

## Evaluation of the toxicity of three plant extracts against the Khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae) under laboratory conditions

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### Evaluación de la toxicidad de tres extractos de plantas contra el escarabajo Khapra *Trogoderma granarium* Everts (Coleoptera: Dermestidae) en condiciones de laboratorio

**RESUMEN.** Este estudio se realizó para evaluar la toxicidad de extractos acuosos, etanólicos y acetónicos de *Lantana camara* L. (Verbenaceae), *Ruta chalepensis* L. (Rutaceae) y *Rhazya stricta* Decne (Apocynaceae), contra larvas del escarabajo Khapra, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), alimentándose de semillas de trigo después de 2, 4 y 6 días (d). Estas plantas comúnmente crecen en condiciones áridas y semiáridas y pueden proporcionar nuevos insecticidas naturales contra las larvas de *T. granarium*. Según los resultados, los extractos de plantas mostraron niveles variables de toxicidad, y los extractos acetónicos proporcionaron la mayor eficacia. El extracto acetónico de *L. camara* mostró un mayor efecto de toxicidad, con LC<sub>50</sub> de 330.6 ppm (después de 2-d) y 110 ppm (6-d), en comparación con 467 ppm (2-d) y 251 ppm (6-d) utilizando *R. stricta*, y 576 ppm (2-d) y 317 ppm (6-d) con *R. chalepensis*. En general, la toxicidad de los extractos acetónicos fue aproximadamente 1,3 veces mayor en comparación con la de extractos acuosos o etanólicos en todo el rango de concentraciones probadas (50-400 ppm). Los extractos acetónicos requirieron seis días para lograr  $\geq 80\%$  de mortalidad de larvas. En conclusión, este estudio sugiere que el extracto acetónico de *L. camara*, *R. chalepensis* y *R. stricta* podría usarse como un método sostenible para controlar el escarabajo Khapra, plaga de granos almacenados.

**PALABRAS CLAVE.** Bioensayo. Biopesticida. *Lantana camara*. *Rhazya stricta*. *Ruta chalepensis*.

**ABSTRACT.** This study was conducted to evaluate the toxicity of aqueous, ethanolic and acetonetic extracts of *Lantana camara* L. (Verbenaceae), *Ruta chalepensis* L. (Rutaceae) and *Rhazya stricta* Decne (Apocynaceae), against larvae of Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), feeding on wheat seeds after 2, 4, and 6 days (d). These plants commonly grow in arid and semi-arid conditions and may provide novel natural insecticides against larvae of *T. granarium*. Results showed that the plant extracts demonstrated varying levels of toxicities, with the acetonetic extracts providing the greatest efficacy. The acetonetic extract of *L. camara* demonstrated a higher toxicity effect, with LC<sub>50</sub> of 330.6 ppm (after 2-d) and 110 ppm (6-d), compared to 467 ppm (2-d) and 251 ppm (6-d) for *R. stricta*, and 576 ppm (2-d) and 317 ppm (6-d) for *R. chalepensis*. Overall, toxicity of acetonetic extracts was about 1.3 fold greater compared with that of aqueous or ethanolic extracts throughout the range of concentrations tested (50-400 ppm). The acetonetic extracts required six days to achieve  $\geq 80\%$  mortality of larvae. In conclusion, this study suggests

that the acetic extract of *L. camara*, *R. chalepensis* and *R. stricta* could be used as a sustainable method for controlling Khapra beetle, pest of stored grains.

**KEYWORDS.** Bioassay. Biopesticide. *Lantana camara*. *Rhazya stricta*. *Ruta chalepensis*.

## INTRODUCTION

Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is one of the most destructive pests of stored grain products (Dwivedi & Shekhawat, 2004; Omar et al., 2012). It can cause the direct loss of stored grains by feeding as well as allowing colonization of damaged grains by secondary pests including other insects and fungi, further deteriorating grain quality (Banks, 1977). Management of this pest is difficult because its larvae feed inside the grains, reducing their exposure to direct insecticidal treatments (Omar et al., 2012). Nevertheless, the protection of stored grains from insect damage currently relies on applying synthetic pesticides, such as fumigation with phosphine or methyl bromide, or dust compounds (Tsumura et al., 1994; Price & Mills, 1998). The widespread use of synthetic pesticides against such pests that attack stored grains have led to the development of insecticide resistance (White, 1995), increased costs and hazards of control and handling, and increased insecticide residues on grains, which can pose risks to human health (Fishwick, 1988). There is a growing awareness of these risks, which has led to the search for safer new methods to control pests of stored-products (Silver, 1994; Mohammed et al., 2019). One such alternative is the use of natural plant products that have insecticidal activity. Natural plant products tend to have low mammalian toxicity, little environmental effect and a wide public acceptance (Odeyemi et al., 2008; Mahmoud et al., 2015; Khaliq et al., 2018).

Several plant extracts have been shown to be effective against stored-product insects (Omar et al., 2012). *Lantana camara* L. (Verbenaceae) grows widely throughout the tropical, sub-tropical and temperate parts of the world (Pung & Srimongkolchai, 2011). Several studies showed that leaves of *L. camara* are a source of insecticidal compounds (Ogendo et al., 2003; Dua et al., 2010), and preliminary studies indicated that the leaves of *L. camara* possess a rich variety of bioactive molecules such as flavonoids, alkaloids, polyphenols and tannins (Sharma et al., 1988; Iqbal et al., 2006), and show promise as a source for new bio-pesticides (Rajashakar et al., 2014). Extracts from seeds and leaves of *Rhazya stricta* Decne (Apocynaceae) were found to inhibit feeding, metamorphosis, fecundity and oviposition, and cause diverse behavioral and physiological disorders for many insects (Ascher, 1993; Chen et al., 1996). The herbaceous plants *R. stricta* and *Ruta chalepensis* L. (Rutaceae) are widely distributed in

the semi-arid and tropical areas, including Saudi Arabia (Migahid, 1978). These plants are known to possess insecticidal activity (Elhag et al., 1996), mammalian toxicity (Adam, 1998), and traditional medicinal value (Al-Yahia et al., 1990). The herbaceous plant *R. stricta* has rich different types of alkaloids (Ahmad et al., 1983), among which many indole alkaloids have cytotoxic activities (Kamil et al., 2000). Abdellaoui et al. (2016) reported that methanolic extract of *R. chalepensis* has a negative effect on feeding and reproductive activities of *Locusta migratoria* L. (Orthoptera: Acrididae). Mejri et al. (2013) showed that this plant had bioactive compounds such as alkaloids, flavonoids, coumarins, tannins, volatile oil, glycosides and terpenes.

Searching for potential bio-pesticides extracted from widely distributed plants adapted to arid and semi-arid conditions against the most destructive pests of stored grain products is the current focus of our research. This study evaluates the insecticidal activity of the extracts of (*L. camara*, *R. stricta* and *R. chalepensis*) against Khapra beetle larvae.

## MATERIAL AND METHODS

### Insect Culture

Khapra beetle adult individuals were collected from wheat flours sold in local grain stores in Jeddah, Saudi Arabia, and identified to species level using identification keys (Beal, 1954, 2003; Halstead, 1986). Laboratory colonies were established and maintained on a wheat flour in glass jars at  $25 \pm 2$  °C and  $65 \pm 5$  % (RH). Each colony was initiated with 80 pairs of adult beetles. These jars were covered with muslin cloth and rubber bands.

### Plant Collection, Identification and Extraction

Aerial parts of *L. camara*, *R. stricta* and *R. chalepensis* were collected from different areas around Riyadh, Saudi Arabia, including Al-Duwadimi, Al-Aflaj and Shagra. These plants were grown without any treatment of pesticides. The collected plants were identified by the specialist staff in the Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University. The collected plants were washed with fresh water and air-dried in a dark laboratory for three weeks, then, ground to a fine powder with electric blender. A 50 g sample of ground powder from each plant species was suspended in 100 ml of distilled water, ethanol or acetone (99.99%) in a conical flask. Flasks were kept at room temperature

for 10 days and covered with aluminum foil. The mixtures were shaken vigorously at 12-hour intervals to ensure proper soaking of the plant products. After ten days, each extract was filtered before drying by evaporation. The residue was weighted and re-dissolved in the 100 ml of corresponding appropriate solvent (water, ethanol and acetone) again for ten days. The resulting mixture was filtered through a double layer of filter paper (Whatman No. 1, GE healthcare UK Limited Buckinghamshire, UK) and the resulting liquid was evaporated using a rotary evaporator at 30 - 40 °C and 3 - 6 rpm for 8 h. The resulting materials were air dried to remove remaining solvents. Stock solutions of plant material extracts were prepared by re-dissolving the extract solids in warm distilled water (0.5 g/500 ml). Experimental solutions with differing concentration of 50, 100, 200 and 400 ppm were prepared from stock solutions.

### Bioassay treatments

Uninfested wheat seeds (15 g) were obtained from the Agronomy Laboratory in the Arid Land Agriculture Department, King Abdulaziz University, Jeddah, Saudi Arabia, and submerged for 1 min in 10 ml of each of the four concentrations of each extract. Seeds were then placed on filter papers in Petri dishes and left to dry before use. Control treatments were prepared by submerging wheat seeds into distilled water. Groups of 30 third-instar larvae were collected from the laboratory colony. Each cohort was introduced to one of the treated groups of seeds under laboratory conditions. The experiment was conducted in the Plant Protection Laboratory at 25 ± 2 °C and 65 ± 5% (RH) in the Arid Land Agriculture Department. The experiment was of a factorial design with three replicates for each concentration of each plant material extract. Larval mortalities were determined at 2, 4 and 6 days (d) after exposure.

### Data analyses

The percentages of Khapra beetles' larvae mortalities were calculated by the following formula:

$$\text{Mortality \%} = (\text{number of dead larvae} / \text{number of introduced larvae}) \times 100$$

The calculated percentages of larvae were subjected to the repeated measures ANOVA within a SPSS version 2.0 (IBM Corporation, 2011). Prior to performing the repeated measures ANOVA on the response variable (larval mortality percentage), the arcsine ( $x/100$ ) was applied to transform all calculated percentages to meet the assumptions of normality and homogeneity of variance. After this, repeated measures ANOVA were performed to assess the effect of the following factors: time (repeated factor), concentration of solvents, plant type, and all interactions on larval mortality. Significance of multi-factor interactions on mortality outputs was tested using Wilks' lambda ( $\lambda$ ) test statistics. If a repeated measure ANOVA is used, the sphericity of the

variance-covariance matrix should be starting by Mauchly's W statistic test. If the assumption of sphericity of the data was not violated, the effect of the repeated measures factor (time) was tested using the F value generated by Sphericity Assumed. However, the figure in this study illustrates untransformed means and standard errors to simplify interpretation and is considered significantly different at  $P \leq 0.05$ . Where significant treatment effects were found, the Fisher's Least Significant Difference (LSD) tests were performed to identify differences in treatment means.

To calculate the lethal concentration demanded to cause 50% mortalities ( $LC_{50}$ ) of larvae, Maximum Likelihood Procedures and Probit analysis (Finney, 1971) were applied by using GW-Basic Software (GW Basic, 1985). Significant differences among  $LC_{50}$  estimates were indicated by failure of 95% CI to overlap.

## RESULTS AND DISCUSSION

All tested plant extracts were toxic to Khapra beetle larvae in a dose-dependent manner, although the extracts of *R. chalepensis* and *R. stricta*, were slow to take effect and their efficacies varied (Table I; Fig. 1). Extract of *L. camara* was the most effective, where the ethanolic and acetonic extracts caused mortality rates of 73.3 and 83% at 400 ppm after 2 d, respectively, and 86.7 and 90% mortalities after 6 d, respectively (Fig. 1). ANOVA test showed higher significant differences among the three investigated plants (Table I) in which the highest percentage mortalities of larvae were caused by extracts of *L. camara* followed by those of *R. stricta* and *R. chalepensis* (Fig. 1). The effectiveness of *L. camara* extract seen in this study may be explained by the presence of a bio-pesticide in this plant. This is consistent with the study of Mvumi & Maunga (2018) who indicated that leaves of *Lantana* have some toxic properties and may be a potential source of biopesticide for use in pest control strategies against aphids with economic and environmental benefits. In addition, there were significant differences among the concentrations of solvents on the percentage mortalities of Khapra beetles larvae (Table I) showing the acetonic extract was the best solvent to produce toxic compounds compared with other solvents (Fig. 1). Remarkably, ANOVA test indicated that the larval mortality depended on the exposure of time, due to significant differences between investigated times on the percentage mortalities (Table I; Fig. 1). The ANOVA test showed a highly significant effect of the interaction between the concentrations of solvents and the exposure times on the mortality percentages (Table I; Fig. 1). However, there were no significant differences when the concentrations of solvents interacted with the used plants (Table I). Mortality percentages were significantly affected by the interaction between plants, the time of exposure, and the solvent concentrations (Table I; Fig. 1).

The acetonic extracts of the three plant extracts were

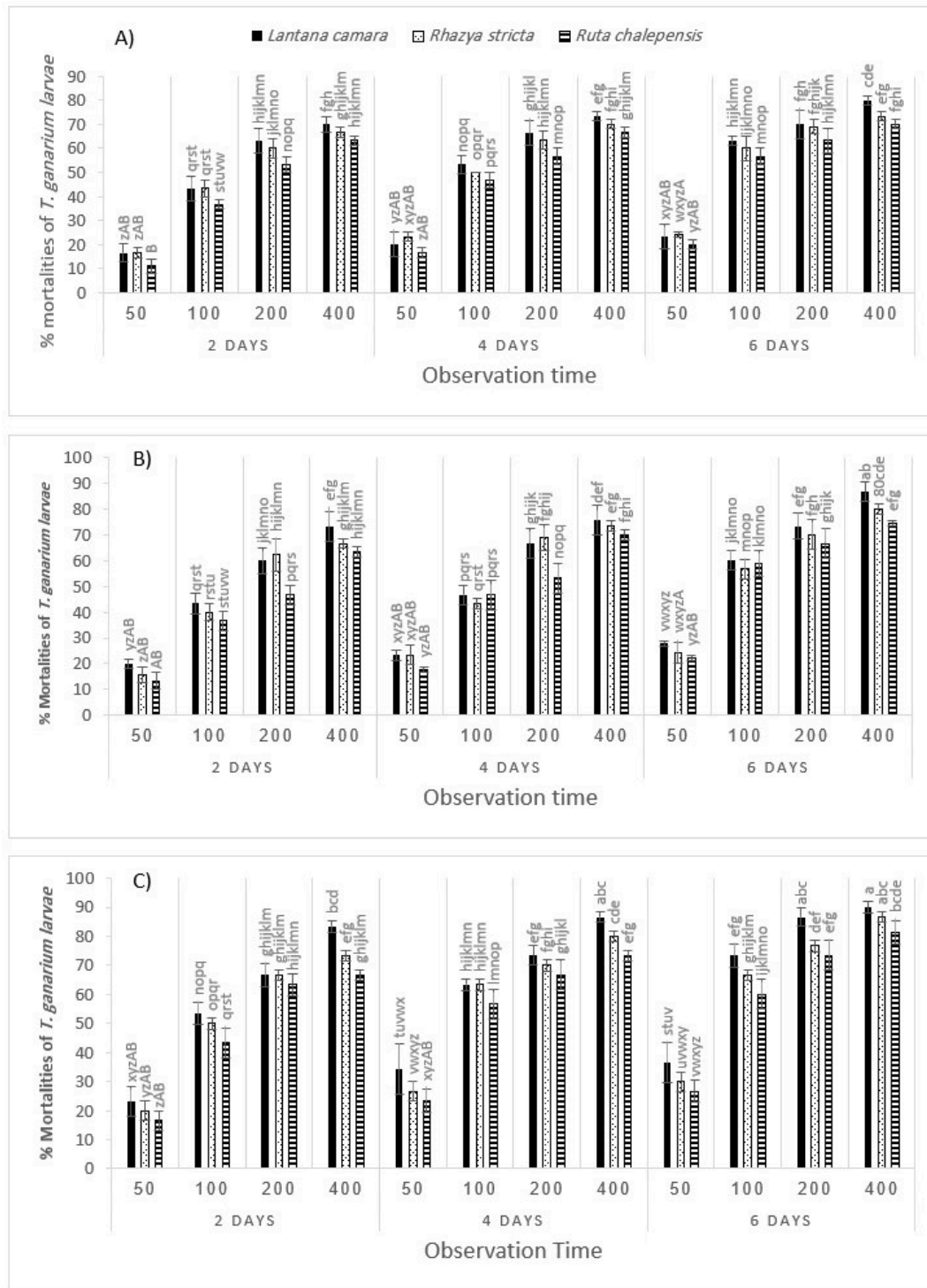


Fig. 1. Percentage means and SE of mortalities of *Trogoderma granarium* larvae after 2, 4 and 6 days of exposure to: A) aqueous, B) ethanolic, and C) acetic extracts of the three plants. Means with the same letter have not significant differences ( $t > 0.05$ ), according to LSD test.

Factors	Degree-of-freedom (factor, error)	F value	P value
Solvent concentration (C)	11	91.04	<0.001
Plant (P)	2	16.56	<0.001
Observation time (T)	2	938.69	<0.001
C * P	22	0.447	0.982
C * T	22	11.59	<0.001
P * T	4	4.41	0.003
C * P * T	44	2.90	<0.001
Error	72		

**Table I. Result of the repeated-measures ANOVA on the percent mortality of Khapra beetle larvae exposed to selected plant extracts.**

generally more toxic than either the aqueous or ethanolic extracts. However, LC<sub>50</sub> values showed that the acetonic extract from *L. camara* was more toxic than other plant extracts in which the acetonic extract solvent had lowest LC<sub>50</sub> values that were calculated as 330 ppm after 2 d and 110.6 ppm after 6 d (Table II).

The ethanolic and aqueous extracts of *L. camara* had LC<sub>50</sub> values of 410.1 ppm, 180.6; and 434.4 ppm, 202.3 ppm after 2 d and 6 d, respectively (Table II). Interestingly, the acetonic/ethanolic or acetonic/aqueous LC<sub>50</sub> ratios for all materials at all levels were consistently 0.6-0.7 and 0.6-0.8, respectively, indicating that the acetonic extracts were mostly 1.3 fold more toxic than either the ethanolic or aqueous extracts (Table III). Mostafa et al. (2012) studied the effect of some plant extracts from *Tamarindus indica* L. (Fabales: Fabaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae), *Eucalyptus* spp. (Myrtales: Myrtaceae), *Switenia mahagoni* (L.) Jacq (Sapindales: Meliaceae) and *Psidium guajava* L. (Myrtales: Myrtaceae) leaves against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). They concluded that the presence of different classes of bioactive compounds such as steroids, phenolic compounds and tannins in different plants extracts used in their study could be responsible for the toxicity against *T. castaneum*.

This study did not determine the mode of action of the tested plant extracts. Several studies documented that extracts of *L. camara* have antifeeding repellent and toxic effect against termites and stored product insects (Yuan & Hu, 2012; Rajasheker et al., 2014). Treating insect with *R. stricta* extract can cripple insect feeding by making the treated food unpalatable; and consequently, insect growth, survival and reproduction are adversely affected (Saxena, 1987). The mortality due to the *R. stricta* extract observed in our study could be a consequence of starvation, due to its antifeeding effect,

rather than a toxic effect. Alqurashi & Bakhshwain (2010) reported that *R. stricta* is toxic to the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) when administered at concentration >400 ppm. Similarly, Viglianco et al. (2008) observed an anti-feeding effect with the chloroform extract of *Aloysia polystachia* (Griseb) (Verbenaceae) against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). However, the toxic effect of *R. stricta* has previously been observed on mosquito larvae (Elhag et al., 1996), *Agrotis ipsilon* Hufn. (Lepidoptera: Noctuidae) and *Hypera brunneipennis* (Boheman) (Coleoptera: Curculionidae) (Elhag et al., 1998). Although the toxic mode of action of *R. stricta* on insects is unknown, it may be attributed to its high content of biologically active alkaloids (Hassan et al., 1997).

Our data shows that Khapra beetles' larvae were less susceptible to death with *R. chalepensis* extracts compared to *L. camara* and *R. stricta* showing the highest mortality percentages, 70, 73.3 and 81.1% after 6 days at 400 ppm for aqueous, ethanolic and acetonic extracts, respectively. This finding is consistent with the results of Alvi et al. (2018) who showed that increasing concentration of leaf and seed acetonic extracts of *R. stricta* caused a higher mortality and repellency of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *T. granarium* under laboratory conditions. Majdoub et al. (2014) found that *R. chalepensis* essential oil a toxic in high concentration against *T. castaneum* (LC<sub>50</sub> = 176.075 µl/l air).

A striking observation on the three plant extracts investigated in the present research was that the length of exposure time of all extracts resulted in increased mortality, indicating that larvae cannot tolerate long exposures to such extracts. Rajapakse (2006) and Ali et al. (2018) were able to obtain a protection against insect pests of stored products by using plant products. They concluded that plant extracts of *Neem A. indica* are more effective than that of *Datura stramonium* L. (Solanales: Solanaceae), due to the repellent and toxicant action against *T. granarium*. The investigated plant extracts in this study could have a practical application in the protection of stored grains against *T. granarium*, due to the environmental safety, low mammalian toxicity, low costs and easy handling of these plant extracts. In conclusion, this study suggests that there is a potential bio-pesticide in the acetonic extract of *L. camara* which warrants further investigation and the identification of the active compounds is required.



Plant species	Assay	LC <sub>50</sub> (95% confidence limits) and extract		
	Time (days)	Aqueous	Ethanolic	Acetonic
<i>Lantana camara</i> L.	2	434.4 (403-768) (1.4±3.9) *	410.1 (401-722) (1.4±3.6)	330.0 (270-528) (1.5±3.3)
	4	305.4 (325-609) (1.4±0.06)	290.2 (222-614) (1.4±2.5)	237.8 (201-383) (1.5±2.7)
	6	202.3 (159-484) (1.4±3.4)	180.6 (156-419) (1.5±3.6)	110.6 (90-194) (1.8±3.2)
<i>Rhazya stricta</i> Decne	2	510.7 (439-877) (1.4±2.2)	480.9 (401-757) (1.5±3.3)	390.7 (301-637) (1.4±1.6)
	4	402.5 (329-688) (1.2±1.8)	327.9 (268-619) (1.3±2.4)	260.7 (201-341) (1.4±1.6)
	6	260.2 (157-504) (1.3±1.7)	230.6 (202-419) (1.4±3.5)	190.8 (108-210) (1.5±1.9)
<i>Ruta chalepensis</i> L.	2	558.0 (496-801) (1.2±2.2)	510.8 (412-711) (1.6±2.4)	445.9 (311-509) (1.4±2.0)
	4	443.7(328-682) (1.3±2.4)	365.9 (210-610) (1.5±2.3)	281.5 (186-472) (1.3±4.3)
	6	317.5 (151-422) (1.3±2.0)	274.7 (126-342) (1.6±2.2)	217.4 (111-242) (1.4±2.5)

**Table II. LC<sub>50</sub> values of *Trogoderma granarium* larvae exposed to three plants extracts.** \*Numbers between lower brackets are the slopes of regression ± SE equation of response (y) on log dose (x) lines.

Plant species	Assay Time (days)	LC <sub>50</sub> ratio	
		Aqueous/Acetonic	Ethanollic/ Acetonic
<i>Lantana camara</i> L.	2	1.32	1.24
	4	1.28	1.22
	6	1.83	1.63
<i>Rhazya stricta</i> Decne	2	1.31	1.23
	4	1.54	1.26
	6	1.36	1.21
<i>Ruta chalepensis</i> L.	2	1.25	1.15
	4	1.58	1.30
	6	1.46	1.26

**Table III. LC<sub>50</sub> ratio of aqueous or ethanolic extract to acetonic extract.**

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