Pakistan Entomologist

Journal homepage: www.pakentomol.com

COMBINED INSECTICIDAL EFFECTIVENESS OF ESSENTIAL OILS OF TWO LOCALLY GROWN PLANT AND ALUMINUM PHOSPHIDE AGAINST *TRIBOLIUM CASTANEUM*

Ch. Muhammad Shahid Hanif[1], *Mansoor-ul-Hasan[1], Muhammad Ashfaq[1] and Nazir Javed[2]

[1]Department of Entomology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

ARTICLE INFORMATION

Received: August 9, 2013
Received in revised form: October 10, 2013
Accepted: October 20, 2013

***Corresponding Author**:
Mansoor-ul-Hasan
Email: mansoorsahi2000@yahoo.com

ABSTRACT

Tribolium castaneum is the most vicious and cosmopolitan pest which causes considerable loss to stored products. It has developed resistance against Phosphine in different parts of the world especially in developing countries. Need of the day is to search the plants derived natural products as an alternative to conventional and synthetic insecticides to overcome environmental hazards and ever-increasing pest resistance against these pesticides. Keeping in view, the current studies were carried out to evaluate the toxicological impact of essential oils (5, 10, 15%) of *Melia azadirach* and *Azadirachta indica*, Phosphine gas (100, 200, 300 ppm) and their different combinations against *T. castaneum.* Mortality percentage was recorded after 24, 48 and 72 hours. *A. indica* caused maximum mean mortality (34.48%) with highest concentration at 72 hrs of exposure, while 74.58% mortality was observed in Phosphine 300 ppm at 72 hrs. Combined application of these essential oils and Phosphine increased mortality significantly; 90.55% and 82.59% mortality was observed in 300 ppm +15% *A. indica* and 300 ppm +15% *M. azadarach* respectively after 72 hrs.

Keywords: Plant extracts, fumigation, stored grain pests, toxicity, exposure time

INTRODUCTION

The post harvest losses of grains have been reported as 10-15% in Pakistan (Ahmad, 1994). The weight losses due to insect pest infestation recorded by the scientists ranged from 1 to more than 2.5% (Ahmad *et al.*, 1992), 3.24% (Khan *et al.*, 1985), 0.45% and 0.72% (Hassan *et al.*, 1994) and 2.03% of wheat in India (Singh and Yadav, 1995). Most common insect pests of stored wheat in Pakistan reported by Irshad and Talpur (1993) are *Rhyzopertha dominica* F, *Tribolium castaneum* (Herbst) and *Sitotroga cerellela*. Zettler (1991) reported that *T. castaneum* is the mostly founded and injurious pest in flour mills, bulks of grains, oilseeds and warehousing facilities. Currently synthetic pesticides are mostly used for the control of stored grain pests but the frequent use of these pesticides has made the pest strains resistant against these pesticides (Subramanyam and Hagstrum, 1995a). Chemical pesticides have been used for a long period of time, but they have some serious drawbacks (Sharaby, 1988), they affect the beneficial insects due to direct toxicity and also affect the fishes and human (Munakata, 1977). Chemical pesticides also have health hazard (Bhaduri *et al*., 1989), resistance against insecticides is developed in insects (Brown, 1968; Georghiou and Taylor, 1977); their cost of application increased, environment has disturbed and they also had harmful effects on non target organisms (Champ and Dyte, 1976; Subramanyam and Hagstrum, 1995b; White and Leesch, 1995; Jembere *et al*., 1995; Okonkwo and Okoye, 1996) and enhanced the environmental and social cost (Pimental *et al*., 1981). Stored grain pests also showed resistance against many chemicals. Phosphine fumigation has become increasingly limited in its use because of resistance developed in stored grain insects to this fumigant, which is now reported from more than 45 countries (Bell and Wilson, 1995).

Many plant powders give effective control against stored grain insects in wheat grains. Toxicity of aqueous methanolic and acetonic extracts of three plants *Azadirachta indica*, *Rhazaya*, *Heliotropium bacciferum* on the khapra beetle was investigated. All extracts showed remarkable toxicities. The toxic effect observed for acetone extract was about 1.4% than observed for either aqueous or methanol extracts (EI-Nadi *et al*., 2001). The plant kingdom could be a rich source of a large

Cite this article as: Hanif, C.M.S., Mansoor-ul-Hasan, M. Ashfaq and N. Javed, 2013. Combined insecticidal effectiveness of essential oils of two locally grown plants and aluminum phosphide against *Tribolium castaneum*. Pak. Entomol., 35(2): 77-81.

number of chemicals which could be developed as control agents against stored grain pests successfully (Aranson *et al*., 1989). Insecticidal effect of many plants against pests of stored grain has been demonstrated (Abubakar *et al*., 2000; Boussalis *et al*., 1999; Fields *et al*., 2001; Huang *et al*., 2000; Tripathi *et al*., 2002). Many plant derived substances showed high physiological and behavioral activities against pests of stored grain, which includes repellent, toxic, antifeedant effects (Aranson *et al*., 1989; Grainge and Ahmed, 1988). Essential oils of aromatic plants which have rich insecticidal properties could be used as alternative insecticides (Dal Bello *et al*., 1996; Regnault-Rogar *et al*., 1993). Several products of floral species have been evaluated to act as repellents, toxicants and antifeedants against a number of Coleopteran species that attack stored grain and their products (Papachristos and Stamopoulos, 2002; Tapondjou *et al*., 2002). Present investigation aimed to assess the insecticidal activities of essential oils, Phosphine gas and their combinations towards *Tribolium castaneum*.

MATERIALS AND METHODS

Collection and Rearing of Insects

Tribolium castaneum population was collected from godowns of flour mills and grain market of Faisalabad. After collection, the insect population was kept in the jars and was covered with muslin cloths. Insect population was regularly checked for their growth, and was sieved and transferred to new uninfested wheat flour diet. Temperature 30 ± 2ºC and relative humidity at 65 ± 5% was maintained in for insect's maximum growth. Homogenous population of equal size and age was sieved out which later on was used for bioassay studies.

Preparation of Plant Extracts and Essential Oils

Weighed amount of fully mature fresh plant leaves of each botanical (*Azadirachta indica* and *Melia azadarach*) were grinded separately after shade drying. For extraction of oil, grinded samples were run on Soxhlet's apparatus with ethanol as solvent in the flask. After that solvent was evaporated leaving the essential oil.

Generation of Phosphine Gas

The Phosphine gas was generated by FAO's method. 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. When it was filled, 5 ml gas was suck out with the help of an air tight syringe and was injected into a sealed desiccators of known volume then 50 ml of gas was taken out from the desiccators and injected into Phosphine meter for measuring gas concentration. With the help of Phosphine meter required concentrations of 100 ppm, 200 ppm and 300 ppm of Phosphine gas were obtained.

Bioassay to evaluate the insecticidal efficacy of Phosphine and essential oils of selected plants against *Tribolium castaneum*

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 adults of *Tribolium castaneum* 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 *Azadirachta indica* and *Melia azadarach* and of Phosphine gas (100, 200 and 300 ppm) were applied. Data regarding mortality of test insects were recorded after 24, 48 and 72 hours. The surviving individuals from the adult bioassay were transferred into the new clean jars containing sterilized wheat flour and grains.

Statistical analysis

Statistical analysis of all collected data of percent mortality was subject to analysis of variance using Statistica-8 software. Means of significant treatments were compared using Tuckey HSD test at $\alpha = 5\%$.

RESULTS

Fumigant effect of plant essential oils against *Tribolium castaneum*

Data regarding percent mortality of *Tribolium castaneum* against *Melia azadarach* (Fig. 1) and *Azadirachta indica* (Fig. 2) revealed that percentage mortality of test insect was time and concentration dependent. It is evident from the figure 1 & 2 that mortality significantly increased with the increase in exposure time and concentration of essential oil. *Azadirachta indica* was found more effective showing maximum mortality (34.48%) with 15% concentration at 72 hrs of exposure, while 23.75% was the highest mortality observed in *Melia azadarach* at the same concentration and exposure time.

Fumigant effect of Phosphine gas

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. 3 indicating significant difference in interaction of time interval and concentrations. It is obvious from the figure that Phosphine gas exhibited minimum mortality 25.43% with 100ppm concentration at 24 hrs of exposure time and it significantly increased up to the maximum of 74.58% with the increase in concentration and exposure time.

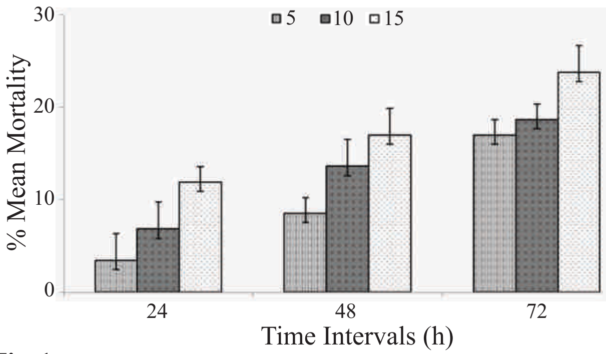

Fig. 1
Comparison of the data regarding percent mean mortality of *Tribolium castaneum* (Herbst) by different concentrations of essential of *Melia azadarch* at various exposure times.

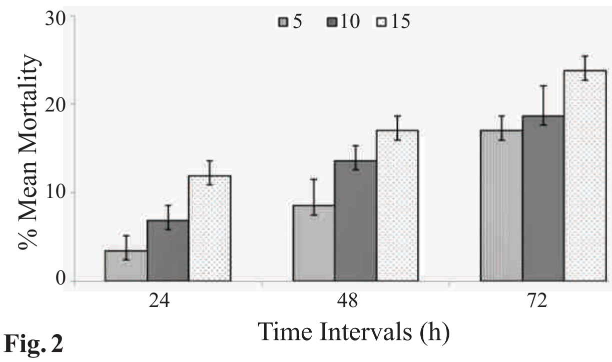

Fig. 2
Comparison of the data regarding percent mean mortality of *Tribolium castaneum* (Herbst) by different concentrations of essential of *Azadirachta indica* at various exposure times.

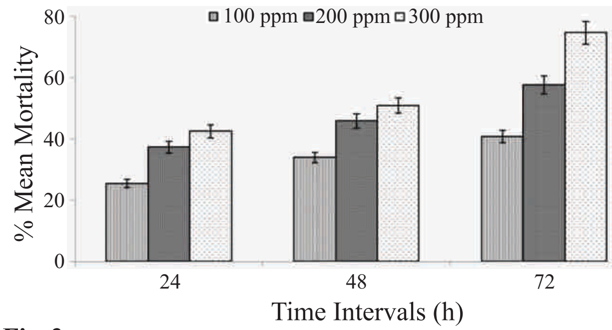

Fig. 3
Comparisons of the data regarding percent mean mortality of *Tribolium castaneum* (Herbst) by Phosphine gas by various concentrations at different exposure periods.

Combined effect of phosphine and plant essential oil

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. *Azadirachta indica* (table. 2) exhibited more percentage mortality than *Melia azadarach* (table. 1) in combination with phosphine. Mortality was recorded 82.59% and 90.55% with 15% *Melia azadarach* +300 pmm and 15% *Azadirachta indica* +300 ppm phosphine gas respectively at 72 hrs of exposure.

Table 1
Percent Mortality of adult of *Tribolium castaneum* against different combination of essential oil of *Melia azadarach* and Phosphine gas.

Phosphine	Plant oil	24 hrs	48 hrs	72 hrs
100 ppm	5%	22.06 ± 1.69	32.22 ± 1.69	33.92 ± 1.39
	10%	27.14 ± 1.69	37.31 ± 1.69	37.31 ± 1.69
	15%	30.53 ± 1.69	42.39 ± 1.69	45.78 ± 1.69
200 ppm	5%	28.84 ± 0.69	35.61 ± 1.69	42.39 ± 1.69
	10%	33.92 ± 2.93	42.39 ± 1.69	50.86 ± 1.69
	15%	40.70 ± 1.69	47.47 ± 1.69	57.64 ± 1.69
300 ppm	5%	41.73 ± 0.18	51.50 ± 0.37	68.14 ± 0.36
	10%	50.17± 0.69	62.55 ± 0.69	73.22 ± 0.36
	15%	61.07 ± 0.37	69.83 ± 0.16	82.59± 1.69

Table 2
Percent Mortality of adult of *Tribolium castaneum* against different combinations of essential oil of *Azadirachta indica* and Phosphine gas.

Phosphine	Plant oil	24 hrs	48 hrs	72 hrs
100 ppm	5%	27.14 ± 1.69	30.53 ± 1.69	35.61 ± 1.69
	10%	30.53 ± 1.69	37.31 ± 1.69	42.39 ± 1.69
	15%	35.61 ± 1.69	42.39 ± 1.69	45.78 ± 1.69
200 ppm	5%	31.56 ± 0.36	37.70 ± 0.15	46.85 ± 0.69
	10%	38.72 ± 0.63	45.17 ± 0.30	52.55 ± 0.30
	15%	45.72 ± 0.36	52.86 ± 0.69	59.33 ± 0.00
300 ppm	5%	45.91 ± 0.18	54.25 ± 0.72	74.58± 0.30
	10%	54.25± 0.30	66.11 ± 0.30	81.39 ± 0.36
	15%	65.94 ± 0.52	74.58 ± 0.62	90.55 ± 0.36

DISCUSSION

Studies were carried out to evaluate toxic effectiveness of plant essential oils, Phosphine gas and their combinations. Many essential oils and their constituents have been studied to possess 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). Both the essential oils assessed in this study exhibited significant, time and concentration dependent mortality against adults of *Tribolium castaneum* however *Azadirachta indica* was found more effective with 34.48% mortality. Previous research testified *Azadirachta indica* as potential for the field evaluation as grain protectant (Rahim, 1998; Lale and Mustapha, 2000; Khan and Marwat, 2003). 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 is combined application of Phosphine gas and essential oils, which not only decline the resistance probability but also enhanced insecticidal effectiveness.

REFERENCES

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

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 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 International Symposium on 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(1): 3-9.

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.

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. In: 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.

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(2): 11.

Huang, H., S.L. Lam and S.H. Ho, 2000. Bio-activities 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.

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, 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. 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.

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

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 *Acanthoscelides obtectus* (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. N.Y. pp. 52.

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.

Regnault-Roger, C., A. Hamraoui, M. Holeman, E. Theron and R. Pinel, 1993. Insecticidal effect of essential oils from Mediterranean plants upon *Acanthoscelides obtectus* (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.

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.

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.

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

Subrammanyam, B. and D.W. Hagstrum, 1995b. Resistance measurement and management.In: Subrammanyam, B. and D. Hagstrum (Eds.), Integrated Management of Insects in Stored Products. M. Dekker, Inc., New York, pp. 331-397.

Tapondjou, L.A., C. Adler and H. Bouda, 2002. Efficacy of powder and essential oil from *Chenopodiumam brosioides* 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 (Rev. Appl. Entomol. (A), 80: 1992; 7560).

Tripathi, A.K., V. Prajapati, N. Verma, J.R. Bahl, R.P. Bansal, S.P.S. Khanjua and S. Kumar, 2002. Bio-activities of the leaf essential oil of C*urcuma longa* (Var. Ch-66) on three species of stored product beetles (Coleoptera). J. Econ. Entomol., 95(1): 183-189.

White, N.D.G. and J.G. Leesch, 1995. Chemical control. In:Subramanyam, B., 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(3): 763-767.