Int J Pharm Bio Sci 2013 July; 4(3): (B) 163 - 170

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIFUNGAL ACTIVITY OF SELECTED PLANT EXTRACTS AGAINST IMPORTANT SEED BORNE FUNGI OF MAIZE

WAHEGAONKAR NILIMA K.[1] AND SHIRURKAR DEEPAVALI D.*[2]

[1] Vasantrao Naik Mahavidyalaya, Aurangabad, Maharashtra, India.
[2] Annasaheb Magar Mahavidyalaya, Hadapsar, Pune, Maharashtra, India.

ABSTRACT

Fifteen different plants used in traditional Indian medicine were examined against eight fungal species viz. *Aspergillus flavus, Aspergillus niger, Aspergillus terreus*, *Aspergillus oryzae, Aspergillus fumigatus*, *Fusarium verticillioides* (Synonym *F. moniliforme*), *Fusarium solani* and *Penicillium sp.* using the agar disc diffusion method. The alcoholic extracts of 15 plants exhibited varying degrees of inhibition activity against the above seed borne fungi. Seven of the 15 plants studied had antifungal activity against above listed fungi while the remaining had no antifungal activity. Extent of activity also differed with the part of the plant used.

KEYWORDS: **Antifungal activity, Maize, Medicinal plants, Seed borne fungi**

SHIRURKAR DEEPAVALI
Annasaheb Magar Mahavidyalaya, Hadapsar, Pune, Maharashtra, India.

INTRODUCTION

Maize (*Zea mays* L.) is one of the main source of cereals for food, forage and processed industrial products. World production of maize is around 790 million tones and it serves as a staple food providing more than one-third of the calories and proteins in some countries[1]. Stored maize is a man-made ecosystem in which quality and nutritive changes occur because of interactions between physical, chemical and biological factors. Fungal spoilage and mycotoxin contamination are of major concern. In India, maize (*Zea mays* L.) is one of the important cereals. Fungal deterioration of stored seeds and grains is a major problem in India. Harvested grains are colonized by various fungal species and during storage grains undergo quantitative and qualitative losses due to mycotoxin production. A significant portion of the agricultural produce in the country and the world over becomes unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of *Aspergillus*[2-4]. There are restrictions on use of pesticide and fungicides[5], hence it is very essential to develop control measures as alternative to chemicals[6]. 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 the synthetic pesticides[7]. Some traditionally useful plants have been shown to exhibit fungi-toxic property[8]. Control measures by using plant extracts are cost effective and non-toxic methods. Efforts have been devoted to search for antifungal material from natural resources by many researchers. In present investigation, antifungal activity of various plant extracts on growth of seed borne fungi had been carried out.

MATERIALS AND METHODS

(i) Plant material and extract preparation

The plant materials of 15 plant species (Table1) were collected from different places. The collected plants were identified and authenticated by a botanist in the Department of Botany, University of Pune, Maharashtra. The plant parts were selected on the basis of the knowledge on their use in different medicine system of health care and as food preservatives[9-10]. The selected parts of different medicinal plants were cleaned and cut into small pieces, used fresh or shade dried at room temperature for fifteen days and then powered with the help of a blender. The powdered material was stored in air tight jars in refrigerator at $4^{o}C$[11]. Finely powdered plant material was dissolved in ethanol to make 100ml of ethanolic extract (5%, 10%, 20%, 30%, 40% and 50% w/v). The mixture was kept undisturbed at room temperature for 24 hours in sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterile Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25ml extract was left in the container. Ethanolic extracts were immediately evaluated for antifungal activity[12-14].

(ii) Isolation of seed borne fungi

Maize grain samples were plated on PDA (Potato dextrose agar), MEA (Malt extract agar), RBA (Rose Bengal agar) and SBM (Standard blotter method) to isolate the frequently occurring important seed borne fungi associated with these grains. The cultures of the isolated fungi were maintained on PDA medium, which served as the test fungi for antifungal activity assay[14]. The stock suspensions of 8 fungal isolates were standardized to10^{6} spores/ml spectrophotometrically at 530nm and were adjusted to 80 to 85% transmittance[15-16].

(iii)Antifungal Assay

Alcoholic plant extracts were screened for their antifungal activity against seed borne fungi of maize by disc diffusion method. Different concentrations of each plant extract (i.e.5%, 10%, 20%, 30%, 40% and 50%) were used for assay. Spore suspensions (0.1ml) were applied on the surface of the PDA plate and spread by using a sterile glass spreader. The sterile discs (5mm diameter, Whatman filter paper No. 42) were soaked in different concentrations of plant part extracts and were placed at equal distance[17]. The test was performed in triplicate. Plates were kept for pre-incubation for 2 hours in refrigerator. The plates were then incubated for 4 days at room temperature. Zone of inhibition in mm was determined after 4 days.

Table – 1
Antifungal activity of plant extracts on seeds borne fungi.

Sr.no.	Vernacular & Botanical Name of the plant	Plant part used	Ethanol extract concentration	*A. flavus* (zone in mm)	*A. niger* (zone in mm)	*A. terreus* (zone in mm)	*A. oryzae* (zone in mm)	*F. solani* (zone in mm)	*F. verticillioides* (zone in mm)	*Penicillium*sp. (zone in mm)
1	Marigold (*Tagetes sp.*)	Flower	Control	-	-	-	-	-	-	-
			5%	-	-	7	-	-	-	-
			10%	-	-	7	-	-	-	-
			20%	-	3	9	6	6	9	
			30%	-	5	10	15	9	11	5
			40%	7	7	12	17	10	15	5
			50%	8	9	13	18	11	25	7
2	Hibiscus (*Hibiscus rosa-sinensis,* L)	Flower	Control	-	-	-	-	-	-	-
			5%	-	-	-	-	-	-	-
			10%	-	-	-	-	-	-	-
			20%	-	-	-	-	5	-	-
			30%	-	5	-	9	7	10	4
			40%	-	9	-	12	9	12	5
			50%	-	9	17	17	10	13	15
3	Ginger (*Zingiberoffcinale*, Rose)	Rhizome	Control	-	-	-	-	-	-	-
			5%	-	2	-	4	-	5	-
			10%		5	-	4	3	4	-
			20%		9	-	6	7	12	-
			30%	2	10	15	7	8	7	-
			40%	7	12	17	11	9	10	-
			50%	10	13	17	14	13	3	-

RESULTS AND DISCUSSIONS

The antifungal activity of 6 plants obtained by the disc diffusion method is shown in Table 1. Ethanolic plant part extracts tested exhibited different degrees of antifungal activity against eight fungal species *Aspergillus flavus, A. niger, A. terreus*, *A. oryzae, A. fumigatus*, *Fusarium verticillioides, F. solani* and *Penicillium sp.* isolated from maize seeds. The data revealed significant reduction in growth of seed borne fungi with different concentrations of ethanolic extract. The different plants showed significant differences in their efficacy. Ethanolic extracts of Marigold flowers showed inhibition of mycelial growth of all species except *A. fumigatus.* Hibiscus flower ethanolic extract was found active against all the test fungi except *A. flavus.* Ginger rhizome and Nirgudi leaves ethanolic extract recorded significant antifungal activity against all the test fungal isolates except *Penicillium sp.* Garlic leaves ethanolic extract inhibited the growth of 5 fungal isolates viz. *A. flavus, A niger, A. oryzae, F. verticillioides* and *Penicillium sp.* while garlic bulb ethanolic extract inhibited the growth of 3 isolates viz. *A niger, A. oryzae* and *Penicillium sp.* Neem leaves ethanolic extract showed inhibitory activity against *A. flavus, A niger*, *A. terreus, A. oryzae* and *Penicillium sp.* Results reveal that out of 15 extracts, 8 extracts from *Cinnamomum* leaves, *Terminalia bellerica* (behada) fruits, *Cynadon dactylon* (harali) leaves, *Phyllanthus neruri* leaves, dry rhizome (sunth) of *Zingiber officinale*, Whole plant of *Solanum xanthocarpum*, *Fenugreek* seeds and bulb of *Allium cepa* (onion) did not possess antifungal activity against the test fungal isolates. The objective of the current research was to study the effect of plant extracts on the mycelial growth of seed borne fungi of maize. The use of biocontrol agents from plant extracts, as alternatives for conventional synthetic pesticides in plant disease control, is reported by many researchers. The antifungal activity of garlic is related to allicin, which is the main biologically active component of garlic extract inhibiting essential enzymes for pathogen infection[18]. Similarly, ajoene (allicin derivative) has also been shown to have strong inhibitory activity against several fungal species including the black mold (*Aspergillus niger*)[19]. The inhibitory activity of garlic extracts against various *Penicillium* sp. such as *Penicillium digitatum*[20], *Penicillium oxalicum* [21], *Penicillium chrysogenum*[22] have been reported by several workers. Khurana *et al.* reported that garlic is most effective in inhibiting spore germination of different fungi[23]. Allicin, thiosulfonate and other compounds showed fungistatic activity against *Aspergillus* spp. such as *A. flavus, A. fumigatus, A. terreus* and *P. chrysogenum*[22]. Several ajoene compounds, derivative of allicin, obtained from garlic with ethanol extraction have been found to be strongly inhibitory against *A. niger*[24-26]. Garlic extract had a positive effect on *Fusarium* inhibition[27]. Misra and Dixit[28] reported the antifungal activity of *Allium sativum* against *Fusarium* spp. Inhibitory effect of extracts of garlic bulbs on the mycelial growth of *Fusarium pallidoroseum* was reported by Jacob and Sivaprakasan[29] and Arya *et al.*[30]. Maximum inhibition in spore germination of *Fusarium solani* f. sp. *melongenae* was exhibited by the extract of *Allium sativum* as reported by Bowers and Locke[31]. Saxena and Mathela[32] in their study on the inhibitory effect of plant extracts on *Fusarium* reported that, *Azadirachta indica*, *Artemisia annua, Eucalyptus globules, Ocimum sanctum* and *Rheum emodi,* showed significant reduction of the pathogen. Neem leaves extract was effective against the fungi *Penicillium digitatum* as reported by Mossini, *et al.*[33]. Neem has a potential and ability to minimize fungal population. Efficacy of Neem was observed by Bhutta *et al*.[34] against five seed borne fungi viz., *Alternaria alternata, Emericellopsis terricola, Fusarium solani, Macrophomina phaseolina and Stemphylium helianthi*. Bhutta *et al*.[35]also reported the effectiveness of seed diffusates of neem in controlling fungi *Fusarium semitectum,* and there was a significant increase in seed germination after elimination of fungi. The efficacy of Neem has been reported and reviewed by Rao *et al*.[36] and similar results were found by Ravishankar & Mamatha[37] on forest seeds. Ginger showed inhibitory effects on *A. niger, Rhizopus* and *Penicillium* spp. in the work of Afzal et al.[38].

Table – 1
continued:Antifungal activity of plant extracts on seeds borne fungi.

Sr.no.	Vernacular & Botanical Name of the plant	Plant part used	Ethanol extract concentration	*A. flavus* (zone in mm)	*A. niger* (zone in mm)	*A. terreus* (zone in mm)	*A. oryzae* (zone in mm)	*F. solani* (zone in mm)	*F. verticillioides* (zone in mm)	*Penicillium*Sp (zone in mm)
4	Nirgudi (*Vitexnegundo*, L)	Leaves	Control	-	-	-	-	-	-	-
			5%	-	-	-	-	-	-	-
			10%	-	-	-	-	-	-	-
			20%	-	-	-	-	-	-	-
			30%	-	6	-	9	9	-	-
			40%	-	8	6	11	9	9	-
			50%	9	11	9	14	13	9	-
5	Garlic (*Allium sativum*, L)	Leaves	Control	-	-	-	-	-	-	-
			5%	-	-	-	-	-	-	-
			10%	-	-	-	6	-	-	-
			20%	-	-	-	7	-	-	-
			30%	6	-	-	7	-	-	-
			40%	7	7	-	8	-	9	6
			50%	8	12	-	13	-	12	10
6	Garlic (*Allium sativum*, L)	Bulb	Control	-	-	-	-	-	-	-
			5%	-	-	-	-	-	-	-
			10%	-	-	-	-	-	-	-
			20%	-	13	-	6	-	-	-
			30%	-	13	-	7	-	-	10
			40%	-	13	-	7	-	-	10
			50%	-	18	-	-	-	-	20
7	Neem (*Azadirechta indica*, Juss)	Leaves	Control	-	-	-	-	-	-	-
			5%	-	-	-	-	-	-	-
			10%	-	-	-	-	-	-	-
			20%	-	-	-	-	-	-	-
			30%	12	-	-	-	-	-	-
			40%	13	-	11	-	-	-	-
			50%	-	12	12	7	-	-	13

CONCLUSION

From above investigation it was revealed that the antifungal activity of the extracts was enhanced by an increase in the concentration of the extract. In vitro evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing ecofriendly management of fungi[39]. Demand for natural extracts is increasing in agricultural industry in order to find effective alternatives to synthetic fungicides[40]. In this context ethanolic extracts of Marigold flower, Hibiscus flower, Ginger rhizome, Nirgudi leaves, Neem leaves, Garlic leaves and bulb gave interesting results. These may be helpful for developing plant based fungicides which are eco-friendly for the management of seed borne fungi and development of commercial formulation of botanicals.

ACKNOWLEDGEMENT

The authors would like to acknowledge BCUD, University of Pune, for financial support. We thank Miss. Harshada Bankar, Miss. Komal Gajamal and Mr. Prakash Shinde for technical assistance.

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