaves and geranium leaves showed a negative antifungal effect but supported the growth of *Fusarium moniliforme.*

DISCUSSION

The results obtained from the present investigation revealed that the highest antifungal activity was exhibited by the rose marry leaves and curry leaves. Cur cumin present in turmeric was found to be active against *Aspergillus flavus, Aspergillus parasiticus and Fusarium moniliforme and pencillium digitatum* [13]. *Marigold* has fungicidal properties due to the presence of thiophenes in its tissues[14]. Earlier studies reported that thio propanal – S –oxide, a compound present in onion had highest inhibitory action against *Aspergiius parasiticus.*

Maximum inhibition of fungal growth was observed in garlic and clove oil, when mixed with maize and *Aspergillus* culture essential oils of some aromatic plants have both fungi static and fungicidal activity against A*spergillus flavus* and A*spergillus parasiticus* and *Aspergillus fumigatus* [15].Presence of powerful sulfur and other numerous phenolic compounds as an antifungal agent in onion[16]. *Vitex negundo* exhibited maximum antifungal activity compared with other plant extract[17]. Extracts of some rice cultivars exhibit antifungal activity against *Macrophomina phascolina* and *Ascochyta rabiei*[18].

The antifungal activity of ethyl acetate extract of *Hypochaeris radicata* leaf showed highest inhibitory activity against the fungus *Aspergillus niger*. It is ailamentous ascomycete in opportunistic infections of human[19]. Maximum antifungal activity against *P.crysogenum* was demonstrated by cold methanol and aqueous extract. Phytochemical analysis indicated the presence of steroids, alkaloids, flavonoids, coumarins and tannins. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, anti allergic, antithrombotic and vasoprotective effects[20-21].

Presence of these active constituents may responsible for the antimicrobial activity[22] reported that water extracts of *Asteraceae* members showed strongest effect on reduction in growth of *A.nigerthan* the species of certain other families. They explained that certain specific compounds of unknown functional group may present in *Asteraceae* member which played role in the inhibition of fungal colonies. Aqueous extracts of *marigold* were found highly effective in suppressing the growth of *Fus.oxysporum*. Various publications have documented the effective antifungal activity of *Asteraceae* members[23-26]. It was further observed that the inhibitory activities of ethyl acetate extract of leaf against *Fusarium* species were significantly greater than that of the standard drug, tetracycline. The present results showed that the rose mary leaves and curry leaves were more effective than the other plant extracts tested.

CONCLUSION

The present study reveals that highest antifungal potential was observed in the rose mary leaves and curry leaves against *Fusarium moniliforme.* Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. Rosemary leaves and curry leaves presents a resourceful reservoir of the various bioactive metabolites and can be of possible use in modern medicine.

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