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Larvicidal Activity of Methanol Extract of Leaves of *Aegle marmelos* (L.) against Four Insect Pests Causing Severe Damage to Grains on Storage

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A B S T R A C T

The methanol extract from leaves of *Aegle marmelos* was investigated to control insect infestation of stored food commodities viz. maize, rice, cowpea and wheat flour during from *Ostrinia nubilalis*, *Spodoptera littoralis*, *Callosobruchus maculates* and *Tribolium confusum*. After introducing the test insects, stored food samples were fumigated with methanol extract from leaves of *Aegle marmelos* at 500 µg/mL (ppm). The methanol extract significantly enhanced feeding deterrence in insects and reduced the grain damage as well as weight loss in fumigated food samples infested with all insects. The methanol extract at different doses significantly reduced oviposition and adult emergence of these insect pests. Regression analysis of data on individuals in treated food samples confirmed that significant reduction of oviposition and adult emergence of *insect pests* decreased with increase in doses. At 10 ppm and after 72 hours of incubation *O. nibilalsis*, *S. littoralis*, *C. maculates* and *T. confusum* the hatching percentage was 80.85%, 79.85%, 81.07% and 78.81% respectively. The hatching of larvae showed a decreasing trend on increasing the concentration of methanol extract of *Aegle marmelos.* At 1000ppm concentration of leaf extract the hatching percentage of *O. nibilalsis*, *S. littoralis*, *C. maculates* and *T. confusum* was 36.85%, 36.50%, 34.08% and 35.24% respectively after 72 hours. At 1000ppm concentration of leaf extract the hatching percentage of *O. nibilalsis*, *S. littoralis*, *C. maculates* and *T. confusum* was 36.85%, 36.50%, 34.08% and 35.24% respectively after 72 hours. The survival percentage of the larvae of all the four insect pests decreased with concentration of methanol extract and with incubation period. At 1000ppm concentration of methanol extract of *Aegle marmelos* the larvae of these insect pests could not survive at any incubation time. 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.

Introduction

Aegle marmelos (L.) is a spinous deciduous and aromatic tree of family Rutaceae with long, strong and axillary spines. This tree grows up to 18mt in height and thickness of tree is about 3- 4ft. Leaves are tri- to pentafoliate, leaflets are ovate and have

typical aroma. Flowers are greenish white in colour and sweet scented. Fruits are large, woody, grayish yellow, 8- 15 celled and have sweet gummy orange coloured pulp. Seeds are compressed, oblong and numerous found in aromatic pulp.

Aegle marmelos (L.) Correa, commonly known as Bael, is a sacred tree for Hindu Religion, 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 viz. 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). Besides the medicinal uses, this plant was also studied for their antimicrobial, antifungal and insecticidal properties (Satyal *et al.,* 2012; Kumar *et al.,* 2008). The effect of leaf extracts of *Aegle marmelos* was also studied against *Anopheles subpictus* (Grassi, 1899) in their oviposition deterrent, ovicidal and repellent activities (Elango *et al.,* 2009). Larvicidal activity of the essential oil of *Aegle marmelos* was also reported against *Culex pipiens* (L., 1758) and *Aedes aegypti* (Vineetha *et al.,* 2009; Kumar *et al.,* 2008).

Cereals and pulses which have great biological and nutritional value in developing countries, are lost up to 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.

In the present investigation the ovicidal and larvicidal activity of methanol extract of leaves of *Aegle marmelos* (L.) were assayed on the Lepidopteran insects viz. *Ostrinia nubilalis* and *Spodoptera littoralis* and Coleopteran insects viz. *Callosobruchus maculatus* and *Tribolium confusum*. These

insects are known to severely damage crops like maize (*Zea mays*), rice (*Oryza sativa*), cotton (*Gossypium herbaceum*), tobacco (*Nicotiana tabaccum*), soybean (*Glycine max*), cowpea (*Vigna unguiculata)*, rice (*Oryza sativa*), wheat (*Triticum aestivum*) and wheat flour on storage.

Materials and Methods

The lepidopteran insects like *Ostrinia nubilalsis* (Europeon corn borer) and *Spodoptera littoralis* (Cotton leaf worm) were reared and maintained on maize and rice grains respectively. Similarly, Coleopteran insects *Callosobruchus maculates* (Cowpea seed beetle) and *Tribolium confusum* (confused flour beetle) were reared and maintained on cowpea (*Vigna unguiculata*) grains and wheat flour respectively. The insects were maintained and reared at 27±2^{0}C and relative humidity (RH) of 80±5% following the methods suggested by Babu *et al.,* 2003; Jenkins *et al.,* 2003; Perez-Mendoza *et al.,* 2004; Arivoli *et al.,* 2011.

Forty adult insects were released separately in 200 g of commodities (maize/rice/ 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± 2^{0}C and RH 80 ± 5%) in darkness to obtain same aged insects. Adult insects were 2- 4 days old when used in the bioassays. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Pupae were transferred to a disposable cup and it is kept inside the cage.

Preparation of methanol extract

Freshly harvested leaves of *Aegle marmelos* were washed under running tap water, blotted with filter paper and dried in shade at room temperature. The dried plant sample was soaked with absolute methanol under reflux condition for the methanolic extract preparation. The sample was homogenized with extraction buffer. The supernatant was collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40^{0}C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The extract was then stored in deep freezer at -20^{0}C until further test.

Fumigation of maize, rice, cowpea and wheat flour by methanol extract of *Aegle marmelos*

The methanol extract of leaves of *A. marmelos* was used to fumigate the Maize, Rice, Cowpea grains and wheat flour samples separately by the method adapted by Shaaya *et al.,* (1997) and Kumar *et al.,* (2007). Five hundred g of samples of each were kept separately in closed plastic containers (35 cm diameter x 16 cm). Care was taken to use uninfested freshly harvested grains and freshly prepared wheat flour. Twenty five individuals of each insect species viz. *Ostrinia nubilalis, Spodoptera littoralis, Callosobruchus maculates* and *Tribolium confusum* of mixed sex were introduced in the containers. Requisite amount of the methanol extract of *A. marmelos* was introduced separately in the plastic containers of each of the varieties by soaking in cotton swab so as to procure concentration of 500 ppm. The containers were made airtight.

The grains and flour inoculated with the test insects without methanol extract 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* 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 samples in the treated and control sets was calculated by fresh weight basis using the formula suggested by Parkin (1956).

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

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.

Mosquito ovicidal bioassay

The ovicidal bioassay was performed according to the method described by Tennyson *et al.,* (2011) and Puspanathan *et al., (2006)* with little modifications. For the ovicidal bioassay, 50 eggs of each species were transferred to each of the three replicates of each concentration. Eggs were exposed to the DMSO and water was treated as control. For determination of LC50 values, a wide numbers of concentrations of the oils were tested against the target species. The number of eggs hatched in control and treatments were recorded and the percentage of ovicidal activity was calculated by the following formula-

$$\text{Percent ovicidal activity} = \frac{\text{Percent of eggs hatched in control} - \text{Percent of eggs hatched in treated}}{\text{Percent of eggs hatched in control}} \times 100$$

Larvicidal bioassay

Screening of the efficacy of methanol extract of *Aegle marmelos* was done by performing bioassay studies against different developmental stages of *Ostrinia nubilalis, Spodoptera littoralis, Callosobruchus maculates and tribolium confusum*. The larvicidal activity of individual extract was assayed following the technique described earlier by Tong *et al.,* (2013) and WHO guidelines (2005). According to the WHO protocol for larvicides testing for laboratory testing, batches of 20 numbers of healthy 4th instar larvae of each species were transferred to the disposable glasses with the depth between 5-10cm having 100ml of water. A series of concentration from the 1000ppm to 10 ppm were used to examine the larvicidal toxicity of the oil. The LC50 values are recorded after 24, 48 and 72 hour exposure. Each concentration was assayed in triplicate along with one negative control group in water and one positive control group with the DMSO. If the pupation occurred in the exposure time or more than 10% larva was died in the control group, the test was repeated. From the data, LC50 values were determined by probit analysis (SPSS 16).

Statistical analysis

All the experiments were conducted in replicates of three and data was recorded 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 marmelos extract* on grain damage as well as weight loss of grains and flour 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).

The data were corrected for the mortalities with the help of Abbott correction factor and were subjected to probit analysis using SPSS software to estimate LC50 values of effective methanol extract against the mosquito. Again, if mortality in the controls was found above 5%, results with the treated samples were corrected using Abbott's formula (Abbott, 1925).

The mortality (%) was corrected using Abbott's formula (1925):

$$Pr = \frac{Po - Pc}{100 - Pc} \times 100$$

Where, Pr = Corrected mortality (%), Po = Observed mortality (%), Pc = Mortality in the control (%).

The results obtained have been presented in Table 1–14.

Results and Discussion

The fumigation efficacy of methanol extract of leaves of *Aegle marmelos* against four insect pests has been presented in Table 1 to 4. From the results it is evident that the methanol extract of leaves of *Aegle marmelos* significantly protected all the four food commodities viz. Maize, Rice, Cowpea and Wheat flour on storage from insect pests such as *Ostrinus nubilalsis, Spodoptera littoralis, Callosobruchus maculatus* and *Tribolium confusum* ($p<0.05$; LSD) (Table 1-4). The feeding deterent index (FDI) of *O. nubilalsis, S. littoralis, C. maculatus* and *T. confusum* on Maize infestation was 92.35%, 97.36%, 96.25% and 98.02% respectively; on Rice infestation was 87.50%, 92.16%, 96.25% and 97.05% respectively; on Cowpea infestation was 90.47%, 96.31%, 92.51% and 97.05% respectively and on Wheat flour infestation was 95.25%, 91.21%, 97.35% and 90.71% respectively.

There was 100% damage of all the four food commodities on storage in control experiment i.e. not fumigated with methanol extract of *Aegle marmekos*. In control experiment there was maximum loss in weight of food commodities.

In case of Maize the loss was 47.75% to 59.50%; of Rice it was 47.45 to 60.48%; of Cowpea 28.45% to 60.49% and of Wheat flour 48.51% to 60.65% (Table 1- 4). All the four insect pests caused minimum damage to food commodities when fumigated with methanol extract of leaves of *Aegle marmelos*.

The damage to maize grains by *O. nubilalsis, Spodoptera littoralis, C. Callosobruchus maculatus* and *Tribolium confusum* was 8.25%, 8.15%3.65% and 1.68% respectively (Table 1). Similarly, the damage to rice grains by *O. nubilalsis, Spodoptera littoralis, Callosobruchus maculatus* and *Tribolium confusum* was 5.25%, 7.25%, 3.65% and 1.68% respectively (Table 2).

A more or less similar pattern of damage was noticed in cowpea and wheat flour by these insect pests after fumigation with methanol extract of *Aegle marmelos* (Table 3 and 4).

In the present study the methanol leaf extract of *A. marmelos* exhibited as botanical fumigant in protection of stored maize, rice, cowpea and wheat flour by enhancing feeding deterrence and reducing grain damage as well

as weight loss of *Ostrinus nubilalsis, Spodoptera littoralis, Callosobruchus maculatus* and *Tribolium confusum*. The findings are in accordance with Kumar *et al.*, (2007) and Varma and Dubey (2001) who investigated that essential oil 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* extract in protection of insect infestation was 24 months thus more than the extract reported earlier.

Plant extracts and essential oils are known to possess repellent, ovicidal and insecticidal activities against various stored grain insects (Hill and Schoonhoven, 1981; Desmarchelier, 1994).

The present findings are also in agreement with the work of Rajesh *et al.*, (2008) who have observed insecticidal activity *Aegle marmelos* essential oil against four stored grain insect pests viz. *Callosobruchus chinensis, Rhyzopertha dominica,Sitophilus oryza*, and *Tribolium castaneum.*

In the present investigation the ovicidal activity of the methanol extract of leaves of *Aegle marmelos* against four insect pests pest, no hatching of larvae were observed till 24 hours. Hatching of larvae were observed from 24 hours to 72 hours.

No further hatching was recorded after 72 hour of treatment. Therefore LC50 value of ovicidal activity was recorded at 72 hour of exposure period (Table 5). At 10 ppm and after 72 hours of *incubation O. nibilalsis, S. littoralis, C. maculatus* and *T. confusum* the hatching percentage was 80.85%, 79.85%, 81.07% and 78.81% respectively. The hatching of larvae showed a decreasing trend on increasing the concentration of methanol extract of *Aegle marmelos*. At 1000ppm concentration of leaf extract the hatching percentage of *O. nibilalsis, S. littoralis, C. maculatus* and *T. confusum* was 36.85%, 36.50%, 34.08% and 35.24% respectively after 72 hours. The LC50 value along with the regression equation is listed in Table 6-9.

The survivability of larvae of these insect pests has been indicated in Table 10. From the result it is evident that the larvae of all the four insect pests viz. *Ostrinus nubilalsis, Spodoptera littoralis, Callosobruchus maculates* and *Tribolium confusum* survived at 10 ppm concentration of methanol extract even after 72 hours of incubation. The survival percentage of the larvae of all the four insect pests decreased with increased concentration of methanol extract and with incubation period. At 1000ppm concentration of methanol extract of *Aegle marmelos* the larvae of these insect pests could not survive at any incubation time (Table 10).

In the present investigation the larvicidal activity of methanol extract of leaves of *Aegle marmelos* was studied against four insect pests viz. *Ostrinus nubilalsis, Spodoptera littoralis, Callosobruchus maculatus* and *Tribolium confusum*. It was found that the larval mortality was directly related to the exposure time and concentration of the methanol extract (Table 11-14).

For *Ostrinus lubilalsis* the LC50 values of the methanol extract at 24h, 48h and 72h was recorded as 185.70ppm, 167.43ppm and 122.45ppm respectively (Table 11). For *S. littoralis* it was 184.68ppm, 166.43ppm and 124.45ppm respectively (Table 12); for *C. maculatus* 185.50ppm, 165.43ppm and 125.55ppm respectively (Table 13) and for *T. confusum* 181.50ppm, 165.43ppm and 126.45ppm respectively (Table 14). The values of sub lethal concentrations are presented in Table 6-9.

Table.1 Fumigant efficacy of methanol extract of *Aegle marmelos* on stored maize against four insect pests at 500ppm

Treatment	*O. nubilalis*			*S. littoralis*			*C. maculatus*			*T. confusum*		
	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)
Extract	8.25^{a} ±0.31	3.26^{a} ±0.21	92.35 ±0.70	8.15^{a} ±0.31	0.71^{a} ±0.34	97.36 ±0.18	3.65^{a} ±0.21	0.70^{a} ±0.71	96.25	1.68^{a} ±0.31	0.48^{a} ±0.15	98.02 ±0.35
control	100.00^{b} ±0.00	59.50^{b} ±0.31		100.00^{b} ±0.00	53.15^{b} ±0.41		100.00^{b} ±0.00	48.75^{b} ±0.13		100.00^{a} ±0.00	47.75^{b} ±0.32	

±: Standard Error; Means within each column followed by different letter are significantly different (*P<0.05, student's t test*)

Table.2 Fumigant efficacy of methanol extract of *Aegle marmelos* on stored Rice against four insect pests at 500ppm

Treatment	*O. nubilalis*			*S. littoralis*			*C. maculatus*			*T. confusum*		
	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)
Extract	5.25^{a} ±0.31	2.65^{a} ±0.21	87.50 ±0.69	7.25^{a} ±0.26	3.15^{a} ±0.25	92.16 ±0.61	3.65^{a} ±0.21	0.71^{a} ±0.31	96.25	1.68^{a} ±0.32	0.51^{a} ±0.31	97.05 ±0.32
control	100.00^{b} ±0.00	60.48^{b} ±0.27		100.00^{b} ±0.00	51.05^{b} ±0.41		100.00^{b} ±0.00	47.95^{b} ±0.12		100.00^{a} ±0.00	47.45^{b} ±0.30	

±: Standard Error; Means within each column followed by different letter are significantly different (*P<0.05, student's t test*)

Table.3 Fumigant efficacy of methanol extract of *Aegle marmelos* on stored cowpea against four insect pests at 500ppm

Treatment	*O. nubilalis*			*S. littoralis*			*C. maculatus*			*T. confusum*		
	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)
Extract	3.25^{a} ±0.31	0.57^{a} ±0.23	90.47 ±0.67	2.15^{a} ±0.25	0.52^{a} ±0.25	96.31 ±0.64	8.55^{a} ±0.21	3.25^{a} ±0.33	92.51	1.75^{a} ±0.31	0.53^{a} ±0.30	97.05 ±0.31
control	100.00^{b} ±0.00	60.49^{b} ±0.23		100.00^{b} ±0.00	28.45^{b} ±0.44		100.00^{b} ±0.00	35.50^{b} ±0.16		100.00^{a} ±0.00	38.32^{b} ±0.22	

±: Standard Error; Means within each column followed by different letter are significantly different (*P<0.05, student's t test*)

Table.4 Fumigant efficacy of methanol extract of *Aegle marmelos* on stored wheat flour against four insect pests at 500ppm

Treatment	O. nubilalis			S. littoralis			C. maculatus			T. confusum		
	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)	Grain damage (%)	Weight loss (%)	FDI (%)
Extract	4.67[a] ±0.31	0.75[a] ±0.21	95.25 ±0.65	3.65[a] ±0.23	0.70[a] ±0.25	91.21 ±0.63	2.67[a] ±0.22	0.75[a] ±0.31	97.35	9.25[a] ±0.30	4.65[a] ±0.31	90.71 ±0.31
control	100.00[b] ±0.00	60.65[b] ±0.27		100.00[b] ±0.00	52.05[b] ±0.42		100.00[b] ±0.00	51.05[b] ±0.17		100.00[a] ±0.00	48.51[b] ±0.21	

±: Standard Error; Means within each column followed by different letter are significantly different (*P<0.05, student's t test*)

Table.5 Hatching percentage of eggs of four insect pests after treatment of different concentration of *Aegle marmelos* leaf extract

No. of individuals	Conc. (ppm)	*O. nubilalis*			*S. littoralis*			*C. maculatus*			*T. confusum*		
150		24 hour	48 hour	72 hour	24 hour	48 hour	72 hour	24 hour	48 hour	72 hour	24 hour	48 hour	72 hour
	10	50.75 ±1.17	80.40 ±1.15	80.85 ±1.17	48.75 ±1.26	79.40 ±1.27	79.85 ±1.07	51.65 ±1.19	75.45 ±1.27	81.07 ±1.31	46.60 ±1.09	78.41 ±1.25	78.81 ±1.17
	100	41.25 ±1.15	63.04 ±1.16	62.46 ±1.17	38.25 ±1.27	61.05 ±1.37	62.08 ±1.08	43.45 ±1.14	59.15 ±1.16	63.41 ±1.32	36.15 ±1.15	60.07 ±1.32	63.05 ±1.17
	500	27.15 ±1.13	35.50 ±1.14	40.15 ±1.17	25.16 ±1.19	35.06 ±1.15	40.10 ±1.21	30.17 ±1.16	36.41 ±1.10	34.25 ±1.30	23.15 ±1.17	34.07 ±1.31	41.15 ±1.17
	1000	19.15 ±1.12	28.65 ±1.17	36.85 ±1.17	21.45 ±1.18	26.61 ±1.12	36.50 ±1.17	17.00 ±1.17	26.11 ±1.17	34.08 ±1.18	20.35 ±1.18	24.50 ±1.14	35.24 ±1.37

Table.6 Ovicidal activity of *Aegle marmelos* extract against *Ostrinia nubilalis*

Ostrinia nubilalis					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
72 hours	279.85	Y = 3.95 + 0.45X	Lower Bound	Upper Bound	71.85
			.343	.516	

Table.7 Ovicidal activity of *Aegle marmelos* extract against *Spodoptera littoralis*

Spodoptera littoralis					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
72 hours	277.85	Y = 3.94 + 0.42X	Lower Bound	Upper Bound	71.83
			.341	.514	

Table.8 Ovicidal activity of *Aegle marmelos* extract against *Callosobruchus maculatus*

Spodoptera littoralis					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
72 hours	281.87	Y = 3.96 + 0.44X	Lower Bound	Upper Bound	72.81
			.343	.516	

Table.9 Ovicidal activity of *Aegle marmelos* extract against *Tribolium confusum*

Tribolium confusum					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
72 hours	276.85	Y = 3.93 + 0.41X	Lower Bound	Upper Bound	71.76
			.341	.513	

Table.10 Survivability larvae of four insect pests after treatment of different concentration of *Aegle marmelos* leaf extract

No. of individuals	**Conc. (ppm)**	***O. nubilalis***			***S. littoralis***			***C. maculatus***			***T. confusum***		
60		24 hour	48 hour	72 hour	24 hour	48 hour	72 hour	24 hour	48 hour	72 hour	24 hour	48 hour	72 hour
	10	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0	100.00	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0	100.00 ±0.0
	100	80.21 ±1.15	70.05 ±1.16	50.15 ±1.17	82.25 ±1.27	75.15 ±1.37	50.08 ±1.08	82.45 ±1.14	72.15± 1.16	51.41 ±1.32	80.05 ±1.15	71.07 ±1.32	50.05 ±1.17
	500	20.15 ±1.13	12.50 ±1.14	10.15 ±1.17	27.16 ±1.19	15.16 ±1.15	12.10 ±1.21	25.17 ±1.16	11.41± 1.10	9.25 ±1.30	30.00 ±1.17	10.07 ±1.31	9.15 ±1.17
	1000	0.0	0.0	0.0	0.0	0.0	0.0	10.00 ±1.17	0.0	0.0	10.0 ±1.18	0.0	0.0

Table.11 Larvicidal activity of *Aegle marmelos* extract against *Ostrinia nubilalis*

Ostrinia nubilalis					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
24 hours	185.70	Y =- 1.53 + 2.85X	Lower Bound	Upper Bound	7.85
			1.986	3.064	
48 hours	168.43	Y = -0.62 + 2.53X	1.95	3.08	9.05
72 hours	122.45	Y = -0.18 + 2.44X	1.76	2.81	5.87

Table.12 Larvicidal activity of *Aegle marmelos* extract against *Spodoptera littoralis*

Spodoptera littoralis					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
24 hours	184.68	Y =- 1.51 + 2.82X	Lower Bound	Upper Bound	7.83
			1.885	3.055	
48 hours	166.43	Y = -0.61 + 2.51X	1.92	3.05	8.05
72 hours	124.45	Y = -0.19 + 2.44X	1.77	2.83	5.89

Table.13 Larvicidal activity of Aegle marmelos extract against *Callosobruchus maculatus*

Callosobruchus maculates					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
24 hours	185.50	Y =- 1.55 + 2.87X	Lower Bound	Upper Bound	7.75
			1.97	3.06	
48 hours	165.43	Y = -0.63 + 2.54X	1.94	3.07	7.05
72 hours	125.55	Y = -0.21 + 2.46X	1.78	2.85	6.87

Table.14 Larvicidal activity of *Aegle marmelos* extract against *Tribolium confusum*

Tribolium confusum					
Time	LC50	Regression Equation	95% confidence level		Chi- square value
24 hours	181.70	Y =- 1.50 + 2.85X	Lower Bound	Upper Bound	7.55
			1.92	3.05	
48 hours	163.43	Y = -0.60 + 2.50X	1.92	3.04	7.05
72 hours	126.45	Y = -0.28 + 2.53X	1.79	2.85	6.85

The extract from fresh leaves of *Aegle marmelos* contains α-pinene, β-myrcene, Bicyclo (3.1.0) hexane, 4- methyl-1-(1-methylethyl, β- terpinyl acetate, β-linalool, 2-cyclohexen-1-ol, 1-methyl-4-(1-methylethyl), Cis-verbenol, crypton, Cis- sabinol, Cis-carveol, 5- isopropenyl-2- methyl-7-oxabicyclo(4.1.0) hepten-2-ol, 3- tetradecyn-1-ol, camphenol, 2,3- pinanediol, 5-isopropenyl-2-methyl-7-oxabicyclo (4.1.0) heptan-2-ol, Dipentene oxide, 2,3-pinanediol, Piperitone oxide, Trans-3(10)-caren-2-ol, Bicyclo-(3.1.0) hexane-6-methanol,2-hydroxy-1.4.4-trimethyl, Caryophyllene oxide and 12-oxabicyclo(9,1,0)dodeca-3,7-diene 1,5,5,8- tetramethyl as carried out by GC- MS technique by Riju shrama *et al.,* (2017).

In the present study, the methanol extract from the leaves of *Aegle marmelos* exhibited insecticidal activities against different developmental stages of the four target insect pests viz. *Ostrinus nubilalsis, Spodoptera littoralis*, *Callosobruchus maculatus* and *Tribolium confusum*. The findings revealed variation in the potentiality of the methanol extract in different development stages of the four insect pests.

The important factors responsible for the insecticidal effect are the major compounds present in the essential oil including its quality and quantity (Suthanont *et al.,* 2010). The compounds including alcohols, aldehydes, fatty acid derivatives, terpenoids, and phenolics may jointly or independently contribute to insecticidal, repellent as well as antifeeding activities (Park and Shin, 2005). The toxicity of essential oil may be attributed to their major constituents (Gleiser and Zygadlo, 2009). Report of GC-MS analysis of *Aegle marmelos* oil in the current study showed β- terpinyl acetate, 5- isopropenyl-2-methyl-7-oxabicyclo (4.1.0) hepten-2-ol and 2, 3-pinanediol as probable major compounds. This result is highly differing from lots of previous studies conducted in other parts of India which implied limonene as the major compound (Satyal *et al.,* 2012; Kumar *et al.,* 2008; Kaur *et al.,* 2006; Garg *et al.,* 2006). Additional study on the same essential oil in other parts of India reported presence of phellandrene, myrcene and eucalyptol as major compound. Again, study on the same essential oil in Egypt by Ibrahim *et al.,* (2015) reported γ- cadinene as major compound. Geographic locations, climatic condition, method of extraction, time of harvesting are some of the factors which influence those variations of the profile of constituents of an essential oil (Ngassapa *et al.,* 2003; Din *et al.,* 2011).

Therefore, extract of *A. marmelos* which is inexpensive, easily available at farm level, environmentally safe with low mammalian toxicity can be recommended as a good alternative to synthetic insecticides against the insect pests.

The findings of present investigation emphasize the efficacy of *Aegle marmelos* as potent ovicide and larvicide against *Ostrinus nubilalsis, Spodoptera littoralis*, *Callosobruchus maculatus* and *Tribolium confusum***.** But further studies regarding the mode of action and field application are necessary to provide a futuristic lead product from *Aegle marmelos* for insect pest control. In summary, *Aegle* marmelos extract may be used as botanical insecticide against different stored grain insect pests causing infestation in stored maize, rice, cowpea and wheat flour. The *Aegle* marmelos extract enhanced feeding deterrence of *Ostrinus nubilalsis*, *Spodoptera littoralis, Callosobruchus maculatus* and *Tribolium confusum*. Therefore, insects were incapable to infest grain and to cause gain damage. *A. marmelos* significantly reduced oviposition and adult emergence from eggs. Moreover, because of the use in traditional medicine in cure of different human diseases,

the *Aegle* oil may be used as semi chemicals 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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