Pakistan J. Zool., vol. 47(4), pp. 1045-1050, 2015.

Antifeedant Effect of Essential Oils of Five Indigenous Medicinal Plants Against Stored Grain Insect Pests

Sohail Akhtar,[1]* Mansoor-ul-Hasan,[1] Muhammad Sagheer[1] and Nazir Javed[2]
[1]*Department of Entomology, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Plant Pathology, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan*

Abstract.- The present study aims at assessing the antifeeding effects of *Azadirachta indica, Melia azedarach, Colocynthis citrullus, Nicotiana tabacum* and *Eucalyptus camaldulensis* against *Tribolium castaneum, Ryzopertha dominica* and *Trogoderma granarium* under laboratory conditions. All treatments showed the prominent feeding deterrence activities. The most potent essential oil against all insect pests was found in *A. indica*, with maximum reduction in weight loss (0.56, 1.02, 1.69%) and feeding deterrence index (75.44, 54.57 and 39.21%) against *T. castaneum, T. granarium* and *R. dominica,* respectively followed by *M. azedarach* (0.63, 1.05 and 1.76%) (67.59, 50.85 and 34.92%), *C. citrullus* (0.65, 1.17 and 1.76%) (65.35, 43.57 and 33.94%), *N. tabacum* (0.7, 1.22 and 1.84) (58.43, 38.87 and 30.28%) and *E. camaldulensis* (0.84, 1.32 and 1.97%) (45.11, 38.98 and 23.18), respectively. It was also observed that weight loss and feeding deterrence index increases with increasing concentration of toxin. Weight loss was (0.37, 0.85 and1.50%) (0.48, 0.96 and 1.61%) (1.26, 1.09 and 1.74%) and (1.72 and 2.39%) at the dose rate of 6%, 4%, 2% and controls against *T. castaneum, T. granarium* and *R. dominica*, respectively. Feeding deterrence index was 71.74, 50.41 and 37.22%, at 6% concentration of toxicant, 62.83, 44.18 and 32.54% at 4% concentration of toxicant and 52.59, 36.65 and 27.17% at 2% concentration of toxicant.

Key words: Antifeedant effect, insect pests, plant essential oils, stored products.

INTRODUCTION

Food safety and security is a key issue particularly in view of rapid increase in world population (Tubiello *et al*., 2007). Pakistan has a population of 177 million which is increasing with the growth rate of 2.07% (Khattak and Shafique, 1986). Saving the produced grains at harvesting can be helpful to meet the food requirements of increasing population. In stored grains, insect damage may account for 10-40% of loss worldwide (Papachristos and Stamopoulos, 2002). In Pakistan the damage caused by *Tribolium castaneum* (Coleoptera: Tenebrionidae) to various stored and food commodities like grain, flour and dried fruits is recorded to be 15-20% (Khattak and Shafique, 1986). The weight loss caused by *T. granarium* (Coleoptera: Dermestidae) in Pakistan is about 2.32% (Khan and Cheema, 1978). The lesser grain borer, *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae) is a threatening pest of stored products and is cosmopolitan in distribution (Potter, 1935). It is mostly in the warmer regions of the world and are highly polyphagous in nature (Edde *et al*., 2005).

Attempts to control stored grain pests mostly relied on synthetic insecticides and fumigants such as methyl bromide (Chakrabarti, 1996), phosphine (Pimentel *et al*., 2007) and sulphuryl fluoride (Bell and Savvidou, 1999). However, drawbacks associated with the use of conventional control strategies (synthetic insecticides) such as effect on non-target organisms (Rajendran and Sriranjini, 2008), human health (Isman, 2006), environmental pollution (Ogendo *et al*., 2003), effects on ozone depletion (Shaaya and Kostyukovsky, 2006) and development of insect resistance and pest resurgence (Sousa *et al*., 2009) have necessitated to look for some biodegradable, safe and environmental friendly sources of pesticides.

Numerous types of insecticidal activities of essential oils have been documented (Papachristos and Stamopoulos, 2002). Along with feeding deterrent effects of various plant essential oil was confirmed (Suthisut *et al*., 2011; Ko *et al*., 2010). *Melia azadirachta* powder has excellent feeding deterrence against stored grain insect pests (Islam, 1983). *Nicotiana tabacum* exhibited feeding deterrence activities against *T. castaneum* (Tiwari *et al*., 1995). *Citrullus colocynthis* L. (Cucurbitaceae)

* Corresponding author: sohailakhter4all@yahoo.com
0030-9923/2015/0004-1045 $ 8.00/0

is a medicinal plant in Africa and Asia (Tavakkol-Afshari *et al*., 2005). Seed extracts of *C. citrullus* inhibit the population of *T. castaneum* at various concentrations (Nadeem *et al*., 2012). Azadirachtin has a unique complex of behavioral and physiological modes of action; it has an antifeedant action on insects (Schmutterer, 1988), whereas *Eucalyptus camaldulensis* has repellant properties against *T. granarium* and *T. castaneum* (Abbasipour *et al*., 2009).

Keeping in the view the importance of medicinal plants, the present study was undertaken to evaluate the antifeedant effect of essential oils extracted from *A. indica*, *M. azedarach*, *C. citrullus N. tabacum* and *E. camaldulensis* against *T. castaneum, R. dominica* and *T. granarium.*

MATERIALS AND METHODS

Plant materials

Plant parts from the locally grown medicinal plants *viz., A. indica, M. azdirachta, C. citrullus, N. tabacum* and *E. camaldulensis* were collected from different locations of Faisalabad, Punjab, Pakistan (longitude, 73°74 East; latitude, 30°31.5 North; altitude, 184 m), and were brought to laboratory of Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad and dried in shade at room temperature. Plants were washed and dried plant parts were powdered using stone electric grinder (Machine No. 20069, Pascall Engineering Co. Ltd.). This powder was then sieved through 40 mesh sieve

Oil extraction

Soxhelt extraction apparatus (Model WHM12295, Daihan Scientific Co., Ltd.) was used to prepare essential oils. Soxhelt thimble was filled with 50 g of fine botanical powder and placed in flask. Acetone was used as solvent in bottom flask. This process of extraction oil from all plant powders was repeated to achieve enough quantity of essential oil based on the nature of plant material. Extracted essential oil was then purified by evaporating solvent by using electric rotary evaporator. Pure extracted essential oils were preserved in glass vials at 4 ºC to prepare the concentrations of 2, 4 and 6% by mixing acetone as solvent.

Test insects

Infested grain samples were collected from Faisalabad. Insect population consisted of adults of *R. dominica* and *T. castaneum* and grubs of *T. granarium*. Collected insects were kept in the jars of 9.5 cm diameter and covered with the muslin cloth. Rearing of the insects was performed in the laboratory for three months to achieve the uniform populations. Insects were regularly checked for their growth and sieved and transferred to jars half filled with uninfested wheat grain or wheat flour diet. Temperature at 30±2 ºC and relative humidity (RH) at 65±5% was maintained for insect maximum growth by using incubator (Model MIR-254, Sanyo).

Bioassay

Weight loss method was adopted for the evaluation of antifeedant activities of essential oils. Weighed amount (5g) of grain (for *T. granarium* and *R. dominica*) and wheat flour (for *T. castaneum*) was treated with 2 ml of 2, 4 and 6% concentration of the essential oil for each treatment. Control was treated with acetone alone. Treated diet was kept in air and the solvent (acetone) was allowed to evaporate for 10 min. Twenty test insects were released in each jar on treated diet. Jars were kept in incubator (Model MIR-254, Sanyo) at 25±2°C and 65±5% RH after covering with a cloth to avoid insect escape. Four replications were performed for each concentration of the essential oils. Data regarding seed damage and weight loss were recorded after 15, 30 and 45 days of treatment.

Count and weight method was followed for calculation of the seed weight loss by *T. granarium* and *R. dominica* (Boxall, 1986)

$$\text{Weight loss (\%)} = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wu\ (Nd - Nu)} \times 100$$

where, Wu, undamaged seeds (weight); Nu, undamaged seeds (number); Wd, damaged seeds (weight); and Nd, damaged seeds (number).

The weight loss (%) of wheat flour by *T. castaneum* in the treated and control sets was calculated by using the formula suggested by Parkin (1956).

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

where, Wi, weight of wheat flour before the experiment; and W, weight of wheat flour after the experiment.

Feeding deterrence was calculated by using the feeding deterrent index following Isman *et al*. (1990).

$$\text{FDI (\%)} = \frac{C - T}{C} \times 100$$

where, C, weight loss in the control diet and T, weight loss in the treated diet.

Statistical analysis

The collected data was subjected to Analysis of Variance (ANOVA) using Statistica software (Stat Soft, 8.0). Means were separated by the Tukey's multiple range test when results were significant ($p < 0.05$).

RESULTS

Antifeedant effect of essential oils

Assessment of weight loss and feeding deterrence index of essential oils of indigenous medicinal plants against *T. castaneum* and *R. dominica* in wheat flour, whereas *T. granarium* in whole wheat treated with the essential oils, in comparison with untreated samples of control was calculated. All plant essential oils caused significant reduction in weight. *A. indica* proved most efficient toxicant where weight loss (0.56, 1.02 and 1.69%) was minimum followed by *M. azedarach* (0.63, 1.05 and 1.76%), *C. citrullus* (0.65, 1.20 and 1.78%), *N. tabacum* (0.71, 1.22 and 1.84%) *E. camaldulensis* (0.84, 1.32 and 1.97%) against *T. castaneum, T. granarium* and *R. dominica*, respectively (Fig. 1). Overall effect of time duration on weight loss was also analyzed. Results showed that the weight loss by *T. castaneum* and *R. dominica* increased as the time period increased. Weight loss was (0.89 and 1.60%) after 45 days, 0.68 and 1.81% after 30 days and 0.47 and 2.02% after 15 days against *T. castaneum* and *R. dominica*, respectively, while in the case of *T. granarium* the weight loss increased from 15 to 30 days but after that there was no significant increase in weight loss because only larval stage of this insect causes damage and within 30 days, all larvae were converted into pupae or adults. Weight loss was 1.24, 1.22 and 1.00% after 45, 30 and 15 days, respectively (Fig. 2). Overall effect of concentration on weight loss was also studied. It was observed that potency of toxin increased the dose rate increased. Weight loss was (0.37, 0.85 and 1.50%), (0.48, 0.96 and 1.61%) and (1.26, 1.09 and 1.74%) against *T. castaneum, T. granarium* and *R. dominica* at the dose rate of 6, 4 and 2%, respectively (Fig. 3). Effect of plant essential oil on the feeding deterrence index showed that the feeding deterrence index was higher against *A. indica* (75.44, 54.47 and 39.21%) followed by *M. azedarach* (67.59, 56.39 and 50.85%), *C. citrullus*

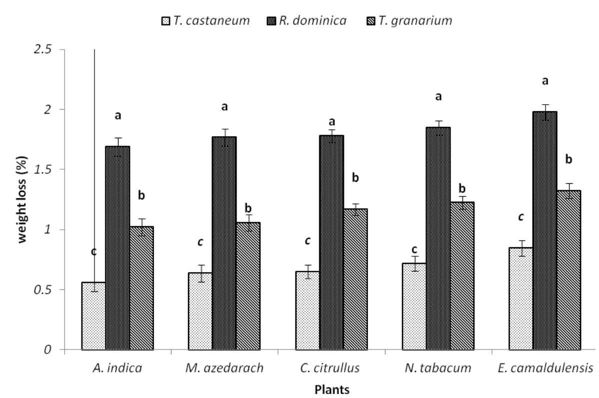

Fig. 1. Mean weight loss (%) of treated food commodities caused by *T. castaneum, T. granarium* and *R. dominica*.

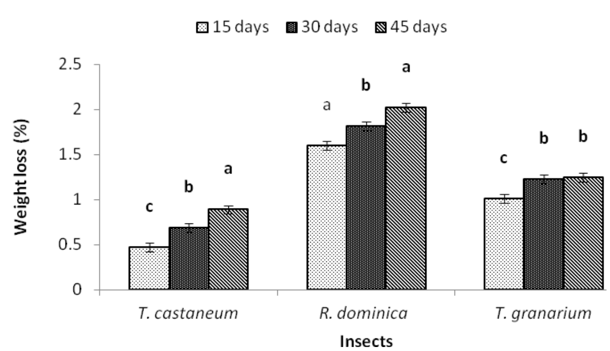

Fig. 2. Mean weight loss (%) of food commodities treated with plant extracts due to infestation of *T. castaneum, T. granarium* and *R. dominica* after different exposure times.

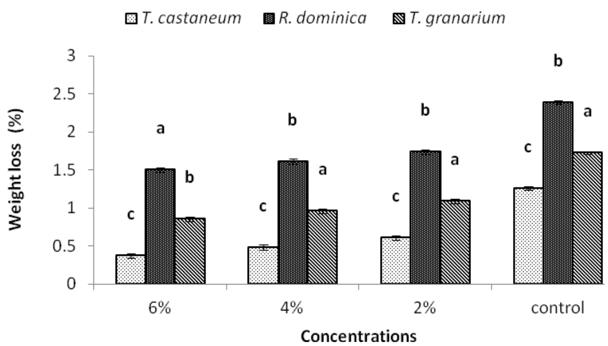

Fig. 3. Mean weight loss (%) of treated food commodities due to *T. castaneum*, *T. granarium* and *R. dominica* against different concentrations of plant essential oils.

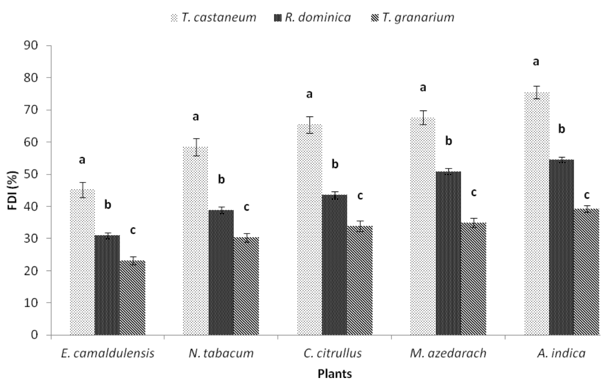

Fig. 4. Mean feeding deterrence indices (%) of *T.castaneum*, *T. granarium* and *R. dominica* on food commodities treated with different plant essential oils.

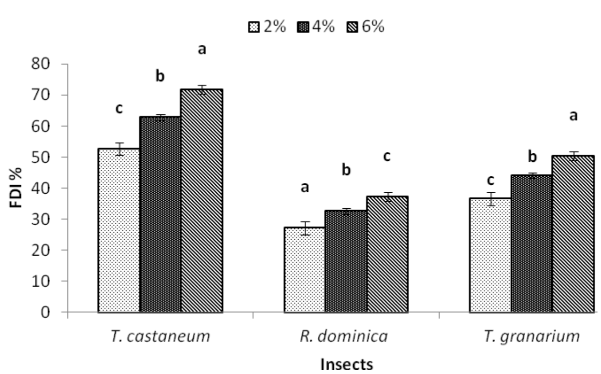

Fig. 5. Mean feeding deterrence indices (%) of *T. castaneum*, *T. granarium* and *R. dominica* on food commodities treated with different plant essential oils at different concentrations.

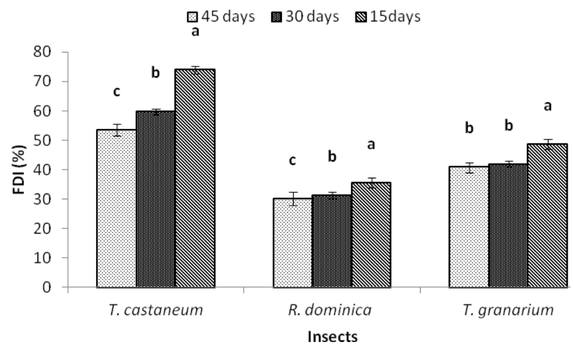

Fig. 6. Mean feeding deterrence indices (%) of *T. castaneum*, *T. granarium* and *R. dominica* on food commodities treated with different plant essential oils after different exposure times.

(65.37, 43.57 and 33.94%), *N. tabacum* (58.43, 38.87 and 30.28%), *E. camaldulensis* (45.11, 30.98 and 23.18%) against *T. castaneum*, *T. granarium* and *R. dominica*, respectively (Fig. 4). Overall effect of time duration on feeding deterrence index revealed that as the duration of exposure increases feeding deterrence index decreases accordingly. Feeding deterrence index was (73.98 and 35.54%), (59.41 and 31.20%) and (53.55 and 30.19%) against *T. castaneum* and *R. dominica* after 15, 30 and 45 days, respectively. In case of *T. granarium* overall effect of time duration on feeding deterrence index revealed that as the time duration increased from 15 to 30 days, feeding deterrence index decreased but after 30 to 45 days there was no significant decrease in feeding deterrence index because only larval stage of this insect causes damage and within 30 days all larvae were converted into pupae or adults and feeding deterrence index was 48.61, 41.85 and 40.78% after 15, 30 and 45 days, respectively (Fig.5). Overall effect of concentration on the feeding deterrence index was also increased with respect to the concentration of tested essential oil. Feeding deterrence index was (71.74, 50.41 and 37.22%), (62.83, 44.18 and 32.54%) and (52.59, 36.65 and 27.17%) against *T. castaneum*, *T. granarium* and *R. dominica* at 6%, 4%, 2% concentration of toxicant, respectively (Fig. 6).

DISCUSSION

The present research work was carried out to assess the antifeeding effect of *A. indica*, *M. azedarach, C. citrullus, N. tabacum* and *E. camaldulensis* in comparison with untreated samples of control against *T. castaneum, R. dominica* and *T. granarium.* All the essential oils showed the prominent feeding deterrence activities. Similar investigations were made by Morgan (2009) who confirmed the feeding deterrence activities of azadirachtin, a triterpene isolated from *Azadirachta indica*. It is a very active antifeedant against 90 % of the more than 600 tested herbivorous and stored product insect species. Contradictory results were obtained in the studies carried out by Huang and Ho (1998) who reported changes in feeding behavior of *T. castaneum* adults when subjected to nutmeg oil (*Myristica fragrans*) but feeding deterrent effect was more prominent in *S. zeamais* as compared to *T. castaneum* adults. This may be due to the fact that different essential oils have different efficiency against different insect pests. Ko *et al*. (2010) showed antifeedant effect of *Litsea salicifolia* at different concentrations of 0, 4, 6, 8 and 10% against these two insect pests. They showed *T. castaneum* was more susceptible than *S. zeamais* These results are somewhat similar to the present study in which highest FDI against *T. castaneum* was 75.44 %. This difference in FDI's value was due to difference in the concentration and nature of toxicants. Feeding action of glycol-alkaloid fraction TGA fraction of *Solanum tuberosum* against *T. granarium* was reported by Nenaah (2011), who showed significant decrease in the food utilization and food consumption rate by khapra beetle at concentrations varying between 20 and 30 mg g^{-1} food with feeding deterrent index reaching 82.40%.

Our results shows that potency increases as the dose rate of toxicant increases. These findings are in accordance with earlier reports made with essential oil of *L. salicifolia* against *S. zeamais* and *T. castaneum* (Ko *et al*., 2010). Feeding deterrence index at the lowest concentration (4%) of *L. salicifolia* against *T. castaneum* was 53.58% which increased to 84.62% with the application of 10% essential oil. Similar trend was also observed by Abbasipour *et al*. (2011) who reported FDI of *D. stramonium* against *T. castaneum* increased from 34.93% to 97.21%, with the increase in concentration from 947 to 3007 mg/l. Recent investigation carried out by Jaya *et al*. (2012) to determine feeding deterrence index of *Coleus aromaticus* against *T. castaneum* resulted in the same effect of concentration ***i.e.***, FDI at 250 ppm was 56.39 % witch increased to 72.31 % at 500 ppm and achieved 100% FDI at 1000 ppm concentration. These results are also in agreement with that of Sarwar (2010), Geng *et al.* (2011) and Nenaah (2011).

The use of plant extracts as grain protectants in storages will help in sustainable control of insect pests of stored cereals and their products; as these plant bio-pesticides are good contact toxicants, anti-feedants, repellents and growth inhibitors.

ACKNOWLEDGEMENTS

This study is financially supported by Higher Education Commission (HEC), Islamabad, Pakistan (Batch-VI) under the Indigenous Ph.D. Fellowship Program (Batch-IV).

REFERENCES

ABBASIPOUR, H., MAHMOUDVAND, M., RASTEGAR, F. AND HOSSEINPOUR, M.H., 2011. Bioactivities of jimson weed extract, *Datura stramonium* L. (Solanaceae), against *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Turk. J. Agric. Forest.*, **35**: 623-629.

BELL, C.H. AND SAVVIDOU, N., 1999. The toxicity of Vikane (Sulfuryl fuoride) to age groups of eggs of the Mediterranean flour moth (*Ephestia kuehniella*). *J. Stored Prod. Res*., **35**: 233-247.

BOXALL, R.A., 1986. *A critical review of methodology for assessing farm-level grain losses after harvest*. Report of the tropical development research institutes G 191. Catham UK. Natural resource institute. 139p.

CHAKRABARTI, B., 1996. *Methyl bromide in storage practice and quarantine*, pp. 237-274

EDDE, P.A., PHILLIPS, T.W. AND TOEWS, M.D., 2005. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height and habitat. *Environ. Ent*., **34**: 1549-1557.

GENG, Z.F., LIU, Z.L., WANG, C.F., LIU, Q.Z., SHEN, S.M., LIU, Z.M., DU, S.S. AND DENG, Z.W., 2011. Feeding deterrents against two grain storage insects from *Euphorbia fischeriana*. *Molecules*, **16**: 466-476.

HUANG, Y. AND HO, S.H., 1998. Toxicity and antifeedant activity of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res.*, **34**: 11-17.

ISLAM, B.N., 1983. Pesticidal action of neem and certain indigenous plants and weeds of Bangladesh. *Proc. 2nd Int. Neem Conf*., pp. 253-290.

ISMAN, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Ent*., **51**:45-66.

ISMAN, M.B, KOUL, O., LUCZYNSKI, A AND KAMINSKI, J., 1990 Insecticidal and antifeedant bioactivities of neem oils and relationship to azadirachtin content. *J. Agric. Fd. Chem*., **38**: 1406-1411

JAYA, P., SINGH, PRAKASH, B. AND DUBEY, N.K., 2012. Insecticidal activity of *Ageratum conyzoides* L., *Coleus aromaticus* Benth and *Hyptis suaveolens* (L.) Poit essential oils as fumigant against storage grain insect *Tribolium castaneum* Herbst. *J. Fd. Sci. Technol*., **51**: 2210-2215

KHAN, D.A. AND CHEEMA, M.A., 1978. Farm products storage and storage losses in the Lahore, Punjab. *Econ. Res. Inst*., **166**: 47.

KHATTAK, S.U.M. AND SHAFIQUE, M., 1986. Varietal susceptibility studies of ten heat cultivars flour to red flour beetle, *Tribolium castaneum. Pakistan J. Zool*., **18**: 257-261.

KO, K., JUNTARAJUMNONG, W. AND CHANDRAPATYA, A., 2010. Insecticidal activities of essential oils from Fruits *of Litsea salicifolia* Roxb. ex Wall. against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *Pakistan J. Zool*., **42**: 551-557.

MORGAN, E.D., 2009. Azadirachtin, a scientific gold mine. *Bioorg. Med. Chem*., **17**: 4096-4105.

NADEEM, M., IQBAL, J., KHATTAK, M.K. AND SHAHZAD, M.A., 2012. Management of *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae) using neem (*Azadirachta indica* A. Juss) and tumha (*Citrullus colocynthis* (L.). *Pakistan J. Zool*., **44**: 1325-1331.

NENAAH, G.E., 2011. Toxic and antifeedant activities of potato glycoalkaloids against *Trogoderma granarium* (Coleoptera: Dermestidae). *J. Stored Prod. Res*., **47**: 185-190

OGENDO, J.O., BELMAIN, S.R., DENG, A.L. AND WALKER, D.J., 2003. Comparison of toxic and repellent effects of *Lantana camara* L. with *Tephrosia vogelii* Hook and a synthetic pesticide against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize grain. *Insect Sci. Applic*., **23**:127-135.

PAPACHRISTOS, D.P. AND STAMOPOULOS, D.C., 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapors on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J. Stored Prod. Res*., **38**: 117-128.

PARKIN, E.A., 1956. Stored Product Entomology (the assessment and reduction of losses caused by insects to stored food stuffs). *Annu. Rev. Ent*., **1**:233-240

PIMENTEL, M.A.G., FARONI, L.R.D.A, TÓTOLA, M.R. AND GUEDES, R.N.C., 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. *Pest. Manage. Sci.,* **63**: 876-881.

POTTER, C., 1935. The biology and distribution of *Rhizopertha dominica* (Fab.). *Trans. R. entomol. Soc. Lond*., **83**, part IV.

RAJENDRAN, S. AND SRIRANJINI, V., 2008. Plant products as fumigants for stored-product insect control. *J. Stored Prod. Res*., **44**: 126-135.

SARWAR, M., 2010. Some possibilities on the effectiveness of plant powders as grain protectants against cowpea weevil, *Callosobruchus maculatus* (Fabricius) Walp (Coleoptera: Bruchidae) infestation in chickpea. *Int. J. Agron. Pl. Prod*., **1**: 45-50.

SCHMUTTERER, H., 1988. Potential of *azadirachtin* containing pesticides for integrated pest control in developing and industrialised countries. *J. Insect Physiol*., **34**: 713-719.

SHAAYA, E. AND KOSTYUKOVSKY, M., 2006. *Essential oils*: *potency against stored product insects and mode of action*. Stewart Postharvest Review [Online].

SOUSA, A., FARONI, L., PIMENTEL, M. AND GUEDES, R., 2009. Developmental and population growth rates of phosphine-resistant and susceptible populations of stored product insect pests. *J. Stored Prod. Res*., **45**: 241-246.

SUTHISUT, D., FIELDS, P.G. AND CHANDRAPATYA, A., 2011. Contact toxicity, feeding reduction, and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. *J. econ. Ent*., **104**:1445-1454.

TAVAKKOL-AFSHARI, J., RAKHSHANDEH, H., ZAMANI, A., MAHDAVI-SHAHRI, N., GHAZI-ZADEH, L. AND VAHEDI, F., 2005. Cytotoxic effect of *Citrullus colocynthis* extract on cell line of Hep2 and L929. *Res. J. Hakim*, **8**: 47-54.

TIWARI, A., LAKSHAMANA KUMAR, M. AND SAXENA, R.C., 1995. Effect of *Nicotiana tabacum* on *Tribolium castaneum* pest control research laboratory, S.S.L. Jain P.G. College, Vidisha, 464 001, MP, India, *Pharma. Biol*., **33**: 348-350.

TUBIELLO, F.N., SOUSSANA, J.F. AND HOWDEN, S.M., 2007. Crop and pasture response to climate change. *Proc. natl. Acad. Sci. USA*, **104**: 19686-19690.

(Received 21 November 2014, revised 12 January 2015)