



BIOLOGICAL AND LETHAL EFFECTS OF SOME VOLATILE PLANT OILS, ALOE AQUEOUS EXTRACT AND SPINOSAD ON COWPEA BEETLE, *Callosobruchus maculatus* (F.)

Habeba M.O. Abu El-Enine*, E.M. Metwally, M.A. El-Deeb and Zeinab A. Mohamed

Plant Prot. Dept., Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

This study was carried out under constant conditions at $30 \pm 0.5^\circ\text{C}$ and $60 \pm 5\%$ RH on cowpea beetle, *Callosobruchus maculatus* (F.) treated with four volatile plant oils (clove, thyme, marjoram and rosemary), aloe aqueous extract and spinosad. The obtained data are summarized as follows: The highest fecundity (63.67 eggs) was recorded in cowpea seeds treated with thyme oil at 0.5% and the lowest one (2.67 eggs) at 2.0% on rosemary oil, compared with (135.67 eggs) in the control. The highest fertility (86.25%) was occurred at 1% of thyme oil, but the lowest one (zero%) was recorded in case of 2% marjoram oil as compared with control (68.38%). There were no larval penetrations (zero) into seeds with all volatile oils at different concentrations except (7.73%) at 0.5% of thyme oil as compared with 11.19% in control. Moreover, the highest fecundity (38.67 eggs) was recorded at 0.5% and decreased to (11.33 eggs) at 2%, the highest percentage of larval penetration (6.22 %) was recorded at 0.5% and decreased to 0.0% in the concentrations of 1.5% and 2%, compared with (11.19%) in control, when treated with aloe aqueous extract. The percentage of eggs reduction was increased by increasing the concentrations used. The highest percentage of reduction (94.25 %) was recorded at the concentration of 2%, while the lowest one (71.09%) was recorded at 0.5%. The highest percentage of weight loss (3.39%) was recorded at 0.5% of clove oil, and the lowest percent (1.15%) was found at 2% of aloe aqueous extract. The highest feeding deterrence index (FDI) was 36.83% as concerns aloe aqueous extract and the lowest one (19.65%) was recorded with clove oil. Adult mortality percentage increased by increasing the concentration of the volatile plant oils used. The highest percentage of mortality (72.22%) was recorded at 2% and 3% rosemary oil after 72 hr. from treatment and 3% of both clove and thyme oils, while the lowest percent (2.78%) was recorded at 0.5% after 24 hr., of clove oil. The highest percentage of mortality (52.78%) was recorded on cowpea seeds treated aloe aqueous extract at 3% after 72 hr., of treatment, while the lowest one (13.89%) was obtained at 0.5% after 48 hr. The highest mortality of spinosad was 80.0% at 70 ppm after 72 hr., whereas the lowest percent (1.67%) was recorded at 40 and 50 ppm after 24 hr. post-treatment, compared with 33.33% at 1 ppm after 24 hr. exposure to malathion (1% powder) and the highest one of 100.00% which was recorded at 8 ppm after 72 hr., exposure period.

Key words: Volatile plant oils, aloe extract, feeding deterrence index (FDI), spinosad, cowpea beetle, *Callosobruchus maculatus* (F.), biological and lethal effects.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most popular leguminous crops used as human food in Egypt. This crop is susceptible to infestation by several insect pests. Cowpea

beetle, *Callosobruchus maculatus* (F.) is one of the most damaging pests of cowpea seeds (Decella, 1981; Jackai and Daoust, 1986). This insect pest causes weight loss, decreased germination potential and diminishes the market value of the crop. Insects not only consume

*Corresponding author: Tel. : +201271722521

E-mail address: habiba.mohamed.omer@gmail.com

seed, but also contaminate it with their metabolic products and body parts.

Essential oils are potential sources of alternative compounds to currently used fumigants; they have low toxicity to warm-blooded animals, high volatility and toxicity to stored grain insect pests (Regnault-Roger and Hamraoui, 1993; Shaaya *et al.*, 1991 and 1997). It could be concluded that plant derivatives might be useful as insecticidal agents for commercial use, increasing benefit for agricultural sectors in developing countries, as these substances are not only of low cost, but also have less environmental impacts in term of insecticidal hazards (El-Sayed, 1986).

Spinosad (metabolite product) is a product of fermentation metabolites of soil actinomycete, *Saccharopolyspora spinosa* with a mixture of the molecules spinosyn A and D. This biocontrol insecticide has a unique mode of action with a very low toxicity to mammals. Spinosad caused high mortality to *C. maculatus* adults and decreased the number of eggs laid by females (Sanon *et al.*, 2010).

Therefore, the present work was carried out to study the effect of some volatile plant oils, aloe aqueous extract and spinosad formulation (Tracer 24% SC) compared with malathion insecticide (Malatox 1% P) against *C. maculatus*.

MATERIALS AND METHODS

This study was carried out in insect rearing chamber of Plant Protection Department, Faculty of Agriculture, Zagazig University.

Rearing Technique of the Tested Insect

Stock culture of cowpea beetle, *Callosobruchus maculatus* (F.) was obtained from the infested cowpea seeds, *V. anguiculata*. The cultures of insect were reared on cowpea seeds for many generations under laboratory conditions at $30 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH.

Tested Materials

Volatile plant oils

The tested volatile plant oils (clove, thyme, marjoram and rosemary) were used in this study. All volatile plant oils were concentrated 100%

and obtained from El Captain Company (CAP PHARM) for Extracting Natural Oils from Plants and Cosmetics (Al-Obour City, Cairo - Egypt) and Pure Misr Company for Medical Oils and Cosmetics. These oils are listed in Table 1.

Aloe aqueous extract

The aloe aqueous extract (concentrated 100%) was obtained from Horticulture Research Institute, Dokki, Giza, Egypt.

Spinosad formulation (metabolite product)

Commercial formulation of spinosad (Tracer 24% SC) was used, as a liquid mixture of ingredients comprising spinosyn A and D (22.8%) and inert ingredients of 77.2%. Spinosad was obtained from Dow Agro Sciences Company, England.

Malathion insecticide

Malathion (Malatox 1% P) was used as the concentrations of 1, 2, 4, 6 and 8 ppm.

Insecticidal Bioassay Tests

Volatile plant oils and aloe aqueous extract

Four volatile plant oils (clove, *Syzygium aromaticum*; thyme, *Thymus vulgaris*; marjoram, *Majorana hortensis* and rosemary, *Rosmarinus officinalis*) were used with acetone solvent and aloe aqueous extract, *Aloe barbadensis* with distilled water as solvent. Bioassay was performed on petri dishes (9-cm diameter) with 25 g of cowpea seeds. The seeds were treated with 2 ml each of acetone or volatile plant oils solution at different concentrations (0, 0.5, 1, 1.5, 2, 3%). Petri dishes containing the cowpea seeds and solution were shaken for 1 min and kept 5 min for deposition of ingredient (Parsaeyan *et al.*, 2012). The solvent evaporated in a few minutes leaving a thin film of volatile oils or aqueous extract. Stock concentrations of each oil or aloe extract in acetone and in water, respectively were prepared periodically on the basis of the volume of the oil and volume of the solvent (V/V).

After treatment, six pairs of insects 1-day old of *C. maculatus* adults were introduced into each petri dish and covered with lid. Each treatment was replicated three times. Control group was included in each experiment. Mortality percentage was recorded at 24, 48 and

Table 1. Certain volatile plant oils, aloe aqueous extract and spinosad formulation compared with malathion insecticide

English name	Scientific name	Part used	Source
Thyme	<i>Thymus vulgaris</i>	Leaves	El Captain Company CAP PHARM Al-Obour City- Cairo-Egypt
Clove	<i>Eugenia carpophyllus</i> (<i>Syzgium aromaticum</i>)	Flowers	Pure Misr Company for Medical Oils and Cosmetics
Marjoram	<i>Majorana hortensis</i>	Leaves	El Captain Company CAP PHARM Al- Obour City- Cairo-Egypt
Rosemary	<i>Rosmarinus officinalis</i>	Leaves	El Captain Company CAP PHARM Al- Obour City- Cairo-Egypt
Aloe	<i>Aloe barbadensis</i> (<i>Aloe vera</i>)	Leaves	Horticulture Research Institute- Dokki – Giza - Egypt
Spinosad	Tracer 24 % SC (trade name)	Formulation	Dow Agro Sciences Company- England
Malathion	Malatox 1% P (trade name)	Powder (1%)	

72 hr., post-treatment. Insects were considered dead; if no movement was observed after probing the antenna with a hot needle (Roghayeh and Pourimiza, 2007). When there was no observed leg or antennal response to a hot needle, insects were considered dead (Khashaveh *et al.*, 2011). In all bioassay primary tests conducted to determine the concentrations that cause mortality between 25-75% for each species separately (Robertson and Preisler, 1992). After treatment the treated seeds with insects were placed in plastic tubes (3.5 cm in diameter × 7 cm in length) to study some biological aspects of cowpea beetle *i.e.*, number of eggs laid per female (fecundity of eggs), egg hatchability (%), larval penetration (%) and reduction of laid eggs (%).

After adult emergence, the efficacy of volatile plant oils due to insect infestation was determined by calculating the weight loss (%) and feeding deterrence index (%) of treatment and control seeds. The feeding deterrence index (FDI) was calculated from the following formula as described by Xie *et al.* (1996):

$$\text{Feeding deterrence index (FDI)} = C - T / C + T \times 100$$

Where:

C = loss of weight (%) in control, T = loss of weight (%) in treatment. The reduction of eggs

was recorded according to El-Lakwah *et al.* (1992).

$$\text{Reduction} = \text{MNEC} - \text{MNET} / \text{MNEC} \times 100$$

Where:

MNEC= Mean No. of eggs laid by adults in the control.

MNET = Mean No. of eggs laid by adults in the treatment.

Spinosad formulation (metabolites product)

Bioassay was performed on petri dishes (9-cm diameter). The bottom and lid of petri dishes were treated with 2 ml each of distilled water or spinosad solution at different concentrations (0, 20, 30, 40, 50, 70 ppm) (V/V). Treating the inside surface of dish lid was necessary to ensure exposure of adults because the cowpea beetle could fly upside on petri dishes surfaces. Petri dishes containing the solution were shaken for 1 min and kept 5 min for deposition of ingredient (Parsaeyan *et al.*, 2012). After treatment, six pairs of 1- day old adult of cowpea beetle, *C. maculatus* were exposed to the thin film of spinosad for 24, 48 and 72 hr., into petri dishes and each dish was covered with lid. Each concentration was replicated three times (Farag, 1996).

Malathion insecticid (1% powder)

The cowpea seeds were mixed with 0.2 mg of powder (W/W) at different concentrations 1, 2, 4, 6 and 8 ppm. Three replicates were used. Mortality percentage was recorded after 24, 48 and 72 hr. post-treatment.

Statistical Analysis

All the obtained results were statistically analyzed using one way and two ways analysis of variance (ANOVA) according to Snedecor (1966). Fisher's least significant difference test (LSD) and the proper "F" value were calculated as described by Fisher (1944 and 1950). Results were recorded as mean \pm standard error (SE). Simple correlation coefficient was calculated according to Hendy, 1969. Percent mortality was corrected using Abbott's Formula (Abbott, 1925).

RESULTS AND DISCUSSION

Biological Effects

Volatile plant oils

Fecundity (Number of eggs / female)

Data presented in Table 2 indicate that the highest mean number of eggs / female (63.67 eggs) was recorded in cowpea seeds treated with thyme oil at 0.5% concentration and the lowest one (2.67 eggs) was obtained with those treated with rosemary oil at 2.0%, compared with (135.67 eggs) in the control. Statistical analysis of variance using F. test cleared that there were highly significant differences between the mean numbers of laid eggs/ female recorded with different concentrations of all tested volatile plant oils.

Percentage of eggs hatchability

Data arranged in Table 2 cleared that the percentage of eggs hatchability was differed between volatile plant oils as well as between concentrations used, the highest and the lowest percent (86.25% and 0.0) were recorded at 1% of thyme oil and 2% of marjoram oil, respectively compared with 68.38% in control. Statistical analysis of variance using F. test proved that there were highly significant differences between the means as concerns the percentage of eggs hatchability occurred with different tested concentrations of thyme, marjoram

and rosemary oils, whereas those regarding clove oil demonstrate insignificant variance.

Percentage of larval penetration

Data given in Table 2 show that the percentage of larval penetration varied, and there was no penetration (0.0%) into cowpea seeds treated with different concentrations of tested volatile plant oils except (7.73%) that was recorded at the concentration of 0.5% of thyme oil, compared with (11.19%) in control. Statistical analysis of variance using F. test cleared that there were highly significant differences between the concentrations as regards the percentage of larval penetration in case of all tested oils.

Reduction of laid eggs

Data presented in Table 2 demonstrate that the reduction of eggs increased by raising the concentration used, the highest percentage (100%) was recorded at seeds treated with 2% marjoram oil, while the lowest one (49.39%) was recorded at 0.5% of thyme oil. Statistical analysis of variance using F. test appeared that there were highly significant differences between the concentrations in respect to the reduction of deposited eggs.

From the aforementioned data, it could be concluded that thyme oil showed the less potential effect, whereas the other tested oils offered complete protection for cowpea seeds against this species of bruchid beetle at all the tested concentrations.

Several authors have previously reported the ovicidal activity of many plant oils on bruchid pests such as Carlos and Cardona (1981) who stated that groundnut oil proved to have an ovicidal action against *C. maculatus*. The author also, suggested that eggs mortality was caused by the physical properties of oil owing to coating and blocking respiration rather than specific chemical effect. Also, Credland (1992) explained the ovicidal effect on terms of asphyxiation of the developing insects as a result of occluding of the funnel structure at the posterior pole of eggs which may be the major route of gaseous exchange. It could be concluded that plant derivatives might be useful as insecticidal agents for commercial use, increasing benefit for agricultural sectors in developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazards (El-Sayed, 1986)

Table 2. Effect of certain volatile plant oils on some biological aspects of the cowpea beetle, *Callosobruchus maculatus* (F.) under constant conditions of 30±1°C and 60 ± 5 % RH

Volatile plant oils	Concentrations (%)	Fecundity (No. of eggs / female)	Eggs hatchability (%)	Larval penetration (%)	Reduction of eggs (%)
Control	0.0	135.67± 7.22	68.38± 3.28	11.19± 2.77	-
	0.5	20.33± 2.96	59.98± 7.09	0.0	87.06 ± 1.45
Clove	1.0	11.67± 1.76	72.39± 3.21	0.0	90.99 ± 0.84
	1.5	10.33± 0.88	63.15± 10.97	0.0	93.12 ± 0.79
	2.0	10.00± 0.58	47.00± 4.56	0.0	94.96 ± 0.28
	LSD 0.05	11.38	20.56	3.90	2.64
	F. Test	**	NS	**	**
	0.5	63.67± 4.81	73.72± 5.90	7.73± 2.13	49.39 ± 4.05
Thyme	1.0	50.00± 3.61	86.25± 2.91	0.0	55.73± 4.04
	1.5	54.00± 1.15	67.75± 3.39	0.0	60.18 ± 3.48
	2.0	52.00± 2.89	59.26± 3.36	0.0	66.80 ± 0.97
	LSD 0.05	13.95	12.36	4.93	9.54
	F. Test	**	**	**	**
	0.5	26.00± 1.15	49.79± 4.63	0.0	85.89 ± 1.74
Marjoram	1.0	6.67± 0.88	44.29± 2.97	0.0	96.73 ± 0.68
	1.5	5.33± 0.33	41.11± 4.84	0.0	96.90± 0.14
	2.0	3.00± 1.73	0.0	0.0	100 ± 0.0
	LSD 0.05	10.68	11.32	3.90	2.64
	F. Test	**	**	**	**
	0.5	16.00± 1.73	59.51± 4.51	0.0	90.64 ± 0.81
Rosemary	1.0	11.67± 2.03	37.78± 6.55	0.0	94.99 ± 1.51
	1.5	3.00± 1.53	11.11± 11.11	0.0	99.29 ± 0.71
	2.0	2.67± 0.88	8.33± 8.33	0.0	99.29± 0.71
	LSD 0.05	11.14	23.04	3.90	2.80
	F. Test	**	**	**	**

- NS indicates that the differences between means are insignificant at 0.05 level of probability.

- * and ** indicates that the differences between means are significant at 0.05 and 0.01 level of probability.

Aloe aqueous extract

Data presented in Table 3 show the effect of aloe aqueous extract, *Aloe barbadensis* on some biological characteristics of *C. maculatus* adults. The obtained results clearly revealed that the number of deposited eggs/ female (insect fecundity of eggs) gradually decreased by increasing the tested concentrations, the highest fecundity (38.67 eggs) was recorded at 0.5% and decreased to 11.33 eggs at 2%. In the meantime, the highest and the lowest percentages of eggs hatchability of 70.39% and 47.58% were recorded at 0.5% and 2%, respectively. The percentage of larval penetration was decreased by increasing concentrations *i.e.* decreased from 6.22% at the concentration of 0.5% to 0.0% in the concentration of 1.5% and 2%, compared with (11.19%) in the control. Also, the percentage of eggs reduction increased by increasing the concentration exhibiting the lowest and the highest percent's of 71.09% and 94.25% at 0.5 and 2%, respectively. Statistical analysis of variance of the data respecting mean number of laid eggs and reduction of eggs proved to be highly significant at 0.01 level of probability, whereas significant differences for larval penetration and insignificant ones for eggs hatchability were detected.

Percentage of seed weight loss

Data given in Table 4 show the effect of the tested volatile plant oils and aloe aqueous extract at different concentrations on the percentage of weight loss of cowpea seeds. The results apparently cleared that the highest average percentage of weight loss (3.39%) was recorded with the seeds treated with clove oil at 0.5%, while the lowest ones (1.18% and 1.15%) with seeds treated with thyme oil and aloe aqueous extract at 2%, respectively compared with 3.55% in untreated control. Statistical analysis of variance using F. test appeared that there were highly significant differences between the botanical materials in respect to the percentage of weight loss of seeds.

These results are in agreement with those obtained by Pandey *et al.* (2011) who found that essential oils based formulations exhibited efficiency as botanical fumigants in the protection of stored pigeon pea seeds up to six months by enhancing feeding deterrence and

reducing seed damage as well as weight loss caused by *C. chinensis* and *C. maculatus*.

Feeding deterrence index (FDI)

Data presented in Table 5 show the influence of four volatile plant oils and aloe aqueous extract on the feeding deterrence index (FDI) of *C. maculatus*. The results cleared that the highest index (50.11%) was recorded with treated seeds at 2% concentration of thyme oil and the lowest one (2.31%) was recorded with seeds treated at 0.5% of clove oil. Generally, the tested volatile oils can be descendingly arranged according to their feeding deterrence index as follows: 31.00%, 30.24%, 27.50% and 19.65% at thyme, rosemary, marjoram and clove oils, respectively compared with 36.83% in respect of aloe aqueous extract which proved to be the highest efficient in deterring insect feeding. Statistical analysis of variance using F. test appeared that there were highly significant differences between the botanical materials respecting the feeding deterrence index at 0.01 level of probability.

Lethal Effects

Volatile plant oils

The obtained results in Table 6 show that the percentage of adults mortality 24 hr., post treatment increased by increasing the concentration of the plant volatile oil used. The highest percentage of adults mortality (61.11%) was recorded at 3% concentration, when treated with rosemary oil and the lowest one (0.0) was recorded at 0.5% on cowpea seeds treated with thyme oil as well as in control experiment. In case of the two other exposure periods (48 and 72 hr.), the same trend of adults mortality percentage was obtained. Highly significant correlation was observed at 0.01 level of probability with each period of exposure to tested plant oils. There were highly significant differences between adult mortality percentages as regards both concentration of oils and exposure period after treatment.

These results are similar with those obtained by some authors such as Frank and Renwick (1983) who mentioned that peanut and coconut oils on cowpea seeds caused more than 65% mortality of *C. maculatus* eggs. El-Sayed (1986) found that the ovicidal effect of groundnut oil was concentration dependent, eggs mortality rates were the highest on cowpea seeds treated at the higher concentration (7.5 ml oil/ kg seeds) as compared with the lower concentration (2.5 ml

Table 3. Effect of aloe aqueous extract on some biological aspects of the cowpea beetle, *Callosobruchus maculatus* (F.) under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Concentration (%)	Fecundity (No. of eggs / female)	Eggs hatchability (%)	Larval penetration (%)	Reduction of eggs (%)
0	135.67± 7.22	68.38± 3.28	11.19± 2.77	-
0.5	38.67± 3.18	70.39± 8.83	6.22 ± 3.17	71.09± 1.69
1.0	31.67± 7.26	55.00± 11.09	2.78 ± 2.78	81.98± 4.04
1.5	15.00± 2.08	49.02± 8.73	0.0	92.43± 0.51
2.0	11.33± 1.20	47.58± 7.14	0.0	94.25± 0.88
LSD 0.05	15.50	25.97	7.10	6.34
F. Test	**	NS	*	**

Table 4. Effect of some volatile plant oils and aloe aqueous extract on the percentage of seeds weight loss resulting from *Callosobruchus maculatus* (F.) infestation at different concentrations under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Concentration (%)	Seeds weight loss at indicated botanical materials (%)					Average irresp. of bot. mat.
	Clove oil	Thyme oil	Marjoram oil	Rosemary oil	Aloe aqueous extract	
0	3.55	3.55	3.55	3.55	3.55	3.55
0.5	3.39	1.99	2.32	1.79	1.43	2.18
1.0	2.18	1.69	1.71	1.57	1.35	1.70
1.5	1.97	1.49	1.60	1.55	1.29	1.58
2.0	1.40	1.18	1.39	1.52	1.15	1.33
Average irresp. of conc.	2.50	1.98	2.11	2.00	1.75	
F. test	Bot. mat. **	Conc. **	Bot. mat. × Conc. **			

Table 5. Effect of some volatile plant oils and aloe aqueous extract on the feeding deterrence index (FDI) of *Callosobruchus maculatus* (F.) at different concentrations under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Concentration (%)	Feeding deterrence index (FDI) at indicated botanical materials (%)					Average irresp. of bot. mat.
	Clove oil	Thyme oil	Marjoram oil	Rosemary oil	Aloe aqueous extract	
0	0	0	0	0	0	0
0.5	2.31	28.16	20.95	33.27	42.57	25.45
1.0	23.91	35.84	34.98	38.67	44.91	35.66
1.5	28.62	40.87	37.86	39.22	46.69	38.65
2.0	43.43	50.11	43.72	40.04	50.0	45.46
Average irresp. of concn.	19.65	31.00	27.50	30.24	36.83	
F. test	Bot. mat. **	Conc. **	Bot. mat. × Conc. *			

Table 6. Mortality percentages of the cowpea beetle, *Callosobruchus maculatus* (F.) adults 24, 48 and 72 hr., post-treatment with the tested volatile plant oils under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Volatile plant oil	Concentration (%)	Percentage of adults mortality after indicated exposure periods		
		24 hr.	48 hr.	72 hr.
Control	0.0	0.00 \pm 0.0	13.89 \pm 2.78	19.45 \pm 2.78
	0.5	2.78 \pm 2.78	13.89 \pm 5.56	22.22 \pm 2.78
Clove	1.0	11.11 \pm 2.78	16.67 \pm 0.0	27.00 \pm 1.15
	1.5	13.89 \pm 5.56	23.00 \pm 1.53	27.78 \pm 2.78
	2.0	27.78 \pm 2.78	41.66 \pm 8.33	66.67 \pm 12.73
	3.0	30.56 \pm 7.35	41.67 \pm 8.33	72.22 \pm 2.78
	LSD 0.05	13.08	16.88	17.54
	F. Test	**	**	**
	r	0.833**	0.680**	0.839**
Thyme	0.5	0.00 \pm 0.0	8.33 \pm 0.0	23.00 \pm 1.53
	1.0	11.11 \pm 2.78	16.67 \pm 4.81	27.78 \pm 7.35
	1.5	13.89 \pm 2.78	16.67 \pm 0.0	36.11 \pm 5.56
	2.0	27.78 \pm 2.78	30.55 \pm 12.11	47.22 \pm 5.55
	3.0	38.89 \pm 2.78	52.78 \pm 2.78	72.22 \pm 10.02
	LSD 0.05	7.00	16.77	18.93
	F. Test	**	**	**
r	0.899**	0.853**	0.955**	
Marjoram	0.5	8.33 \pm 0.0	13.89 \pm 2.78	19.56 \pm 1.55
	1.0	11.11 \pm 2.78	16.67 \pm 4.81	30.55 \pm 2.78
	1.5	16.67 \pm 4.81	36.11 \pm 7.35	55.56 \pm 11.11
	2.0	25.00 \pm 4.80	38.89 \pm 11.11	58.34 \pm 8.33
	3.0	33.33 \pm 4.81	50.00 \pm 0.0	66.67 \pm 4.81
	LSD 0.05	11.06	18.17	19.25
	F. Test	**	**	**
r	0.872**	0.837**	0.901**	
Rosemary	0.5	13.89 \pm 2.78	19.45 \pm 2.78	41.67 \pm 12.73
	1.0	22.22 \pm 2.78	33.33 \pm 4.81	41.67 \pm 12.73
	1.5	47.22 \pm 12.11	52.78 \pm 7.35	63.89 \pm 7.35
	2.0	55.56 \pm 5.56	61.11 \pm 5.56	72.22 \pm 5.55
	3.0	61.11 \pm 5.56	66.67 \pm 9.62	72.22 \pm 5.55
	LSD 0.05	18.83	18.50	26.63
	F. Test	**	**	**
r	0.775**	0.857**	0.892**	

- ** indicates that correlation is highly significant at 0.01 level of probability.

oil / kg seeds). Shaaya *et al.* (1997) recorded the highest mortality against four stored product insects after 3 hours of exposure to vapor of peppermint oil in fumigant chamber. Adedire and Lajide (1999) stated that the volatile plant oils caused mortality due to the strong choky odors which may have exerted a toxic effect by disrupting normal respiration activity of weevils, thereby resulting in asphyxiation and subsequent death.

On the contrary, these results partially disagree with the findings of Magd El-Din (2003) who reported that eucalyptus oil caused 100% mortality of *C. chinensis* adults after 96 hr., of treatment with 4000 ppm.

Aloe aqueous extract

As clearly shown from the results compiled in Table 7, the highest percentage of adults mortality of *C. maculatus* (52.78%) was recorded on cowpea seeds treated with aloe aqueous extract at the concentration of 3% after 72 hr., of treatment and the lowest one (13.89%) at 0.5% after 48 hr. of treatment, compared with the control wherein no mortality. There were highly significant differences between mortality percentages recorded with the exposure periods of 48 and 72 hr., post-treatment at the concentrations used.

Spinosad formulation (Tracer 24% SC)

Data presented in Table 8 indicate that the highest mortality of cowpea beetle, *C. maculatus*

adults was 80.0% at 70 ppm after 72 hr., of treatment by spinosad and the lowest percent (1.67%) at the concentrations of 40 and 50 ppm after 24 hr., compared with no mortality in the control. There were highly significant differences between mortality percentages at the two exposure periods post-treatment of 48 and 72 hr., as well those detected with the concentrations used. Statistical analysis reveals that highly significant correlations between mortality percentages and concentrations were observed at 0.01 level of probability in case of 24 and 48 hr., exposure periods, whereas that regarding 72 hr., exposure period proved to be statistically significant at 0.05 level of probability.

Malathion (Malatox 1% P)

Data tabulated in Table 9 show that the adults mortality of the cowpea beetle, *C. maculatus* was completely occurred (100 %) after 72 hr., of treatment at 8 ppm of malathion and the lowest percent (33.33%) at 1 ppm after 24 hr., of treatment, compared with (2.78%) in the control experiment. There were highly significant differences between mortality percentages at the three tested exposure periods of treatment as well as between those recorded in the concentration used. Statistical analysis reveals that highly significant correlation was observed at 0.01 level of probability with each period of exposure tested.

Table 7. Mortality percentages of the cowpea beetle, *Callosobruchus maculatus* (F.) adults 24, 48 and 72 hr., post-treatment with aloe aqueous extract under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Concentration (%)	Percentage of adults mortality after indicated exposure periods		
	24 hr.	48 hr.	72 hr.
0	0.0±0.0	0.0 ± 0.0	8.33± 0.0
0.5	0.0±0.0	13.89 ± 2.78	36.11± 0.35
1	0.0±0.0	25.00± 4.81	38.89± 2.78
1.5	0.0±0.0	27.78± 7.35	41.67± 4.81
2	0.0±0.0	44.44± 10.01	49.99± 8.33
3	0.0±0.0	47.22± 2.78	52.78± 5.55
LSD 0.05	0.0	17.48	17.13
F. Test	-	**	**
r	-	0.812**	0.759**

Table 8. Mortality percentages of the cowpea beetle, *Callosobruchus maculatus* (F.) adults 24, 48 and 72 hr., post-treatment with spinosad formulation (Tracer 24% SC) under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Concentration (ppm)	Percentage of adults mortality after indicated exposure periods		
	24 hr.	48 hr.	72 hr.
0	0.00±0.0	10.00±0.	30.00±0.0
20	0.00±0.0	50.00±0.0	56.67± 1.67
30	0.00±0.0	48.33± 0.67	65.00± 7.64
40	1.67± 1.67	50.00±0.0	56.67± 1.67
50	1.67± 1.67	50.00± 5.00	65.00± 7.64
70	21.67± 12.02	51.67± 1.67	80.00± 5.77
LSD 0.05	15.41	6.96	15.70
F. Test	NS	**	**
r	0.825**	0.722**	0.563*

Table 9. Mortality percentages of the cowpea beetle, *Callosobruchus maculatus* (F.) adults 24, 48 and 72 hr., post-treatment with malathion (Malatox 1% P) under constant conditions of $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH

Concentration (ppm)	Percentage of adults mortality after indicated exposure periods		
	24 hr.	48 hr.	72 hr.
0	2.78± 2.78	5.55± 2.78	11.11± 2.78
1	33.33± 4.81	46.67± 6.67	53.33± 6.67
2	58.33± 9.62	46.67± 6.67	60.00± 11.55
4	63.89± 2.78	60.00± 11.55	66.67± 13.33
6	66.67± 13.33	73.33± 6.67	93.33± 6.67
8	86.67± 6.67	93.33± 6.67	100± 0.0
LSD 0.05	23.66	22.47	25.41
F. Test	**	**	**
r	0.866**	0.889**	0.864**

REFERENCES

- Abbott, W. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18 (1): 265- 267.
- Adedire, C.O. and L. Lajide (1999). Toxicity and oviposition deterrence of some plant extracts on cowpea storage bruchid, *Callosobruchus maculatus* (F.). *J. Plant Dis. Prot.*, 106 (6): 647-653.
- Carlos, C. and D. Cardona (1981). Control of aryl seed weevils with cooking oil. *J. Agric. Puerto Univ.*, 65: 295- 298.
- Credland, P.F. (1992). The structure of bruchid eggs may explain the ovicidal effects of oils. *J. Stored Prod. Res.*, 28: 1-9.
- Decella, J. (1981). Bruchidae related to grain legumes in the Afro-Tropical Area. *Entomol.*, 19: 193-198.
- El-Lakwah F.A., A.A. Darwish and O.M. Khaled (1992). Effectiveness of dill seed powder on stored product insects. *Ann. Agric. Sci. Moshtohor*, (34): 2031- 2037.
- El-Sayed, M.A. (1986). Effectiveness of oils in protecting stored cowpeas against weevils. *Agric. Res. Rev.*, 64 (1): 1-11.
- Farag, E.M.E.A. (1996). Plant extracts and their effects against *Sitophilus oryzae* (L.) and *Sitophilus granarius* (L.). M.Sc. Thesis. Fac. Sci., Cairo Univ., Giza, Egypt.
- Fisher, R.A. (1944). *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh and London.
- Fisher, R.A. (1950). *Statistical Methods for Research Workers*. 2nd Rev. Ed., Oliver and Boyd, London.
- Frank, J.M. and J.A.A. Renwick (1983). Effectiveness of oils in protecting stored cowpea from the cowpea weevil (Coleoptera: Bruchidae). *J. Econ. Entomol.*, 76: 634-636.
- Hendy, L. (1969). *Experimental Statistics*. Dar El-Maaref, Egypt, 369 (in Arabic language).
- Jackai, L.E.N. and R.A. Daoust (1986). Insect pests of cowpea. *Annual Rev. Entomol.*, 31: 95- 119.
- Khashaveh, A., M. Ziaee and M.H. Safaralizadeh (2011). Control of pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in different cereals using spinosad dust in storage conditions. *J. Plant Prot. Res.*, 51 (1): 77-81.
- Magd El-Din, M. (2003). Biological effects of some medical and aromatic plants on the cowpea weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *J. Egypt. Ger. Soc. Zool.*, 42 (E) Entomol., 1-10.
- Pandey, A.K., S. Pooja, U.T. Palni and N.N. Tripathi (2011). Use of essential oils of aromatic plants for the management of pigeon pea infestation by pulse bruchids during storage. *J. Agric. Technol.*, 7 (6): 1615-1624.
- Parsaeyan, E., M. Saber and S. Vojoudi (2012). Lethal and sublethal effects from short-term exposure of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) to diatomaceous earth and spinosad on glass surface. *Acta Entomologica Sinica*, 55 (11): 1289-1294.
- Regnault-Roger, C. and A. Hamraoui (1993). Efficiency of plants from the south of France used as traditional protectants of *Phaseolus vulgaris* L., against its bruchid *Acanthoscelides obtectus* (Say). *J. Stored Prod. Res.*, 29: 259-264.
- Robertson, J.L. and H.K. Preisler (1992). *Pesticide Bioassays with Arthropods*. CRC Press, Boca Roton, Florida., 121.
- Roghayeh, Y.I. and A.A. Pourimiza (2007). Effect of spinosad on adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pak. J. Biol. Sci.*, 10 (15): 2505- 2509.
- Sanon, A., N.M. Ba, C.L. Binso-Dabire and B.R. Pittendrigh (2010). Effectiveness of spinosad (naturalytes) in controlling the cowpea storage pest, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J. Econ. Entomol.*, 103 (1): 203-210.
- Shaaya, E., U. Ravid, N. Paster, B. Juven, U. Zisman and V. Pissarev (1991). Fumigant toxicity of essential oils against four major stored- product insects. *J. Chem. Ecol.*, 17: 499-504.

Shaaya, E., M. Kostjukovski, J. Eilberg and C. Sukprakarn (1997). Plant oils as fumigants and contact insecticides for control of stored-product insects. J. Stored Prod. Res., 33 (1): 7- 15.

Snedecor, G.W. (1966). Statistical Methods Applied to Experiments in Agriculture and

Biology. 5th Ed. Iowa State Univ., 534.

Xie, Y.S., R.P. Bodnaryk and P.G. Field (1996). A rapid and simple flour- disc bioassay for testing substances active against stored product insects. The Canadian Entomol., 128: 865-875.

التأثيرات البيولوجية والمميتة لبعض الزيوت النباتية الطيارة والمستخلص المائي للصبان ومستحضر سبينوساد على حشرة خنفساء اللوبيا (*Callosobruchus maculatus* (F.))

حبيبة محمد عمر أبوالعنين – السيد مجاهد متولي – محمد على الديب – زينب عبدالله محمد

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

أجريت هذه الدراسة تحت الظروف الثابتة ($30 \pm 1^\circ\text{C}$ ، $60 \pm 5\%$ رطوبة نسبية) بهدف تقدير الصفات البيولوجية ونسبة الموت لحشرة خنفساء اللوبيا على بذور اللوبيا نتيجة المعاملة بأربعة من الزيوت النباتية الطيارة (القرنفل، الزعتر، البردقوش، حصابان) والمستخلص المائي للصبان ومستحضر سبينوزاد، وقد أوضحت النتائج أن أعلى متوسط لعدد البيض للأنتي الواحدة (٦٣،٦٧ بيضة) كان عند المعاملة بزيت الزعتر في التركيز ٠،٥% بينما كان أقل متوسط لعدد البيض (٢،٦٧ بيضة) عند المعاملة بزيت حصابان بتركيز ٢% مقارنة بالكنترول (١٣٥،٦٧ بيضة)، سُجلت أعلى نسبة لفقس البيض (٨٦،٢٥%) في حالة المعاملة بزيت الزعتر عند التركيز ١% بينما كانت أقل نسبة للفقس (صفر%) عند المعاملة بزيت البردقوش عند تركيز ٢% مقارنة بالكنترول (٦٨،٣٨%)، لوحظ عدم وجود نسبة اختراق لليرقات مع جميع الزيوت المستخدمة عند التركيزات المختلفة، فيما عدا زيت الزعتر حيث سُجلت نسبة اختراق (٧،٧٣%) عند التركيز ٠،٥% مقارنة بالكنترول (١١،١٩%)، وفي حالة المستخلص المائي للصبان فقد سجل أعلى متوسط لعدد للبيض (٣٨،٦٧ بيضة) عند تركيز ٠،٥% بينما أقل عدد (١١،٣٣ بيضة) فقد تم تسجيله عند تركيز ٢% مقارنة بالكنترول، وسُجلت أعلى نسبة اختراق (٦،٢٢%) عند تركيز ٠،٥% وانخفضت إلى (صفر%) عند التركيزين ١،٥%، ٢% مقارنة بالكنترول (١١،١٩%)، وقد وجد أن نسبة النقص في البيض يزيد مع زيادة التركيز المستخدم، وأعلى نسبة للنقص في عدد البيض (٩٤،٢٥%) كانت عند تركيز ٢%، بينما أقل نسبة (٧١،٠٩%) سُجلت عند تركيز ٠،٥%، وقد سُجلت أعلى نسبة للفقد في الوزن (٣،٣٩%) عند المعاملة بزيت القرنفل بتركيز ٠،٥%، بينما أقل نسبة للفقد (١،١٥%) فقد سُجلت عند المعاملة بالمستخلص المائي للصبان في تركيز ٢%، ولقد سجل أعلى معدل لمنع التغذية (٣٦،٨٣%) عند المعاملة بالمستخلص المائي للصبان بينما أقل معدل (١٩،٦٥%) تم تسجيله عند المعاملة بزيت القرنفل، لوحظ زيادة نسبة الموت في الحشرات الكاملة لخنفساء اللوبيا مع زيادة التركيز المستخدم وفترة التعريض للزيوت النباتية الطيارة المستخدمة، وقد سُجلت أعلى نسبة للموت في الحشرات الكاملة (٧٢،٢٢%) عند التركيزين ٢% و ٣% بعد المعاملة بزيت حصابان وتركيز ٣% في كل من زيت القرنفل والزعتر بعد ٧٢ ساعة من المعاملة، بينما أقل نسبة للموت (٢،٧٨%) فقد سُجلت بعد ٢٤ ساعة من المعاملة بزيت القرنفل عند التركيز ٠،٥% بالمقارنة بالكنترول، سُجلت أعلى نسبة للموت (٥٢،٧٨%) عند التركيز ٣% لمستخلص الصبان المائي بعد ٧٢ ساعة، بينما كانت أقل نسبة (١٣،٨٩%) عند تركيز ٠،٥% بعد ٤٨ ساعة، فيما يخص تأثير المستحضر البكتيري (سبينوزاد) فقد ثبت أن أعلى نسبة للموت في الحشرات الكاملة (٨٠%) كانت عند التركيز ٧٠ جزء في المليون بعد ٧٢ ساعة من التعريض، بينما سُجلت أقل نسبة للموت (١،٦٧%) عند تركيز ٤٠ و ٥٠ جزء في المليون بعد ٢٤ ساعة، وبالمقارنة بمبيد الملاثيون (١% مسحوق) فقد سُجلت أقل نسبة موت (٣٣،٣٣%) بعد ٢٤ ساعة عند التركيز ١ جزء في المليون، وأعلى نسبة للموت (١٠٠%) عند التركيز ٨ جزء في المليون بعد ٧٢ ساعة.

المحكمون :

- ١- أ.د. عبدالبديع عبدالحامد غانم
- ٢- أ.د. سعد سالم محمد حسنين

أستاذ الحشرات الاقتصادية – كلية الزراعة – جامعة المنصورة.
أستاذ الحشرات الاقتصادية المتفرغ – كلية الزراعة – جامعة الزقازيق.