Pak. J. Agri. Sci., Vol. 52(3), 709-715; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

COMPARATIVE INSECTICIDAL EFFECTIVENESS OF ESSENTIAL OILS OF THREE LOCALLY GROWN PLANTS AND PHOSPHINE GAS AGAINST *Trogoderma granarium*

Ch. Muhammad Shahid Hanif[1], Mansoor-ul-Hasan[2], Muhammad Sagheer[2]*, Shahzad Saleem[3], Kazam Ali[2] and Sohail Akhtar[4]
[1]Department of Entomology, College of Agriculture BZU, Bahadur Campus Layyah, Pakistan
[2]Department of Entomology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Biosciences, COMSATS Institute of Information Technology, Sahiwal, Pakistan
[4]Department of Entomology, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan
[*]Corresponding author's e-mail: sagheersharif@yahoo.com

The aim to plan this investigation was to evaluate insecticidal activities of three essential oils extracted from locally grown plants, *Melia azadarach, Datura stramonium* and *Azadirachta indica* at various concentrations viz., 5%, 10%, 15% along with phosphine gas at a dose rate of 100, 200, 300 ppm and their combinations against the grubs of *Trogoderma granarium*. Results depicted considerable toxicity of essential oils and phosphine gas alone which was considerably increased when test insects were exposed to the combination of these two. In essential oil alone assay, *D. stramonium* showed highest mean mortality of 31.99% at maximum concentration (15%) after 72h of exposure. While 57.63% mean mortality was observed against phosphine with 300 ppm of concentration after 72h. Combined application of essential oil (%) and phosphine (ppm) significantly increased mean mortality such as 86.47% (300ppm + 15% *D. stramonium)*, 83.03% (300ppm + 15% *A. indica)* and 76.24% (300ppm + 15% *M. azadarach)* after 72h of exposure. Results regarding mortality were found to be directly related to concentration and exposure time.
Keywords: Biocidal, botanical extracts, phosphine, fumigation and khapra

INTRODUCTION

Post-harvest losses of stored grains have been reported as 10-15% in Pakistan (Ahmad, 1994) but storage losses assessed by different workers vary greatly. Wheat grain losses due to insect pest infestation have been estimated up to 10% (Ramzan *et al.,* 1991; Khan *et al.,* 2010). Ahmad *et al.* (1992) gave a comprehensive review of post production losses of food grains occurring in Pakistan and stated that losses estimated by different workers varied greatly i.e.10-15% (Ahmad,1986) and 3.24% (Khan *et al*.,1985). Hassan *et al*. (1994) studied that during storage losses occurred due to insect pests ranged between 0.45-0.72 percent. Similar studies carried out in India showed that storage losses of wheat due to insect pest infestation are 2.03%. Singh and Yadav, 1995 reported losses of wheat 0.2% at national reserve level in India and in China 7-13% in rural household storage (Weifen *et al*., 2003). Among these insect pests, Khapra beetle (*Trogoderma granarium* Everts) is recorded to be the most damaging and destructive pest of stored products throughout the world (Burges, 2008; Mark *et al*., 2010). A broad range of stored commodities such as pulses, cereals and cereal products, oil seed and many feeding stuffs of animal are economically damaged by the grubs of *T. granarium* (Haines, 1991; Lale, 2002).

Synthetic pesticides like methyl bromide (White and Leesch, 1996), Phosphine (Sadomov, 1984), Sulphuryl fluoride (Bell and Savvidou, 1999) and some other fumigants are regularly used for the management of stored grain pests but these are also making the pest strains resistant (Sousa *et al*., 2009). These pesticides also affect the non target organisms, fish and humans due to their direct toxicity (Isman *et al*., 2006) and environmental pollution and ozone depletion (Ogendo *et al*., 2003).

Essential oils which are extracted from aromatic plants have rich toxicological properties, could be used as alternative insecticides (Dal-Bello *et al*., 1996; Regnault-Rogar *et al*., 1993) which have been evaluated in a number of studies (Boussalis *et al*., 1999; Abubakar *et al*., 2000; Huang *et al*., 2000; Fields *et al*., 2001; Tripathi *et al*., 2002). Many biopesticides showed high physiological and behavioral actions against stored grain insect pests, which include repulsive, lethal, antifeedant and growth regulatory effects (Aranson *et al*., 1989; Grainge and Ahmed, 1988. Recently, low concentrations (0.01%) of protein-rich pea flour against several stored-product insect pests have shown adult mortality and reduce reproduction (Fields *et al*., 2001). Main advantage of using botanicals is that they had medicinal value, specific in action, environment friendly, less expensive, easy to use and easily biodegradable.

In this scenario, a study was much needed to control increasing resistance in *T. granarium* against pesticides. Present investigation was aimed to assess the insecticidal activities of essential oils, phosphine gas and their combinations against *T. granarium* commonly known as Khapra beetle.

MATERIALS AND METHODS

***Collection and rearing of insects*:** Mix population of *Trogoderma granarium* was collected from godowns, flour mills and grain market of Faisalabad (Longitude 73°74 East; Latitude 30°31.5 North; Altitude: 184 m). After collection, the insect population was kept in the jars and was covered with muslin cloths. Insect population was regularly observed for their growth, and was sieved and put to new uninfected wheat flour diet for getting homogenous population. Temperature 30±2°C and relative humidity at 65±5% was maintained in for insect's highest growth. One week old grubs were used for bioassay studies.

***Preparation of plant essential oils*:** Weighed amount of fresh leaves of each plant (*Azadirachta indica*, *Datura stramonium* and *Melia azadarach*) were grounded separately after shade drying. For extraction of oil, grounded samples were run in Soxhlet's apparatus (Model WHM12295, DAIHAN Scientific Co., Ltd.) (Sluiter *et al*., 2005). 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. These pure extracted essential oils were preserved in glass vials at 4 ºC to prepare the concentrations of 5, 10 and 15% by mixing acetone as solvent.These concentrations were used for subsequent experiments.

***Generation of phosphine gas*:** The phosphine gas was generated by FAO's method (Anonymous, 1975). The apparatus for generation of phosphine gas consisted of a 5 liter beaker, a collection tube (cylinder), an inverted funnel, Phostoxin® (Aluminum phosphide) tablets and muslin cloth. The tube for collection of gas was sealed from one side with air tight rubber stopper and then was filled with 5% sulphuric acid (H_2SO_4) solution. Half of the beaker was also filled by 5% H_2SO_4 solution. The gas collecting tube was placed carefully into the beaker over the inverted funnel in such a way that there is no loss of H_2SO_4 solution from the collection tube, while dipping into the beaker. Before generating phosphine gas all air in collection tube was removed within collection tube. Then Phostoxin tablets (wrapped in muslin cloth) were placed under inverted funnel. Phosphine (PH_3) gas was then collected in gas colleting tube inverted over the funnel. As the funnel filled with generated gas the level of solution goes down. After that, gas was taken with the help of syringes and injected into Phosphine meter for measuring gas concentration. With the help of Phosphine meter required concentrations of 100, 200 and 300ppm of phosphine gas were obtained.

***Toxicological impact of essential oils against Trogoderma granarium*:** Each experiment was carried out in petri dishes lined with filter papers. Different concentrations (5, 10 and 15%) of essential oils of *D. stramonium*, *A. indica* and *M. azadarach* were applied on filter paper and left the filter paper to air dry. Twenty grubs of *T. granarium* were released separately in each petri dishe and enclosed. Each treatment was replicated thrice. Completely Randomized Design (CRD) was followed for the experiment. Data was recorded after 24, 48 and 72h.

***Phosphine fumigation against T. granarium*:** Bioassay of phosphine gas was carried out in jars with lids having fixed rubber stopper. Concentrations of phosphine were measured using a phosphine meter. Twenty grubs of *T. granarium* were released separately in these jars. Each experiment was replicated three times using Completely Randomized Design. Mortality was recorded after 24, 48 and 72 hours. The surviving individuals from the bioassay were transferred into the new clean jars containing sterilized wheat flour and grains.

***Toxicological impact of combination (phosphine and essential oils) against T. granarium*:**
Different combinations of phosphine and plant essential oils were used in this bioassay study. Filter papers treated with different dilutions of essential oils were placed inside the jars. Twenty grubs of *T. granarium* were released in each jar and were covered tightly with the lids having fixed rubber stoppers. Different concentrations (5, 10 and 15%) of essential oils of *A. indica*, *D. stramonium* and *M. azadarach* and of phosphine gas (100, 200 and 300ppm) were applied. Data regarding mortality of test insects were recorded after 24, 48 and 72h.

***Statistical analysis*:** Statistical analysis of all collected data was subjected to analysis of variance using Statistica-8 software. Means of significant treatments were compared using Tuckey-HSD test at α=5%.

RESULTS

***Toxicological effect of plant essential oils against Trogoderma granarium*:** Data regarding percent mean mortality of *Trogoderma granarium* against *Melia azadarach* (Fig. 1), *Azadirachta indica* (Fig. 2) and *Datura stramonium* (Fig. 3) revealed that percentage mortality of test insect was time and concentration dependent. This direct relation is evident from the Figures 1-3 that mortality significantly increased with the increase in exposure time and concentration of essential oils. *D. stramonium* was found most effective exhibiting maximum mean mortality (31.99%) with 15% concentration at 72h of exposure; whereas, 29.31 and 30.53% was the highest mean mortality observed in *A. indica* and *M. azadarach,* respectively at the same concentration and exposure time.

Considering toxicity of *M. azadarach*, the highest mortality was recorded at 15% concentration of plant oil after 72 hrs of application which is statistically different from all the other treatments (Fig. 1). Minimum mortality was observed at concentration of 5% after 24 hours. Results significantly showed that with the increase in dose rate and time of exposure, mortality of *Trogoderm granarium* was increased. Similar trend was also observed in case of *A. indica* and *D. stramonoium*

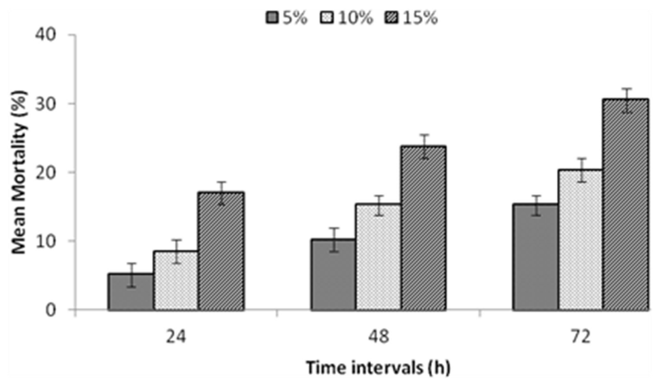

Figure 1. Percent mean mortality of *Trogoderma granarium* grubs with essential oil of *Melia azadarach.*

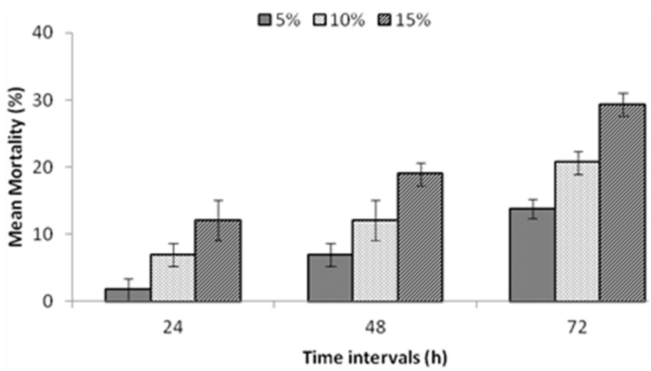

Figure 2. Percent mean mortality of *Trogoderma granarium* grubs against essential oil of *Azadirachta indica.*

***Fumigant effect of phosphine gas against Trogoderma granarium*:** Data concerning percentage mortality of phosphine gas with three concentrations of 100, 200 and 300 ppm and interaction of time interval and concentration are given in Fig. 4. It is indicating the significant difference in interaction of concentrations and time interval. It is obvious from the figure that phosphine gas exhibited minimum mortality 18.65% with 100 ppm concentration at 24 hrs of exposure time and it significantly increased up to the maximum of 57.63% with the increase in concentration and exposure time.

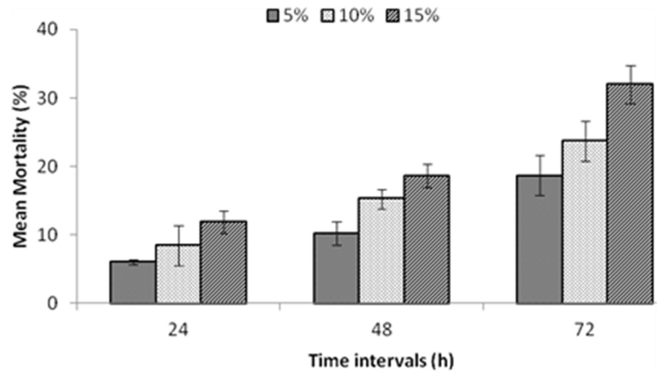

Figure 3. Percent mean mortality of *Trogoderma granarium* grubs against essential oil of *Datura stramonium*

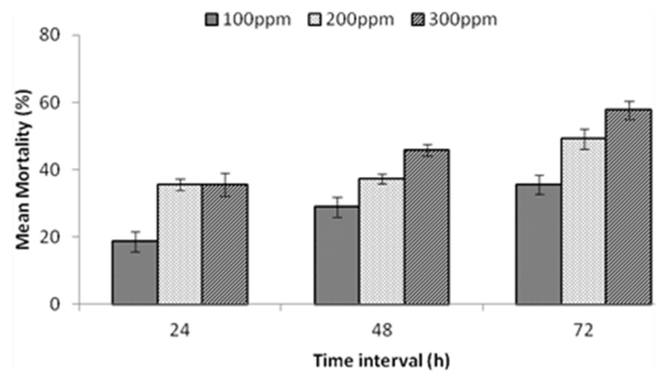

Figure 4. Percent mean mortality of grubs of *Trogoderma granarium* at different concentration of Phosphine

***Combined effect of phosphine and plant essential oil against Trogoderma granarium*:** For the evaluation of combined mortality effects, different concentration combinations of essential oils and phosphine gas were applied with the same exposure time as was in phosphine gas and essential oil alone treatments. From the observations recorded, it is clear that percentage mortality was increased in combined application of phosphine gas and essential oil as compared to when both were applied alone (Table 1). *D. stramonium* exhibited more percent mean mortality than *A. indica* and *M. azadarach* in combination with phosphine. Mortality was recorded 86.47%, 83.03% and 76.24% with 15% *D. stramonium* along with 300 ppm, 15% *A. indica* and 300 ppm and 15% *M. azadarach* and 300 ppm of phosphine gas, respectively at 72h of exposure. Mortality of test insect was found directly proportional to exposure time and concentrations of essential oils and phosphine gas.

Table 1. Percent mean mortality of *T. granarium* grubs against different combinations of essential oils and Phosphine.

Phosphine Conc. (ppm)	Essential oil Conc. (%)	*Datura stramonium* and Phosphine			*Azadirachta indica* and Phosphine			*Melia Azadarach* and Phosphine		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
100	5	27.14 ±1.69	30.53 ±1.69	35.60 ±1.69	22.06 ±1.69	25.45 ±1.69	30.53 ±1.72	15.52 ±1.72	20.69 ±1.69	24.14 ±1.39
	10	30.53 ±1.69	37.31 ±1.69	42.39 ±1.69	32.22 ±1.69	32.22 ±1.69	37.31 ±2.93	22.41 ±1.69	25.86 ±1.72	31.03 ±1.69
	15	35.61 ±1.69	42.39 ±1.69	45.78 ±1.69	30.53 ±1.69	37.31 ±1.36	40.70 ±1.69	27.58 ±0.69	29.31 ±1.69	36.20 ±1.79
200	5	32.22 ±1.69	39.00 ±2.93	45.78 ±1.69	27.07 ±0.76	33.59 ±0.37	40.69 ±0.69	23.72 ±1.69	28.84 ±2.93	35.61 ±1.69
	10	37.31 ±0.63	47.17 ±1.30	52.56 ±1.69	32.22 ±0.69	42.39 ±0.69	46.76 ±0.38	28.84 ±1.72	37.31 ±1.69	42.39 ±1.69
	15	47.17 ±1.36	52.56 ±1.69	61.03 ±1.09	42.69 ±0.69	46.95 ±1.12	55.94 ±0.69	37.31 ±1.69	45.78 ±1.69	52.56 ±1.69
300	5	44.92 ±0.83	55.61 ±0.36	66.21 ±0.66	41.03 ±0.60	51.23 ±0.60	60.67 ±0.33	35.13 ±0.21	45.45 ±0.37	55.94 ±0.69
	10	56.15 ±0.90	68.14 ±0.36	74.61 ±0.59	50.17 ±0.45	61.84 ±0.29	70.18 ±0.31	45.45 ±0.36	57.74 ±0.59	64.15 ±0.30
	15	72.88 ±0.69	77.30 ±0.87	86.47 ±0.36	67.80 ±0.69	73.16 ±0.33	83.03 ±0.36	63.06 ±0.37	67.14 ±0.36	76.24 ±0.65

DISCUSSION

Studies were carried out to evaluate toxic effectiveness of plant essential oils, phosphine gas, and their combinations against *Trogoderma granarium.* Many essential oils and their constituents have been studied to have potential as alternative compounds to currently used insect-control agents (Shaaya *et al*., 1991; Huang *et al*., 2000; Rozman *et al*., 2007; Rajendran and Sriranjini, 2008; Batish *et al*., 2008; Sahaf *et al*., 2008; Cosimi *et al*., 2009). All the essential oils assessed in this study exhibited significant mortality against grubs of *Trogoderma granarium.* However, *Datura stramonium* was found more effective with 31.99% mortality at maximum concentration and exposure time. Similar trend was also observed by Manzoor *et al*. (2011) who studied the toxic effects of *Melia Azedarach*, *Mentha longifolia*, *Myrtus communis*, *Cymbopogon citratus* and *Datura stramonium* and found *D. stramonium* most toxic with 21.43% mortality among all essential oils. Formerly conducted investigations have also documented significant insecticidal activities of *D. stramonium* (Abbasipour *et al*., 2011), *M. oleifera* (Anita *et al*., 2012), *E. camaldulensis* (Negahban and Moharramipour, 2007) and *N. sativa* (Chaubey, 2007).

Percentage of mortality recorded was significantly high when insects were kept in contact with essential oil for longer period of time. This result confirms the conclusion drawn by Manzoor *et al*. (2011) who studied the insecticidal effects of *D. stramonium* against *T. castaneum* and found 6.67, 13.33 and 20.00% mortality at 72, 120 and 168 hr with the same increasing trend. Furthermore insecticidal potential calculated was directly proportional to concentration. Mahfuz and Khanam (2007) were also in agreement this experiment who reported decrease in lethal dose (0.681 to 0.289 mg/cm2) of *D. stramonium* extract against *T. confusum* with increase (24 to 72 hr) in exposure time.

Similarly, data regarding phosphine gas evidenced for its matchless insecticidal activities; however, various previous researches accounted the prevalence of resistance to phosphine in stored product insect pests (Taylor, 1991; EI-Lakwah *et al*. 1995). One possible solution to overcome the increasing resistance was combined application of phosphine gas and essential oils, which not only decline the resistance probability but also enhanced the insecticidal effectiveness.

REFFRENCES

Abbasipour, H., M. Mahmoudvand, F. Rastegar and M.H. Hosseinpour. 2011. Bioactivities of jimsonweed extract, *Datura stramonium* L. (Solanaceae), against *Tribolium castaneum* (Coleoptera: Tenebrionidae).Turk J. Agric. For. 35: 623-629.

Abubakar, M.S., E.M. Abdurahman and A.K. Haruna. 2000. The repellent and antifeedent properties of *Cyperrus articulatus* against *Tribolium castaneum* (Herbst). Phytotherapy Res.14: 281-283.

Ahmad, F., M.R. Khan and M. Ahmad. 1992. Post harvest losses of food grains and their contamination. Pak. Entomol. 14: 103-112.

Ahmad, M., M.R. Khan, A. Iqbal and M. Hassan. 1986. Farm level storage losses of wheat by insect pests in Samundri Tehsil. Pak. Entomol. 8: 41-44.

Ahmad, M. 1994. Grain storage manage. News Letter 1(2):1.

Anita, S., P. Sujatha and P. Prabhudas. 2012. Efficacy of pulverised leaves of *Annona squamosa* (L.), *Moringa oleifera* (Lam.) and *Eucalyptus globulus* (Labill.) against the stored grain pest, *Tribolium castaneum* (Herbst.) Recent Res. Sci. & Tech. 4: 19-23.

Anonymous. 1975. Recommended methods for detection and measurement of resistance in agricultural pests to pesticides. Tentative methods for adults of some major pest species to stored cereals with methyl bromide and phosphine. FAO Plant Prot. Bull. 23:12-35.

Aranson, J.T., B.J.R. Philogene and P. Morand. 1989. Insecticides of plant origin. Amer. Chem. Soc. Washington, D.C; pp.387.

Batish, D.R., H.P. Singh, R.K. Kohli and S. Kaur. 2008. Eucalyptus essential oil as a natural pesticide. For. Ecol. Manage. 256: 2166-2174.

Bell, C.H. and N. Savvidou. 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.

Bell, C.H. and S.M. Wilson. 1995. Phosphine tolerance and resistance in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). J. Stored. Prod. Res. 31: 199-205.

Bhaduri, N., D.P. Gupta and S. Ram. 1989. Effect of vegetable oils on ovipositional behaviour of *Callosobruchus chinensis* Fab. pp. 81-84. In: Proc. 2nd Int. Symp. Bruchids and Legumes (ISBL-2). Okayama, Japan.

Boussalis, A.M., E.G. Ferraro, V.S. Martino, D.J. Pinzon-Coussio and J.C. Alvarez. 1999. Argentine plants as potential source of insecticidal compound. J. Ethnopharmacol. 67: 219-223.

Brown, A.W.A. 1968. Insecticides resistance comes of age. Bull. Entomol. Soc. America. 14: 3-9.

Burges, H.D. 2008. Development of the khapra beetle, *Trogoderma granarium*, in the lower part of its temperature range. J. Stored Prod. Res. 44: 32-35.

Champ, B.R. and C.E. Dyte. 1976. FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Prot. Bull. 25: 49-67.

Chaubey, M.K. 2007. Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored-product beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Afr. J. Agric. Res. 2: 596-600.

Cosimi, S., E. Rossi, P.L. Cioni and A. Canale. 2009. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). J. Stored Prod. Res.45: 125-132.

Dal-Bello, P.G., L. Lopez and C.M. Fabrizio. 1996. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. J. Stored Prod. Res. 37: 77-84.

EI Nadi, A.H., E.A. Elhag, A.A. Zaitoon and M.A. Al-Doghairi. 2001. Toxicity of three plants extracts to *Trogoderma granarium* Everts (Coleoptera: Dermestidae). Pak. J. Biol. Sci. 4: 1503-1505.

El-Lakwah, F.A., M.M. Khattab, G.H. Rady and Z.A. Halawa. 1995. Selection of the cowpea beetle, *Callosobruchus maculatus* (F.) for resistance to phosphine, carbon dioxide and their mixture in the laboratory and biological observations on the resistant strains. Ann Agric. Sci. 33: 843-63.

Fields, P.G., Y.S. Xie and X. Hou. 2001. Repellent effect of pea (*Pisum sativum*) fractions against stored-product insects. J. Stored Prod. Res. 37: 359-370.

Georghiou, G.P. and C.E. Taylor. 1977. Pesticide resistance as an evolutionary phenomenon. Proc. 14th Int. Cong. Entomol., pp.759.

Grainge, M. and S. Ahmed. 1988. Handbook of Plants with Pest-Control Properties. Wiley-Interscience Publications, New York, N.Y.

Haines, C.P. 1991. Insects and Arachnids of tropical stored products: Their biology and identification (a training manual), 2nd Ed. Natural Resources Institute, Chathan Kent, UK; pp.246.

Hassan, M., M.T. Farooq, F. Ahmad and F.R khan. 1994. Asseement of storage losses in wheat due to insect pests in Multan and Bahawalpur wheat storage. Grain Storage Manage. Newsletter 1:11.

Huang, H., S.L. Lam and S.H. Ho. 2000. Bioactivities of essential oil from *Ellitaria cardamomum* (L.) Maton. To *Sitophilus oryzae* and *Tribolium castaneum*. J. Stored Prod. Res. 36: 107-117.

Irshad, M. and S.U Talpur. 1993. Interaction among three co-existing species of stored grain insect pests. Pak. J. Zool. 25:131-133.

Isman, M.B. 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol. 51: 45-66.

Jembare, B., D. Obeng-Ofori, A. Hassanali and G.N N. Nyamasyo. 1995. Products derived from the leaves of *Ocimum kilimanndscharicum* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insect pests. Bull. Entomol. Res. 85: 361-367.

Khan, I., S. Afsheen, N. Din, S. Khattak, S.K. Khalil and Y.H.Y. Lou. 2010. Appraisal of different wheat genotypes against angoumois grain moth, *Sitotroga ceralella* (Oliv.). Pak. J. Zool. 42: 161-168.

Khan, M.R., A.H. Sabir and M. Ahmad. 1985. Storage losses to wheat in Faisalabad Tehsil. Pak. Entomol. 7: 5-10.

Khan, S.M. and A.A. Marwat. 2003. Deterrent/repellent effect of different plant parts of Neem and Kanair against lesser grain borer "*Rhyzopertha dominica* F". J. Pak. Entomol. 25:131-136.

Lale, N.E.S. 2002. Bioactivity and limitations against widespread use of neem products as alternatives to synthetic insecticides for the management of insect pests of stored agricultural products in Nigeria. Nigerian J. Exp. App. Biol. 3:115-124.

Lale, N.E.S. and A. Mustapha. 2000. Efficacy and acceptability of neem seed oil and primiphos methyl applied in three storage devices for the control of *Callsobruchus maculatus*. Z. Pflanzenk. Planzens. J. Plant Dis. Prot. 107: 399-405.

Mahfuz, I. and L.A.M. Khanam. 2007. Toxicity of some indigenous plant extracts against *Tribolium confusum* Duval J. bio-sci. 15: 133-138.

Manzoor, F., G. Nasim, S. Saif and S.A. Malik. 2011. Effect of ethanolic plant extracts on three storage grain pests of economic importance. Pak. J. Bot. 43: 2941-2946.

Mark, A.C., D.L. Severtson, C.J. Brumley, A. Szito, R.G. Foottit, M. Grimm, K. Munyard and D.M. Groth. 2010. A rapid non-destructive DNA extraction method for insects and other arthropods. J. Asia-Pacific Entomol. 13: 243-248.

Munakata, K., 1977. Insect antifeedants of *Spodoptera litura* in plants, pp. 185-196. *Hedin*. P.A. Host plant resistance to pests.ACS symposium series No. 62. Amer. Chem. Soc. Washington.

Negahban, M. and S. Moharramipour. 2007. Fumigant toxicity of *Eucalyptus intertexta*, *Eucalyptus sargentii* and *Eucalyptus camaldulensis* against stored-product beetles. J. Appl. Ent. 131: 256-261.

Ogendo, J., M. Kostyukovsky, U. Ravid, J. Matasyoh, A. deng, E. Omolo, S. Kariuki and E. Shaaya. 2008. Bioactivities of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. J. Stored Prod. Res. 44: 328-334.

Okonkwo, E.U. and W.J. Okoye. 1996. The efficacy of four seed powders and essential oils as protectants of cow pea and maize grain against infestation by *Callosobruchus maculates* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. Int. J. Pest Manag. 42: 143-146.

Papachristos, D.P. and D.C. Stamopoulos. 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapors on *Acanthoscelideso btectus* (Say) (Coleoptera: Bruchidae). J. Stored Prod. Res. 38: 117-128.

Pimental, D. 1981. An overview of pest management (Mimeograph). Department of Entomology, Section of Ecology and Systematic, Cornell University, Ithaca NY; p.52.

Pugazhvendan, S. R., K. Elumalai, P. R. Ross and M. Soundararajan. 2009. Repellent activity of chosen plant species against *T. castaneum* (Herbst). World J. Zool. 4: 188-190.

Rahim, M. 1998. Biological activity of Azadirachtin-enriched neem kernel extracts against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in stored wheat. J. Stored Prod. Res. 34:123-128.

Rajendran, S. and V. Sriranjini. 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. 43: 126-135.

Ramzan, M., B.K. Judge and P.S. Madan. 1991. Losses caused by storage pests in different wheat varieties under normal storage condition. Ind. J. Res. Punjab Agric. Univ. 2: 63- 67.

Regnault-Roger, C., A. Hamraoui, M. Holeman, E. Theron and R. Pinel. 1993. Insecticidal effect of essential oils from Mediterranean plants upon *Acanthoscelideso btectus* (Say) (Coleoptera: Bruchidae), a pest of kidney bean (*Phaseolus vulgaris* L.). J. Chem. Ecol. 14: 1965-1975.

Rozman, V., I. Kalinovic and Z. Korunic. 2007. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res. 43: 349-355.

Sadomov, E. 1984. Granivorous beetles. Rastitelna Zashchita 36: 32-34.

Sahaf, B.Z., S. Moharramipour and M.H. Meshkatalsadat. 2008. Fumigant toxicity of essential oil from Vitex pseudo-negundo against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.). J. Asia-Pac. Entomol. 1: 175-179.

Shaaya, E., U. Ravid, N. Paster, B. Juven, U. Zisman and V.Pisarrev. 1991. Fumigant toxicity of essential oils against four major stored-product insects. J. Chem. Econ. 17: 499-504.

Sharaby, A. 1988. Evaluation of some Mytraceae plant leaves as protectants against the infestation by *Sitophilus oryzae* L. and *Sitophilus granarius* L. Insect Sci. Appl. 9: 465-468.

Sharma, R.K. 1999. Efficacy of neem products against storage pests in maize. Ann. Agri. Res. 20: 198-201.

Singh, D. and T.D. Yadav. 1995. Studies on the incidence of insect pest, practices and losses in stored wheat in Haryana. Indian J. Entomol. 57: 107-115.

Sluiter, A., R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton. 2005. Determination of extractives in biomass. Laboratory Analytical Procedure (LAP), 1617.

Sousa, A., L. Faroni, M. Pimentel and R. Guedes. 2009. Developmental and population growth rates of phosphine-resistant and susceptible populations of stored product insect pests. J. Stored Prod. Res. 45: 241-246.

Subramanyam, B. and D.W. Hagstrum. 1995. Resistance measurement and management. In: B. Subramanyam and D.W. Hagstrum (eds.), Integrated Management of Insects in Stored Products. Marcel Dekker, New York, pp. 331–397.

Tapondjou, L.A., C. Adler and H. Bouda. 2002. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six stored product beetles. J. Stored Prod. Res. 38: 395-402.

Taylor, R.W.D. 1991. Resistance to grain fumigants and future prospects for their use. Pestic. Outlook (UK) 2: 22-4.

Tripathi, A.K., V. Prajapati, N. Verma, J.R. Bahl, R.P. Bansal, S.P.S. Khanjua and S. Kumar. 2002. Bioactivities of the leaf essential oil of C*urcuma longa* (Var. Ch-66) on three species of stored product beetles (Coleoptera). J. Econ. Entomol. 95: 183-189.

Weifen, Q.J., Z.X. Credland, P.F. Armitage, D.M. Bell, C.H. Cogan and E. Highley. 2003. Advances in stored product protection. Proceed. 8th Int. Working Conf. Stored Prod. Protect., York, U.K.

White, N.D.G. and J.G. Leesch. 1996. Chemical control. In: B. Subramanyam and D.W. Hagstrum (eds.), Integrated management of insects in stored products. Marcel Dekker, Inc., New York, pp.287-330.

Zettler, J.L. 1991. Pesticide resistance in *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) from flour mills in the United States. J. Econ. Entomol. 89: 763-767.