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RESEARCH ARTICLE

EFFECT OF FUMIGATION OF BOTANICALS ON SEED MYCOFLORA AND SEEDLING GROWTH

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ABSTRACT

Fungal deterioration of stored grains is a chronic problem in the Indian storage system. When fungi associate with grains, they often reduce both the quality and yield of grains (Violeta *et al*., 2003). Mycotoxins produced by these fungi are hazardous for human beings and animals. In the present study, maize grains are fumigated by dry powders of leaves of *Azadirecta indica* (neem), seeds of *Trachyspermum ammi* (ajwain/ ajowan) and *Anethum graveolens* (Synonyms, *Peucedanum graveolens*) (Shepa/dill seeds), peels of *Citrus sinensis* (lemon), *C. medica* (sweet lime) *and C. reticulate* (orange) and stored in cotton bags for 30 days. The results indicated that fumigation of botanicals controlled growth of seed borne fungi viz *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus Fusarium moniliforme*, *Fusarium solani* and *Penicillium sp*. All the natural product fumigation treatments applied were also significantly effective in enhancing seed germination, seedling length and chlorophyll content. The findings emphasize on toxicity of fumigation of botanicals against fungi attacking stored grain and strengthen the possibility of using them as an alternative to chemicals for preserving stored grains.

INTRODUCTION

Maize (*Zea mays* L.) is one of the main cereals as a source of food, forage and processed products for industry. World production of maize is around 790 million tons. As a staple food it provides more than one-third of the calories and proteins in some countries (Chulze, 2010). In developing countries maize is a major source of income to farmers among whom many are resource-poor. Maize cultivation in the world is limited by diseases which cause grain loss of about 11% of the total production. The control of maize diseases is very important as a complementary technology to boost maize production. Various approaches have been used over many decades to control maize diseases (Tagne, 2008). 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 (Chulze, 2010). Effective and efficient control of seed borne fungi can be achieved by the use of synthetic chemical fungicides but cannot be applied to grains for reasons of pesticide toxicity (Harris *et al*., 2001). Chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon., 2005). Wide use of pest control methods, which are eco-friendly and effective, can be used as fungicides (Mohana *et al.,* 2011). Exploitation of botanicals in crop protection and prevention of biodeterioration caused by fungi is very essential. In view of these, the author screened some botanicals against seed-borne fungi. Chemical fungicides are costly, environmentally hazardous with non-target effects hence biocontrol strategies are gaining importance in controlling the fungi. Fumigation will be one of the effective and valuable control strategies for the preservation of grains and other stored commodities for many years to come. It is the cheapest method available for disinfesting grain (Winks, 1990).

MATERIALS AND METHODS

Sources of botanicals

Different plant parts were collected from local areas. Fresh and healthy leaves of *Azadirecta indica* (neem), seeds of *Trachyspermum ammi* (ajwain/ ajowan) and Ane*thum graveolens* (Synonym, *Peucedanum graveolens*) (Shepa/dill seeds) were collected from local market. Peels of *Citrus sinensis* (lemon), *C. medica* (sweet lime) *and C. reticulate* (orange) were collected from juice shops. All botanicals were air dried at room temperature for 3-4 days and finely powdered using a blender.

Fumigation of seeds

Grains of maize varieties were collected from market, farmers and store houses. 1kg seeds of each variety were divided into two lots and each seed lot was placed in cotton bag. One of the lots of collected seeds of maize varieties was fumigated with different botanicals. A wooden box was bifurcated into upper and lower compartments by means of 2 mm size porous metallic sieve (Krishnamurthy and Shashikala, 2006). Surface of lower compartment was covered by aluminum sheet and with hot plate. 500 g maize seeds of different varieties were placed on upper compartment and dry powders of botanicals (50 g) were taken separately and burnt in lower shelf in such a way that only fumes came out of the botanicals and maize lot was exposed to the fumes through porous metallic sieve. This process was carried out until all botanicals were burnt out completely. Then exposed maize grains were stored in cotton bags at room temperature for 30 days.

Detection of seed Mycoflora

For determination of seed mycoflora under treated and non-treated (control) condition, agar plate method and blotter technique was used as described by International Seed Testing Association, ISTA (1976). For isolation of seed borne mycoflora, pre-sterilized corning glass Petri-plates of 9 cm diameter were poured with 25 ml of autoclaved media. On cooling of the medium, 05 seeds per Petri-plate were spaced at equal distance aseptically. Fifty seeds were tested for each treatment. The plates were incubated at room temperature (25 ± 2^0c) under natural day night conditions. Seeds were examined for fungal growth after seven days of incubation and fungi were identified under microscope.

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Germination studies

Treated and non-treated seeds (5seeds/plate) were placed equidistantly on the Petriplates with wet cotton and germination paper (Dhavale, 1988 and Janardhan *et al*. 2011). The plates were kept wet by frequent spraying of water. The plates were incubated at room temperature (25 ± 2^0c) under natural day night conditions. After 5 days the seed germination was recorded. All experiments were carried out in triplicates.

Pot culture studies

Treated and non-treated seeds (5seeds/pot) were placed equidistantly in plastic pots containing sterile soil. After 14 days seedling growth in terms of root and shoot length was measured and chlorophyll content was estimated by Arnon's method (1949).

Statistical analysis

All the experiments were performed in four replicates and the results were averaged. Analysis of Variance (ANOVA) technique has been applied to observe the statistical significance of botanicals on germination %, total length and total chlorophyll content of seedlings as well as significance of varieties with respect to germination %, total length and total chlorophyll content of seedling. P value, 0.05 is considered to be statically significant.

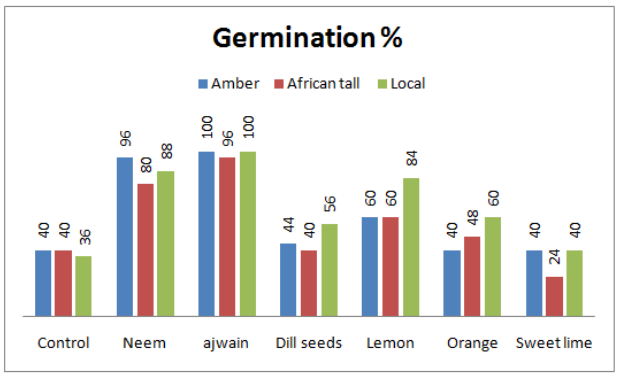
RESULTS AND DISCUSSION

Detection of mycoflora

Under control condition (non-treated seeds) on different agar media and blotter method eight species of fungi were found to be associated with seeds of three different maize varieties like Amber, Local, and African tall with different frequency. These species were identified as *Aspergillus flavus, Aspergillus oryzae, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus Fusarium moniliforme, Fusarium solani* and *Penicillium sp.* In treated seeds frequency of fungal species was found to be negligible.

Germination studies

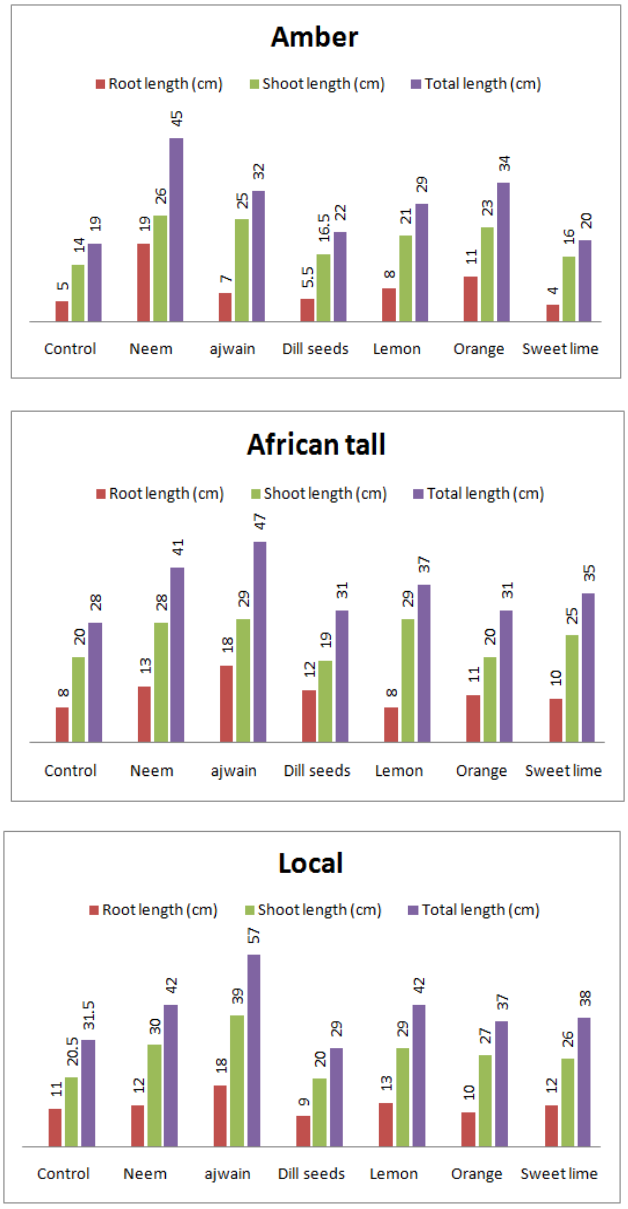
Amber variety showed enhancement in germination percentage when seeds were fumigated with leaves of *Azadirecta indica* (neem), peels of *Citrus sinensis* (lemon),seeds of *Trachyspermum ammi* (ajwain/ ajowan) and *Anethum graveolens* (Shepa/dill seeds) while germination % was same as control when seeds were fumigated with peels of *C. medica* (sweet lime) *and C. reticulate* (orange). Germination % was significantly increased in African tall variety when seeds were treated (fumigated) with all botanicals except peels of *C. medica* (sweet lime). Fumigation treatments of all botanicals were very effective to enhance germination% in Local variety. Fumigation treatment of ajwain seeds and neem leaves recorded highest germination % in all varieties of maize (Graph 1).



Graph 1 - Effect of fumigation of botanicals on germination %.

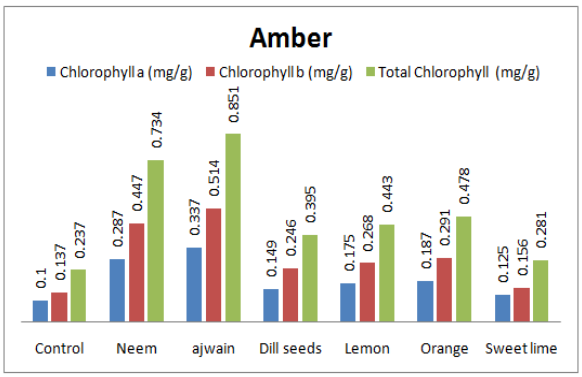
Pot culture studies

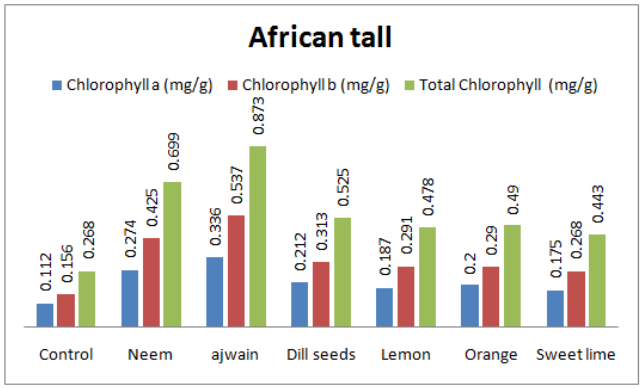
Estimation of chlorophyll content and root, shoot and total length was recorded in 14 days old seedling. Root length of seedlings varied according to botanicals but in all varieties shoot length and total length is augmented in treated seedling (Graph 2).

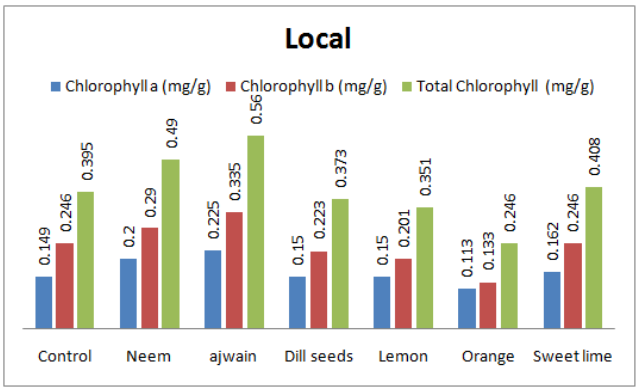


Graph 2 - Effect of fumigation of botanicals on seedling length (in cm).

Significantly enhanced in Chlorophyll a, chlorophyll b and total Chlorophyll content was recorded in all varieties of maize when seeds were fumigated with all mentioned botanicals (Graph 3). Exceptional increase in chlorophyll content was noted in seedlings with seeds fumigated with ajwain seeds and neem leaves.







Graph 3 - Effect of fumigation of botanicals on chlorophyll content (in mg/g) of seedling.

P value < 0.05 is considered to be statistically significant. The results show that there exists statistical significance between Germination and botanical fumigation treatment, Germination and maize varieties, Total length of seedling and botanical fumigation treatment, Total length of seedling and maize varieties and Total chlorophyll content and botanical fumigation treatment. Total chlorophyll content and maize varieties are statistically non-significant (Table 1).

Table 1. Statistical Analysis of data

Sr no.	Variables	F Value	P Value	Analysis/Result
1	Germination and botanical fumigation treatment	35.53	0.00000058 < α = 0.05	Significant
2	Germination and maize varieties	3.966	0.047616 < α = 0.05	Significant
3	Total length of seedling and botanical fumigation treatment	6.12	0.0038 < α = 0.05	Significant
4	Total length of seedling and maize varieties	8.06	0.0060 < α = 0.05	Significant
5	Total chlorophyll content and botanical fumigation treatment	8.085	0.001174 < α = 0.05	Significant
6	Total chlorophyll content and maize varieties	3.36	0.069 > α = 0.05	Non-Significant

Seed-borne fungi render serious problems in agriculture, environment pollution, human and animal health, as well as in economic aspects. Botanicals are easily available for farmers. The fumigation treatments of botanicals applied were significantly effective in the management of seed-borne fungi during storage. Synthetic antifungal chemicals have been used for preservation of stored grains. Considering health and economic problems, these natural plant products may efficaciously replace toxic chemicals and offer alternative method for management of seed borne fungi. These treatments are simple to apply and cost effective for management of seed borne fungi during storage. This study was aimed to document extent of adoption of management practices of seed borne fungi during storage. Fumigation with botanicals for control of seed borne fungi was practiced to justify the effectiveness of this method, so that the poor farmers of our country would practice this method in their house without facing any technological distresses for seed storage. The authors have tried to make this methodology easy to practice without any kind of special training to the poor farmers in the country. In relation to above work effect of fumigation of botanicals on seed mycoflora and seedling growth references are very scanty. There is a need to work more on this line which is very promising, easy to practice and cost effective.

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