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Efficiency of Different Plant Foliar Extracts on Grain Protection and Seed Germination in Maize

P. Usha Rani and P. Devanand
Biology and Biotechnology Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 007, (A.P), India

Corresponding Author: Pathipati Usha Rani, Biology and Biotechnology Division, Indian Institute of Chemical Technology, Taranaka, Hyderabad 500 007 (A.P). India Tel: +91-40-27193148, +91-40-23413013 Fax: +91-40-27160387, 27160757

ABSTRACT

A study was undertaken to explore the pesticidal potential of certain Indian vegetable plant leaves for the post-harvest protection of maize seeds from stored pests and seed borne fungi. The crude extracts and purified fractions from leaves of papaya, *Carica papaya* L., ivy gourd, *Coccinia indica* Wight and Arn., bitter gourd, *Momordica charantia* L., curry leaf, *Murraya koenigii* L., chilli plant, *Capsicum annuum* L. and brinjal, *Solanum melongena* L. were evaluated for their capacity to control storage deterioration due to infestation by two major pests of maize, rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and red flour beetle, *Tribolium castaneum* H. (Coleoptera: Tenebrionidae). All crude extracts and the solvent eluted fractions were tested for their insecticidal activity in the vapor and contact form. The effects of treatment on maize viability and antifungal activity against the fungi associated with the germinating seedlings were also recorded. The crude extracts of *C. annuum, M. charantia, S. melongena, C. papaya* and *M. koenigii* caused significant mortality to both pests in fumigation method and the extracts of *C. annuum* and *M. charantia* exhibited a delayed toxicity (i.e., 72 h) in contact form. Ethyl acetate eluted fractions of *C. annuum, S. melongena* and *M. charantia* showed 100% mortality to *T. castaneum* and *S. oryzae* within 24 h of treatment in vapor form. The maize seeds treated with *C. papaya, C. indica, M. charantia* and *S. melongena* had germinated completely (100%). While the extracts *M. koenigii, M. charantia* and *S. melongena* were effective in reducing the incidence of seed born fungi, *Aspergillus flavus* during germination period.

Key words: Botanical pesticides, stored product pests, *Aspergillus flavus,* maize seed germination, antifungal activity

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop of India that is cultivated in all the three seasons; rainy, winter and summer. The harvested grains are stored by farmers for considerable periods to provide food reserve and seed material for planting in various types of storage structures made of mud, bamboo strips, *Cajanus Cajan* reeds, Palm leaves or paddy straws (Sinha and Sinha, 1992). These traditional storage methods inevitably provide suitable conditions for the growth and metabolism of insects, rodents and microorganisms responsible for quality loss in stored grains. Insects are a problem in stored grain throughout the world because they reduce the quality and quantity of grain (Cao and Hart, 2002) along with playing an important role in the

development and distribution of fungal inocula (Hell *et al.*, 2000). The major genera commonly encountered on maize in topical regions are *Fusarium*, *Aspergillus* and *Penicillium* (Khosravi *et al.*, 2007). Prevention or minimization of post-harvest losses is as important as efforts to increase yields. Therefore, control of pests attacking stored food grain and food material has become a major concern today. Seventy four percent of the insect pests attacking stored maize are coleopteron insects and the most damaging species of storage insects are in the genera of *Sitophilus* and *Tribolium* (Pinto *et al.*, 1997).

In recent years many workers have given greater attention to the control stored grain pests using vegetable, essential and mineral oils (Law-Ogbomo and Enobakhare, 2007). Botanical products are one of the most prominent alternatives for pest control in current and future requirements (National Research Council, 2000) and the effectiveness of many botanicals against stored grain insects has been demonstrated (Dunkel and Sears, 1998; Govindan and Nelson, 2009; Usha Rani and Rajasekharreddy, 2010). Khoshnoud *et al.* (2008) reported that the using extract of *Verbascum cheiranthifolium* Schard. for control of *Sitophilus oryzae* L. Plant products are well known to have a range of useful biological properties against insect pests and essentially play a tremendous role in protecting seed born pathogens and improving the quality and field emergence of plant seeds (Shah *et al.*, 1992). Given that plant derived materials are easily biodegradable and hence less likely to contaminate the environment, botanical pesticides are easily far superior to synthetic pesticides. Research on the evaluation of indigenous plants for stored product protection is thus very necessary to help farmers use locally available, environmentally friendly control tactics to limit post-harvest losses of their produce.

Keeping the tremendous value of plant derived pesticides in mind, we carried out a detailed study of the toxic effects of six indigenous plants against two major pests of stored maize., i.e., rice weevil, *Sitophilus oryzae* L. and red flour beetle, *Tribolium castaneum* H. All the plants selected for evaluation are locally grown and are known to contain several medicinal properties. However, the detailed study of their use in controlling the pests of stored maize has not been investigated before. Further, as there has been convincing evidence in recent times that many plants contain substances capable of inhibiting spore germination (Adamu *et al.*, 2006; Satish *et al.*, 2009; Feng and Zheng, 2007) as well as increased seed germination (Wakjira *et al.*, 2005; Akinkurolere *et al.*, 2006) root and shoot lengths of rice (Parimelazhagan and Francis, 1999), we also investigated the effects on seed germination capabilities, plant growth and the potency to control the seed pathogenic fungi.

MATERIALS AND METHODS

Plant material: Experiments were conducted in the laboratory, Biology and Biotechnology Division, Indian Institute of Chemical Technology, Hyderabad, India during May-September 2006 at 28±2°C and 65±5% r.h. and 14 h photoperiod to evaluate the bio-efficacy of six indigenous edible plants in protecting the stored maize from pest attack. Extracts from these plants and their partially purified fractions were screened for their toxicity followed by their effects on seed germination and seed viability. A total of 6 plants were selected for the present study namely Papaya, *Carica papaya* (L.) (Caricaceae), Ivy gourd, *Coccinia indica* (Wight and Arn) (Cucurbitaceae), Bitter gourd, *Momordica charantia* (L.) (Cucurbitaceae), Curry leaf, *Murraya koenigii* (L.) (Rutaceae), Chilli plant, *Capsicum annuum* (L.) (Solanaceae) and *Solanum melongena* (L.) (Solanaceae).

Insect material: The tested insect species, *S. oryzae* and *T. castaneum* were obtained from Directorate of Maize Research Station (DMRS), ANGRAU, Hyderabad. India. The pest insects were

reared on sterilized maize (*Zea mays* L.) and the cultures maintained at 28±2°C and 65±5% r.h. and L14:D10 in the laboratory of Indian Institute of Chemical Technology (IICT), Hyderabad, India. Initially, 50 pairs of 1-2 day-old adults were placed in a jar containing their respective food grains (1 kg). The jars remained sealed for a maximum period of 7 days to allow mating and oviposition. Parental stocks were then removed and the remaining contents (diet and eggs laid) of each jar used to infest the fresh seeds respective of each species. The subsequent progenies of the beetles were used for all experiments. Adult beetles, 1-2 weeks old, were used for the experiments.

Preparation and extraction of plant material: Plant material was ground to 2.0 mm particle size using electric mixer (Usha Lexus, India). The dried, ground leaves of plant materials were extracted sequentially at room temperature for about 18-20 h with acetone (Fig. 1). The resulting extract was then subjected to solvent evaporation at 45°C using rotary evaporator (Heidolph Laborota 4000) and stored at -20°C. The yield of each extraction is shown in Table 1, *C. annuum* with minimum yielded of 7.1% and *S. melongena* produced maximum of 9.8% compare with other test extracts. The final product was weighed and re-dissolved in acetone to obtain required dilutions of the plant extract for the experimental use.

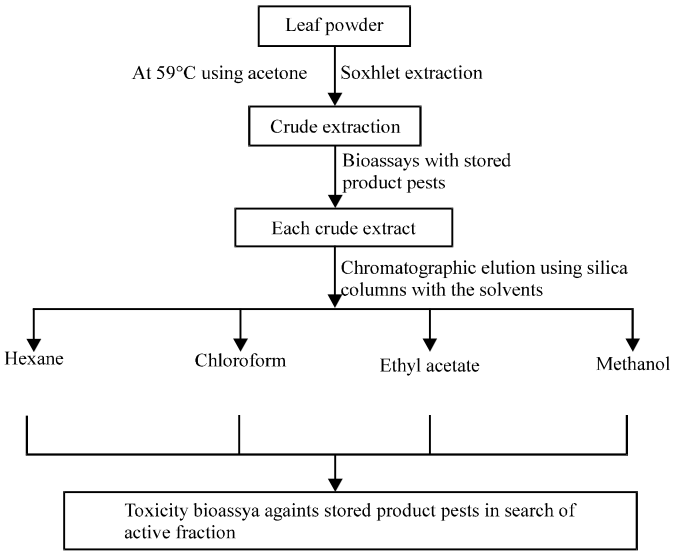

Fig. 1: Extraction process of plant crude extracts

Table 1: List of plant species tested

Family	Test material	Tissue collected	Yield (%)[a]
Caricaceae	*Carica papaya* L.	Leaf	8.1
Cucurbitaceae	*Coccinia indica* Wight and Arn	Leaf	7.8
Cucurbitaceae	*Momordica charantia* L.	Leaf	9.0
Rutaceae	*Murraya koenigii* L.	Leaf	9.2
Solanaceae	*Capsicum annuum* L.	Leaf	7.1
Solanaceae	*Solanum melongena* L.	Leaf	9.8

[a]Test plants, (dry weight of acetone extract / dry weight of test plant) ×100

Partial purification of crude leaf extracts: The active crude leaf extracts were separated into different fractions in column chromatography using silica gel (100-200 mesh) as adsorbent. The crude extract was eluted with solvents of increasing polarities i.e., hexane, chloroform, ethyl acetate and methanol and concentrated to dryness in a rotary evaporator, weighed and re-dissolved in acetone to required dilutions. All the crude extracts and semi-purified fractions were stored in freezer at-20°C until use (Fig. 1).

Vapor toxicity of the tested plant extracts: The vapor toxicity of the tested plant extracts was evaluated according to a method described by Usha Rani and Udaya Lakshmi (2007). In brief, small airtight glass containers (7 cm height x 6.8 cm diameter) (200 cc capacity) used as fumigation chambers were filled with 30 g of maize seeds, the diet of the tested insects. The samples were applied individually to a small ball of absorbent cotton weighing 300 mg and attached underneath the aluminum screw cap of each container. For each plant extract, six concentrations of crude plant extract (10, 20, 40, 60, 80 and 100 mg/200 cc) as well as five concentrations (2, 4, 6, 8 and 10 mg/100 cc) of each of the chromatographed fraction were used. For each plant extract treatment (crude extracts or chromatographed fractions) an additional absorbent cotton ball treated only with acetone served as control. Twenty unsexed adults of each species (1-2 weeks old) were released in to the chamber and the container sealed. All tests were carried out at 28±2°C temperature and 65±5 % r.h and L14:D10. Mortality was ensured by probing insect body with a slender paintbrush. Dead insects were counted every 24 h for a total of 72 h post treatment. There were five replicates per treatment while the tests were repeated 3 times on different date each time to avoid any day-to-day variation. The LC_{50} values were calculated by probit analysis for each species and treatment combination (Finney, 1971).

Contact toxicity of the tested plant extracts: The contact toxicity tests were carried out according to the bioassay procedure described by Usha Rani and Udaya Lakshmi (2007). Acetone diluted samples of the crude leaf extracts of each plant were applied at concentrations of 10, 20, 40, 60, 80 and 100 mg/ 30 g diet using a micro applicator. The treated diet was thoroughly shaken soon after treatment to ensure uniform coating of the seeds with the plant extract solutions. After allowing the solvent to evaporate for about 15 min (at 28±2°C and 65±5% r.h.) after treatment, 20 adult insects of each species were released separately into each jar containing the treated diet. Experimental conditions, mortality counts, number of treatments and statistical analysis were the same as those described in the former paragraph.

Seed germination after exposure to tested plant extracts: To study the impact of the botanical extracts on the maize seeds, germination tests were conducted in laboratory assays. Surface sterilized maize seeds were treated with all plant extracts at different concentrations (15, 30, 60, 100 and 150 µg/seed) or solvent alone (control), similar to that of contact application method. In another set, fresh maize seeds were exposed to test insect (*S. oryzae* and *T. castaneum*) infestation. All the experimental sets were allowed to remain in the laboratory conditions (28±2°C and 65±5% r.h. and L14:D10) up to 90 days, to study the seed viability of these treated grains by germination assay. For this, twenty seeds from each treatment set were collected randomly and placed in a petri dish (9 cm diameter) lined with a wet filter paper (Whatman No. 1). The Petri dishes were sealed with parafilm to prevent moisture loss and contamination and placed in the environmental chamber (Labtech, Korea). The temperature was maintained at 25±1°C and the

dishes exposed to constant light conditions by means of 2 standard fluorescent light tubes (at least 40 μmol m^{-2} sec for 24 h day^{-1}). Germination was checked daily up to a period of 12-16 days. Seed germination was defined as when the radicle or shoot had extended 1 mm beyond the seed coat or caryopsis, respectively (Steinmaus *et al.,* 2000). Recording ceased when there was no change in seed germination counts for more than 4 days. The seed germination in all the treated as well as control petri dishes was noted. The shoot length was also measured at regular intervals and compared between the concentrations, treatments and the controls. These were expressed as total percent germinated. The experiments were repeated on 5 different days (N = 20 seeds/replicate, 12 replicates N = 240 seeds).

***In vitro* antifungal activity to tested plant extracts:** Antifungal tests were performed with a few modifications from the method described by Adamu *et al.* (2006). The seed-borne fungi (*Aspergillus flavus*) used for this investigation were isolated from naturally infected south Indian, maize seeds. The *in vitro* tests were carried out to measure the effects of the leaf extracts from six plants on the seed borne fungi. Potato Dextrose Agar (PDA) medium was used in the study. The leaf extracts were mixed with sterile molten PDA to obtain final concentrations 5, 10 and 15 mg in 100 μL acetone. The solution in each Petri dish was gently swirled and allowed to solidify. The extract-amended medium in the petri dishes were inoculated at the center with 5 mm inoculum-disc of *A. flavus* and all the plates were carefully sealed with masking tape to avoid any aerial contamination and incubated at 25±2°C for 15 days. The medium with inoculum disc treated only with acetone (100 μL) served as control. Each experiment was replicated six times on three different days (18 replicates). Percentage inhibition of mycelial growth by the leaf extracts was calculated using the formula (Dixit *et al.,* 1978):

$$\text{MGI (\%)} = \frac{A_C - A_T}{A_C} \times 100$$

Where:
%MGI = % Inhibition of mycelial growth
A_C = Diameter (mm) of control
A_T = Diameter (mm) of test

Data analysis: Mortality counts were corrected for control mortality as suggested by Abbott (1925) where necessary. Statistical analysis of the toxicity data was performed using probit analysis to determine the LC_{50} (Finney, 1971). All experimental data were subjected to a one-way ANOVA to determine differences between three or more samples using Sigmastat v 3.5. Means were separated using the Tukey`s HSD test at the 5% level. All figures were plotted using Origin (ver 8.0) plot software.

RESULTS

Vapor toxicity of the tested plant extracts: Almost all the plant extracts (except *C. indica*) showed toxicity towards the adults of *S. oryzae* and *T. castaneum* and treatments resulted in 100% toxicity of the test insects in vapor form Table 2 and 3. It was observed that in all these treatments *T. castaneum* was more tolerant than *S. oryzae*. In this fumigation method, a strong difference in insect mortality was found as the concentration and exposure period increased. *C. annuum* and *M. charantia* caused significant toxicity ($p<0.05$) towards both insects at a concentration of

Table 2: Vapor toxicity of plant leaf extracts against *Sitophilus oryzae* L. by fumigation method

	Toxicity (%) ± SD (100 mg/200 cc)[b]				
Plant name	24 h	48 h	72 h	LC_{50} (95% FL)[a] (mg/20 g diet)	χ^2 (df)
C. papaya	75.6±5.8e	89.6±3.1d	100±0.0a	46.6 (38.1-59.1)	3.96 (5)
C. indica	53.4±5.0d	61.4±4.7c	71.2±1.6b	65.7 (57.3-77.3)	2.42 (5)
M. charantia	100±0.0a	-	-	37.4 (20.3-65.0)	8.97 (5)
M. koenigii	70.3±6.3f	80.4±3.3a	89.2±2.6d	51.2 (42.9-63.1)	1.16 (5)
C. annuum	100±0.0a	-	-	21.2 (17.1-35.7)	5.25 (5)
S. melongena	85.8±6.0c	100±0.0e	-	48.7 (38.0-84.7)	6.51 (5)

[a]FL-fiducial limits; after 72 h of treatment. [b]Each column followed by the same letter are not significantly different from another (One-Way ANOVA; Tukey HSD test at, <0.05)

Table 3: Vapor toxicity of plant leaf extracts against *Tribolium castaneum* H. by fumigation method

	Toxicity (%) ± SD (100 mg/200 cc)[b]				
Plant name	24 h	48 h	72 h	LC_{50} (95% FL)[a] (mg/20 g diet)	χ^2 (df)
C. papaya	80.6±5.5a	83.6±5.0a	99.2±1.2e	56.0 (48.1-67.2)	5.35 (4)
C. indica	46.4±4.1c	52.2±5.0c	62.0±2.3c	82.5 (63.7-100)	0.79 (4)
M. charantia	78.8±4.3a	94.0±3.5d	100±0.0e	42.4 (27.7-70.5)	9.75 (4)
M. koenigii	69.8±4.1d	85.0±3.5a	90.0±1.4a	72.1 (60.5-90.6)	6.52 (4)
C. annuum	100±0.0e	-	-	24.8 (12.1-42.0)	12.92 (4)
S. melongena	83.6±5.0a	97.2±2.7d	100±0.0e	47.1 (37.9-71.0)	1.44 (4)

[a]FL- fiducial limits; after 72 h of treatment. [b]Each column followed by the same letter are not significantly different from another (One-Way ANOVA; Tukey HSD test at, <0.05)

100 mg/200 cc within 24 h of treatment. But *S. melongena* and *C. papaya* were also effective in producing 100% toxicity but only after 72 h of treatment.

Vapor toxicity of the solvent eluted-chromatographic fractions from the crude plant extracts revealed their effectiveness against the pest insects (Table 4, 5). The chromatographic fractions of the ethyl acetate elute of *C. annuum, S. melongena* and *M. charantia* plant foliar extracts caused 100% mortality of *S. oryzae* (Table 4) and *T. castaneum* (Table 5) within 24 h of treatment at 10 mg/200 cc concentration. The methanol eluted fraction of *M. charantia*, *C. indica* and *S. melongena* crude leaf extracts significantly ($p<0.05$) affected survival of *S. oryzae* adults and *T. castaneum* showed less susceptibility to these extracts at a 10 mg/200 cc concentration after 72 h of treatment. Hexane and chloroform eluted fractions from *M. charantia* leaf crude exhibited 100% toxicity against *S. oryzae* and *T. castaneum*, respectively at a concentration of 10 mg/200 cc, when compared with other plant extracts. The adults of *S. oryzae* showed more susceptibility towards pure ethyl acetate and methanol-eluted fractions of *M. charantia, C. annuum, S. melongena* and *C. indica* compared with other test insect *T. castaneum* in fumigation method. However, the ethyl acetate eluted fraction of all plant crude extracts appeared to be potent fumigants of all the stored product insects tested in this investigation, suggesting that ethyl acetate soluble chemicals are responsible for the potential toxic effects of the extracts.

Contact toxicity of the tested plant extracts: The contact application of test materials resulted in mortality of the treated insects. However, in all these treatments, the time required for total insect kill was about 72 h. At a concentration of 100 mg/20 g the leaf crude extracts of *C. annuum* and *M. charantia* produced 100% mortality of *S. oryzae* and > 90% of *T. castaneum* after 72 h

Table 4: Vapor toxicity of purified chromatographic fractions from plant leaf extracts against *Sitophilus oryzae* L. by fumigation method

	Toxicity (%) ± SD (100 mg/200 cc)[b]				
Fractions	24 h	48 h	72 h	LC_{50} (95% FL)[a] (mg/20 g diet)	χ^2 (df)
Hexane					
C. papaya	18.8±2.3a	28.6±2.4b	31.4±1.5d	>10	0.17 (4)
C. indica	11.2±2.2d	18.0±2.8a	24.2±1.1c	>10	3.27 (4)
M. charantia	77.8±4.2c	84.2±2.9c	100±0.0e	3.52 (2.80-4.16)	0.57 (4)
M. koenigii	66.6±4.3b	81.2±2.1c	89.0±2.4b	5.87 (4.12->10)	5.96 (4)
C. annuum	27.6±4.9e	31.8±1.1b	38.6±1.4a	>10	25.7 (4)
S. melongena	20.0±3.5a	23.2±2.0a	33.0±1.3d	>10	2.19 (4)
Chloroform					
C. papaya	80.2±4.4a	86.4±2.4a	95.0±2.4f	4.35 (3.99-4.71)	2.81 (4)
C. indica	19.2±2.4c	26.8±2.1d	34.8±1.3d	>10	0.28 (4)
M. charantia	31.4±2.5b	34.0±3.2c	49.2±2.4b	>10	80.4 (4)
M. koenigii	22.8±1.6c	29.0±1.8d	31.2±2.5e	>10	6.22 (4)
C. annuum	61.4±3.4e	78.8±2.5f	81.4±2.1a	5.81(4.02-8.61)	9.79 (4)
S. melongena	20.2±2.7c	30.0±2.0d	31.6±1.9e	>10	0.14 (4)
Ethyl acetate					
C. papaya	33.4±4.1d	41.6±2.1c	47.8±2.0a	>10	0.29 (4)
C. indica	69.8±1.3b	80.8±2.0e	90.6±2.1e	9.09 (8.43- 9.95)	22.6 (4)
M. charantia	80.4±1.9c	100±0.0f	-	4.42 (3.29- 6.31)	80.4 (4)
M. koenigii	25.0±3.5e	36.6±2.4d	46.0±2.8a	≥10	2.08 (4)
C. annuum	100±0.0a	-	-	2.51 (1.51-3.28)	15.9 (4)
S. melongena	100±0.0a	-	-	3.12 (0.52-4.86)	49.1 (4)
Methanol					
C. papaya	21.0±2.2e	36.4±2.4f	40.4±1.5c	>10	0.43 (4)
C. indica	81.2±3.1d	95.8±3.7c	100±0.0d	3.76 (1.52-5.53)	15.7 (4)
M. charantia	100±0.0c	-	-	2.82 (0.75-4.22)	42.1 (4)
M. koenigii	20.0±1.9e	29.2±2.6a	37.0±1.8a	>10	1.52 (4)
C. annuum	29.3±2.0a	32. 8±3.2d	42.0±2.2c	>10	0.82 (4)
S. melongena	80.8±3.0d	94.6±1.6c	100±0.0 d	4.12 (2.95-5.60)	10.6 (4)

[a]FL- fiducial limits; after 72 h of treatment. [b]Each column followed by the same letter are not significantly different from another (One-Way ANOVA; Tukey HSD test at, <0.05)

of treatment (Table 6, 7) in this bioassay. The time of exposure, insect species and the test chemical concentration played an important role in generating the toxicity separately. Apart from this, the leaf extracts of *C. annuum* and *M. charantia* also exhibited considerably good amount of toxicity (50%), against *S. oryzae* (27.6 and 47.4 mg/20 g) and *T. castaneum* (32.0 and 44.6 mg/20 g) diet. Adults of *S. oryzae* and *T. castaneum* showed higher susceptibility to *C. annuum* and *M. charantia* leaf extracts compared to other test extracts after 72 h of treatment.

Seed germination: The effect of plant extract treatments on maize seed germination was studied and the results are presented in Fig. 2. These studies revealed the significant impact that these plant extracts have on seed germination. The seed treatment with *C. papaya*, *C. indica*, *M. charantia* and *S. melongena* at all the tested concentrations (15 to 150 μg seed^{-1}) resulted in successful and normal germination that is comparable with that of the normal seed (CON II) (Fig. 2). However, the application of lower concentrations (15 to 60 μg/seed) of *M. koenigii* and

Table 5: Vapor toxicity of purified chromatographic fractions from plant leaf extracts against *Tribolium castaneum* by fumigation method

	Toxicity (%) ± SD (100 mg/200 cc)[b]				
Fractions	24 h	48 h	72 h	LC_{50} (95% FL)[a] (mg/20 g diet)	χ^2 (df)
Hexane					
C. papaya	30.8±2.1f	35.4±3.3b	41.8±3.5c	>10	1.00 (4)
C. indica	21.0±1.8d	32.2±3.6a	34.4±2.8d	>10	0.94 (4)
M. charantia	40.6±3.7e	46.2±2.0e	55.0±4.8e	>10	0.50 (4)
M. koenigii	33.2±4.5 a	45.2±3.9e	50.6±3.7f	>10	0.52 (4)
C. annuum	20.4±2.4d	25.8±3.5c	33.6±2.4d	>10	4.44 (4)
S. melongena	20.8±2.1d	29.2±2.0a	37.0±1.9a	>10	3.46 (4)
Chloroform					
C. papaya	14.8±2.1c	29.0±2.4d	31.4±2.5e	>10	1.27 (4)
C. indica	17.0±2.2c	21.0±1.4d	28.4±3.4c	>10	0.10 (4)
M. charantia	80.6±4.6a	81.0±3.5a	100±0.0b	5. 82 (4.06-7.02)	22.0 (4)
M. koenigii	21.4±2.0e	32.6±1.6c	42.0±2.8f	>10	3.25 (4)
C. annuum	63.8±4.2f	81.6±2.6a	91.8±2.5a	6.54 (4.41-9.62)	18.0 (4)
S. melongena	24.8±3.8b	38.8±2.2e	41.8±1.5f	>10	1.32 (4)
Ethyl acetate					
C. papaya	72.2±3.4a	81.2±2.1b	98.8±2.0a	5.70 (5.36-6.04)	3.43 (4)
C. indica	34.0±5.9c	52.6±3.1a	61.2±2.5c	9.53 (6.65->10)	1.82 (4)
M. charantia	100±0.0e	-	-	3.57 (2.51-4.48)	14.1 (4)
M. koenigii	69.4±2.7a	81.0 ± 2.1b	91.8±2.6d	7.76 (7.13-8.53)	7.53 (4)
C. annuum	100±0.0e	-	-	2.80 (1.46-3.80)	25.8 (4)
S. melongena	94.8±4.4d	100 ± 0.0c	-	3.29 (1.97-5.10)	2.43 (4)
Methanol					
C. papaya	19.2± 3.6b	32.2±2.9b	43.6±2.7e	>10	0.34 (4)
C. indica	12.6±4.9c	30.8±2.1b	32.0±1.2d	>10	0.39 (4)
M. charantia	33.4±3.5e	45.0±3.3a	52.0±2.2c	>10	0.07 (4)
M. koenigii	18.8±2.0b	20.2±1.5c	32.8±3.4d	>10	1.24 (4)
C. annuum	41.0±2.1d	45.6±1.5a	57.6±1.6c	≥10	1.10 (4)
S. melongena	37.4±4.1d	42.6±2.6a	58.8±2.3c	≥10	3.83 (4)

[a]FL-fiducial limits; after 72 h of treatment. [b]Each column followed by the same letter are not significantly different from another (One-Way ANOVA; Tukey HSD test at, <0.05)

Table 6: Contact toxicity of plant leaf extracts against *Sitophilus oryzae* by direct contact application method

	Toxicity (%) ± SD (100 mg/200 cc)[b]				
Plant name	24 h	48 h	72 h	LC_{50} (95% FL)[a] (mg/20 g diet)	χ^2 (df)
C. papaya	37.2±3.6b	41.6±2.8a	46.0±2.9e	> 100	3.19 (5)
C. indica	48.8±2.4a	50.8±1.0c	54.6±1.3c	99.3 (71.8->100)	2.20 (5)
M. charantia	76.6±4.3c	82.8±4.8d	97.6±1.8a	47.4 (32.3-78.8)	8.86 (5)
M. koenigii	63.0±5.0e	69.4±2.2b	75.4±2.4d	59 .3 (51.0-70.2)	2.25 (5)
C. annuum	91.6±2.5d	98.6±1.9e	100±0.0a	27.6 (13.5-44.7)	11.72 (5)
S. melongena	39.8±3.2b	46.8±5.4f	61.4±1.8b	70.1 (55.1-97. 6)	2.19 (5)

[a]FL-fiducial limits; after 72 h of treatment. [b]Each column followed by the same letter are not significantly different from another (One-Way ANOVA; Tukey HSD test at, <0.05)

C. annuum extracts caused a normal germination of maize, but the same plant extracts at higher concentrations (100 and 150 μg seed^{-1}) caused 30-35% inhibition of seed germination. Seed germination was significantly ($p<0.001$) reduced when untreated maize seeds were exposed to *S. oryzae* and *T. castaneum* (CON I) (Fig. 2a-f).

Table 7: Contact toxicity of plant leaf extracts against *Tribolium castaneum* by direct contact application method

	Toxicity (%) ± SD (100mg/200cc)[b]				
Plant name	24 h	48 h	72 h	LC_{50} (95% FL)[a] (mg/20 g diet)	χ^2 (df)
C. papaya	20.2±3.3b	34.8±3.4d	40.2±1.5f	> 100	5.22 (5)
C. indica	39.4±2.9e	47.4±3.3e	57.2±3.5d	94.2 (53.7->100)	0.12 (5)
M. charantia	78.2±2.5a	86.0±3.5c	94.0±3.0a	44.6 (26.6-88.4)	14.31 (5)
M. koenigii	45.2±3.6c	62.0±3.9f	68.4±2.8c	73.5 (61.7-92.5)	2.95 (5)
C. annuum	85.4±3.3d	90.4±1.9b	100.0±0.0e	32.0 (16.9-49.7)	15.66 (5)
S. melongena	43.0±4.1c	53.8±3.6a	61.6±2.4b	88.6 (81.3->100)	2.79 (5)

[a]FL- fiducial limits; after 72 h of treatment. [b]Each column followed by the same letter are not significantly different from another (One-Way ANOVA; Tukey HSD test at, < 0.05)

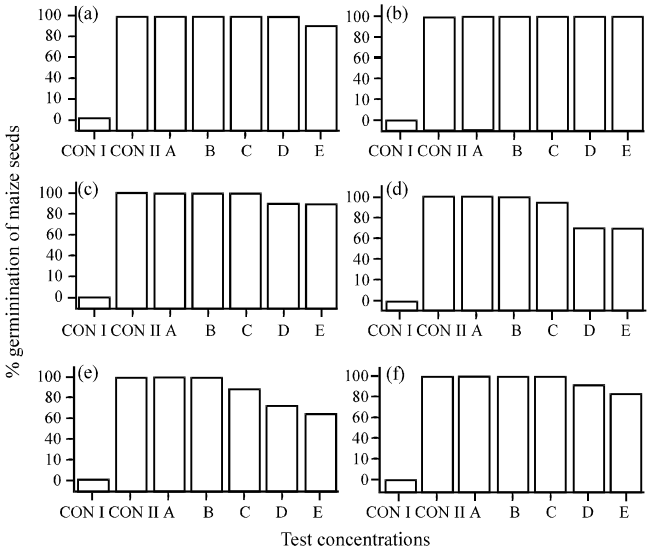

Fig. 2: Effect of different plant foliar extracts on percentage germination of maize seeds. (a) *Carica papaya* L., (b) *Coccinia indica* L., (c) *Momordica charantia* L., (d) *Murraya koenigii* L., (e) *Capsicum annuum* L. and (f) *Solanum melongena* L. Each data (Mean±SD) are compared, and are not significantly different from one another if treatment bars indicated by same letter (one-way ANOVA; $p<0.05$, Tukey's HSD test; N = 20 seeds/replicate, 12 replicates N = 240 seeds). NS = No significant difference. A-15, B-30, C-60, D-100 and E-150 µg/seed.CON I- fresh maize seeds infested with test insects; CON II- fresh maize seeds treated with solvent alone

***In vitro* antifungal activity:** The effects of the test extracts on mycelial growth of major seed-borne fungi, *A. flavus* of maize seeds are presented in Fig. 3. The findings showed that the *A. flavus* was inhibited significantly ($p<0.001$) by the extracts of *M. koenigii*, *M. charantia* and *S. melongena* as compared to the other plant extracts and control (Fig. 3a-c). The percentage inhibition of mycelial growth of the fungi varied with the type of leaf extracts and extract concentration. *M. koenigii* and *M. charantia* leaf extracts gave the highest inhibition (33.0 and 36.6 mm) while the *C. papaya* extract gave the lowest inhibition (10.4 mm) at a concentration of 15 mg.

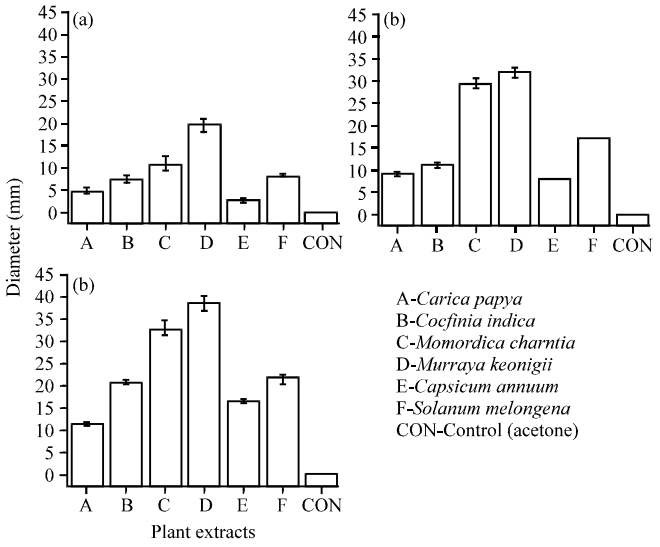

Fig. 3: Inhibitory effects of different plant foliar extracts on *Aspergillus flavus in vitro* method. (a) At 5 mg conc, (b) At 10 mg conc and (c) At 15 mg conc. Each data (Mean±SD) are compared and are significantly different from one another (one-way ANOVA; $p<0.05$, Tukey's HSD test; 18 replicates/treatment)

DISCUSSION

The results obtained indicate the competence of the plant leaf extracts and its chromatographic fractions in controlling pest infestation in maize storage by *S. oryzae* and *T. castaneum* adults. The foliar extracts of *M. charantia, C. annuum* and *S. melongena* appeared to be significantly ($p<0.001$) effective in protecting the stored maize from the two pest insects tested, followed by *M. koenigii, C. papaya* and *C. indica* extracts in vapor toxicity mode. The insecticidal activity varied with insect species, concentrations of the test compounds, exposure time and mode of application. Leaf crude extracts as well as solvent-eluted chromatographic fractions were highly toxic to both the insects at 100 and 10 mg concentrations, respectively, while all the tested plant extracts were effective in vapor form (with in 24 h of treatment) ($p<0.001$). In the contact method, concentration and exposure time played an important role in producing the lethal effects; while the compound at the lower dose failed to exhibit toxic symptoms at 24 h, the percentage mortality was enhanced with increased duration of exposure to these extracts (after 72 h of treatment). These plants extracts produced toxic effects in the vapor form, which is advantageous for the control of stored product pests as vapors are able to penetrate deeper into containers. Another major advantage of the plant compounds tested in the present study is their high volatility, which is a desirable characteristic for insecticidal preparations acting as fumigants for the control of stored product pests (Konstantopoulou *et al.*, 1992; Regnault-Roger and Hamraoui, 1995; Ahn *et al.*, 1998). However, although the results of the present study were promising with regards the use of the leaf extracts of different plants for the protection of stored maize, further investigation about the chemical isolation and identification of the bioactive constituents of the tested plant extracts is required before accepting such preparations as grain protectants.

It is also shown here that the storage of maize seeds for prolonged periods such as 90 days after treating with various botanical concentrations do not have any adverse effect on the seed viability

(Fig. 2, $p<0.001$), which is an important aspect for the use of botanicals for storage pests. This concurs with studies by 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. Maize seeds treated with all test extracts and unexposed to insects showed significant germination equal to the control (seeds treated with solvent alone) ($p<0.001$) whereas *M. koenigii* and *C. annuum* treated maize seeds with higher concentrations (100 and 150 μg/seed) showed inhibitory effects on seed germination. This finding is supported by Chung and Miller (1995) and Sunderraj *et al.* (1996), who found that the degree of inhibition increased with increased extract concentration. However, in our study we found that the *C. papaya, C. indica, M. charantia* and *S. melongena* treated maize seeds showed good germination potential equal to that of solvent treated seeds. The ability of the plant extracts to increase seed germination could be attributed to the inhibition of the incidence of the seed borne fungi that could have killed the embryo of the seeds. This result is consistent with that of Parimelazhagan and Francis (1999) who established that leaf extracts of *Cerastium viscosum* L. increased seed germination and improved seedling development of rice seeds. In the present trials it is clear that using treated botanical extracts as natural pesticides in maize storage has 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.

Pre and post harvest bio-deterioration and spoilage of grains due to infestation by insects and microorganisms may cause losses of up to 100% (Satish *et al.*, 2009). The species of *Aspergillus* has been reported to cause significant loss in seed quality and nutritional quality of grains (Koirala *et al.*, 2005). The development of disease resistance and problems due to environmental pollution make the use of chemical pest management problematic (Gamliel and Yarden, 1998). Therefore, an urgent and important aspect is the development of alternative control treatments based on plant extracts (Stephan and Koch, 2002). The efficacy of different plant leaf extracts against major seed borne fungi, *A. flavus* of maize seeds were tested *in vitro*. The experimental results on seed borne fungus, *A. flavus* indicate that certain extracts are promising in inhibiting the seed borne fungal growth and the inhibition depends on both concentration and test material. Most of the plant extracts have been reported to inhibit postharvest fungi in *in vitro* conditions (Bajwa *et al.*, 2004; Satish *et al.*, 2009; Parekh and Chanda, 2008; Zakaria, 2010). The leaf extracts of *M. charantia, M. koenigii* and *S. melongena* gave the best result for reducing mycelial growth of tested phytopathogenic fungi, *A. flavus* in *in vitro* conditions ($p<0.001$). This may be because the bioactive compounds of the leaves of this plant may differ in quantity and quality compared to other tested plants. In this study, we conclude that the crude leaf extracts of *M. charantia, M. koenigii* and *S. melongena* were effective in inhibiting the fungal growth on the germinating seeds, which is again a positive and important result obtained in this investigation. These results are highly promising for the future exploitation of these plants extracts as stored maize protectants.

It is concluded that the use of these plant extracts as natural pesticides in maize storage significantly reduce the insect damage, seed borne fungal growth on maize with least or no adverse effects on seed germination of treated maize up to 3 months. The botanical treatment did not cause any change in grain color or odour since the compounds are effective in vapor form without contacting the grain. Hence, the grain value in the market will not be affected (decreased). For the practical use of these plant extracts and their active ingredients as novel grain protectants, further research is required as far as safety issues for human health are concerned. Other areas requiring attention are the development of cost effective formulations with improved efficacy, insecticidal

potency and stability against the stored product pests. The use of plant materials in pest control could become important supplements or alternatives to synthetic pesticides. Therefore, it is important that appropriate technology is developed to promote a direct preparation of traditional pesticides at the farm level for those poor farmers who have no access to commercial pesticides or cannot afford.

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