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Short Communication

Effect of Plant Extracts on Seed Mycoflora of Maize

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Maize (*Zea mays* L.), an important cereal crop in the North East region of India, is invariably attacked by microbes during storage resulting in heavy losses. Since its maturity time coincides with the monsoon, seed moisture becomes high attracting many storage micro-organisms. Storage problems have been the bottleneck in successful development of seed sector in this humid region. Use of chemical fungicides for the control of microbial spoilage of food grains are reported to be effective [1] but they are not widely adoptable owing to high cost, residual problems and toxicity to animals. Hence, the emphasis is on the use of botanical sources of pesticides as one of the future strategies. Information on the utilization of indigenous plant materials in minimizing the seed storage mycoflora of maize is meagre. Thus, an attempt was made to find the effectiveness of some indigenous plant extracts in reducing the incidence of mycoflora on maize seeds during storage.

A study was undertaken to control the seed mycoflora of maize seeds during storage with extracts of five indigenous plants namely *Artemisia parviflora, Cymbopogon* sp., *Goniothalamus sesquipedalis, Plectranthus ternifolius* and *Vitex negundo* at three different concentrations (0.1%, 0.2% and 0.4%). Fresh leaf extracts from these plants were prepared through hydro-distillation of leaves. Freshly harvested seeds of maize variety RCM-I-2 were sun-dried for five days on sunny days (at seed moisture content of 13.5%) and 300 g seeds were treated with different plant extracts separately. Seeds were mixed thoroughly with the plant extracts and sealed separately in sterilized self locking polythene bags (300μ gauge) and stored under ambient conditions for three months. Untreated seeds sealed in sterilized polythene bags served as control. Seed mycoflora were examined after three months of storage following standard moist blotter technique and agar plate methods [2]. In sterilized Petri-plates (15cm) lined with three blotting papers, 20 seeds were placed aseptically with uniform spacing with four replications and incubated at 27±1°C. Observations and identification of seed mycoflora were made after eight days of incubation. Pure culture isolations were made on Czapek-Dox-Agar medium and the fungal population was expressed in terms of per cent occurrence for each fungal species with the following formula.

$$\text{Per cent occurrence} = \frac{\text{No. of seeds on which growth of fungal species detected}}{\text{Total number of seeds examined}} \times 100$$

Number of fungal colonies found in each Petri-plate was counted and analyzed by

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adopting split-plot design with treatments as main factor and concentrations as sub-factor. Total number of fungal species associated with seeds under different treatments were also recorded.

Fifteen fungal species *viz., Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, A .glaucus, A. niger, A. teneus, A. wenti, Chaetomium* sp., *Curvularia lunata, Mucor* sp., *Penicillium implicatum, P. rubrum, Periconia* sp. and two forms of sterile mycelium were found associated with maize seeds stored for three months under ambient conditions (Table 1). The fungal occurrence (per cent) was found less when the seeds were treated with various plant extracts than the untreated seeds. Varied effects were noted among the different extracts and concentrations. With increase in concentration, there was reduction in the percentage of fungal occurrence. Among the extracts, *P. ternifolius* was found to be most effective in controlling the storage fungi at all concentrations tried followed by *V. negundo* and *Cymbopogon* sp. Occurrences on *P. ternifolius* extract treated seeds at 0.1, 0.2 and 0.4 per cent were 21.00, 19.50 and 14.50 per cent respectively as compared to 44.50 per cent in control. In seeds treated with *P. ternifolius*, majority of fungal species were checked. Variations in efficacies of different plant extracts could be due to different antifungal constituents.

Extracts of *A. parviflora* and *Cymbopogon* sp. though less effective, were found to be better than the untreated control. All the plant extracts showed lesser fungal occurrence with increase in concentration. At 0.4 per cent concentration, the fungal occurrence in *V. 'negundo, Cymbopogon* sp., *A. parviflora* and *G. sesquipedalis* treated seeds were 19.50 per cent, 22.50 per cent, 29.00 per cent and 35.00 per cent respectively as compared to 44.50 per cent in control. It is in conformity with the findings of El-Shami *et al.* [3] wherein, the higher doses of extract of *Allium cepa* had higher inhibiting effect on the mycelial growth and spore germination of *Fusarium oxysponum f.* sp. *niveum;* and those of ethanol extracts of ten plant species [4] against five pathogenic fungi tested under laboratory conditions.

The higher efficacies of plant extracts on some mycoflora than on others might be due to difference in locations of the fungi in different layers of the seeds as found in rice [5] or due to differential antifungal properties of the plant extracts against each fungal species.

Occurrence of fungal colonies during seed storage was lower on plant extract treated seeds than on the untreated seeds (Table 2). Lowest number of fungal colonies was recorded from seeds treated with *P. ternifolius* (4.02) followed by *V. negundo* (4.50), *Cymbopogon* sp. (5.00), *A. parviflora* (6.33) and *G. sesquipedalis* (7.33) as compared to control (9.50). Occurrence of fungal colonies also decreased with increasing concentration which conforms to earlier findings in mustard seed [6], where the efficacy was highest when used in crude form.

Number of fungal species recorded from *P. ternifolius* treated seeds was also lowest (7 species) in comparison with other treatments and control (Table 3). Eleven fungal species isolated from seeds treated with both *Cymbopogon* sp. and *V. negundo* whereas, *A. parviflora* (12 species) and *G. sesquipedalis* (13 species) were almost similar with control (13 species). Similarly, Khan and Kumar [7] also found reduction in seed mycoflora of wheat treated with leaf extracts of *Azadirachta indica.* Among the plant extracts tested, *A. parviflora* and *G. sesquipedalis* leave extracts showed less or no effect in inhibiting some of the fungal species. It suggests that the active ingredients and antifungal properties of different plant extracts might not have the same effect against different fungal species. Extracts of *Tagetes erecta* was also reported to be less effective against *Alternaria brassicicola* [4].

Seed treatment with extracts of locally available plants like *P. ternifolius* and *V. negundo* in maize seeds before storage, could reduce the fungi associated with the seeds during storage under ambient conditions for three months. Such treatments could be more effective when used in combination with dry storage conditions like moisture-vapour-proof packaging, desiccated and dehumidified storages. Such studies are of importance with the present day organic agriculture systems. Chemical analysis and identification of active ingredients in the effective

Table 1. Effect of plant extracts on occurrence of different fungal species on maize seeds during storage

Fungi associated/plant extract	Per cent fungal occurrence															
	Artemisia parviflora			*Cymbopogon* sp.			*Goniothalamus sesquipedalis*			*Plectranthus ternifolius*			*Vitex negundo*			
	0.1%	0.2%	0.4%	0.1%	0.2%	0.4%	0.1%	0.2%	0.4%	0.1%	0.2%	0.4%	0.1%	0.2%	0.4%	Control
Aspergillus candidus	2.50	2.00	2.00	2.00	1.50	1.50	4.00	4.50	4.50	0.50	-	-	3.20	4.00	3.00	4.00
A. clavatus	1.00	-	0.50	-	-	0.50	2.30	2.50	2.00	1.50	1.50	2.50	0.50	0.50	0.50	4.00
A. flavus	3.50	2.30	2.00	-	-	-	3.00	3.00	2.50	-	-	-	1.30	-	0.50	3.00
A. fumigatus	-	-	1.00	3.00	2.00	3.00	-	-	-	0.50	0.50	-	-	-	0.50	4.50
A. glaucus	-	-	-	1.00	0.50	-	-	-	-	-	-	-	-	-	-	-
A. niger	2.00	3.20	0.50	1.00	1.50	1.00	3.20	3.00	3.00	3.50	3.00	0.50	3.00	0.50	-	2.00
A. tereus	-	-	0.50	-	-	0.50	-	-	0.50	-	0.50	-	-	-	-	-
A. wenti	1.50	1.00	1.00	-	-	-	0.50	0.50	-	-	-	-	-	0.50	0.50	0.50
Chaetomium sp.	1.00	-	0.50	2.00	-	0.50	2.00	1.00	1.00	-	-	0.50	1.00	1.00	-	1.50
Curvularia lunata	3.00	3.00	4.50	3.50	3.00	-	3.00	3.00	3.50	-	-	0.50	1.50	1.50	-	2.00
Dark sterile mycelium	4.50	6.00	5.50	3.00	3.00	3.00	5.00	5.50	5.50	5.50	6.00	4.00	3.50	-	2.00	-
Mucor sp.	3.00	2.00	-	2.00	1.00	1.50	2.00	-	-	1.50	-	-	0.50	-	1.00	-
Penicillium implicatum	1.00	1.00	-	-	-	-	-	0.50	2.00	-	-	-	1.50	3.00	3.00	6.00
P. rubrum	0.50	-	-	-	2.50	2.00	1.00	2.00	3.50	-	-	-	2.50	2.50	3.00	4.50
Perinoconia sp.	-	-	-	-	-	-	2.00	-	0.50	-	-	-	1.50	-	-	-
White sterile mycelium	5.00	5.00	6.00	3.00	4.50	4.50	6.50	6.30	6.50	5.50	5.50	3.50	3.50	5.50	5.50	6.50
Total	34.00	31.00	29.00	32.00	24.00	22.50	38.50	35.80	36.00	21.00	19.50	14.50	25.50	20.30	19.50	44.50

plants would also be useful for isolation and further studies.

Table 2. Effect of plant extracts on the occurrence of total fungal colonies on maize seed during storage

Plant extract/dose	Occurrence of fungal colonies per 25 seeds			
	0.1%	0.2%	0.4%	Mean
Artemisia parviflora	7.0	6.0	6.0	6.33
Cymbopogon sp.	6.0	5.0	4.0	5.00
Goniothalamus sesquipedalis	7.5	7.5	7.0	7.33
Plectranthus ternifolius	4.5	4.0	3.6	4.02
Vitex negundo	5.5	4.0	4.0	4.50
Mean	6.1	5.3	4.9	5.43
Control	9.5			
C.D. at 5%				
Plant extract			0.36	
Dose			0.28	
Plant extract x dose			0.56	

Table 3. Effect of plant extracts on the number of fungal species associated with maize seed

Plant extract/dose	Number of fungal species			
	0.1%	0.2%	0.4%	Mean
Artemisia parviflora	13	10	12	12
Cymbopogon sp.	10	10	12	11
Goniothalamus sesquipedalis	13	12	14	13
Plectranthus ternifolius	7	6	8	7
Vitex negundo	13	11	10	11
Mean	11.2	10	11	11
Control	13			

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