Botanical treatment for grain protection and their effects on Seed Germination and Seedling Performance of stored maize

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Apart from being known for having insecticidal activity, several plants and their materials also show effects on seed viability and plant growth. The crude acetone extracts from the seeds of *Piper cubeba* L, coriander, *Coriandrum sativum* L. and the shade dried leaves of the aquatic weed, *Eichhornia crassipes* Mart., wood-apple, *Limonia acidissima L,* the Indian Tamarind, *Tamarindus indica* L., the coconut palm, *Cocos nucifera* L., Indian Badam, *Terminalia catappa* L., the Indian Cherry *Syzygium cumini* L. and Ivy Gourd, *Coccinia indica* Wight& Arn. Were evaluated for finding their influence on germination capabilities and seedling growth of treated *Zea mays* L. seeds. The effect of extract on germination, root and shoot growth and inhibition of seed borne fungi *Aspergillus flavus* have been observed in Petri dish bioassays at a concentrations of 0.5, 1.25 and 2.5 mg /g maize seed. In general the extracts had no adverse affects on seed germination and the fungal growth was also quantitatively similar to that of the controls. Present investigation reveals the importance and potential of plant extracts and their allelopathic effects on stored grain.

Key words: Plant products, Seed germination, Seedling growth, Antifungal.

INTRODUCTION

In India maize is stored for longer period for seed purpose and during this period it is vulnerable to pest and disease infestations. The application of naturally occurring plant products, which are ecologically friendly to agricultural practice, is a promising method of stored product pest control in recent years. Several botanical extracts has been proved to prevent or regulate the pest and disease attack (Usha Rani 2007). Essential oils of thyme and oregano are effective fumigants against fungi, which attack stored grains. This property strengthens the probability of their use as alternative chemicals in the storage of grains. Certain plant essential oils and their components showed inhibitory activity to *A. flavus* in Maize (Belmont, and Carvajal, 1998).

Particularly in the event of methyl bromide ban/ going to ban in several countries there is a growing need for the development and use of natural pesticides. One of the major disadvantages of the use of plant based insect control agents on stored food commodities is their possible adverse effect on seed germination. According to Jood *et al*. (1993) the sorghum grains treated with plant products were normal in colour, appearance and texture after 6 months, but their seed viability and seedling growth adversely affected by either insect infestation or plant products. Majority of plant extracts are safe and a careful utilization of these compounds on food commodities is beneficial in several ways. The plant based chemicals when used as either fumigants or contact toxicants often show high toxicity besides being economical and environmentally safe as well as biodegradable. However, studies are needed to explore the adverse effects if any associated with the use of the botanical pesticides, such as germination capacity, nutritional value and seedling growth of the treated grain are essential before recommending their use.

In the present study experiments were conducted to evaluate the impact of allelopathic potential of certain plant crude extracts on germination, growth and fungi associated with the germination of maize (*Zea mays* L.). The effects of nine plant extracts on maize kernel protection against *Aspergillus flavus* were studied. Tests were conducted to determine optimal levels of dosages for maize protection, residual effects and toxicity of the extracts to maize plants.

MATERIALS AND METHODS:

Plant extract

Laboratory experiments were conducted to evaluate the effects of various botanical insecticides on the seed germination and seedling performance of stored maize. The plant extracts used were, seed extract of tail pepper, *Piper cubeba* L and the mature leaves of the aquatic weed common water hyacinth, *Eichhornia crassipes* Mart., the dog wood apple, *Limonia acidissima* L, the Indian Tamarind, *Tamarindus indica* L., the coconut palm, *Cocos nucifera* L., Indian Badam, *Terminalia catappa* L., the Indian Cherry *Syzygium cumini* and the Kovai Fruit *Coccinia indica* Wight& Arn. and the seed oil of coriander, *Coriandrum sativum* were harvested from naturally growing population in the campus of the Indian Institute of Chemical Technology, Hyderabad, India. The plant materials were shade dried at room temperature 28±4°C for 72 h, grounded in an electrical mixer-grinder, and extracted with soxhlet using acetone as solvent. The resulting crude extract is concentrated in a rotary

Table1. Allelopathic effects of various plant extracts on shoot and root length of treated *Zea mays* (10 day old) at different concentrations.

Test Species	Mean (%) inhibition[a]					
	Shoot growth			Root growth		
	0.5[b]	1.25[b]	5.0[b]	0.5[b]	1.25[b]	5.0[b]
E. crassipes (root)	5.77	0.98 a	6.73	6.66	1.14	17.79
E. crassipes (leaf)	6.73	1.93 a	3.85	5.64	2.84	1.42
L. acidissima	6.03	1.21 a	3.61	4.63	1.56	10.76
S. cumini	10.49	2.87 ac	-2.87	35.74	39.30	8.88
T. indica	0.98	4.91 c	19.53	3.07	4.05 a	76.77
P. cubeba	31.78	14.18	3.33	21.94	15.17	9.07
C. nucifera	15.40	17.61	23.21	-13.07	-32.08	2.07
T. catappa	7.45	10.75	14.90	-32.32	-41.51	-4.61
C. indica	22.13	3.16 a	2.11	6.15	4.07 a	30.61
C. sativum	22.47	4.62 c	0.05	3.15	10.28	14.91

[a] Each datum represents the mean percentage inhibition = [(control-extracts)/control] X 100

Values followed by the same letter with in a column are not significantly different (P< 0.05, tukey test). (N=10/ replicate, total 10 replicates for each treatment).

[b] indicate the extracts treated at mg/g concentration.

- negative inhibition.

evaporator, re-dissolved in a known amount of acetone to obtain a 50% stock solution. The extracts were stored in the refrigerator for subsequent experiments. Further dilutions were made to prepare test solutions (w/v) just before their use in the experiments.

Seeds of maize (DM 5000) were provided by Directorate of Maize Research (Indian Council of Agricultural Research), hyderabad.

Seed germination and incidence of seed bore fungi bioassays

The viability of treated and control seeds were tested one day, ten days, 1 month and 6 months after application. For this assay, maize seeds were separately treated with different plant extracts, at the rate of 0.5, 1.25 and 5 mg per gm seeds. The control seeds were treated with the solvent. The seeds were air-dried for 2–3 hours. Then 25 seeds from each treatment and control group were placed separately in plastic containers and stored under laboratory conditions for 6 months. There were 4 replicates for each treatment. Simultaneously incidence of the seed borne fungi was tested for each treatment. The germination of treated seeds and incidence of the seed borne fungi were evaluated after one day, ten days, one month and six months for each treatment. Each group of seeds was placed on moist filter paper in Petri dishes. Plates were incubated in a growth chamber at 25°C under 10 h light periods daily. After 7 days, seed germination and incidence of seed born fungi were measured and compared to that of controls. The inhibition percentage calculated by using following formulae. Inhibition percentage (%) = [(control-extracts)/ control] X 100

Seedling growth:

The effect of prolonged botanical treatment on seedling growth of the maize grain was evaluated at one day, 10 days, one month and six months after treatment.

The treated maize grains were germinated on the moist filter paper for 24 hours as described earliar. 10 uniform seedlings were selected and transformed in to the petri dish (150 X 30 mm) lined with two sheaths of moist whattman No# 1 filter paper. The seedlings were grown for seven days in a growth chamber at 25 °C under 10 h light periods daily. After 7 days, shoot and root length of the treated seeds were measured and compared with that of controls. The inhibition was calculated by using the following formulae:

Inhibition percentage (%) = [(control-extracts)/control] X 100.

Experimental design and analysis

Repeated measurements on each variable were obtained from the same experimental units over time. All the data collected were first homogenised using appropriate logarithmic and square root transformations (Gomez and Gomez, 1984) before being subjected to analysis of variance (ANOVA), repeated measures analysis and means separated using Tukey's test.

Table 2. Allelopathic effects of various plant extracts on shoot and root length of treated *Zea mays* (one month old) at different concentrations.

Test Species	Mean (%) inhibition[a]					
	Shoot growth				Root growth	
	0.5[b]	1.25[b]	5.0[b]	0.5[b]	1.25[b]	5.0[b]
E. crassipes (root)	16.00	4.00	14.00	2.94	35.29 c	5.88 b
E. crassipes (leaf)	2.00	3.00 a	11.00	36.76	63.24	55.88
L. acidissima	11.97	11.11	39.32	-8.33	33.33 b	29.17 b
S. cumini	0.00	100.00	100.00	1.89 a	100.00	100.00
T. indica	7.69 a	17.09	18.80	2.00 a	18.00	6.00 a
P. cubeba	22.79 b	27.94	1.47 a	57.14	34.69 c	30.61 b
C. nucifera	4.48	30.60	2.24 b	5.88	9.80	37.25
T. catappa	23.00 b	5.00 b	3.00	25.00	33.33 b	10.42
C. indica	36.90	2.38 a	-4.76	-2.44	21.95 a	7.32 a
C. sativum	7.69 a	5.77 b	1.92 ab	5.00	22.50 a	0.00

[a] Each datum represents the mean percentage inhibition = [(control-extracts)/control] X 100

Values followed by the same letter with in a column are not significantly different (P< 0.05, tukey test). (N=10/ replicate, total 10 replicates for each treatment).

[b] indicate the extracts treated at mg/g concentration.

- negative inhibition.

RESULTS

Seed germination and Incidence of seed borne fungi

Almost all the plant extracts utilized in the experiments were effective in promoting the seed germination and none of the extracts hindered the germination capacity of the treated maize seeds. There was significant difference between the percentage seed germinated in treated and controls. In one day stored seeds, very few botanicals like *E. crassipes, L. acidissima, S. cumini, C. sativum* and *C. nucifera* showed the inhibition at higher concentrations. The negetive effects of the treated botanicals on seed germination is highly insignificant in this investigation. Irrespective of concentration, all the treated botanicals either promoted or did not effect the seed germination for 10 days, 1 month and 6 months after treatment in stored maize.

In parallel to seed germination, the incidence of seed borne fungi was also studied. One day old seeds, treated with *E. crassipes* root and leaf extracts showed significent suppression of the incidence of seed borne fungi at all concentrations. *S. cumini* and *C. sativum* caused a total inhibition of fungal development on maize kernels. A residual effect of these two extracts were detected up to 6 months . No phytotoxic effect on germination and growth was found with *C. sativum* and *S. cumini* after one month of treatement. *T. indica* and *P. cubeba* at optimal protective dosage of 0.5 mg/g (v/v) was fungistatic on *A. flavus* with a residual effect that lasted for 6 months.

L. acidissima showed inhibition of incidence of seed borne fungi at lower doses only without effecting the seed germination. The effects produced due to the treatment were similar and comparable to that of the controls and suggest that the extracts did not have any adverse action against the average seed germination.

Seedling growth- Shoot growth

In 10-day-old treated seeds, crude extracts of botanicals caused a mild inhibition of shoot growth. The degree of inhibition was a function of extract concentration. The percentage of shoot growth inhibition ranges from 0.96 to 30% in maize seedlings. *P. cubeba* showed significant inhibition even at lower concentrations and the least inhibition was found in *E. crassipes* root treatment at 1.25 mg/g (Table 1). The lower extract concentrations of *S. cumini, C. indica* and *C. sativum* (0.5 mg / g) caused inhibition of the shoot growth, while at higher concentrations their inhibition was insignificant (20 %) as shown in the table 1. The *T. catappa* and *C. nucifera* extracts exhibited dose dependent shoot growth inhibition and their inhibition increased with the increase of concentration.

In one month treated seeds, the inhibitory potential of the extracts, however, was found to vary with the specific species (Table 2). There was no effect on shoot growth at lower concentration of *S. cumini* extract; whereas, at higher concentrations (1.25 and 5 mg / g) extract completely inhibited the growth (Table 2).

The lengths of the maize shoot were inhibited significantly by all the applied concentrations of *T. indica* extracts and were in a concentration – dependent manner. *E. crassipes* root, *T. catappa* and *C. indica* showed greatest inhibition at lower concentrations and their inhibition effect decreased with increase in concentration (Table 2). *C. nucifera* and *P. cubeba* showed high shoot growth

Table 3. Allelopathic effects of various plant extracts on shoot and root length of treated *Zea mays* (six months old) at different concentrations.

Test Species	Mean (%) inhibition[a]					
	Shoot growth				Root growth	
	0.5[b]	1.25[b]	5.0[b]	0.5[b]	1.25[b]	5.0[b]
E. crassipes (root)	16.00	4.00	14.00	2.94	35.29 c	5.88 b
	0.5[b]	1.25[b]	5.0[b]	0.5[b]	1.25[b]	5.0[b]
E. crassipes (root)	3.73	1.50 ab	0.68	6.48 b	2.35 a	5.77 a
E. crassipes (leaf)	-61.10	-22.34	-32.27	6.01 b	6.90	-33.61
L. acidissima	1.85 cd	-3.09 bc	18.00 c	-33.25	-28.48	-2.51
S. cumini	0.37 a	21.22	38.55	-2.53 a	0.89	17.56 c
T. indica	0.79 ab	3.79 c	4.37 a	-1.62 a	-21.61	-39.28
P. cubeba	1.48 bc	1.39 a	4.75 a	0.67	4.05	14.63 b
C. nucifera	1.95 cd	11.00 d	11.90 b	4.14	13.95 b	16.03 bc
T. catappa	9.76	11.77 a	17.76 c	2.09	14.39 b	11.63
C. indica	2.33 d	0.94 a	16.11	-15.41	8.75	6.59 a
C. sativum	0.46 a	10.47 d	12.19	6.67 b	2.31 a	6.88 a

[a] Each datum represents the mean percentage inhibition = [(control-extracts)/control] X 100
Values followed by the same letter with in a column are not significantly different ($P< 0.05$, tukey test). (N=10/ replicate, total 10 replicates for each treatment).
[b] indicate the extracts treated at mg/g concentration.
- negative inhibition.

inhibition at 1.25 mg / g while least inhibition observed in 0.5 and 5 mg / g concentrations (Table 2). *L. acidissima* extract was comparatively less toxic, with only the highest concentration of 5 mg / g significantly suppressing the shoot growth (Table 2)

Fig.1: Effect of botanical extracts on maize seedling growth of 10 days old treated *Zea mays*: A, Control maize seedling; B, Root and shoot inhibition due to *C. indica*; C, Root growth promoted due to *C. nucifera*; D, Seedling growth retardation due to *P. cubeba*.,

The botanicals had negligible effect on the shoot growth of 6 months old treated seeds and the inhibition ranged from 0.68 to 38.55 %. The greatest inhibition was found with the extract of *S. cumini* (Fig 2 d) at 5 mg / g and the least was caused by *E. crassipes* root at 1.25 mg/g. The leaf extracts of *E. crassipes* promoted the shoot growth at all the concentrations tested (Fig 2 b), while all other plant extracts inhibited it in a concentration dependent manner.

Root growth

When *C. nucifera* and *T. catappa* treated seeds after 10 days when allowed to germinate they promoted the root growth ranging from 13 to 41 % at the lower concentrations tested (0.5 and 1.25 mg / g) (Fig 1 c). The length of the maize root was inhibited significantly by all the applied concentrations of *E. crassipes* root, *L. acidissima, C. indica,* (Fig 1 b) *C. sativum* and *T. indica* extracts and these extracts increased the root growth inhibition in a concentration –dependent manner. In contrast, *S. cumini* and *P. cubeba* (Fig 1 d) extracts decreased the root growth inhibition in a concentration–dependent manner. Greatest root growth inhibition was observed in *T. indica* (76.77%) followed by *C. indica* (30.61%) and the least inhibition was found in *E. crassipes* root (1.14%) as shown in the table 1.

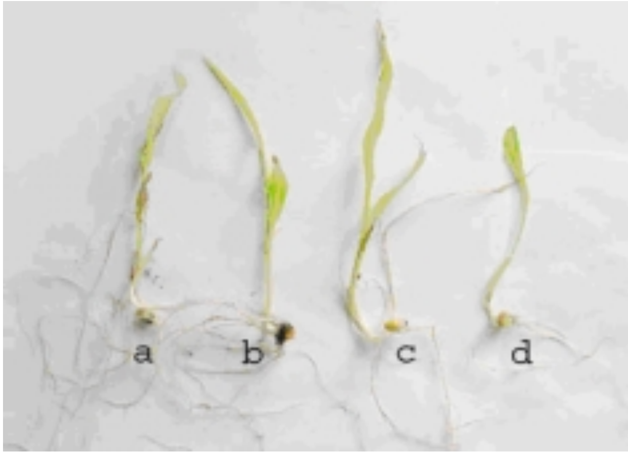

Fig. 2: Effect of botanical extracts on maize seedling growth of 1 month old treated *Zea mays*: a, control maize seedling; b, root growth promoted due to *L. acidissima*; c, root and shoot promoted due to *C. indica* leaf; d, growth retardation due to *E. crassipes leaf* extract.

In one month treated grain, botanical extracts showed significantly high toxicity and root growth inhibition ranging from 2% to 100% (Table 2). There was no effect on root growth at lower concentration of *S. cumini* extract, whereas, at higher concentrations (1.25 and 5 mg / g) the extract had completely inhibited the root growth. Similar results were obtained with *E. crassipes* leaf extract, which showed greater inhibition at higher concentrations (Table2). However leaf extract of *E. crassipes*, *P. cubeba* and *T. catappa* caused inhibitions even at lower concentrations (Table 2). A distinct response was observed in botanical extracts of *E. crassipes* leaf (Fig 2 d), *T. indica*. *L. acidissima* (Fig 2 b), *T. catappa, C. indica* (Fig 2 c) and *C. sativum* plants. At 1.25-mg/ g, these extracts inhibited the root growth, while at 0.5 and 5 mg/ g, the inhibition was insignificant.

In six months treated seeds, the effect of botanicals on the root growth was negligible. A distinct response was observed with *E. crassipes* leaf extract treatment. At higher concentration, the extract had promoted the shoot growth, while at lower concentrations, the extract inhibited the root growth (Table 3). In contrast, *S. cumini* and *C. indica* promoted the shoot growth at lower concentration and inhibited at the higher concentrations tested (Table 3). The extracts of *P. cubeba, C. nucifera* and *T. catappa* increased the root growth inhibition in a concentration-dependent manner. *L. acidissima* (Fig 3 c) and *C. indica* promoted significant root growth at all concentrations.

Fig. 3: Effect of botanical extracts on maize seedling growth of 6 months old treated *Zea mays*: a, Control maize seedling; Root and shoot growth promoted due to treated with b, *E. crassipes* leaf; c, *L. acidissima;* d, seedling inhibition due the treatment of *S. cumini* leaf extract

DISCUSSION

From the results of these trials, it was found that the use of treated botanical extracts as natural pesticides in maize storage have negligible adverse effects on seed germination. The botanical treatments caused only marginal changes in seed viability, which should have virtually no impact on the local market value of treated grain.

Results indicate that the seed viability of treated seeds was influenced by the type of plant extract treatment and storage duration. *E. crassipes, L. acidissima, S. cumini* and *C. nucifera* inhibited the germination at higher concentrations in the grain after 24 hours post treatment. This finding is supported by Sundarraj *et al*. 1996, who found that the degree of inhibition increased at higher concentrations.

The storage of maize seeds up to a prolonged period such as 6 months after treating with various botanicals does not have any adverse effect on the seed viability, which is an important aspect of use of botanicals for storage pests. This concurs with, Pandey *et al*. (1986) and Kasa and Tadese (1995) who reported that the use of crude powders of 17 botanical plant species on sorghum had no effect on seed germination.

The experimental results on seed borne fungus indicate that certain extracts are promissing in inhibiting the seed borne fungal growth and the inhibition depends on both concentration and duration of the storage.

In one day old treated seeds, *E. crassipes* root and leaf extracts showed significant suppression of the incidence of seed borne fungi at all concentrations. *S. cumini* and *C. sativum* caused a total inhibition of fungal development on maize kernels. A residual effect of these two extracts were detected up to 6 months. Nidiry (1999) also obtained a similar result with tomato seed extract, which reduced the mycelial growth of *Colletotrichum gloeosporioides*. The degree of inhibition increased with the extract concentration. No adverse phytotoxic effect on treated seed germination and seedling growth was detected with *C. sativum* and *S. cumini* even after one month of treatement. At optimal protective dosage of 0.5 mg/g (v/v), *T. indica* and *P. cubeba* were fungistatic on *A. flavus* with a residual effect that lasted for 6 months and *L. acidissima* showed inhibition of seed born fungal growth at lower doses only without effecting the seed germination and it appears that these plant materials contain chemicals having fungicidal properties.

The crude extracts were more effective in reducing the fungal growth. This is an indication that dilution of the extracts reduced toxic effects of the leaf extracts on the seed-borne fungi. The result agrees with the findings of Zaman *et al* (1997), who also found that the efficacy of *S. cumini, T. indica* and *P. cubeba* extracts on seed borne fungi of mustard, declined with increase in dilution and are effective up to 6 months. It has also been revealed in this investigation that the botanical treatments, storage duration and their interaction effects influenced the seedling growth.

It appears that these botanical extracts contain chemicals that are effective only on root growth enhancement but failed to promote the shoot growth. This kind of observations were also made by Leather and Einhellig (1985).

In 1-month-old treated seeds, *S. cumini* showed total inhibition of radicle and hypocotyl growth of seedling, which was negatively correlated to the seed germination. The results of this study add support to previous studies by Chung and Miller (1995), who found that mixture of certain plant extracts significantly reduced hypocotyl length at all concentrations. The plant extracts like *E. crassipes* leaf, *P. cubeba, T. catappa* and *C. indica* showed greater seedling growth inhibition in comparison to 10 days old seeds. No linear relationships were detected between the concentration of extract applied and the percentage of seedling growth inhibition. The effects lasted for only one month.

The magnitude of depression of inhibition decreased with storage duration of treated seeds. In 6 months treated seeds, except *S. cumini,* all other treated botanicals showed negligible seedling growth inhibition. In fact the leaf extracts of *E. crassipes, L. acidissima, C. indica* and *T. indica* treated and stored for 1 month inhibited the seedling growth moderately, while 6 month old treated grain enhanced to a small extent.

From the results of these trials, it can be concluded that the use of certain botanical extracts as natural pesticides in maize storage significantly reduce the seed borne fungal growth with no adverse effects on seed germination and seedling growth of treated maize seeds up to 6 months. Further tests are under progress to investigate the effects of botanical treatments up to the corn production. Studies on the levels of residues remaining on treated food and their potential adverse effects when consumed should be evaluated before the use of these treatments are institutionally promoted as part of a sustainable insect pest management system for farm level storage.

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