

Toxicity and repellent activity of *Cymbopogon citratus* (D.C.) Stapf. and *Murraya koenigii* Sprang. against *Callosobruchus maculatus* (F.) (Coleoptera; Bruchidae)

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ABSTRACT

Essential oils of *Cymbopogon citratus* (Lemongrass) and *Murraya koenigii* (Curry leaf) were tested for their toxicity and repellent activity against *Callosobruchus maculatus* (F.) in stored cowpea. In the contact toxicity bioassay, lemongrass oil at a concentration of 0.15 g/l caused 100 % mortality and the number of eggs laid was zero. Curry leaf oil also showed similar activity at a concentration of 0.75 g/l. In fumigant toxicity bioassays lemongrass oil at 1.5-g/l concentration and curry leaf oil at 7.5-g/l concentration caused 100 % bruchid mortality and reduced their oviposition and F₁ adult emergence. In contact toxicity bioassay, the lowest LC₅₀ value of 0.026 g/l was observed for lemongrass and the LC₅₀ value of curry leaf was 0.240 g/l. The results indicated that lemongrass oil was more effective as a contact toxicant on bruchids than curry leaf oil. In olfactometer and choice chamber bioassays, the % responses of bruchid decreased with increasing doses of both oils. Only 7.0 % bruchids settled in at the dosage of 160 mg in choice chamber for both lemongrass and curry leaf oils. These results suggested that the essential oils of lemongrass and curry leaf could be used as alternatives to develop less toxic treatment system to protect stored cowpea.

Key words: *Callosobruchus maculatus*, *Cymbopogon citratus*, *Murraya koenigii*, Repellency, Toxicity.

INTRODUCTION

Cymbopogon citratus (D.C.) Stapf. (lemongrass) and *Murraya koenigii* Spreng. (curry leaf) are native spice plants abundant in Sri Lanka. The essential oil of lemongrass is commercially produced using leaves, yielding 0.2 % - 0.3 % oil (Paranagama 1991). This oil has an intense lemon like odour and taste. The bulk of the oil is utilized in the isolation of citral a & b, which are used in flavor and fragrance industries. Citral a & b are the main constituents (78 %) of lemongrass oil and a significant proportion of myrcene and limonene (10 %) are also present (Paranagama 1991). The antibacterial property of lemongrass oil has previously been reported against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis* and *Mycobacterium avium* (Nettasingha and Paskaranathan 1976). Fungicidal activity the lemongrass has been reported against soil borne fungi such as *Pythium aphanidermatum* and *P. debryanum* (Jayasinghe *et al.* 1999). Various preparations containing this essential oil are currently being used as repellents against houseflies, tsetse fly and mosquitoes (Osmani *et al.* 1972; Tiwari *et al.* 1966). Powdered lemongrass leaf has

previously been reported to have a significant effect on the reduction of oviposition of *Callosobruchus maculatus* (Rajapakse and Emden 1997).

In Sri Lanka, curry leaves (Karapincha) are extensively used for culinary purposes. The juice of fresh leaf suppresses blood cholesterol level and is given as a remedy for diarrhoea and dysentery (MacLeod and Peris 1982). A number of studies concerning the composition and quality of curry leaf have been reported (Paranagama 1991; Wong and Tie 1993). The studies on combined gas chromatography - mass spectrometry revealed that the essential oil of curry leaf contains 53 compounds. The major constituents in the curry leaf oil has been reported to be β -caryophyllene (23.3 %), β -phellandrene (18.9), (E)- β -ocimene (12.7 %), β -thujene (5.8 %), α -humulene (4.3 %) and β -bisabolene (3.14 %) (Paranagama 1991). The toxicity of crude curry leaf oil had been studied against *Callosobruchus chinensis* in green gram and chickpea seeds with LC₅₀ and LC₉₀ values of 4.672 and 5.148 mg/l respectively (Namarata *et al.* 1997).

One of the most widespread species of *Callosobruchus* (Bruchidae) is the southern cowpea weevil, *C. maculatus* (F.) which is an insect closely associated with the family Leguminosae. Cowpea

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[*Vigna unguiculata* (L.) Walp.] is one of the main sources of protein for the people in developing countries. However infestation of *Callosobruchus* species in stored grains results in heavy qualitative and quantitative post harvest losses. A loss of 50 % of cowpea could result during 3-4 months storage due to infestation by *C. maculatus* (Caswell 1981).

The current pest control method recommended by the Department of Agriculture in Sri Lanka is the use of pirimiphos-methyl (Actellic), either as a 0.25 % solution, which is sprayed on to storage bags containing cowpea seeds, or as a 2 % dust on stored seeds used for planting purposes (Anon. 1997). However this method is not cost effective and involves the risk of health hazards to consumers (Anon. 1991). Alternatively, environment friendly, comparatively less toxic and less expensive insecticides are urgently needed in stored grain insect control. The insecticidal properties of essential oils are becoming more popular among the farmers, mainly in the developing countries due to the volatile nature and edible properties of the essential oils.

The aim of the present study was to investigate the toxicity and repellency of essential oils of *C. citratus* and *M. koenigii* against the common cowpea bruchid, *C. maculatus*.

MATERIALS AND METHODS

Insects

Callosobruchus maculatus (F.) were obtained from, Entomology Division, Horticultural Crop Research and Development Institute, Peradeniya, Sri Lanka. Rearing of weevils was carried out at the Department of Chemistry, University of Kelaniya, under prevailing conditions of 28 ± 3 °C and 75 ± 5 % RH. using newly harvested, fresh cowpea seeds [*Vigna unguiculata* (L.) Walp.].

Clear, transparent 1 L plastic bottles (with 8-cm diameter screw lid) were used to breed insects. A hole (3 cm diameter) was made on the lid of each bottle and a fine gauze (25 mesh/ cm²) was pasted on the hole from inside of the lid to provide sufficient ventilation for the bruchids. Twenty-five pairs of adult bruchids were placed in each container, with 250 g of seeds. Under the above experimental conditions, a new generation of bruchids emerged after 3-4 weeks from eggs laid by the introduced females. Five-ten hours old adults were obtained from this culture. Insect handling and sexing was done according to the method described by Bandara and Saxena (1995).

Plant material

Leaves of *M. koenigii* were air dried for 3-4 days and subjected to steam distillation for 3 h (Paranagama, 1991). The essential oil was extracted with CH₂Cl₂ and dried on anhydrous Na₂SO₄. The extract was concentrated in a rotary evaporator at 35 °C and the remaining solvent was evaporated using a N₂ stream. Essential oil of *C. citratus* and pirimiphos methyl (Actellic) were purchased from EOAS Organics (Pvt) Ltd., Rathmalana, Sri Lanka and Chemical Industries Ltd., Colombo, Sri Lanka respectively.

Contact toxicity bioassay

Contact toxicity bioassay was performed using the method described by Huang *et al.* (1999). A series of treatments (0.0015 g/l - 0.150 g/l of lemongrass oil and 0.015 g/l - 0.75 g/l of curry leaf) were prepared separately in ethanol and applied evenly on the inner surface of glass vials (6.5 ml) and the screw caps. The solvent was evaporated using N₂ gas. Five pairs of 5-10 hour old bruchids were introduced into each vial and the cap screwed tightly on to the vial. After 24-hour incubation period, bruchids were transferred to clean vials with 50 untreated, fresh cowpea seeds. Ethanol treated samples and samples without any treatment were used as the control for comparison purposes. A series of doses (4×10^{-10} - 100×10^{-4} g/l) of pirimiphos methyl were prepared separately and the contact toxicity of pirimiphos methyl was carried out with *C. maculatus* to calculate the LC₅₀ value. The experimental design was a Completely Randomized Design (CRD) with six-replicates per treatment.

Fumigant toxicity bioassay

Fumigant toxicity bioassay was carried out using the method described by Bandara and Senevirathne (1993). A Whatman no 1 filter paper strip (1.5-cm diameter) was impregnated with an appropriate concentration of the essential oil (0.08-1.50 g/l lemongrass or 0.75-7.50 g/l curry leaf oil) and the solvent was allowed to evaporate for 10 minutes. Subsequently the filter paper was placed on the undersurface of the screw cap of a glass vial (6.5 ml), and five pairs of 5-10 hour old bruchids were introduced into the vial. The neck of the vial was blocked with a metal mesh (25 mesh/cm²) to avoid contact of insects with the test substance and the cap of each vial was screwed tightly and incubated for 24 h. Thereafter, the insects were transferred into a clean vial with 50 fresh, untreated cowpea seeds. Parallel

experiments with pirimiphos methyl (1.3×10^{-6} - 16×10^{-4} g/l), ethanol and control were also conducted. Experimental design was a CRD with six replicates per treatment.

Mortality of the bruchids and the number of eggs laid were recorded for 10 days in both contact and fumigant toxicity tests. The numbers of F_1 adults were also counted.

Repellent activity of volatile constituents

Olfactometer bioassay

The Y shaped Olfactometer was used for the bioassay (Bandara, 1997). Filter paper strips (2.5 cm x 3.0 cm) treated separately with different doses (10, 20, 40, 80 and 160 mg) of test volatile oil was placed in a plastic container (300 ml) connected to one arm of the Y tube (baited arm). A filter paper strip treated with the same amount of ethanol was placed in another plastic container connected to the second arm (non-baited arm) of the Olfactometer. A round-bottomed flask (100 ml) with 50 unsexed adult bruchids was connected to the third arm of the Y tube and kept in darkness. The fourth arm, which was at the meeting point of the Y tube, was connected to a vacuum pump to regulate the airflow inside the Olfactometer (Figure 1). Five different doses of volatiles from each plant were used separately. The baited and the non-baited arms of the Y tube were positioned towards a 40 W fluorescent light source. All the bioassays were conducted between 7 a.m.-11 a.m. The baited and the non-baited arms were

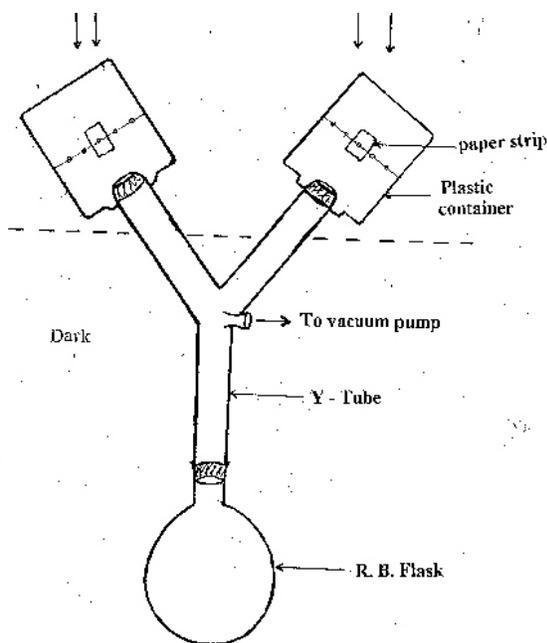


Fig. 1. Setup for Olfactometer

interchanged in subsequent replicates. The number of test insects moved to the baited arm and non-baited arm were counted after 30 minutes. Each dose of volatile oil was replicated six times.

Choice Chamber bioassay

The Choice Chamber consisted of eight transparent plastic bottles (300 ml), placed equidistant to each other. The bottles were connected to a large transparent bottle (1l) placed in the center of the chamber through glass tubes (1 cm diameter and 8 cm long). The experimental apparatus was placed in a plastic basin having a diameter of 42 cm and the height of 18 cm and the sidewalls were covered with black paper (Figure 2).

Five different doses (10, 20, 40, 80 and 160 mg) of the volatile samples were placed on filter paper strips (2.5 cm x 5.0 cm) separately and the solvent was allowed to evaporate. Each strip was placed in an appropriate bottle containing 50 cowpea seeds. Two bottles containing 50 cowpea seeds without any treatment and a filter paper strip treated with only

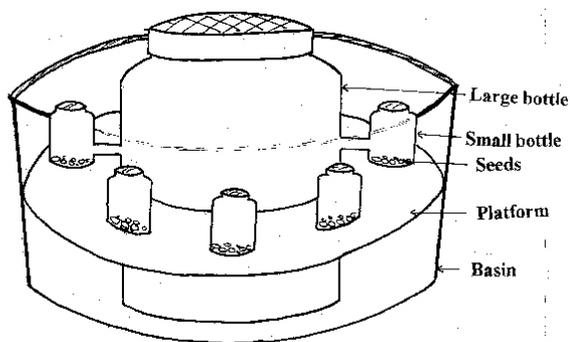


Fig. 2. Setup for choice chamber

ethanol were considered as the controls. Two hundred and fifty adult bruchids (unsexed, 1-3 d old) were introduced to the central bottle, and the chamber was placed in a dark room. After 24 h, the number of bruchids moved in to each bottle was recorded. The bioassay was replicated six times.

Effect of the volatile oils on seed viability

Fifty cowpea seeds were fumigated with volatile of lemongrass and curry leaf at 1.5 g/l and 7.5 g/l and ethanol in 60 ml glass vials for 30 days with untreated controls. The samples of both treated and untreated were placed on a moistened filter paper (20 cm diameter) to facilitate germination of seeds. The number of seeds germinated in each sample was recorded for 10 days. The experimental design was a CRD with 6 replicates.

Statistical analysis

The data obtained for percentage mortality, mean eggs laid and F_1 emergence were recorded on 24 h, 3 days and 30 days after treatment respectively in both fumigant and contact toxicity bioassays. Data obtained after test oil treatments during toxicity or repellent bioassays were analyzed statistically using One-way ANOVA and the means were compared using Tukey's pair wise comparison test. LC_{50} values of lemongrass, curry leaf volatiles and standard insecticide, pirimiphos methyl were calculated with the aid of Probit Analysis computer software program.

RESULTS

The percentage mortality was 100 at 0.150 g/l treatment of lemongrass oil and 0.75 g/l of curry leaf oil (Table 1). The results of ethanol and control samples were not significantly different from each other ($P > 0.05$). The mean F_1 adult emergences in

both volatile treatments decreased with increasing concentration of both volatile.

In the fumigant toxicity bioassay, 100 % mortality was observed at concentrations of 1.5 g/l of lemongrass and 0.75 g/l of curry leaf oils (Table 2). Control and ethanol treated samples displayed only 2 % mortality. The mean number of eggs laid reduced to 42.5 at the concentration of 0.11 g/l of lemongrass oil while 1.50 g/l of curry leaf oil was required to visualize the same effect. The emergence of F_1 generation was reduced with increasing concentration of both oils. The highest mean number of eggs laid (approximately 90) and F_1 adults emergence (approximately 61) were observed in control and ethanol treated samples. The LC_{50} values for lemongrass and curry leaf oil indicated that contact toxicity of lemongrass has the highest effect on bruchid mortality (Table 3).

During the Olfactometer bioassay, both oils demonstrated strong repellent activity on *C. maculatus*. Repellent effect of lemongrass oil (at doses ranging from 80 and 160 mg) was significantly

Table 1. Effect of leaf lemongrass and curry leaf oils on *C. maculatus* during contact toxicity bioassay

Treatments (g/l)	Lemongrass oil			Curry leaf oil		
	%Mortality	Mean # eggs laid/ 50 seeds	Mean F_1 emerged/ 50 seeds	%Mortality	Mean # eggs laid/ 50 seeds	Mean F_1 emerged/ 50 seeds
0.0015	3 ± 2 ^a	89.3 ± 4.2 ^a	67.3 ± 5.8 ^a	-	-	-
0.0080	25 ± 3 ^b	71.8 ± 4.9 ^{ab}	47.3 ± 4.6 ^b	-	-	-
0.0150	28 ± 3 ^b	46.3 ± 7.7 ^c	27.3 ± 4.3 ^c	2 ± 2 ^P	73.3 ± 2.9 ^P	63.2 ± 2.1 ^P
0.030	60 ± 7 ^c	22.8 ± 6.3 ^d	13.2 ± 4.7 ^d	-	-	-
0.750	93 ± 3 ^d	4.2 ± 1.9 ^c	1.5 ± 0.9 ^{de}	7 ± 2 ^{Pq}	53.7 ± 3.2 ^{qr}	43.5 ± 2.4 ^q
0.150	100 ± 0 ^d	0.0 ± 0.0 ^e	0.0 ± 0.0 ^e	15 ± 4 ^{qr}	42.0 ± 4.2 ^r	30.7 ± 3.2 ^r
0.375	-	-	-	93 ± 3 ^s	2.0 ± 1.0 ^s	0.0 ± 0.0 ^s
0.750	-	-	-	100 ± 0 st	0.0 ± 0.0 st	0.0 ± 0.0 ^s
Ethanol	2 ± 2 ^a	79.0 ± 4.0 ^{ab}	66.2 ± 3.6 ^a	2 ± 2 ^P	79.0 ± 4.0 ^P	66.2 ± 3.6 ^P
Control	2 ± 2 ^a	80.8 ± 4.2 ^{ab}	61.2 ± 3.0 ^a	2 ± 2 ^P	80.8 ± 4.2 ^P	61.2 ± 3.0 ^P

Each data point represents the mean of 6 replicates ± S.E., Each column followed by the same letters are not significantly different according to One-way ANOVA and Tukey's pair wise comparison test.

Table 2. Effect of leaf lemongrass and curry leaf oils on *C. maculatus* during fumigant toxicity bioassay

Treatments (g/l)	Lemongrass oil			Curry leaf oil		
	%Mortality	Mean # eggs laid/ 50 seeds	Mean F_1 emerged/ 50 seeds	%Mortality	Mean # eggs laid/ 50 seeds	Mean F_1 emerged/ 50 seeds
0.08	3 ± 2 ^a	93.6 ± 7.0 ^a	55.7 ± 6.5 ^a	-	-	-
0.11	18 ± 3 ^b	42.5 ± 2.5 ^a	31.0 ± 2.1 ^b	-	-	-
0.15	43 ± 3 ^c	38.0 ± 3.8 ^b	28.8 ± 3.4 ^b	-	-	-
0.35	88 ± 5 ^d	13.3 ± 2.1 ^c	6.7 ± 2.1 ^c	-	-	-
0.75	98 ± 3 ^{ef}	0.0 ± 0.0 ^d	0.0 ± 0.0 ^{cd}	13 ± 2 ^P	53.7 ± 4.9 ^P	44.5 ± 3.3 ^P
1.50	100 ± 0 ^f	0.0 ± 0.0 ^d	0.0 ± 0.0 ^{cd}	17 ± 3 ^P	42.2 ± 4.3 ^{Pq}	34.5 ± 4.0 ^{Pq}
2.30	-	-	-	32 ± 3 ^q	36.0 ± 2.6 ^{qr}	27.0 ± 3.3 ^{qr}
3.80	-	-	-	42 ± 7 ^{qr}	27.3 ± 4.9 ^{rs}	18.5 ± 3.4 ^{rs}
7.50	-	-	-	100 ± 0 ^s	2.3 ± 1.3 ^t	1.0 ± 0.7 ^u
Ethanol	21 ± 21 ^a	90.0 ± 6.5 ^a	66.2 ± 3.6 ^a	2 ± 2 ^t	90.0 ± 6.5 ^k	66.2 ± 3.6 ^k
Control	2 ± 2 ^a	89.6 ± 6.8 ^a	61.2 ± 3.0 ^a	2 ± 2 ^t	89.6 ± 6.8 ^k	61.2 ± 3.0 ^k

Each data point represents the mean of 6 replicates ± S.E., Each column followed by the same letters are not significantly different according to One-way ANOVA and Tukey's pair wise comparison test.

($P > 0.05$) higher than curry leaf oil treatments. In the Choice Chamber bioassay, the numbers of bruchids moved to each container decreased with increasing

Table 3. The LC₅₀ values of contact and fumigant toxicity assays for lemongrass and curry leaf volatiles and standard insecticide pirimiphos methyl

Bioassay	LC ₅₀ values (g/l) ^a		
	Lemongrass	Curry leaf	Pirimiphos methyl
Contact toxicity	0.026	0.240	3.27 X 10 ⁻⁹
Fumigant toxicity	0.286	4.330	5.03 X 10 ⁻⁵

The LC₅₀ values were calculated using Probit analysis computer programme. a = on 24 h after treatment

Table 4. Effect of leaf lemongrass and curry leaf oils on *C. maculatus* during olfactometer test 30 mins after introduction.

Doses (mg)	Percentage responded	
	lemongrass	Curry leaf
10	47.7 ± 1.8 ^a	51.4 ± 1.5 ^a
20	42.4 ± 1.5 ^b	42.4 ± 1.2 ^b
40	35.1 ± 1.2 ^c	32.1 ± 0.7 ^c
80	18.8 ± 1.5 ^d	30.3 ± 0.8 ^d
160	9.5 ± 0.4 ^d	16.9 ± 0.7 ^e

Each data point represents the mean of 6 replicates ± S.E., Each column followed by the same letters is not significantly different according to One-way ANOVA and Tukey's pair wise comparison test.

doses of each essential oil (Table 5). All doses of essential oils significantly ($p < 0.05$) repelled bruchids and the results revealed more than 93 % repellent activity at 160 mg for both oils. Repellency effect of lemongrass was significantly higher than that of curry leaf oil at doses lower than 20 mg. The oviposition was significantly ($p < 0.05$) reduced with the increasing dose of oil treatment, when compared to the control and ethanol treatments. There were no significant effect of essential oils and the ethanol on the germination of cowpea seeds, when compared to the control (Table 6).

Table 5. Effect of leaf lemongrass and curry leaf oils on *C. maculatus* during choice chamber bioassay 24 h after introduction.

Dose (mg)	Percentage responded		Percentage eggs laid	
	lemongrass	Curry leaf	lemongrass	Curry leaf
10	13.5 ± 4.1 ^a	22.3 ± 3.8 ^P	6.2 ± 1.2 ^a	23.9 ± 4.1 ^P
20	12.1 ± 3.8 ^a	15.3 ± 4.2 ^q	0.9 ± 0.2 ^b	11.9 ± 2.1 ^q
40	5.7 ± 0.4 ^b	7.4 ± 1.2 ^r	0.3 ± 0.3 ^{bc}	4.9 ± 0.4 ^r
80	5.1 ± 0.3 ^{bc}	4.0 ± 0.2 ^s	0.0 ± 0.0 ^c	2.1 ± 0.2 ^s
160	4.0 ± 0.2 ^c	0.0 ± 0.0 ^t	0.0 ± 0.0 ^c	0.3 ± 0.2 st
Control	29.4 ± 2.1 ^d	20.2 ± 5.4 ^u	45.6 ± 3.7 ^d	46.6 ± 6.5 ^u
Ethanol	29.2 ± 2.3 ^d	22.5 ± 6.2 ^u	46.8 ± 4.9 ^d	30.2 ± 5.2 ^u

Each data point represents the mean of 6 replicates ± S.E., Each column followed by the same letters is not significantly different according to One-way ANOVA and Tukey's pair wise comparison test.

Table 6. Percentage germination of cowpea seed treated with volatile oils of lemongrass and curry leaf after 30 days fumigation

Treatments	% germination
Lemongrass 1.5 g/l	72.5 ± 2.6 ^a
Curry leaf 7.5 g/l	71.5 ± 3.5 ^a
Control	77.2 ± 3.6 ^a
Ethanol	74.0 ± 3.0 ^a

Each data point represents the mean of 6 replicates ± S.E., Each column followed by the same letters is not significantly different according to One-way ANOVA and Tukey's pair wise comparison test.

DISCUSSION

Several researchers have previously reported the effects of essential oils or essential oil bearing plants on *Callosobruchus* species (Rajapakse and Emden 1997; Bandara and Senevirathne 1993; Loganathan and Ahangama 1996; Ivbijaro 1990). In 1997, Rajapakse and Emden reported that a mixture of powdered *C. citratus* with cowpea seeds at rates of 200-300 g/kg reduced the number of eggs laid by *Callosobruchus* species. Jayasinghe *et al.* (1999) reported the repellent effect of lemongrass oil against tsetse fly, houseflies and mosquitoes. Namarata *et al.* (1997) reported the fumigant and contact toxicity effects of curry leaf volatiles against *C. chinensis*, with the LC₅₀ value of 4.672 mg/l.

Powder of wild ginger rhizome (*Zingiber purpureum*) has been tested against *C. maculatus* and it was indicated that the rhizome powder could prevent the bruchid infestation of cowpea seeds for up to 40 days (Bandara and Senevirathne 1993). Dharmasena (1999) has explained the ovicidal effect of neem and mee oil (7.5 ml/kg) on bruchids. The same survey also revealed that neem and mee oil did not affect the germination of cowpea seeds. Don Pedro (1996) reported that citrus peel oil treated with cowpea at a rate of 7 ml/kg caused 100 % mortality of bruchids one hour after application and a slightly higher dose of 10 mg/ml reduced oviposition or larval emergence of *C. maculatus*.

In the present study, curry leaf and in particular, lemongrass oil demonstrated contact toxicity and fumigant toxicity against *C. maculatus*. The toxicity data revealed that lemongrass oil is more effective than curry leaf oil. The repellent effect of lemongrass oil at lower doses (10 and 20 mg) was higher than that of the curry leaf oil at the same dose. But at the higher doses of 40-160 mg, fairly similar repellent effect was observed due to both oils during Choice Chamber bioassay.

The results of seed germination indicated that the oils have no adverse effect on the germination of seeds even after 30 days of treatments. Several

researchers have previously studied the germination of cowpea seeds after treatment with wild ginger preparations and castor and soybean oils, and the results suggested that there were no significant negative effects of these plant preparations on the seed viability (Bandara and Senevirathne, 1993; Pacheco *et al.*, 1995).

Present results revealed that essential oils of lemongrass and curry leaf could be used as alternatives to develop less toxic treatment system(s) to protect stored cowpea. The active components of essential oils of lemongrass and curry leaf are being identified using Gas Chromatography and combined Gas Chromatography-Electroantennogram Detector analysis. This study will help to develop and formulate an efficient as well as cost effective treatment system(s), in order to lengthen the storage life of cowpea and other stored grains.

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