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Insecticidal Efficacy of Wild Medicinal Plants, *Dhatura alba* and *Calotropis procera*, against *Trogoderma granarium* (Everts) in Wheat Store Grains

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Authors' Contribution
SAK did the experiments and wrote the manuscript. MHR, AA and MS conceived and designed the experiments. AAK wrote the manuscript and analyzed the Data. ZH edited the manuscript.

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Medicinal plants, Biopesticides, Khapra beetle, Store grains.

ABSTRACT

Trogoderma granarium (Everts) is one of the world's most destructive pests of cereals, oilseed, malting, and other foodstuffs. Current studies were aimed to investigate the effect of essential oils of two different medicinal plants (*Dhatura alba* and *Calotropis procera*) against two different strains of *T. granarium*. Plant extracts were obtained by Rotary Shaker apparatus by using acetone as solvent. Four different concentrations 5%, 10%, 15% and 20% were prepared by diluting in acetone. The data regarding mortality, growth regulation and repellency of *T. granarium* was observed. Repellency of plant extracts was tested using area preference method while for the growth regulatory potential of plant extracts, the adults insects were released in wheat grains treated with different concentrations of plant extracts. All the experiments were carried out in an incubator at 30±2°C and 65±5% R.H using Completely Randomized Design (CRD). The results for repellency remained more significant for *C. procera* extract as compared to *D. alba*. While for growth the *C. procera* showed more significant differences. In case of mortality both *D. alba* and *C. procera* were found equally effective against *T. granarium*.

INTRODUCTION

About 90% grains formed for regular use of human and animals come from cereals like rice, wheat and maize (McFarlane, 1989), whereas insect pests are of an excessive worth for highest causes of loss of grains throughout the storage (Scotti, 1978). Khapra beetle (*Trogoderma granarium*) is one of the most destructive insect pests of stored grains. It is economically important pest causing both qualitative and quantitative losses due to its huge population, in addition to changing the grain into frass and damages may be too much (up to 70%). Thus it is necessary to control this pest by using suitable methods like suing biotic and a-biotic factors, but the most usable and famous/common method for controlling is use of insecticides (Haq *et al*., 2014). But the problem comes up in way that this pest creates resistance against insecticides and damage remains same instead of control. The notable reasons of resistance are survival of this pest long time without food in minimal moisture contents and its cryptic nature of the larvae that live in cervices and cracks (Eliopoulos, 2013). Larvae of *T. granarium* cause severe damage after come out from diapause when it gets suitable conditions. The damaged seeds become unhealthy and found unfit for human use (Arain *et al*., 2006). *Trogoderma granarium* infested grains lost their market value, because larvae during feeding consume quite a lot of nutrients (Jood and Kapoor, 1993). The series of losses take place due to infestation of *T. granarium* over a period of 1 to 10 months in the range from 0.2 to 2.9% (Irshad *et al*., 1988).

Many practices have been evaluated to manage this pest including chemicals as most common and cheap method but the chemicals are not environment friendly and have bad impacts on grain quality and leave many poisonous ingredients in grains (Champ and Dyte, 1977; Georghiou and Tejeda, 1991). Beside their harmful effects toward human health, these chemicals have been noticed to be resistance against stored grain insect pests so, required to be replaced with some alternate sources of control. The numerous plants are neem, eucalyptus, dhatura, chrysanthemum, moringa, ginger, capsicum and many other weeds like Aak weed and many other naturally occurring plants are included. It is need of the day that, the reduced use of hard chemistry insecticides and their integration with botanicals and other softer chemistries may be included in IPM modules (Khan *et al*., 2014; Khaliq

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et al., 2014). The representative samples of *T. granarium* from different geographical region are found to be sensitive to esfenvelarate (synthetic pyrethroid). However it is dependent on enzymatic activities and doses of applied chemicals (Shakoori *et al*., 2018). Currently a wide range of botanicals have been discovered which are eco-friendly and have no side effects on grain's life and human health (Regnault-Roger *et al*., 1993; Park *et al*., 2003). Plant source are getting better attention as prophylactic measures for the control of pests in storage, due to safety to other than target pests (Jood *et al*., 1996). Insecticidal effects of many plants beside pests of stored grains have been established. Many plants show their physiological and behavioral activities like; contact, repellent and antifeedant, against pests (Grainge and Ahmed, 1998). Botanical insecticides are one of the most imperative groups of harmless and effective insecticides which are minus toxic to mammalian and are eco-friendly. Naturally occurring botanicals and medicinal plants are found to be cheaper, easily available and quite incorporable in the integrated-pest management packages. Moreover, the industrialized nations of the world are also stressing upon the adoption of organic farming for the preservation of the world health as well as for the development of sustainable agriculture. Therefore, it was important to exploit some botanicals for the organic control of stored-food insect pests. The current study was aimed to investigate two strains (Bhakkar and Faisalabad) of *Trogoderma granarium* by using *Dhatura alba* and *Calotropis procera* to observe their repellency, growth regulation and mortality of *T. granarium*.

MATERIALS AND METHODS

The present study was carried out in the Grain Research, Training and Storage Management Cell (GRTSMC) of the Department of Entomology, University of Agriculture Faisalabad during 2015-2016.

Collection of the insects

The adults and larvae of *T. granarium* were collected from grain market of Faisalabad district and were reared in laboratory to get homogenous population for further use in experiments.

Rearing and colony maintenance of insects

The insect culture was maintained in earthen pots (2.5 kg capacity) and plastic containers (1.5 kg). The pots were covered with organdy cloth, tightened with rubber bands to avoid the escape of insects. The insects were allowed to multiply in natural environment as in traditional structure and observed at regular intervals. The food medium was sterilized in an oven for 28 h at 60°C and thoroughly washed thereafter with water to remove any contamination or other insects present in the materials and carefully dried under sunlight, ensuring 13-14 % moisture content. The sterilized food was then preserved in air tight glass jars (1000 ml) in order to impede further infestation. Newly emerged 3rd instar larvae of *T. granarium* were used in the experiments.

Collection and preparation of botanical extract

The leaves of *Dhatora alba*, and *Calotropis procera* were collected from range land areas of district Bhakkar (Punjab). The leaves of tested plants were washed out using tape water. After shade drying the leaves were grinded in electrical grinder to bring these in the form of powder. The leaf extraction was made using Soxhlet's Extraction Apparatus by dipping 50 g of powder in 250 ml acetone, following procedure as described by Valladares *et al*. (1997) and Ahmad *et al*. (2006). Chemical extracts obtained were saved in clean and airtight lid glass flasks. The samples were stored in the refrigerator at 4°C for further use.

Insect bioassays

From the stock solution, various concentrations (5, 10, 15 and 20%) of each extract were prepared by using acetone as a diluting solvent. The prepared concentrations of these extracts were applied on sterilized wheat. After the evaporation of the solvent the air dried wheat (200g) was transferred into the treatment jars. The bioassays for each treatment were replicated thrice.

For repellency bioassay, same concentrations were applied on filter paper and twenty larvae of *T. granarium* were used for each treatment. The data was observed with the interval of 24 h and percent repellency was calculated. For insecticidal bioactivity and growth regulatory studies 30 grubs were released in sterilized glass jars containing wheat grains. Data for percent mortality was recorded with the interval of 24, 48 and 72 h and data regarding larval duration, percent pupation and percent adult emergence were also recorded with same pattern.

Statistical analysis

Before the analysis of data, normality was assessed using Kolmogrorov-Smirnov normality tests. Parametric tests were performed on normal data. The effect of different concentrations and plant extracts were analyzed with ANOVA using GLM and means were compared with Tuckey's HSD test at 5%. All statistical analyses were performed using computer software Stastix 8.1®.

RESULTS

The mean mortality for *D. alba* in Bhakkar strains

was maximum (56.96) at 20% concentration and the minimum mortality (14.71) at 5%. Which were statistically different from other two concentrations 10% and 15% having mortalities (24.27) and (38.83), respectively. While *C. procera* showed maximum mortality (55.96) at 20% concentration and the minimum mean mortality (12.71) at 5%. Which were statistically at par with concentrations 10% having mean mortality (24.27) and 10% concentration was statistically at par 15% concentration having mortality (42.38). The mean mortality of Faisalabad strains according to different concentrations of *D. alba* the maximum mean mortality (57.44) was found at 20% concentration and the minimum mean mortality (15.44). Which were statistically different from other two concentrations 10% and 15% having mortalities (33.16) and (42.87) respectively but these two concentrations was not significantly different. While the data regarding mean mortality according to different concentrations of *C. procera* in Faisalabad strains the maximum mean mortality (57.44) was found at 20% concentration and the minimum mean mortality (15.84). Which were statistically at par with concentrations 10% having mean mortality (33.16) and 10% concentration was statistically at par 15% concentration having mortality (42.87) (Fig. 1).

The mean repellency of different concentrations of *D. alba* in Faisalabad strains was significantly differed from each other. The highest repellency showed by the 5% concentration which's mean was 41.49 followed by 15% concentration with mean value of 25.76 and at 10% concentration mean repellency was 40.97and at 20% concentration the mean repellency was 16.02. While the mean repellency of different concentrations of *C. procera* was significantly differed from each other. The highest repellency showed by the 5% concentration with mean of 51.49 followed by 15% concentration with mean value of 35.76 and at 10% concentration mean repellency was 50.97 and at 20% concentration the mean repellency was 26.02. The mean repellency of different concentrations of *D. alba* in Bhakkar strains was significantly differed from each other. The highest repellency showed at 20% concentration (mean 51) followed by 15% concentration (mean 50), at 10%, 5% concentration the mean repellency was 35 and 26 respectively. However, the mean repellency of different concentrations of *C. procera* was significantly differed from each other. The highest repellency showed at 20% concentration (mean 50) followed by 15% concentration (mean 34), at 10%, 5% concentration the mean repellency was 48.33 and 25, respectively (Fig. 1).

The mean mortality by *D. alba* in Bhakkar strains was observed for different time intervals. Maximum mean mortality (36.93) was found in 72 h while exposure time 24 h gave minimum mean mortality (25.2). Which have no significantly differed to other time interval that was 48 hours having mean mortality (32.5); while for *C. procera* the maximum mean mortality (32.53) was found at 72 h of observation, while exposure time 24 h gave minimum mean mortality (25.28). Which have significant difference with other time interval that was 48 h having mean mortality (36.93). Data regarding the mean mortality by *D. alba* in

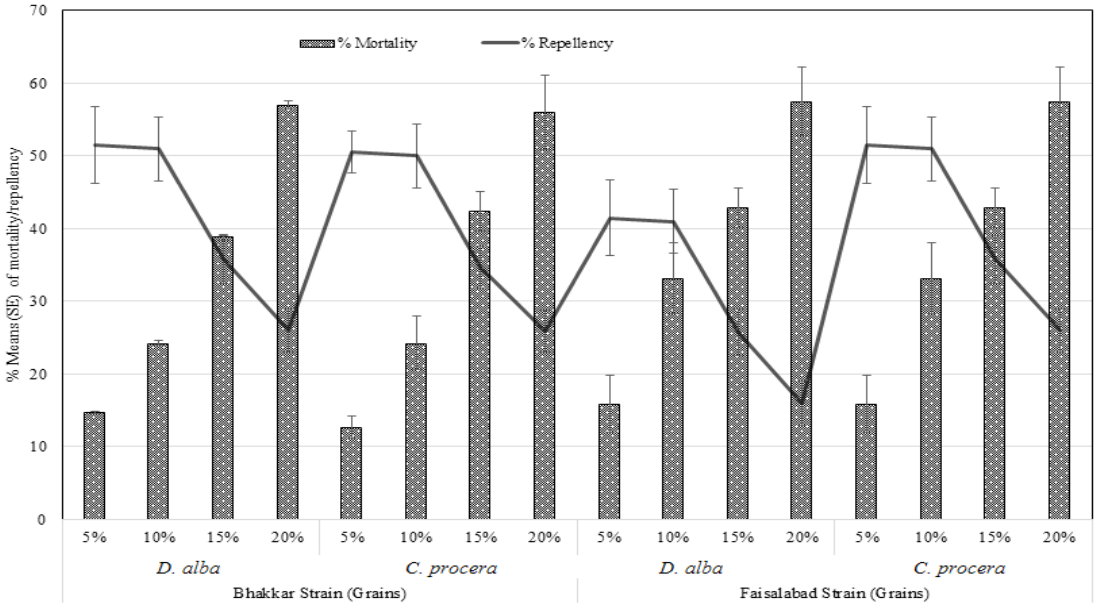

Fig. 1. Percent mortality and repellency of *T. granarium* by different concentrations of *D. alba* and *C. procera.*

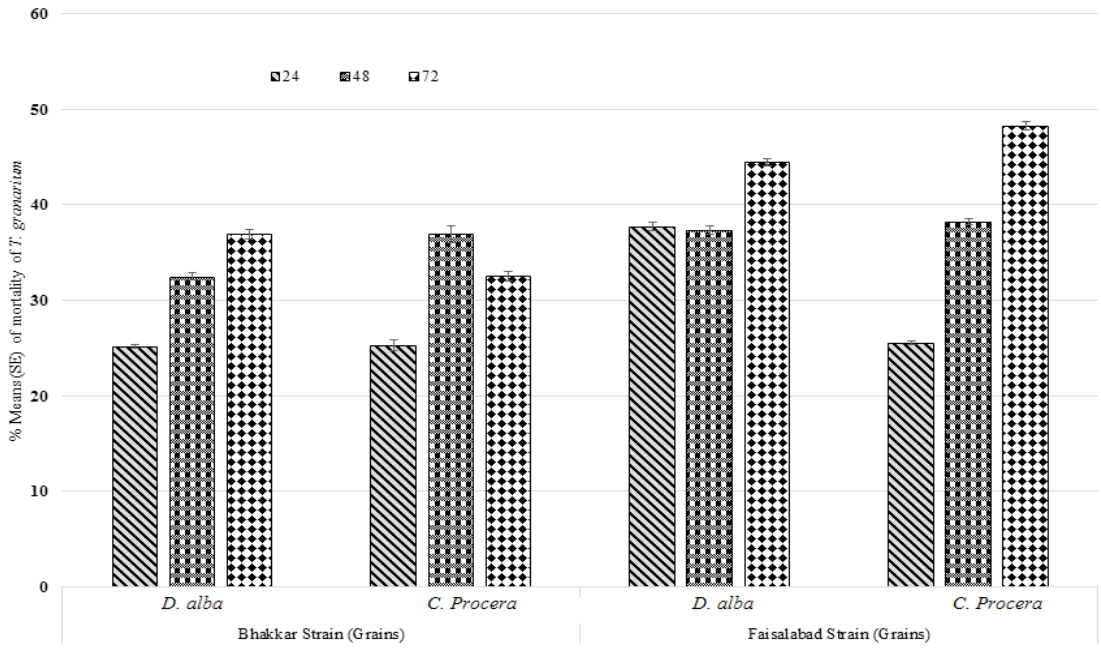

Fig. 2. Percent mortality of *T. granarium* by different exposure times of 15 % concentration of *D. alba* and *C. procera* extracts.

Table I.- Percentage of pupation and adult eclosion of *T. granarium* by different concentrations of *D. alba* and *C. procera* extracts.

Treatments/ Stage/ Concentration	Bhakkar strain (grains)				Faisalabad strain (grains)			
	D. alba		*C. procera*		*D. alba*		*C. procera*	
	Pupation (%)	Adult eclosion (%)	Pupation (%)	Adult eclosion (%)	Pupation (%)	Adult eclosion (%)	Pupation (%)	Adult eclosion (%)
5 %	77.78±1.11d	68.89±1.11c	83.33±1.92d	77.78±2.94d	70.78±1.11d	58.89±1.11c	83.33±1.92d	77.78±2.94d
10 %	65.56±2.94c	55.56±1.11b	75.56±1.11c	68.89±1.11c	60.56±2.94c	45.56±1.11b	75.56±1.11c	68.89±1.11c
15 %	57.78±1.11b	52.22±1.11b	66.67±1.92b	58.89±1.11b	50.78±1.11b	42.22±1.11b	66.67±1.92b	58.89±1.11b
20 %	51.11±1.11a	41.11±1.11a	56.67±1.92a	48.89±1.11a	43.11±1.11a	31.11±1.11a	56.67±1.92a	48.89±1.11a

The values in columns with different lettering are significantly different at P<0.05.

Faisalabad strains observed for different time intervals. Maximum mean mortality (44.48) was found in 72 h while exposure time 24 h showed minimum mean mortality (37.33). Which have significantly no difference with other time interval *i.e.* 48 h having mean mortality (37.72). For *C. procera* the mean mortality was maximum (48.28) was found at 72 h while exposure time 24 h provided minimum mean mortality (25.54). Which have significant difference with 48 h observations having mean mortality (38.18) (Fig. 2).

The mean percent pupation by *D. alba* in Bhakkar strains of different concentrations was significantly differed from each other. The highest percent pupation showed @ 5% concentration (mean 77.78%) followed by 10% concentration (mean 65.56%) and @ 15% , 20% concentration the percent pupation was 57.78% and 51.11%, respectively; while the mean percent pupation by *C. procera* of different concentrations was significantly differed from each other. The highest percent pupation showed @ 5% concentration (mean 83.33%) followed by 10% concentration (mean 75.56%) and @ 15%, 10 % concentration the percent pupation was 66.67% and 56.67%, respectively. Same trend was also found for *D. alba* and *C. procera* in Faisalabad strains. For both botanicals *i.e. D. alba* and *C. procera* and in two different strains of *T. granarium* at different concentrations were significantly differed from each other as shown in Table I.

DISCUSSION

The maximum repellency showed in Bhakkar strains for extracts comparisons of *D. alba* and lesser was observed when treated with different concentrations of *C. procera*. For time intervals check mortality the maximum repellency showed by *D. alba*. The current observations are showing that with increased concentrations, the repellency also increased and vice versa. The assorted behavioral and physiological effects such as repellency shown in this study, and toxicity, oviposition deterrence and reproduction inhibition are in agreement with Bodnaryk *et al*. (1999) who suggested the active principles may constitute a complex of chemicals while applications are done with different concentrations and observed at different time intervals. Delobel *et al*. (1999) have isolated a 37 amino acid protein from pea seed that are toxic to *Sitophilus* spp. However, they have not determined if it is also repellent to stored-product insects. For growth regulation of *T. granarium* a 70 days process was done for both strains. The results for Bhakkar strain were maximum for *C. procera* and also in Faisalabad strain but different in counts, which are concede with those of El-Nadi *et al*. (2001) and Al-Moajel (2004).

CONCLUSION

It is concluded that the tested biochemicals may be utilized as alternative to hard chemistries in insect control modules of stored grains. In addition to that, their less persistency may lead to safe protection of stored products.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Ahmad, U.A., Zuha, M.S., Nabil, H.H., Bashier, H.H., Muafi, K., Zhongping, H. and Youling, G., 2006. Evaluation of insecticidal potentialities of aqueous extracts from *Calotropis procera* Ait. against *Henosepilachna elaterii* Rossi. *J. appl. Sci*., **6**: 2466-2470.

Al-Moajel, N.H., 2004. Testing some various botanical powders for protection of wheat grain against *Trogoderma granarium* Everts. *J. biol. Sci*., **4**: 592-597. https://doi.org/10.3923/jbs.2004.592.597

Arain, M., Ahmad, A.T. and Afzal, M., 2006. Preliminary studies on khapra beetle *Trogoderma granarium* Everts. infertation in wheat under lab condition. *Pak. Entomol*., **28**: 27-29.

Bodnaryk, R., Fields, P., Xie, Y. and Fulcher, K., 1999. Insecticidal factors from field pea. *U.S. Patents*, **5**: 820-955.

Champ, B.R. and Dyte, C.E., 1977. FAO global survey of pesticide susceptibility of stored grain pests. *FAO Pl. Protec. Bull.,* **25**: 49-67.

Delobel, B., Grenier, A., Gueguen, J., Ferrasson, E. and Mbailao, M., 1999. *Utilisation d'un polypeptide derive´ d'unealbumine PA1B de le´gumineuse comme insecticide*. French Patent Pending.

El-Nadi, A.H., Elhag, E.A., Zaitoon, A.A. and Al-Doghairi, M.A., 2001. Toxicity of three plants extract to *Trogoderma granarium* Everts (Coleoptera : Dermestidae). *Pak. J. biol. Sci.,* **4**: 1503-1505. https://doi.org/10.3923/pjbs.2001.1503.1505

Elipoulos, P.A., 2013. New approaches for tackling the Khapra beetle. *CAB Int. Rev*., **8**: 1-13. https://doi.org/10.1079/PAVSNNR20138012

Georghiou, G.P. and Tejeda, L.A., 1991. T*he occurrence of resistance to pesticides in arthropods*. FAO, Rome.

Grainge, M. and Ahmed, S., 1988. *Hand book of plants with pest-control properties*. Wiley-Interscience, New York, pp. 273-282.

Haq, Z.M., Afzal, M., Khan, A.A., Raza, M.A., Irfanullah, M., Khan, M.A. and Kamran, M., 2014. Impact of phytopesticides on *Trogoderma granarium* Everts (Coleoptera: Dermestidae) in stored wheat. *World appl. Sci. J*., **31**: 1722-1733.

Irshad, M., Khan, A. and Baloch, U.K., 1988. Losses in wheat in public sector storage in Rawalpindi region during 1984-1985. *Pak. J. Agric.,* **9**: 136-140.

Jood, S. and Kapoor, A.C., 1993. Protein and uric acid contents of cereals grains as affected by insect infestation. *Fd. Chem*., **46**: 143-146. https://doi.org/10.1016/0308-8146(93)90027-D

Jood, S., Kapoor, A.L. and Singh, R., 1996. Evaluation of some plant products against *Trogoderma granarium* (Everts) in Sorghum and their effects on nutritional composition and organoleptic characteristics. *J. Stored Prod. Res.,* **32**: 345-352. https://doi.org/10.1016/S0022-474X(96)00026-4

Khaliq, A., Khan, A.A., Afzal, M., Tahir, H.M., Raza, A.M. and Khan, A.M., 2014. Field evaluation of selected botanicals and commercial synthetic insecticides against *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) populations and predators in onion field plots. *Crop Protec.,* **62**: 10-15. https://doi.org/10.1016/j.cropro.2014.03.019

Khan, A.A., Afzal, M., Qureshi, J.A., Khan, A.M. and Raza, A.M., 2014. Botanicals, selective insecticides and predators to control *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) in citrus

orchards. *Insect Sci.*, **21**: 717-726. https://doi.org/10.1111/1744-7917.12173

McFarlane, J.A., 1989. *Guidelines for pest management research to reduce stored food losses caused by insect and mites*. Bulletin No. 22, Overseas Development Natural Resources Institute, London, England.

Park, C., Kim, S.I. and Ahn, Y.J., 2003. Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored product insects. *J. Stored Prod. Res.,* **39**: 333-342. https://doi.org/10.1016/S0022-474X(02)00027-9

Regnault-Roger, C. and Hamraoui, A., 1995. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). *J. Stored Prod. Res*., **31**: 291-299. https://doi.org/10.1016/0022-474X(95)00025-3

Regnault-Roger, C. and Hamraoui, A., 1993. Efficiency of plants from the south of France used as traditional protectants of *Phaseolus vulgaris* L. against its bruchid *Acanthoscelides obtectus* (Say). *J. Stored Prod. Res.*, **29**: 259-264. https://doi.org/10.1016/0022-474X(93)90008-R

Scotti, G., 1978. *Les insectes ET les acariens des céréales stokées*. ITCF/AFNOR, Paris, pp. 238.

Shakoori, F.R., Riaz, T., Ramzan, U., Feroz, A. and Shakoori, A.R., 2018. Toxicological effect of esfenvalerate on carbohydrate metabolizing enzymes and macromolecules of a stored grain pest, *Trogoderma granarium. Pakistan J. Zool.,* **50**: 2185-2192. http://dx.doi.org/10.17582/journal.pjz/2018.50.6.2185.2192

Valladares, G., Dafago, M.T., Palacois, S. and Carpinella, M.C., 1997. Laboratory evaluation of *Melia azedarach* (Meliaceae) extracts against the elm leaf beetle (Coleoptera: Chrysomelidae). *J. econ. Ent.,* **90**: 747-750. https://doi.org/10.1093/jee/90.3.747