OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2020.XX.XX

Research Article
Determinations of the Effect of Three Natural Oils on *Rhyzopertha dominica* (Coleoptera: Bostrichidae) under Laboratory and Store Conditions

Magda M.A. Sabbour

Department of Pests and Plant Protection, Agriculture Division, National Research Center, 33rd El Bohouth St., Dokki, Giza, Egypt

Abstract

Background and Objective: The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is a very harmful insect pests to cereals grain including wheat. Three oils tested on this pests they are thyme, frankincense and turmeric under laboratory and store conditions. **Materials and Methods:** The tested nano-oils were experimented at tested concentration (0.5%) for their insecticidal activities against the 3rd instar larvae of tested insects. After 7 days of exposure, accumulative mortality percentages were calculated in the treated and untreated control. Also, the tested nano-oils were sprayed to the foam granules and were mixed with wheat (2 g foam/100 g wheat) for testing the oviposition inhibitory effects of tested oils. The experiment was designed to test the persistent effect of tested oils against tested species on foam as surface protectant after 90 day intervals. **Results:** Results showed that the accumulative mortality percentage was gradually increased after increasing the period of exposure to foam treated with the thyme, frankincense and turmeric tested. Nano-oils showed a highest accumulative mortality (99.0, 89, 87%) when treated with thyme, frankincense and turmeric, respectively. The reduction of the eggs laid per female was scored 99.3% eggs/female after treatments with nano-thyme. Under store conditions the percentage of infestations was significantly decreased to 2, 7 and 10% after treating the stocks sacs with thyme, frankincense and turmeric, respectively as compared to 99% in the stock sacs control (untreated). **Conclusion:** The three natural oils and its nano-compositions decreased the infestations numbers of *R. dominica* under laboratory and store conditions.

Key words: Natural oils, *Rhyzopertha dominica*, thyme, frankincense, turmeric

Citation: Magda M.A. Sabbour, 2020. Determinations of the effect of three natural oils on *Rhyzopertha dominica* (Coleoptera: Bostrichidae) under laboratory and store conditions. Asian J. Biol. Sci., 13: XX-XX.

Corresponding Author: Magda M.A. Sabbour, Department of Pests and Plant Protection, Agriculture Division, National Research Center, 33rd El Bohouth St., Dokki, Giza, Egypt

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (F.) is a major cosmopolitan insect pest of stored wheat. The females lay eggs on the outside of the wheat kernel and the first-instars hatch and bore into the kernel. The larvae feed and develop inside the kernel and upon reaching the adult stage, bore out of the kernel and create a large exit hole. Because the majority of the development occurs inside the kernel, *R. dominica* is difficult to kill with contact insecticides applied directly to stored wheat[1-3] and insect contamination in food commodities is an important quality control problem of concern for food industries. In industrialized countries like Canada and Australia there is zero tolerance for insects in food grains[4,5]. The *R. dominica* is a widespread pest of stored products-especially cereals and found around the world. The adult moths do not feed. The immature stages of the stored products larvae are the most harmful and destructive stages. The feeding pollutes stored products with faeces and webbings and causing spoil of the product[5,6]. Plants played a significant role in integrated pest management strategies because they possess an important source of insecticides[7,8]. Essential oils are distinguished by a low toxicity to mammalian, high volatility and toxicity to stored product insects. The essential oils used against a large variety of organisms and showed a highly effect in biological activities against insect pests especially the stored products this, inclusive the fumigation, insecticidal activities, oviposition inhibitory toxicity, antifeeding and repellent.

Essential oils are distinguished by a low toxicity to mammalian, high volatility and toxicity to stored product insects[8,9]. Essential oils display a broad spectrum of biological activities against insect pests especially of stored products including; fumigation, toxicity, insecticidal activities, repellent, antifeeding, oviposition inhibitory[10-13]. Each of nano-pesticides, nano-fungicides and nano-herbicides have been utilized in agriculture[14-16]. Nano-encapsulated pesticide formulation is able to minimize the dosage of pesticides and human exposition to them, which is environmentally friendly for crop protection. Botanical essential oils have efficient stored product pest management properties. Therefore, this study investigated some botanical oils comparing with nano-botanical oils, against *Ephestia kuehniella* under laboratory and during storage.

Nano-encapsulated pesticide formulation is able to minimize the dosage of pesticides and human exposition to them, which is environmentally friendly for crop protection. Botanical essential oils have efficient stored product pest management properties. Therefore, this study investigated some botanical oils comparing with nano-botanical oils, against *R. dominica* under laboratory and during storage.

MATERIALS AND METHODS

Rearing of insects: *Rhyzopertha dominica* were used in the experiments. The target insects were reared under laboratory conditions at National Research Centre, Agriculture Division during May, 2019 on beans seeds and wheat seeds, respectively. All cultures and experiments were held at 26±2°C and 70-80% Relative Humidity (RH) with 16 h light and 8 h dark.

Tested oils: Three essential oils were used in the bioassay tests, thyme, frankincense and turmeric. The essential oils were obtained by steam distillation of dried plants[17].

The tested oil emulsions were prepared as follows; 5 drops of Triton-X-100 as emulsifier were mixed thoroughly with 5 mL of each tested oil and then water was added to obtain the desired concentrations (2%) in percent of v/v. The emulsifier was mixed at the corresponding concentrations and used as check. Insecticidal activity of tested oils: The experiment was planed to evaluate the premier as well as the continual effect of the tested oils on the stored product weevils as accumulative mortality during sequential, times (24, 48, 96 and 168 h). Foam granules about 1 cm in diameter were treated at time (0 time) with tested oils (2% conc.) dried and provided with heat sterilized rice seeds (100 g each) fastened each with a string. Then all treatments were used immediately as non-choice test. The foam particles treated with the evaluated natural oils which were mixed with stored seeds (2 g foam/100 g seeds)[15,16]. A pair of newly emerged weevils was placed with treated or untreated rice seeds in glass jars (250 mL capacity) covered with muslin. The number of dead weevils in each jar was counted every day and the percentages of mortality were corrected using the Abbott[18] formula. The tested fungi were diluted in distilled water (v/v) to obtain 6 concentrations (16, 8, 4, 2, 1 and 0.5x108 spores/mL) at the rated 16.5x107 spores/mL. They were mixed at 4.25x108 spores/mL with the tested oils (0.05% conc.), then sprayed to the seeds. A pair of newly emerged weevils was placed with treated or untreated broad seeds in glass jars (250 mL capacity) covered with muslin. The number of dead weevils in each jar was counted every day and the percentages of mortality were corrected using the Abbott formula. The LC_{50} was calculated through the probit analysis[19]. This experiment was achieved under the National Research Centre laboratory conditions of 27±2°C and 60-65% RH. The experiment was replicated 4 times.

The nano-encapsulation is a process through which a chemical is slowly, but efficiently released to the particular host for insect pests control. "Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH"[20].

The nano-encapsulation process was carried out by polymerization technology. The tested oils were used as a core material and Urea (U) and Formaldehyde (F) as shell materials. Sulfuric acid solution (10% w/w) was prepared in our laboratory to control the pH (4.4) of emulsion and tween-80 (polysorbate 80) were used as emulsifier (Merck, Germany). The obtained suspension of nano-capsules was cooled down to ambient temperature, rinsed with deionized water, filtered and finally dehydrated by freeze-drying by using a LIO-5P, which is a freeze dryers for laboratory use (Apparatus Cinque Pascal, Trezzano SN, Milan, Italy). Nano-emulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol to create stable droplets which that increase the retention of the oil and cause as low release of the nano-materials. The release rate depends upon the protection time, consequently a decrease in release rate can prolong insect pests protection time[21]. For each tested bulk essential oils, 4 concentrations were prepared 3, 1.5, 0.5 and 0.2%. While, in case of nano-essential oils, the tested concentrations were 0.1, 0.5, 0.05 and 0.005%. Three drops of emulsifier (Triton-X-100) was mixed with water and used as check. The tested oils (Bulk and Nano) were experimented at tested concentrations for their insecticidal activities against the 3rd instar larvae of target pets. According to Abd El-Aziz[22], the foam granules were sprayed with the tested oils (Bulk and Nano) and were mixed with wheat (2 g foam/100 g wheat). For each tested concentration, 4 glass jars as replicates were used. Thereafter, 10 third instar larvae were introduced into each glass jar and was covered with muslin for suitable ventilation. In case of untreated control, 12 replicates were kept under the same conditions without any treatments. After seven days of exposure, mortality (%) were calculated in the treated and untreated control. All tests were carried at 27±2°C and 65±5% Relative Humidity (RH). The dead larvae numbers were estimate in each jar was used and the mortalities (%) were determined. Each experiment was replicated 4 times.

The tested oils (Bulk and Nano) were sprayed to the foam granules and were mixed with wheat (2 g foam/100 g wheat) for testing the oviposition inhibitory effects of tested oils[22]. In no-choice test, 2 pairs of mixed sex of mediterranean flour moth adults (2-3 days old) were placed with treated or untreated wheat grains with foam particles in glass jars (250 cc capacity) covered with muslin. Adults female moths were left to lay their eggs, hen the deposited eggs numbers treated or untreated the grains/ female were estimated in each of the tested jars. Four glass jars as replicates for each tested concentration were used and the test was repeated three times. The persistence effect of tested oils (Bulk and Nano) on foam as surface protectant was evaluated after storage interval 120 days (4 months) against *E. kuehniella* moth's emergence. Hundred gram of heat sterilized wheat grains were introduced to gunny sacks (10×10 cm each) closed each with a string. The foam granules (about 1 cm in diameter) were sprayed with treatments, dried and provided as a layer between sacks. Then, 2 pairs of newly emerged moths were placed in a jar (2 capacity with 2 gunny sacks). Then remove the moths which was died after lying egg. The count the new emerged adult moths after 120 days about (4 months).

Statistical analysis: Data were displayed to Analysis of Variance (ANOVA) and means were compared by a least significant different test. The data was analyzed by using Analysis of Variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the Least Significant Difference (LSD) test at 5% level[23]. The data was analyzed by using Analysis of Variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the Least Significant Difference (LSD) test at 5% level[23].

RESULTS

Tested oil on target pests accumulations: During the first week of board the accumulations mortality of *R. dominica* adults oils showed a highest accumulative mortality obtained (51.2, 34.3, 44.3%) after two days of treated seeds with thyme, frankincense and turmeric, respectively. After seven days the accumulative mortality of the corresponding oil calculated (90.0, 89.0 and 87.3%), respectively as compared to 0.1 in the control (untreated) (Table 1).

When the target insect pests *R. dominica* adults treated with the nano-oils after two days the accumulation mortality obtained 53.9, 36.92 and 33.2% after treated with nano-oils of thyme, frankincense and turmeric, respectively as compared to 0 in the control. After 7 days of treatments of the nano-oils of the corresponding oils the accumulative mortality reached to 98.3, 83.8 and 90.9 as compared to 0.5% in the control (Table 2).

Table 1: Accumulations mortality of target pests adults during the first week of broad bean seeds in treated foam with different oils

Tested oil	Time (days)	*Rhyzopertha dominica*
Thyme	0	36.9
	2	51.2
	4	62.2
	7	90.0
Frankincense	0	27.6
	2	34.3
	4	56.1
	7	89.0
Turmeric	0	30.2
	2	44.3
	4	57.1
	7	87.3
Untreated	0	00.0
	2	00.0
	4	00.2
	7	00.1
F-test (LSD 5%)		24.0 8.8

Table 2: Accumulations mortality of target pests adults during the first week of broad bean seeds in treated foam with different nano-oils

Tested oil	Time (days)	*Rhyzopertha dominica*
Nano-thyme	0	36.9
	2	53.9
	4	643.0
	7	98.3
Nano-frankincense	0	29.5
	2	36.9
	4	48.5
	7	83.8
Nano-frankincense	0	31.1
	2	33.2
	4	55.3
	7	90.9
Untreated	0	0.0
	2	0.0
	4	0.3
	7	0.5
F-test	38.9	
LSD (5%)	19.7	

Effect of tested oils on number of laid eggs/female and adult emergence (F1) (%) of target pests during storage periods: The reduction of the eggs laid per female was scored 99.3% eggs/female after treatments with nano-thyme.

Table 3 showed the effect of oils and its nano on number of laid eggs/female and adult emergence (F1)(%) of *R. dominica* during storage periods . The percentages of eggs laid /female significantly decreased to 99.3, 93.2 and 90.7 after nano-thyme, nano-frankincense and nano-turmeric treatments as compared to 100% in the control (untreated).

Effect of nano-tested oils on number of laid eggs/female and adult emergence (F1)(%) of target pests during storage periods. Table 4 showed that the nano-compositions of the three tested oil affect on the means number of eggs laid per female. The mean number significantly decreased to 10.3±0.0, 20.4±1.21 and 20.4±5.38 eggs/female after nano-thyme, nano-frankincense and nano-turmeric as compared to 297.9±7.89 eggs/female in the control. The percentages of adult emergence significantly decreased to 0.1% in case of nano theme treatments as compared to100 in the control. Also, the malformation of the nano-thyme, nano frankincense and nano turmeric reached to 98, 99 and 98% as compared to 0 in the control (Table 4). Table 4 showed that the nano-compositions of the 3 tested oil affect on the means number of eggs laid per female. The mean number significantly decreased to 10.3±0.0, 20.4±1.21 and 20.4±5.38 eggs/female after nano-thyme, nano frankincense and nano turmeric as compared to 297.9±7.89 eggs/female in the control. The percentages of adult emergence significantly decreased to 0.1% in case of nano theme treatments as compared to 100 in the control. Also, the malformation of the nano-thyme, nano-frankincense and nano-turmeric reached to 98, 99 and 98% as compared to 0 in the control (Table 4).

Table 3: Effect of tested oils on number of laid eggs/female and adult emergence (F1)(%) of target pests during storage periods

Tested materials	Reduction of eggs laid/female (%)	Adult emergence (F1) (%)	Malformation (%)
Nano-thyme	99.3	1	100
Nano-frankincense	93.2	9	100
Nano-turmeric	90.7	6	99
Control	100	100	0
F-test	38.7		
LSD (5%)	19.9		

Table 4: Effect of nano-tested oils on number of laid eggs/female and adult emergence (F1)(%) of target pests during storage periods

Tested materials	No. of eggs/♀±S.E.	Adult emergence (F1) (%)	Malformation (%)
Nano-thyme	10.3±0.0	0.1	98
Nano-frankincense	20.4±1.21	2	99
Nano-turmeric	20.4±5.38	1	98
Control	297.9±7.89	100	0
F-test	36.7		
LSD (5%)	18.7		

DISCUSSIONS

Results showed that the reduction in number of eggs laid/female decreased to 10.3±0.0, 20.4±1.21 and 20.4±5.38 eggs/female after nano-thyme, nano-frankincense and nano-turmeric as compared to 297.9±7.89 eggs/female in the control, this meet of those who reported that the entomopathogenic fungus after being treated with *B. bassiana* and botanical oils, *N. sativa, T. distichum* and *B. carterii* when compared to the control group[22-24]. Many studies found that natural edible oils have a potency control agents against *S. granarius* and also play a serious remarkable effect in stored-grain protection[24]. Researchers[22-24] mentioned that, nano-thyme, nano-frankincense and nano-turmeric clove and eucalyptus oil vapors impaired the fecundity of brachid beetles data proved promising oviposition deterrence toxicity and suppression of eggs and adult emergence[19].

Lisansky[25] recorded that the LD_{50} for some formulations of *B. bassiana* was reduced to 97% after the addition of coconut oil. It was suggested that the cutinophilic properties of the oil could allow a greater number of fungal conidia to penetrate the mouth parts of insects. Oil transporter could to distribute the inoculum over the stored product insect pests thin skin membranes, which are more easily penetrated by entomopathogenic fungi[25]. The increase in the pathogenicity of *B. bassiana* combined with mustard oil to *C. maculatus* beetles may be attributed to some degradation occurring at the structural level of the integument, which could have facilitated the penetration of the cuticle by the germ tube of the fungus. Similar results were obtained by Hassan and Charnley[26] in Manduca sexta treated with *M. anisopliae* and the chitin-synthesis inhibitor dimiline. It was reported that synergistic effects of a combined application of *B. bassiana* and the chloronicotinyl insecticide imidaclopride on *Diaprepes abbreviatus* L. (Coleoptera: curculionidae)[26]. Similar results obtained by multiple studies[13,27]. Chander and Ahmed[28] reported that the applications of the different doses of natural essential oils like the *Acorus catamus* seeds oils of green beans to protect them from the dangerous pest infestation. Abd El-Aziz[22] reported that foam sprayed with clove oil (5%) and placed between sacks caused the highest mortality to *C. maculatus*. Similar results obtained by various studies[26-29]. The fungus *B. bassiana* and plant extracts reduced insect infestations of *Cassida vittata* and *Callosobruchus maculatus*. Sabbour and Abd El-Aziz[2] and Sabbour[30,31] used two entomopathogenic fungi alone or in combination with modified diatomaceous earth to control of Bruchidius incarnates and *Rhyzopertha dominica*. Accordingly Hanafi[32] who reported that biological and natural insecticides were more functional in preventing seeds losses by pests in stores in cases which contain the premier levels of pest infestation was relatively low.

We recommended to use the natural oils as bio-products which do not pollute the environments.

CONCLUSION

The results of this work research indicate that some the natural essential oils are very important and useful for managing and control the harmful pests in enclosed stores places because of their effective repellant and fumigant action. Moreover, the natural oils are highly eclectic to pests. Incorporation of natural oils as a controlled release nano-materials formulation which has a preventive rapid vaporization and degradation. The present experiments showed that the essential oils tested obviously very important and also, showed that the tested nano-oils have been found significantly efficient against tested insects as more insect mortality, a smaller number of eggs, less adult emergence, high percentage of adult malformation and less percentage of weight loss after 90 days of storage. These results based on the criteria that help the farmers to decrease the loss of stored seeds and give a good method to stored the grains.

SIGNIFICANCE STATEMENT

This study discover that the natural oils used and the nano-oils will be beneficial for store insect pest's control inside the stores. This study will help the researcher to uncover the critical areas of pests control and make their researches on the nano natural essential. The essential oils thyme, frankincense and turmeric proved a highly significant effects on *Rhyzopertha dominica* under laboratory and store conditions and protect the seed from the harmful infestations.

REFERENCES

1. Rajendran, S., 2002. Postharvest Pest Losses. In: Encyclopedia of Pest Management, Pimentel, D. (Ed.). Marcel Dekker, Inc., New York, pp: 654-656.
2. Sabbour, M.M. and S.E. Abd El-Aziz, 2014. Control of *Bruchidius incarnatus* and *Rhyzopertha dominica* using two entomopathogenic fungi alone or in combination with modified diatomaceous earth. Elixir Entomol., 68: 22239-22242.

3. Sabbour, M.M., 2018. Pathogenicity of imidacloprid and its nano against *Rhyzopertha dominica* (coleoptera: bostrichidae) under laboratory and store conditions integrated protection of stored products. IOBC-WPRS Bull., 130: 185-191.
4. White, N.D.G., 1995. Insects, Mites and Insecticides in Stored Grain Ecosystems. Jayas, D.S., N.D. White and W.E. Muir (Eds.). Marcel Dekker, New York, USA., pp: 123-168.
5. Pheloung, P. and F. Macbeth, 2002. Export Inspection: Adding Value to Australia's Grain. In: Stored Grain in Australia 2000: Proceedings of the Australian Postharvest Technical Conference, Adelaide, 1-4 August 2000, Wright, E.J., H.J. Banks and E. Highley (Eds.)., CSIRO Stored Grain Research Laboratory, Canberra, Australia, pp: 15-17.
6. Athanassiou, C.G., N.G. Kavallieratos, B.J. Vayias, J.B. Tsakiri, N.H. Mikeli, C.M. Meletsis and Z. Tomanović, 2008. Persistence and efficacy of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) and diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.)(Coleoptera: Bostrychidae) on wheat and maize. Crop Prot., 27: 1303-1311.
7. Golob, P., C. Moss, M. Dales, A. Fidgen, J. Evans and I. Gudrups, 1999. The Use of Spices and Medicinals as Bioactive Protectants for Grains (FAO Agricultural Services Bulletin 137). Food and Agriculture Organization, Rome, Italy, ISBN-13: 9789251042946, Pages: 239.
8. Regnault-Roger, C., 1997. The potential of botanical essential oils for insect pest control. Integ. Pest. Manage. Rev., 2: 25-34.
9. Regnault-Roger, C. and Hamraoui, 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.
10. Abd El-Aziz, S.E. and M. Sharaby, 1997. Some biological effects of white mustard oil, *Brassica alba* against the cotton leafworm, *Spodoptera littoralis* (Boisd.). Anz. Schadlingskde Pflanzenschutz Umweltschutz, 70: 62-64.
11. Sabbour, M.M. and S.E. Abd El-Aziz, 2010. Efficacy of some bioinsecticides against *Bruchidius incarnatus* (Boh.) (Coleoptera: Bruchidae) infestation during storage. J. Plant Prot. Res., 50: 28-34.
12. Ketoh, G.K., H.K. Koumaglo and I.A. Glitho, 2005. Inhibition of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) development with essential oil extracted from *Cymbopogon schoenanthus* L. Spreng. (Poaceae) and the wasp *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae). J. Stored Prod. Res., 41: 363-371.
13. Sabbour, M.M., S.E. Abd El-Aziz and E. Shadia, 2007. Efficiency of some bioinsecticides against broad bean beetle, *Bruchus rufimanus* (Coleoptera: Bruchidae). Res. J. Agric. Biol. Sci., 3: 67-72.
14. Magda, S. and M.M. Hussein, 2015. Usage of the nano phosphorous fertilizers in enhancing the corn crop and its effect on corn borers infestations after fungi treatments. Int. J. ChemTech Res., 8: 167-173.
15. Magda, S. and M.M. Hussein, 2016. Determinations of the effect of using silca gel and nano-silica gel against *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato fields. J. Chem. Pharm. Res., 8: 506-512.
16. Sabbour, M.M. and S.E.A. El-Aziz, 2016. Efficacy of three essential oils and their nano-particles against *Sitophilus granaries* under laboratory and store conditions. J. Entomol. Res., 40: 229-234.
17. Guenther, G., 1961. The Essential Oils VIII. Robert E.D. Nastrand Company Inc., Toronto.
18. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
19. Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, London, UK.
20. Vidyalakshmi, R., R. Paranthaman and J. Indhumathi, 2009. Amylase production on submerged fermentation by *Bacillus* spp. World J. Chem., 4: 89-91.
21. Sakulku, U., O. Nuchuchua, N. Uawongyart, S. Puttipipatkhachorn, A. Soottitantawat and U. Ruktanonchai, 2009. Characterization and mosquito repellent activity of citronella oil nanoemulsion. Int. J. Pharmaceut., 372: 105-111.
22. Abd El-Aziz, S.E., 2001. Persistence of some plant oils against the bruchid beetle, *Callosobruchus maculatus* F., Coleoptera: Bruchidae during storage. Arab Univ. J. Agric. Sci. Egypt, 9: 423-432.
23. Sokal, R.R. and F.J. Rohlf, 1981. Biometry: The Principles and Practice of Statistics in Biological Research. 2nd Edn., W.H. Freeman Publisher, San Francisco, USA., ISBN-13: 9780716712541, Pages: 859.
24. Shaaya, E., M. Kostjukovski, J. Eilberg and C. Sukprakarn, 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. J. Stored Prod. Res., 33: 7-15.
25. Lisansky, S., 1989. Biopesticides fall short of market projections. Perform. Chem., 16: 387-396.
26. Hassan, A.E.M. and A.K. Charnley, 1989. Ultrastructural study of the penetration by *Metarhizium anisopliae* through Dimilin-affected cuticle of *Manduca sexta*. J. Invertebr. Pathol., 54: 117-124.
27. Vidhyalakshmi, R., R. Bhakyaraj and R.S. Subhasree, 2009. Encapsulation "The future of probiotics"-A review. Adv. Biol. Res., 3: 96-103.
28. Chander, H. and S.M. Ahmed, 1986. Efficacy of oils from medicinal plants as protectants of green gram against the pulse beetle *Callosobruchus chinensis*. Entomon, 11: 21-28.
29. Bhaskar, P.S., O. Koul and K. Tikku, 1976. Non-toxic protectant against stored grain insect pests. Bull. Grain Technol., 14: 190-193.

30. Sabbour, M., 2009. Evaluation of two entomopathogenic fungi against some insect pests infesting tomato crops in Egypt. IOBC/WPRS Bull., 49: 273-278.
31. Sabbour, M.M., 2006. Effect of some fertilizers mixed with bioinsecticides on the potato tuber moth *Phthorimaea operculella* infesting potato in the field and store. Pak. J. Biol. Sci., 9: 1929-1934.
32. Hanafi, A., 1999. Integrated pest management of potato tuber moth in field and storage. Potato Res., 42: 373-380.