Efficiency of certain insecticides against the small bean beetle, *Bruchidius incarnatus* (Boh.).

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Abstract:

The efficiency of five insecticides were evaluated against **the small bean beetle, *Bruchidius incarnates.*** Result indicated that the tested insecticides could be descendingly arranged according to their effeciency as follows: Malathion, Neemazal, Clove oil, K.Z. oil, and Agrien with LC_{50} values of 6.26, 100.00, 439.00, 618, and 1267 ppm, respectively. It is clear that malathion (organophosphoures pesticides) was the most toxic compound, whereas Agrien (biocide, *(Bacillus thurengensis*) was the least toxic one. Botanical pesticides and mineral oil occupied an intermediate position between with. Type formulation and concentrate of malathion play an important role in toxicity on adult stage of *B. incarnates*. Malathion 57% E.C. exhibited that highest activity compared with the malathion 5% D. and malathion 1% D. The LC_{50} values were 6.26, 13.3, and 45.8 ppm for Malathion 57% E.C., Malathion 5% D., and malathion 1% D., respectively. While the corresponding LC_{90} values were 34, 119 and 367 ppm, respectively. Obtained data indicated that the total residues in dry seeds of broad bean at all the different intervals from treatment were below the permissible limit (MRLs = 2 ppm).

Introduction:

The faba bean, *Vicia faba* (L.) belongs to the legumes family Leguminaceae isone of the oldest cultivated legumes in the world

and it is the most important food legume in Egypt. Legume seeds are considered a main source of protein for human and animal nutrition **(Smartt, 1976)**. Faba bean is composed of about 28% protein, 48% carbohydrates and 3.5% minerals **(EL Hardallu, 1981).**

Faba bean is subjected to the attack by several insect pests and diseases from the early developmental phase to postharvest time; post-harvest losses are mainly due to the attack by several storage pests. *Bruchidius incarnatus* (Bruchidae: Coleoptera) is the most important storage pest among 20 species of insect pests of faba bean recorded in Egypt **(Hawtin, *et al*., 1982; and Abd El-Aziz and Ismail, 2000).**

In Egypt, considerable yield losses of faba bean occur during storage due to the attack of Coleopterous beetles belonging to the family Bruchidae viz, *Bruchidius incarnatus* Boh. and *Callosobruchus maculatus* (F.). The infestation by these beetles causes serious losses in weight and deterioration in quality and the germination percentage of the stored faba bean seeds.

Chemical control of *B. incarnatus* (Bruchidae: Coleoptera) can be achieved by several chemical, different formulations and modes of action which include contact chemicals, dust, wettable powder and powder pesticides such as dimethoate (Rogar®), malathion, and carbaryl (Sevin®) and by fumigation with phostoxin **(Bushara, 1982).** The chemical control is effective, quick, secure and economical but it has some major drawbacks: negative impact on products and environment; constant danger of intoxication for humans and animals; presence of residues in different parts of the plants; appearance, at the pest species, of resistance to pesticide **(Rombke and Moltmann, 2000).** Many investigators have studied the

effectiveness of organophosphorus insecticides against stored product insect e.g. **(Abo-El ghar and Badawy, 1961; Ahmed and Delmon, 1979; Abdel-Kader et al.,1982; and Patoural and Joyeb, 1988).**

Alternative techniques to control weevils have received increasing attention, including growth resistant varieties of beans, artificial cooling, inert powder, oils and powders of repellents and other integrated management methods for insect pests **(Mazzonetto and Vendramim, 2003; and Maldonado, *et al.*, 1996).** These alternative control methods should always be applied with in a rational management program and be economically feasible, adapted to the size and the need of the storage.

Oils gave multiple effects on storage bruchids, such as increased adult mortality, lowering oviposition rate or interfering with larval development and adult emergence **(Messina and Renwick, 1983).**

Pesticide residue are substances or mixtures of substances in food for man or animals resulting from the use of pesticide including any specified derivatives, such as degradation and conversion products, metabolites, reaction products and impurities considered to be of toxicological significance **(EC, 2008)**. Pesticide residues most commonly found in food samples of vegetal origin are pesticides that are intentionally applied to the plants to attack invertebrate pests (insecticides, acaricides, etc.) and plant diseases (fungicides). The current study was carried out to evaluate toxicity of malathion and its alternatives against *B. incarnatus* adults on faba bean seeds under laboratory conditions and to determine of Malathion residues on broad bean seeds Vicia faba during storage.

Materials and Methods:

Stock culture: A laboratory culture of broad bean beetle, *B. incarnatus* was established from the naturally infested faba bean seeds and maintained for three generation under laboratory conditions. Healthy faba bean seeds (*vicia faba* L.) were first deep frozen for 30days to kill off any prior infestation and were then kept under laboratory conditions for ten days before infestation. Newly emerged adults were kept in glass jars of 2 litter capacity containing about 100 grams of broad bean seeds and covered with muslin.

Insecticides: five insecticides including one organophosphours pesticides with three formulation (Dust, Wettable powder, and Emlsifable concentrate), one biocide, two essential oils, and one mineral oil were used in this study. The common name, trade name, type of formulation, concentration, and chemical group besides for each pesticide are presented in (Table 1).

Table (1). Profile of insecticides used in the study.

No	Common name	Trade name	Type of formulation	Conc.%	Chemical group
1	Malathion	Malathion	E.C.	57	organophosphate
2	Malathion	Malacid	Dust	5	organophosphate
3	Malathion	Malathion	Dust	1	organophosphate
4	*Bacillus Thuringiensis*	Agerine	W.P.	6.5	Biocide*(B.t.)*
5	*Eugenia caryophyllata*	Clove oil	E.C.	100	Botanical pesticide
6	*Azadirachta indica*	Neemazal	E.C.	1	Botanical pesticide
7	Mineral oil	K.Z oil	E.C.	94	Mineral oil

Bioassay

Residual film method was used for adult bioassays. A half ml of water solution of each tested insecticide at the selected concentrations was spread on a Whatman No. 1 filter paper (7cm i.d.), which was placed in a petri dish, using 1 ml pipette, in

progressively decreasing spiral to ensure the chemical distribution andallowed to dry for approximately half an hour .Twenty normally active adult beetles were released in each petri dish, confined by plastic rings, and incubated at 28 ± 5º C and R. H. 60± 5 % and mortalities were recorded after 24 hours. For each bioassay at least five concentrations, giving mortalities between 0%-100 percent, were tested. Four replicates were set for each concentration. Water was used as a control. Data were considered acceptable if the mortalities observed in controls were less than 20%. Mortality was adjusted using Abbott's formula (**Abbott, 1925)** and Concentration-mortality regression lines were analyzed using a computer program modified from the method of (**Finney, 1971)** to estimate the LC_{50}, the confidence limits and the slopes of LCp lines.

Determination of Malathion residues in broad bean seeds *Vicia faba*

Chemicals

Acetone, methylene chloride, acetonitrile, and n-hexane were of HPLC reagent grade (Sigma–Aldrich, Steinheim, Germany). Florisil (60-100 mesh) was used for column chromatography, sodium chloride and anhydrous sodium sulfate **(El-Nasr Co., Egypt).** Malathion standard solution was obtained from the Central Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

Sampling and Storage

Three kg of faba bean seed treated with Malathion 1% dust was randomly collected 1, 2, 3, 4, 5, 6, 7, and 8 weeks after harvest. Sample was subdivided and then representative subsamples of 50 g were stored at -20°C until analysis.

Extraction procedure

Fifty grams of dry seeds samples was transferred into a stain-less steel jar blender and homogenized with 150 ml of acetone for 2 min. The macerate was filtered through a clean cotton pad into a graduated cylinder. A known volume (100 ml) of the extract was shaken successively with 100, 50 and 50 ml of methylene chloride in a separating funnel after adding 10 ml saturated sodium chloride solution. The combined organic phases were dried by filtration through anhydrous sodium sulfate (activated over night at 105°C). Extract was evaporated just to dryness using a rotary evaporator operating at 40 °C according to **Tang, *et al.*, (2005).**

Cleanup procedure

Clean up was carried out using column chromatography on florisil activated using methylene chloride: n-hexane: acetonitrile (50:48.5: 1.5) as eluting solvent. The residue was dissolved in 10 ml of the eluting solvent, then quantitatively transferred and filtered through a chromatographic column (2.5 cm i.d.) packed with a 7 cm layer of florisil. The column was previously conditioned with n-hexane before elution process. At the posterior end of the column, a small piece of glass wool was put to act as a mechanical support to prevent solid layer from running with the eluting liquid. Rinsing the residue was repeated several times with approximate 5 ml portions of the eluting solvent, and each washing was added to the column just before the preceding fraction has completely entered the column. Elution was continued till a total volume of 200 ml of the eluting solvent was used at a flow rate of about 1.5 ml / min. The eluents were collected in a 500 ml rotary flask and evaporated under vacuum to dryness using a rotary evaporator operating at 40 °C. The residue was dissolved in 10 ml of ethyl acetate (GC grade) and

poured into a 10 ml-measuring flask for GC determination.

Apparatus and chromatography

The gas chromatography used was a Agilent 6890 equipped with a flame photometric detector (FPD) with phosphorus filter. A fused silica capillary (PAS -17o1), column containing 14% cyanopropilsyloxane as stationary phase (30 m) length x 0.32 mm internal diameter (i.g.) x 0.25 μm film thickness), was used for the separation in the GC.

GC operating conditions were the following: Injector and detector temperatures were 240 °C; initial oven temperature, 200 °C for 2 min, raised at 5 °C/min. and then held at 240 °C for 2 min. The carrier gas was nitrogen at 3 ml/min. and hydrogen and air were used for the combustion and 100 ml/min, respectively. A gas chromatograph programed for external standardization using the peak area was used. Under these operating conditions the retention time of Malathion was 2.200 min

Recovery assays

Known quantities of Malathion dissolved in ethyl acetate were added to control samples of dry seeds at fortification levels of 0.1, 0.5, and 1 ppm. Extraction and cleanup were carried out as described above. Simultaneous processing frequently checked the recovery of the overall method.

4. Results and Discussion

4.1. Toxicity of the tested insecticides on adult stage of *B. incarnates* under laboratory conditions.

Data in Table (2) and Fig. (1) indicated that Malathion exhibited the highest activity compared with the other toxicants K.Z. oil and Agerine at LC_{50} and LC_{90} levels. The LC_{50} values were 6.26, 100.00, 439.00, and 618 ppm for Malathion, Neemazal , Clove oil, and K.Z. oil, respectively. While the corresponding LC_{90} values were 34, 541, 2674, and 4097 ppm after harvest. The toxicity of Nemazal was 13.3, and 21.99 % that of Malathion at the LC_{50} and LC_{90} levels, respectively. While the toxicity of Agerine was 1.05, and 2.05 % that of Malathion at the LC_{50} and LC_{90} levels, respectively. On ground of potency levels, compared with Agerine, the toxicity of Malathion was 95.26 and 48.46 fold that of Agerine at the LC_{50} and LC_{90} levels, respectively. The toxicity Neemazal was 12.67, and 10.66 fold of Agerine at the LC_{50} and LC_{90} levels, respectively. It was found that Agerine has very low toxicity against adult stage of *B. incarnatus.*

It is obvious, as could be seen in Fig. (1) and Table (2), that malathion had the steepest toxicity line and Agerine had the flattest one while, neemazal, Clove oil, and K.Z. oil lie in between; this reflect the superiority of malathion and inferiority of Agerine. These results are in agreement with those obtained by **Shalaby and Ebadah (2005**) who studied the efficiency of profenofos, chlorpyriphos, chlorpyriphos-methyl and Malathion insecticides against the egg stage (that takes as indicated effect of adults) of bean bruchids, *Bruchidius incarnatus* (Schm.) beetles infesting broad bean pods in field. The results revealed that the percent of infestation reduction were 93.6, 91.1, 95.0 and 89.1 % in seed treated by the pervious insecticides, respectively after one day of application. After fifteen days from spraying, the percent infestation reduction were, 62.0, 61.7, 51.6 and 30.1 %,

respectively. **Thuy *et al.*, (1994)** reported that the use of chemical protectants such as malathion and Sumithion gave good control of pests.. **Sabbour and Abd-El-Aziz (2007)** tested the efficacy of botanical oils and microbial agents (bacteria and fungi) on the broad bean beetle, *Bruchus rufimanus* in laboratory and field. The LC50 of the fungi *Beauveria bassiana*, *Metarhaizum anisopliae* and *Verticillium lecanii*, were 141, 138 and 146 spore/ ml, respectively. While, in case of *Bacillus thuringiensis*, the LC50 was 176 ug/ml, under laboratory conditions. Accumulative mortality percentage of *B. rufimanus* beetles increased gradually by increasing the period of exposure to foam treated with the different tested oils. Nigella oil had the highest accumulative mortality (74.2%), followed with mustard (60.1%). While the cumulative mortality in case of clove and onion oils were almost equal with, 33.7 and 30.9%, respectively. **Ibrahim (2012)** showed that plant oils were effective in decreasing insect infestation of bruchids on chickpea. **Fouad (2013)** found that the cinnamon oil had the highest toxicity rate on *B. incarnatus* adult fallowed by clove, camphor, mustard and the lowest effect was by castor oil. Who or He concluded that essential oils of camphor, cinnamon, clove and mustard have potential for use in the integrated management of *B. incarnatus* adult.

Neemazal, Clove oil, K.Z. oil, and Agrien could be considered promising alternatives to conventional insecticides for use against many pest insects. These findings are in agreement with those reported by many investigators. **Allam (2003)** suggested that the use of some effective alternatives such as thiocyclam – H- oxalate, Naturol oil and K.Z. oil in controlling *Aphis craccivera* in compatible

program with chemical insecticides instead of conventional individuals insecticides. **Lutfallah *et al.*, (2004)** concluded that use of oil could be recommended as an element of integrated pest management for their safety and economic consideration.

Table (2): Toxicity of Malathion, Neemazal, Clove oil, K.Z. oil , and Agerine on adult stage of *B. incarnates* under laboratory conditions.

Insecticides	LC_{50*}	LC_{90}	Slope	Toxicity index at LC_{50}	LC_{90}	Number of folds compared with Agerine 6.5% LC_{50}	LC_{90}
Malathion 57%	6.26	34	1.75	100	100	95.26	48.46
Neemazal 1%	100	541	1.78	13.3	21.99	12.67	10.66
Clove oil	439	2674	1.64	3.02	4.45	2.88	2.16
K. Z oil	618	4097	1.5	2.15	2.9	2.05	1.4
Agerine 6.5%	1267	5767	2.04	1.05	2.06	1.00	1.00

*ppm

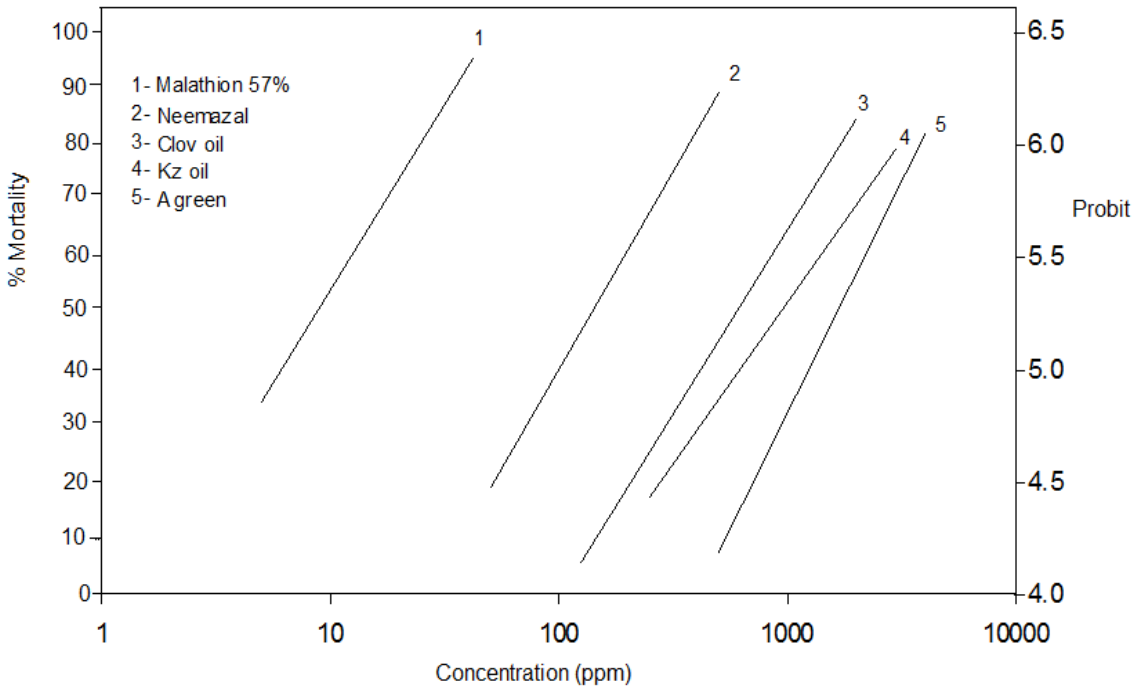

Fig. (1) Toxicity of Malathion, Neemazal, Clove oil, K.Z. oil , and Agerine on adult stage of *B. incarnates*

Effect of type formulation malathion pesticide on toxicity on adult stage of *B. incarnates* under laboratory conditions.

Data presented in Table (3) and Figure (2) revealed that the type of formulation and concentrate of malathion play an important role in toxicity on adult stage of *B. incarnates*. Malathion 57% E.C. exhibited that highest activity compared with the otherformaulation; malathion 5% D. and malathion 1% D. at LC_{50} and LC_{90} levels. The LC_{50} values were 6.26, 13.3, and 45.8 ppm for Malathion 57% E.C., Malathion 5% D., and malathion 1% D. respectively. While the corresponding LC_{90} values were 34, 119 and 367 ppm.

It is obvious, as could be seen in Fig. (2) and Table (3), that malathion 57% E.C. had the steepest toxicity line and Malathion 1% D. had the flattest one; malathion 5% D. lie in between; this reflect the superiority of malathion 57% E.C. and inferiority of Malathion 1% D.

Table (3): Effect of formulation type on the toxicity of malation on adult stage of *B. incarnates*

Insecticides	LC_{50}	LC_{90}	Slope	Toxicity index at LC_{50}	Toxicity index at LC_{90}	Number of folds compared with Malation 1% LC_{50}	Number of folds compared with Malation 1% LC_{90}
Malathion 57% EC	6.26	34	1.75	100	100	7.32	10.79
Malathion 5% D	13.3	119	1.38	47.07	28.57	3.44	3.08
Malathion 1% D	45.8	367	1.45	13.67	9.26	1.00	1.00

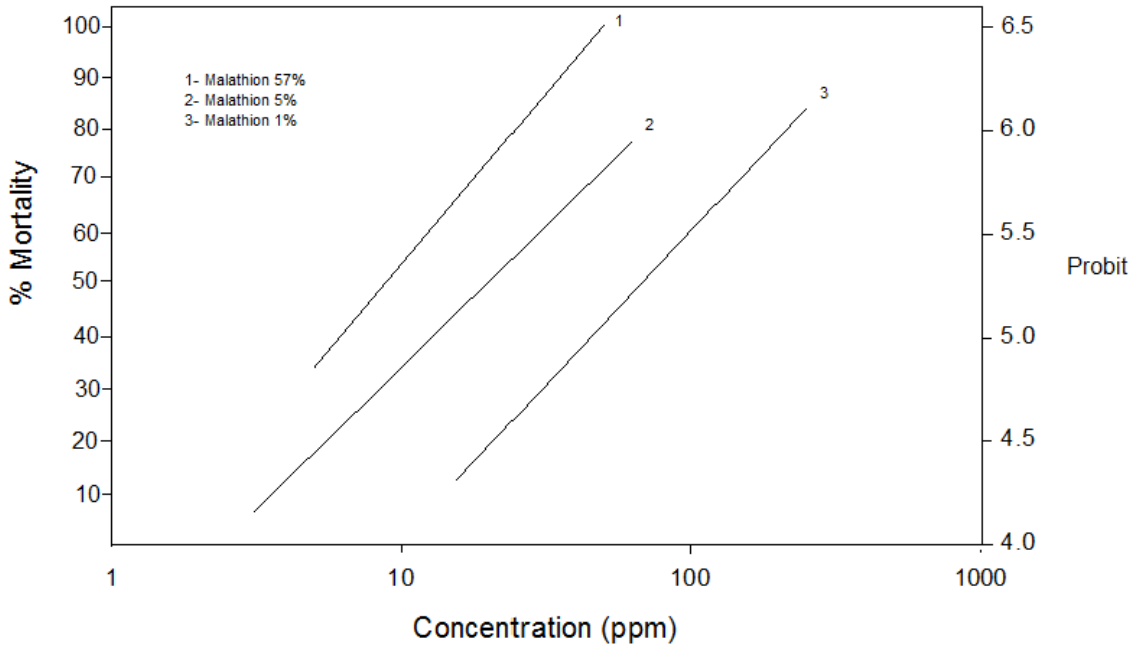

Fig. (2) Toxicity of malation 57% E.C.; (dust 5% W/W).; and (dust 1% W/W) on adult stage of *B. incarnates*.

Determination of malathion residues in faba bean seeds, *Vicia faba* during storage.

Data presented in Table (4) revealed that the mean recovery percentage for malathion in broad bean seeds were 97.66, 92.00, and 89.33 at the fortification levels 1.0, 0.5, 0.1 (Mg /Kg), respectively. This results are in agreement with **Khani *et al.*, (2011)** who reported that recoveries the mean recovery percentage for malathion were 91.1 and 104.12 % for two fortification levels; 0.1 and 1 mg/kg, respectively. In some samples, residues of malathion showed different level of contamination, and in some samples no residues were detected. Finally, the results showed that this method has good accuracy, precision and sensitivity for determination of residues of malathion. **Tang *et al.*, (2005)** determined nine organophosphorus pesticide residues in cereals and kidney beans by capillary gas chromatography with flame-photometric detection. The method was rapid, simple, and reliable.

Table (4): Recovery percentages of Malathion dust 1% in faba bean seeds.

Fortification levels (mg/kg)	Mean recoveries for 3 replicates (%)
1.0	97.66
0.5	92.00
0.1	**89.33**
Average	92.99

Residues of malathion in dry seeds of broad bean are shown in table (5). Data obtained in the table indicated that the total residues at the different intervals were below the permissible limit.

Table (5): Malathion residues (ppm) in dry seeds of faba bean during periods after treatment.

Treatment	Residues at the indicated periods of harvest		
	Just after treatment	One month	Two month
Dry seeds	0.0150*	UN**	UN

* ppm **UN : Un detected

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