Insecticidal Effect of Leaf Extract of *Aegle marmelos* (L.) On Some Insect Pests Of Stored Grain

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ABSTRACT-

Experiments was conducted out to determine the potential of using the leaves extract of *Aegle marmelos* to control of insect infestation of stored gram from *Callosobruchus chinensis* (L.) (Bruchidae) and wheat from *Rhyzopertha dominica* (F.) (Bostrychidae), *Sitophilus oryzae* (L.) (Curculionidae) and *Tribolium castaneum* (Herbst) (Tenebrionidae). After introducing the test insects, stored gram and wheat samples were fumigated with leaf extract of *Aegle marmelos* at 500 µg/mL (ppm). The leaf extract significantly enhanced the feeding deterrence in insects and reduced the grain damage as well as weight loss in fumigated gram and wheat samples infested with all insects except *T. castaneum*. The extract of leaf at different doses significantly reduced oviposition and adult emergence of *C. chinensis* in treated cowpea seeds. The oil protected stored gram from *C. chinensis* and wheat from *R. dominica* and *S. oryzae* for two years. Limonene (88 %) was found to be the major component in the extract of leaf through GC-MS analysis. Regression analysis of data on individuals in treated cowpea confirmed that significant reduction of oviposition and adult emergence of *C. chinensis* decreased with increase in doses. The findings emphasize the efficacy of *A. marmelos* leaf extract as fumigant against insect infestations of stored grains and strengthen the possibility of using it as an alternative to synthetic chemicals for preserving stored grains.

Key words: *Aegle marmelos*, *Callosobruchus chinensis*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum*, oviposition

I. INTRODUCTION:-

Cereals and pulses are of great biological and nutritional value in developing countries, are lost upto 20-60 per cent by storage insect pests during storage (Arthur and Throne 2003; Babu et al. 2003; Shaaya et al. 1997). Post-harvest deterioration causes economic losses due to obvious decay and adverse changes in the odour, taste, appearance and nutrition value (Phillips and Burkholder 1984; Mondal and Port 1994; Arlian et al. 1996). In addition, the arthropods transfer bacteria and microscopic fungi of pathogen importance on stick on their bodies or disseminate them via faeces (Wilbur and Mills 1978, Hubert et al. 2004). During recent years considerable attention has been paid towards exploitation of plant materials in protection of food commodities from insect infestations. Extracts of some plant species viz. *Lantana camara* (Saxena et al. 1992), *Illicium verum* (Ho et al. 1995), *Tithonia diversifolia* (Adedire and Akinneye, 2004) have been reported to possess strong insecticidal activity against different storage insects. Plant derived products namely, azadirachtin from *Azadirachta indica*, pyrethrin from *Chrysanthemum cinerariaefolium*, carvone from *Carum carvi* and allyl isothiocynate from mustard and horseradish oil have been received global attention due to their pesticidal properties and potential to protect several food commodities (Hartmans et al. 1995; Ward 1998; Varma and Dubey 1999; Athanassiou et al. 2005). Essential oils produced by different plant genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties (Mishra and Dubey 1994; Varma and Dubey 1999; Dubey et al. 2004; Holley and Patel 2005). The volatility and biodegradability of flavour compounds of angiosperm will be advantageous if they are developed as pesticide insecticide (French 1985). There may be least chance of residual toxicity by treatment of food commodities with volatile substances of higher plant origin.

Aegle marmelos (L.) Correa (Rutaceae), commonly known as Bael, is a sacred tree for Hindu Religon, native to northern India, but is found widely throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China (Bailey 1963). All parts of the tree namely, root, leaf, trunk, fruit and seed are used for treatment of many different diseases. The constituents of *Aegle* are used in heart diseases (Kakiuchi et al. 1991), inflammatory and wound healing (Udupa et al. 1994). Leaves of *A. marmelos* have been reported as hypoglycemic effect (Santhoshkumari and Devi 1990; Sharma et al. 1996). The essential oil from the leaves of *A. marmelos* is known to exhibited antifungal properties (Renu et al. 1986; Rana et al. 1997).

Keeping these facts in mind, in the present investigation, essential oil of *A. marmelos* was tested as an *in vivo* fumigant to protect stored gram from insect pest *Callosobruchus chinensis* (L.) (Bruchidae) and wheat from *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). The effect of *Aegle* oil applications on mortality, oviposition and adult development of *C. chinensis* has also been determined.

II. MATERIALS AND METHODS:-

Plant Collection

The plant material was collected from college garden. The leaves were collected by hand plucking and soaked in distilled water for the preparation of aqueous extract.

Rearing of test insects.

Rearing of *C. chinensis* was maintained on cowpea seeds (*Vigna unguiculata* (L.) Walp.) (Babu et al. 2003; Jenkins et al. 2003). *R. dominica*, and *S. oryzae* were maintained on wheat grains (*Titricum aestivum* (L.) while *T. castaneum* on wheat flour at 27± 2°C and RH 80 ± 5% (Perez-Mendoza et al., 2004). Forty adult insects were released separately in 200 g of commodities (wheat/cowpea/wheat flour) in plastic containers covered by muslin cloth. After 24 hours, adult insects were removed and the commodities were incubated in a temperature/humidity controlled cabinet (27± 20C and RH 80 ± 5%) in darkness to obtain same aged insects. Adult insects were 2- 4 days old when used in the bioassays.

Preparation of Aqueous Extract

About 500 g of the plant material was soaked for about 12 hours in 1 liter of distilled water for the preparation of the aqueous extract. The liquid extract was concentrated under reduced pressure in a rotary evaporator.

Chemical characterization.

The leaf extract of *A. marmelos* leaves was subjected to GC-MS analysis. GC-MS analysis was performed on Perkin Elmer Turbomass/Auto XL Instrument (Perkin Elmer, Inc. USA) at 70 eV with EQUITY-5 capillary column (60m X 0.32 mm X 0.25 μm). Helium was used as carrier gas @ 10 psi column head pressure. Injector and detector temperature were at 220 °C and 280°C, respectively. Split ratio was 1:50. The oven temperature was programmed from 100 to 280°C @ 3 °C/min with initial and final holdup time 2 min. The compounds were identified by comparing their retention times, mass spectra with authentic standards and the fragmentation patterns of the MS with NIST and Wiley GC-MS computer library of essential leaf extract.

Fumigation of wheat and gram samples by the essential leaf extract of *A. marmelos*.

The leaf extract of *A. marmelos* was used to fumigate the wheat and gram samples separately by the method adapted by Shaaya et al. (1997) and Kumar et al. (2007). Five hundred g of wheat samples (var. Malviya 234, 10.93 % moisture) were kept separately in closed plastic containers (35 cm diameter x 16 cm). Care was taken to use uninfested freshly harvested wheat grains. Twenty five individuals of each insect species, i.e. *R. dominica*, *S. oryzae* and *T. castaneum* of mixed sex were introduced in the containers. Requisite amount of the oil of *A. marmelos* was introduced separately in the plastic containers by soaking in cotton swab so as to procure concentration of 500 ppm. The containers were made airtight. The freshly harvested uninfested gram samples (var. PUSA 362, 10.75 % moisture) were also similarly provided with 25 individuals of *C. chinensis* and fumigated with the oil of *A. marmelos* at 500 ppm. The wheat and gram inoculated with the test insects without oil treatment served as controls. After 24 months of storage at laboratory conditions in a temperature/humidity control cabinet (27 ± 2°C and RH 80 ± 5%) in darkness. The efficacy of *A. marmelos* leaf extract on insect infestation was determined by calculating grain damage (%), weight loss (%) and feeding deterrence (%) of treated and control sets. The grain damage was determined by counting feeding injuries and emergence holes on the surface of the grains. The weight loss (%) of wheat and gram samples in the treated and control sets was calculated by fresh weight basis using the formula suggested by Parkin (1956).

$$Weight\ loss\ (\%) = \frac{\mathrm{WI} - \mathrm{W}}{\mathrm{WI}} \times 100$$

Where WI and W represents the weight of grains before and after the experiment, respectively
Feeding deterrence was calculated using the feeding deterrent index following Isman (1990):

$$\text{Feeding deterrent index (FDI) [\%]} = \frac{C - T}{C + T} \times 100$$

Where C and T is the weight loss in the controls and in the fumigated sets, respectively.

Effect of essential leaf extract on mortality, oviposition and adult development of bruchids on cow pea.

The leaf extract of *A. marmelos* was tested for its *in vivo* effects on insect mortality, oviposition and adult emergence on *C. chinensis* following Bright et al. (2001) and Kumar et al. (2007). A stock solution of the essential leaf extract was prepared by dissolving 100 µl of leaf extract in 1.9 ml of acetone. Twenty five seeds of cow pea (*Vigna unguiculata* (L.) Walp.) were filled in glass vials (6.3 X 2 cm diameter) and treated separately with different dose i.e. 100, 10, 1.0 and 0.1 µl of the leaf extract. The seeds were then dressed by continuous shaking for five minutes for proper mixing of the leaf extract on the seeds. For control sets the seeds were dressed in requisite amount of acetone in place of the leaf extact. The treated samples were kept in temperature-humidity control cabinet (27±2 °C and RH 80± 5%). After 24 hours five bruchids of mixed sex were introduced in each vial separately. Requisite control sets were kept for each treatment set. After 24 hours the mortality of insects was observed in each vial and all insects (live and dead) were removed. The number of eggs laid on seeds of treated and control seeds were counted after three days of starting the experiment. The number of adult insects emerged in cow pea samples in each treated and control set was counted after 30, 45 and 90 days of storage.

Statistical analysis.

All the experiments were repeated thrice and data was reported as mean value ± SE. The statistical analysis was performed by one way analysis of variance and means were compared by least significance difference test ($P< 0.05$) using the SPSS statistical software package (SPSS, ver. 10.0; Chicago. IL, USA). Further, the data was subjected to Student's 't' test to analyzed the effect of *Aegle* leaf extract on grain damage as well as weight loss of wheat and gram with control. The correlation coefficient was calculated between dose-mortality, dose-oviposition, dose-adult emergence, mortality-oviposition and oviposition-adult emergence using software Origin (Origin 6.0 Northampton, MA, USA).

III. RESULT:-

The GC-MS analysis of the *A. marmelos* leaf extract depicted the presence of following compounds viz. α pinene (0.28 %), sabinene (0.14 %), limonene (88.57 %), ocimene (2.29 %) and p caryophyllene (0.06 %). Limonene was found to be major component in *Aegle* leaf extract. As it evident from Table 1 that oil significantly protected stored gram from *C. chinensis* and wheat samples from *R. dominica and S. oryzae* for the first two years ($P<0.05$; LSD). The feeding deterrent index of the leaf extract for *C. chinensis*, *R. dominica* and *S. oryzae* was 91.51, 97.26 and 98.02 % respectively while it was -6.18 % against *T. castaneum*. There was 100 % grain damage in *T. castaneum* while 7.0, 3.67 and 1.67 % grain damage was found in *C. chinensis. R. dominica* and *S. oryzae* infested grains respectively. However, significant reduction in weight loss was found in fumigated gram and wheat against the test insects except *T. castaneum.*

The effect of *Aegle* leaf extract on mortality, oviposition and adult development of *C. chinensis* in treated cowpea samples is presented in Table 2. *C. chinensis* showed 71.41 % mortality at 100 µl dose of leaf extract. Oviposition deterrent activity of the oil for *C. chinensis* enhanced with dose. The oviposition was reduced to 56.25 % at 100 µl leaf extract dose. The reduction of hatching was also directly proportional to leaf extract dose. *Aegle* leaf extract checked more than 70 % of adult emergence of *C. chinensis* at different doses.

IV. DISCUSSION:-

In the present study the essential leaf extract of *A. marmelos* exhibited as botanical fumigant in protection of stored gram and wheat by enhancing feeding deterrence and reducing grain damage as well as weight loss of *C. chinensis, R. dominica* and *S. oryzae*. The findings are in accordance with Kumar et al. (2007) and Varma and Dubey (2001) who investigated that essential leaf extract of *Cymbopogon martinii*, *Caesulia axillaris* and *Mentha arvensis* protected stored gram and wheat from *C. chinensis, S. oryzae* and *T. castaneum* for first 12 months of storage. In the present investigation the shelf life of the *Aegle* leaf extract in protection of insect infestation was 24 months thus more than the leaf extract reported earlier. Plant leaf extracts are known to possess repellent, ovicidal and insecticidal activities against various stored grain insects (Hill and Schoonhoven 1981; Desmarchelier 1994). The adult mortality might be attributed to the contact toxicity or to the abrasive effect on the pest cuticle (Mathur et al. 1985), which might also interfere with the respiratory mechanism of insect (Schoonhoven 1978; Agarwal et al. 1988; Kim et al. 2003). Results of present and earlier studies indicate that essential leaf extract might be useful for managing coleopterous insects in enclosed spaces such as storage bins, glasshouse and buildings etc. because of their fumigant action. In addition, the interesting finding of the present study was the dissimilar performance of the *A. marmelos* leaf extract against the test insects as well as food commodities. The same dose level of *A. marmelos* leaf extract did not provide same level of protection against different commodities. The structure of the grains, texture, dust deposition and degree of adherence to the kernel affect the efficacy of insecticidal products (Subramanyam and Roseli 2000, Korunic 1997; Vayias and Athanassiou 2004). Thus, the dose should be prescribed according to the target species and the commodities.

In the present study the mortality, oviposition and adult emergence of *C. chinensis* were found vary significantly with different doses of *Aegle* leaf extract. 42 *Callosobruchus* females generally prefer smooth seed varieties for oviposition (Haines 1991). Therefore, the cow pea was selected for oviposition and adult.

Table1.

Fumigant efficacy of *A. marmelos* Leaf extract on stored gram and wheat infested with four insect pests at 500 ppm

Leaf extract	Gram			Wheat								
	C. chinensis			R. dominica			S. oryzae			T. castaneum		
	Grain Damage (%)	Weight loss (%)	FDI Index (%)	Grain Damage (%)	Weight loss (%)	FDI Index (%)	Grain Damage (%)	Weight loss (%)	FDI Index (%)	Grain Damage (%)	Weight loss (%)	FDI Index (%)
A. marmelos	7.00^{a} ± 0.30	2.64^{a} ± 0.21	91.51 ± 0.21	3.67^{a} ±0.33	0.72^{a} ±0.17	97.26 ±0.66	1.67^{a} ±0.30	0.49^{a} ±0.09	98.02 ±0.36	100.00^{a} ±0.00	54.80^{a} ±0.12	-6.18^{a} ±0.27
Control	100.00^{b} ±0.00	59.50^{b} ±0.29		100.00^{b} ±0.00	52.07^{b} ±0.46		100.00^{b} ±0.00	48.96^{b} ±0.15		100.00^{b} ±0.00	48.45^{b} ±0.32	

±: Standard error

Means within each column followed by the different letter are significantly different (P<0.05, Student's *t* test)

Table 2. Effect of *A. marmelos* leaf extract on adult mortality, oviposition and adult emergence in *C. chinensis* on treated cow pea

Dose of Leaf extract (μl)	Per cent adult mortality	Per cent reduction in egg number	Per cent reduction in adult Emergence (Days after treatment)		
			30 DAT	45 DAT	90 DAT
100	71.41 ± 7.10^{a}	56.25 ± 2.08^{a}	72.42 ± 0.15^{b}	62.68 ± 1.50^{a}	63.29 ± 1.30^{a}
10	49.99 ± 7.10^{ab}	47.92 ± 2.40^{a}	70.90 ± 1.82^{ab}	46.26 ± 2.60^{b}	41.76 ± 3.35^{b}
1.0	42.85 ± 7.10^{b}	34.03 ± 1.40^{b}	69.09 ± 3.35^{ab}	31.30 ± 3.00^{c}	37.97 ±3.34^{c}
0.1	14.28 ± 0.00^{c}	22.22 ± 3.02^{c}	65.45 ± 1.82^{a}	25.36± 1.50^{c}	25.31 ± 3.30^{c}

±: Standard error

DAT: Days after treatment

Means within each column followed by the different letter are significantly different (P<0.05, LSD).

emergence trails in place of gram. *C. chinensis* females laid significantly fewer eggs on cowpea treated with different doses of *Aegle* leaf extract than control sets, indicating that oil deterred *C. chinensis* females from ovipositing. Furthermore fewer insects were hatched in treated cowpea than the control at 30, 45 and 90 DAT (days after treatment). Since *C. chinensis* females deposit their eggs on the seed surface (Haines 1991), neonate larvae have to penetrate through the outer skin of the cowpea to reach the endosperm where the biologically active compounds from the *Aegle* leaf extract might have deterred them. The reduction in adult emergence could either be due to egg-mortality or larval mortality or even reduction in hatching of the eggs. Oviposition inhibitors have the advantage of attacking a pest at the start of its life cycle. The insect is deterred from laying its eggs on the foodstuff, thus preventing the pest population from increasing.

The insecticidal constituents of many essential oils are mainly monoterpenoids (Coats et al. 1991; Konstantopoulou et al. 1992; Regnault-Roger and Hamraoui 1995; Kim et al. 2003). Monoterpenoids have been reported to inhibit reproduction of stored insects at several steps of the cycle: inhibition in oviposition, ovicidal effects, a larvicidal effect on neonate larvae before the penetration of the seeds or a larvicidal effect on larvae settled within the seed, thus inhibiting the emergence of imagos. A strong inhibition of oviposition was produced by carvacrol, thymol and terpineol. α pinene strongly inhibited the larval penetration and less effective to oviposition inhibition against the storage insect pest, *Acanthoscelides obtectus* (Regnault-Roger and Hamraoui, 1995). In the present study limonene was the major component of the leaf extract of *A. marmelos* and it may be responsible for fumigant toxicity, oviposition deterrent, inhibition of adult development, and feeding-deterrent activities of test insects. The mode of toxicity for monoterpenoids is believed to be via competitive inhibition of acetylcholinesterase (Ryan and Byrne 1988).

Compounds with feeding deterrents are generally toxic to insects or cause physiological disturbances of development or oviposition (Nawrot and Harmantha 1994). For example azadirachtin is well known antifeedant and also interferes with insect growth (Siddig 1980). *A. marmelos* leaf extract was mixture of a number of components that would reduce the chances for the development of insect resistance, which could be due to the diffusion of the gene selection process (Begon et al. 1999; Davies 1992). Although animal toxicity with *A. marmelos* leaf extract were not conducted in the present study, however, concerns for residue of essential oil pesticides on food grains should be mitigated by the growing body of evidence that many essential constituents acquired through the diet are actually beneficial to human health (Huang et al. 1994).

V. SUMMARY

Aegle leaf extract may be used as botanical insecticide against different stored grain insect pests causing infestation in stored wheat and pulses. The *Aegle* leaf extract enhanced feeding deterrence of *C. chinensis*, *R. dominica* and *S. oryzae*. Therefore, insects were incapable to infest grain and to cause gain damage. *A. marmelos* significantly reduced oviposition and adult emergence of *C. chinensis* in treated cowpea. Moreover, because of the use in traditional medicine in cure of different human diseases, the *Aegle* leaf extract may be used as semiochemicals mediating phytopesticide to protect stored food commodities in developing countries, for which some farmers may not have easy access to chemical insecticides.

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