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Antifungal activity of selected plant derived oils and some fungicides against seed borne fungi of maize.

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ABSTRACT

Two fungicides and eight different plant derived oils were examined against 8 seed borne fungi namely, Aspergillus flavus, A. niger, A. terreus, A. oryzae, A. fumigatus, Fusarium moniliforme, F. solani and Penicillium sp. isolated from maize grains. Antifungal activity was tested on PDA medium using well diffusion method. The plant derived oil exhibited varying degrees of inhibition activity against the fungi. Oils of Nutmeg, Eucalyptus, Cinnamon and Clove showed maximum antifungal activity against all the eight fungal species. Oils of Neem, Sesame, Pongam and Negundo did not exhibit any antifungal activity. Mancozeb inhibited mycelial growth of all the test fungi significantly. Bavistin also inhibited all the test fungi except F. solani and Penicillium sp.

Keywords: Antifungal activity, oils, seed borne fungi, maize

INTRODUCTION

Fungal deterioration of stored seeds and grains is a chronic problem in the Indian storage system because of the tropical hot and humid climate. Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins [1, 2].

More than 25% of the world cereals are contaminated with known mycotoxins and more than 300 fungal metabolites are reported to be toxic to man and animals [3]. The main toxic effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immuno-suppression [4,5]. Even though effective and efficient control of seed borne fungi can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity [6,7,8]. Thus, there is a need to search alternative approaches to store cereal grains for human consumption, that are eco-friendly and not capital intensive. Spices and essential oils have been reported to have antimicrobial activity and some also to inhibit aflatoxin formation [9,10,11,12].

The aim of this study was to assess the antifungal activity of some chemicals and selected plant derived oils against seed borne fungi of maize.

MATERIALS AND METHODS

Isolation and Identification of Fungi:

To isolate the important seed borne fungi associated with Maize grains, the samples were plated on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Martin's Rose Bengal Agar and SBM (Standard blotter method) [13]. The pure cultures of these fungi were maintained on PDA medium, which served as the test fungi for antifungal activity assay.

List of investigated chemicals and plant derived oils.

Eight plant derived oils (Pongam, Sesame, Nutmeg, Cinnamon, Clove, Eucalyptus, Neem and Negundo) and two commercially used chemicals; Bavistin and Mancozeb were obtained from the market. Different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) for Bavistin and Mancozeb were used for investigation of antifungal activity while the oils were used as received from the market.

Antifungal activity assay:

Antifungal activity assay was carried out by agar well diffusion method [14]. With sterile cork borer of size 5mm. 48 hours old cultures grown on potato dextrose agar (PDA) were used for preparing spore suspension for inoculation. 0.1 ml of test fungal spore suspension was spread on sterile agar plates. Appropriate wells were made on agar plate by using cork borer and 50 µl of test oil and fungicide of different concentrations were loaded. Plates were sealed with cling film and kept for pre-incubation for 30 min in refrigerator and then were incubated for 4 days at room temperature (28°C). The antifungal activity was evaluated by measuring zone of inhibition of fungal growth surrounding the well with test oil/chemical. The experiment was carried out in 5 replicates.

RESULTS AND DISCUSSION

Many reports have been documented for the antimicrobial activity of essential oils and plant extracts [15,16,17,18]. Plant oils have been reported to different degrees of antifungal activity against species of *Aspergillus*, the most common storage fungus [19,20].

The present study to evaluate antifungal activity of the eight different plant derived oils and two known antifungal synthetic chemicals available in market were tested against the eight fungal species *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. oryzae*, *A. fumigatus*, *Fusarium moniliforme*, *F. solani*, *Penicillium sp.* isolated from maize grains. The results revealed that Bavistin, Mancozeb and some of the oils tested exhibited different degrees of antifungal activity against different seed borne fungi of maize.

Table 1 - Antifungal activity of plant derived oils against seed borne fungi.

Name of oil	ZONE OF INHIBITION in mm							
	<i>A. oryzae</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. terreus</i>	<i>F. moniliforme</i>	<i>F. solani</i>	<i>Penicillium sp.</i>
Control	0	0		0	0	0	0	0
Eucalyptus	21.5	16	19	12	12	15	19	0
Clove	59	21	43.5	33	53.5	38.5	44	0
Cinnamon	38	39	22.5	49	59	41	67.5	44
Nutmeg	24	36	20	27	41.5	19	15.5	32
Neem	0	0	0	0	0	0	0	0
Nirgudi	0	0	0	0	0	0	0	0
Karanj	0	0	0	0	0	0	0	0
Sesame	0	0	0	0	0	0	0	0

* Values are mean of 5 replicates

The antifungal activities of eight plant derived oils obtained by the well diffusion method are shown in Table 1. The results indicated that only four oils exhibited different degrees of antifungal activities against seed borne fungi of maize.

Cinnamon oil and nutmeg oil showed significant inhibitory effect on fungal growth against all test fungi. Cinnamon oil was more effective antifungal agent than nutmeg oil against *F. solani*, followed by *A. terreus*, *A. fumigatus*, *Penicillium sp.*, *F. moniliforme*, *A. flavus*, *A. oryzae* and *A. niger*. With nutmeg oil zone of inhibition was maximum

against *A. terreus* subsequently followed by *A. flavus*, *Penicillium sp.*, *A. fumigatus*, *A. oryzae*, *A. niger*, *F. moniliforme* and *F. solani*.

Table 2 - Antifungal activity of fungicides against seed borne fungi

concentration in %	ZONE OF INHIBITION in mm															
	<i>A. oryzae</i>		<i>A. flavus</i>		<i>A. niger</i>		<i>A. fumigatus</i>		<i>A. terreus</i>		<i>F. moniliforme</i>		<i>F. solani</i>		<i>Penicillium sp.</i>	
	Ba	Ma	Ba	Ma	Ba	Ma	Ba	Ma	Ba	Ma	Ba	Ma	Ba	Ma	Ba	Ma
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10%	12	0	13	10	19	0.8	23	14	19	11	0.9	0.9	0	0	0	0
0.20%	20	0	14	10	22	0.9	25	16	22	13	10	10	0	14	0	0
0.30%	22	0	15	13	24	10	26	17	22	15	11	11	0	15	0	10
0.40%	23	11	16	14	24	10	26	20	23	16	13	13	0	16	0	12
0.50%	23	13	18	15	28	11	27	22	23	17	15	15	0	16	0	12

* Values are mean of 5 replicates

Ba – Bavistin

Ma – Mancozeb

Eucalyptus oil and clove oil showed activity against all fungi except *Penicillium sp.* Among these two oils results of clove oil were better than eucalyptus oil against all fungi. Eucalyptus oil exhibited maximum antifungal activity against *A. oryzae* followed by *A. niger* and *F. solani* then by *A. flavus*, *F. moniliforme*, *A. terreus* and *A. fumigatus*. Clove oil also revealed larger zone of inhibition against *A. oryzae* followed by *A. terreus*, *F. solani*, *A. niger*, *F. moniliforme*, *A. fumigatus* and *A. flavus*. Oils of neem, Sesame, Pongam and Negundo did not exhibit any antifungal activity against any of the test fungi.

Similar results with Cinnamon, Clove and Eucalyptus oils were obtained by Singh R., [21] and Singh R. and Rai B. [22] who reported high antifungal activity of oils of *Cinnamomum cyminum*, *C. martini*, *C. citrates*, *E. globulus*, *C. zeylanicum*, *Ocimum sanctum* etc. . Essential oil of Cinnamon, Peppermint, Basil, Origanum, Clove and thyme were proved to cause total inhibition of *A. flavus* on maize kernels [23]. Monte-Belmont and M. Carvajal [24], reported use of essential oils of *Cinnamomum zeylanicum* for protection of stored maize grains against *A. flavus*. The distilled oil of *C. zeylanicum* showed complete inhibition of *Aspergillus* [25,26]. Verma et al. [27], reported that essential oils of *Syzygium aromaticum*, *Citrus limon* and *Mentha piperita* exhibited highest antifungal activity on the growth of *niger*. Overall inhibition in mycelial growth was observed with all concentrations (0.1 to 0.5 %) of Mancozeb. Bavistin did not control growth of *F. solani* and *Penicillium sp.* but *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. oryzae*, *A. fumigatus*, *F. moniliforme* were controlled by Bavistin. As compared to Bavistin the activity was less with Mancozeb (Table 2).

Efficacy of the oils when compared with two fungicides such as Mancozeb and Bavistin revealed that, Mancozeb inhibited mycelial growth of all the test fungi but inhibition of mycelial growth by clove, cinnamon and nutmeg oils was much more than Mancozeb. Effective inhibition of mycelial growth in *A. fumigatus*, *A. terreus* and *F. moniliforme* was significant by eucalyptus oil as compared to Mancozeb. Clove, cinnamon and nutmeg oil showed large zones of inhibition against test fungi like *A. oryzae*, *A. flavus*, *A. fumigatus*, *A. terreus* and *F. moniliforme* as compared to Bavistin. Bavistin could not control growth of *Penicillium sp.* but it was controlled by cinnamon and nutmeg oil.

CONCLUSION

In accordance with the earlier reports the results of the present work suggested that some essential oils can be applied as mold inhibitors to prevent growth of toxigenic fungi in stored food. Plant derived oils viz. Cinnamon, Clove, Eucalyptus and Nutmeg can be used as effective and promising fungal inhibitors to control fungal infestation during storage of grains as an ecofriendly management of seed borne fungi. Seeds treated with fungicide are unfit for human consumption and animal feed whereas seeds are treated with above mentioned oils are non-hazardous for human consumption and animal feed.

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