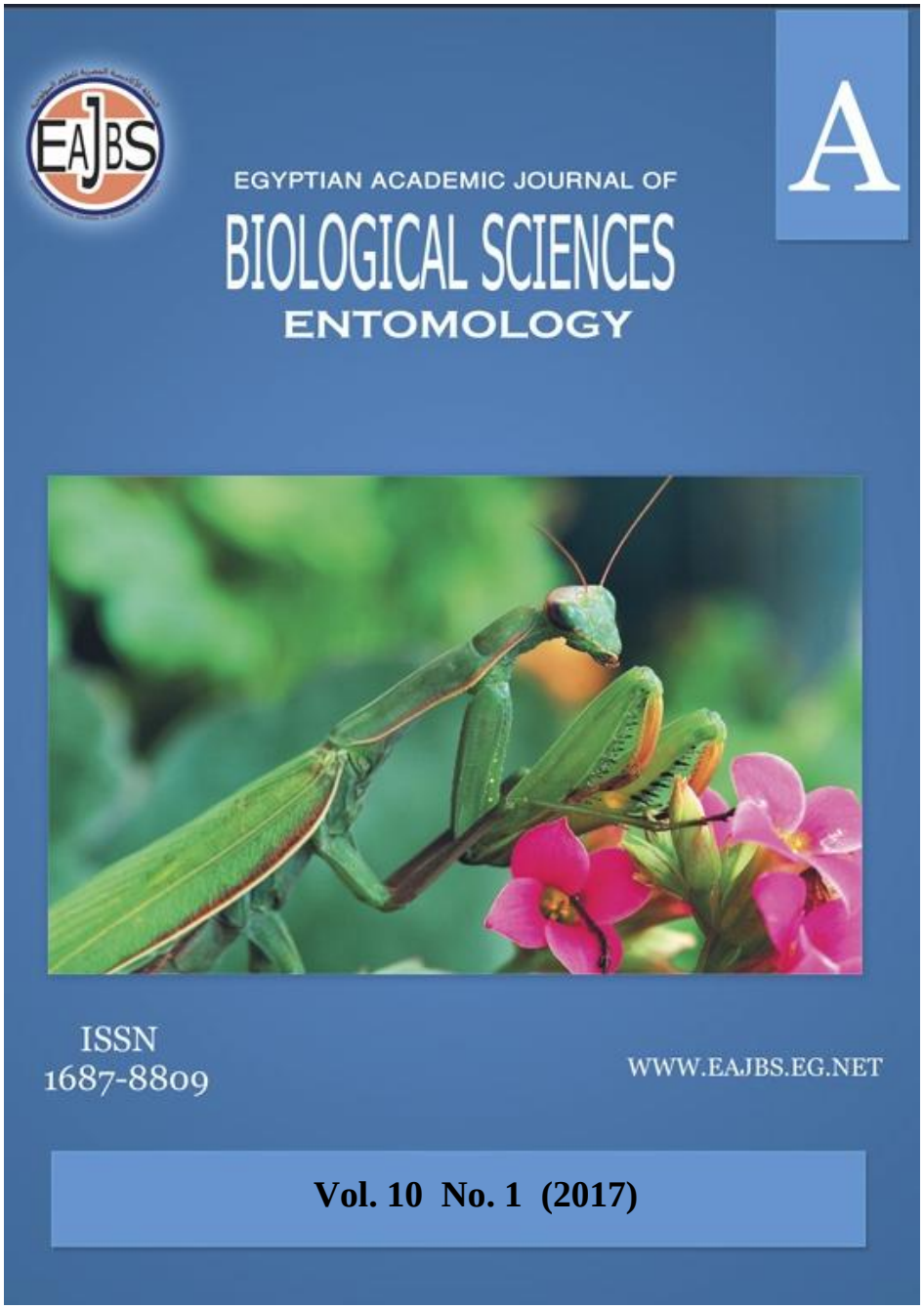

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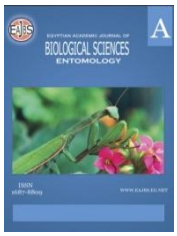

Efficacy and Residual Activity of Some Plant Oils, Abamectin bio-insecticide and Their Mixtures Against Cowpea Seed Beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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ABSTRACT

This study was carried out to evaluate toxicity, persistence, and biological effect of Fenugreek, Ginseng oils, Abamectin and their mixtures. This evaluation was conducted against *Callosobruchus maculatus.* Results showed that Abamectin was the strongest material that still kills adults of *C. maculatus,* prevent egg laying and adult emergence till the end of the storage period at $Lc_{95.}$ oils and mixtures reduced adult emergence to varied periods. Mixing oils to Abamectin helping in using it without suffering from the danger of its high concentration usage. All treatments were not affect seed germination significantly.

INTRODUCTION

Good seed is a basic requirement in seed production. The successful production of any crop depends on the quality of seeds sown. During storage the quality of seeds get deteriorated in a number of ways of which infestation by the storage pests contribute a bulk share.

Cowpea (*Vigna unguiculata* L.) is one of the families Fabaceae; cultivated in numerous parts of the world. Its value's lies in its high protein content and its ability to fix atmospheric nitrogen, which allows it to grown in many parts of the world and improve poor soils. It is used as human food due to its high protein content and also as a fodder for livestock feeds (Fery 2003).

The cultivated area of cowpea in Egypt according to the latest statistics of Ministry of Agriculture (2014) gives 35,817 green pods with an average of 3.4 tons per acre, while 8993 tons dry seeds produced with an average of 0.98 tons per acre. Cowpea is remarkably affected by viruses, nematodes, fungi, bacteria and insect (Reuben *et al*. 2006); however, one of the most injurious pests is the *Callosobuchus maculatus*.

C. maculatus (Bean beetle) is a species of beetle known commonly as the cowpea weevil; belonging to order Coleoptera and family Bruchidae (Lale 1991).

The larvae of this species feed on the seed of legumes, hence the name bean beetle. *C. maculatus* utilized as a model organism for research due to its rapid generation and ease of maintenance.*C. maculatus* is known to result in 100% loss of stored cowpea (Tiroesele *et al*., 2015).

In order to decrease serious losses during storage, various techniques and

control methods have been developed and more are still being developed. The use of plant products was confirmed as environmentally friendly and safer instead of synthetic insecticides. Also, various living microorganisms or their products are used to reduce populations of insects. *Streptomyces* strains are major source of insecticidal natural products. Recently, different compounds separated from microorganisms such as Abamectin which was isolated from *Streptomyces avermitilis* have been documented as the major source in bio-pesticide industry (Xie 1998 and Shi 2000). The objective of this study of this study is to evaluate the effIcacy of Fenugreek, Ginseng oil, Abamectin and their mixture as protectant versus cowpea beetle.

MATERIALS AND METHODS

Plant materials

Seeds of Cowpea (*Vigna unguiculata* L.) cultivar Teba and *Vicia faba* (Giza 2 variety) were used as tested plant.

Tested insect

A laboratory colony established and maintained on cowpea seeds kept under laboratory conditions of 27± 3°c and 65 ±5% RH in Stored Grains pest Research Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. Adult of 2 days old Cowpea beetle (*Callosobruchus maculatus*) was used as tested insect.

Oils and bio insecticide

Fenugreek and Ginseng oils used as protectants materials were obtained from the Egyptian market, Abamectin is a mixture of 80% Avermectin B_{1a} and 20% avermectin B_{1b}. it is an insecticidal and anthelmintic compounds derived from various laboratory broths fermented by the soil bacterium *Streptomyces avermitilis*. As well as, tow mixtures were prepared as follow:

Mix.1. consists of LC_{20} Abamectin + 2 LC_{95} Fenugreek, Mix. 2. consists of LC_{20} + 2 LC_{95} Ginseng. One ml of Fenugreek or Ginseng oils was diluted in 10 ml of petroleum ether (El-Lakwah and El-Kashlan, 1999). Serial dillutions of Abamectin were prepared using distilled water. The experiments in each set were designed to estimate LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} and LC_{99} (for *Callosobruchus maculatus*) were estimated after 24 hrs from insect exposure (Bliss, 1935).

Toxicological studies

Serious of gradual concentrations from each of tested oils and Abamectin were evaluated to determine the efficiency of these compounds. A sample of 10 gm of disinfected Cowpea seeds was placed in a glass tube (3 x7.5 cm) and separately mixed with each oil or abamectin at their different concentration and covered tightly. The tubes were shaken vigorously to ensure uniform coating of Cowpea seeds with the tested oil. The solvent was allowed to evaporate for 30 minutes by using an electric fan. Three replicates for every treatment were infested by 25 adults of the tested insect. The tubes were covered secured with elastic bands and kept in the incubator under constant conditions. Mortality counts of *C. maculatus* adults were recorded in all experimental treatments after 1, 2, 3 and 5 days from exposure. Percentage of mortality was taken and were calculated according to Abbott‘s formula, 1925. The experiments in each set were designed to provide concentration of mortality regression lines for tested materials against *C. maculatus* according to the method described by Finney, 1971. The slope values of established lines, LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} and LC_{99} (for insects) were estimated after 24 hrs from insect exposure (Bliss, 1935).

Biological studies

Five grams of cleaned cowpea seeds were mixed with the tested materials at the percentage values which mentioned before were placed in glass tube (each treatment had three replicates). Two pairs of newly emerged adults (2 days old) and were placed in each tube and covered tightly. After 10 days the insects were removed and the number of hatched and non-hatched eggs was recorded. Percent hatchability was determined and similar the reduction in hatchability was determined. The collected Cowpea seeds bearing deposited eggs were placed in clean glass tubes and kept in an incubator under the afore mentioned constant conditions till progeny emergence. Emerging adults were counted and discarded for a period of 10 days.

Residual activity of tested materials at tested storage periods

Each tested concentration of $2LC_{95}$ of Fenugreek, $2LC_{95}$ of Ginseng, LC_{95} of Abamectin, Mix.1 and Mix.2 was mixed to500 gm of cowpea seeds. Twenty-five adults of *C. maculatus* were placed to 10 gm of Cowpea seeds of each of the three replicates in the presence of untreated control was to study the residual effect of the tested treatments. All treatment was kept in glass jar, covered tightly under laboratory conditions at 27 ±3ºC. Treated seeds were stored for 3 months.

Seed germination

Twenty-five treated Cowpea seeds were taken from each of the treatments mentioned before and were placed in Petri dishes. Each Petri dish was lined with two layers; the first was cotton layer and the second was filter paper both were soaked with water and then 25 Cowpea seeds were placed on the paper. After three days, germinated grains were recorded. Similar three replicates of untreated Cowpea seeds were used as control. Percentage of germination was calculated and percentage of reduction was determined**.** All treatments were repeated three times with the untreated control. The above steps were repeated at the end of the storage period to determine the percentage of germination.

RESULTS AND DISCUSSION

Mortality percentage

The mortality percentage increase with the increase of rate of concentration (ml/kg) and by increasing the days of exposure to oils and Abamectin. The estimated toxicity values of LC_{25}, LC_{50}, LC_{75}, LC_{95} and LC_{99} after one days of exposure with tested materials are presented in Table (1).

Table 1: Mortality percentage of Cowpea weevil adults after different

Tested materials	Rate of treatments ml/kg	Mortality % after indicated periods (days)				LC_{25}	LC_{50}	LC_{75}	LC_{90}	LC_{95}	LC_{99}
		1	2	3	5						
Fenugreek	2.0	25.53	33.67	41.73	58.61	2.22	3.28	4.86	6.91	8.53	12.67
	3.0	38.03	44.71	61.73	73.91						
	4.0	54.61	62.58	78,38	87.33						
	5.0	78.72	89.34	96.83	100						
	6.0	90.10	98.72	100	100						
Ginseng	1.0	19.15	27.42	43.24	60.33	1.68	2.83	4.77	7.65	10.14	17.18
	2.0	24.82	31.73	55.61	71.4						
	3.0	27.66	41.28	67.82	80.13						
	4.0	65.96	77.33	89.23	95.82						
	5.0	97.16	100	100	100						
Abamectin	0.003	20	37.92	51.33	69.02	0.004	0.01	0.03	0.06	0.09	0.20
	0.01	38.66	60.17	88.66	98.99						
	0.02	69.33	82.88	95.33	100						
	0.03	78.66	90.63	98.72	100						
	0.1	97.33	100	100	100						

According to LC_{95} values, the toxicity of the tow tested oils and Abamectin on the *C. maculatus* adults could be arranged as follows: Abamectin > Fenugreek > Ginseng. This is agree with (Ketoch *et al.*, (2005), Udo. 2011, Sereme *et al.* 2013, El-Razik and Sayed (2014), Hafez *et al*., (2014).

Biological studies

Except for Fenugreek oil number of eggs and hatchability decreases by increasing concentration of the tested materials. The Lc_{95} causes complete reduction with Ginseng and Abamectin. Reduction also was 100% with Mix.2 and 99.29% with Mix.1. Fenugreek oil differs from other treatments at which number of eggs and hatchability increase by increasing concentration. Reduction was 54.71% at Lc_{50} and 39.29% at Lc_{95} Table (2) Fig. (1). (Don-Pedro, 1989) reported that Eggs on the coconut oil treated seeds were found to be less firmly joined than on the controls, suggesting that the oil may inhibit larval penetration into the seed. Hafez *et al.* (2014) found that both ethyl oleate and *A. calamus* oil inhibit egg lying on the untreated seeds. *Streptoverticillium* were the most potent actinomycetes affecting the biological and physiological criteria of the different insect species Bream *et al*. (2001).

Table 2: Efficacy of the tested plant powders on the oviposition and hatchability of eggs of *Callosobruchus maculatus* adults.

Treatment	Conc.	**Mean no. of eggs /2 female**				
		hatched eggs	**unhatched eggs**	**Total no. of eggs**	**Hatchability**	**Reduction**
		No.	**No.**		**%**	**%**
Fenugreek oil	LC_{50}	37.5	10.5	48	78.13	54.71
	LC_{95}	51	6.75	57.75	88.31	39.29
Ginseng oil	LC_{50}	7	3	10	70	91.67
	LC_{95}	_	0	0	0	100
Abamectin	Lc_{50}	2.3	0	2.3	100	97.26
	Lc_{95}	0	0	0	0	100
Mix.1	Fenugreek $2LC_{95}$+abamectin LC_{20}	0.6	0	0.6	100	99.29
Mix.2	Ginseng $2LC_{95}$+ Abamectin LC_{20}	0	0	0	0	100
control		84	5	89	94.38	0

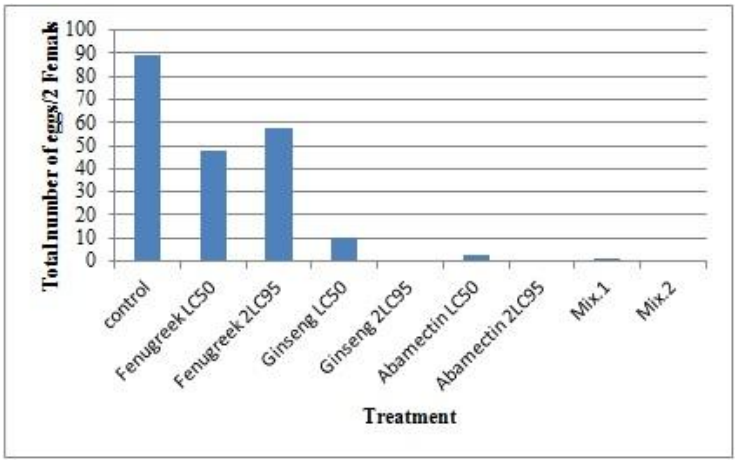

Fig. 1: Effect of tested materials on egg lying of *C. maculatus* emergence

Reduction in number of emerged adults increases by increasing concentration with all treatments. Lc_{95} reduced adult emergence with Ginseng and Abamectin.

Reduction also was 100% with Mix.1 and Mix.2, while Fenugreek oil reduced adult emergence up to 96.10% at Lc_{95}. Reduction of Lc50 with Fenugreek, Ginseng and Abamectin was 94.81%, 92.21%, 98.70% Table (3) and Fig.(2). plant oils combined with Abamectin have synergistic effect against *C. maculatus* adults. Palm oils inhibited progeny emergence of *C. maculatus* at 20ml treatment level (Udo, 2011). Combination of both *allium sativum* oil and fruit powder of *Casicum annum* Miller revealed reduction of *C. maculatus* (F1) Mailafiya *et al.* (2014).

Table 3: Efficacy of the tested three tested oils, Abamectin and mixtures on percentage adult emergence of *Callosobruchus maculatus*.

Treatment	**Conc.**	**Progeny emergence**				
		Female No.	**Male No.**	**Total No. (F_1 emerged adults)**	**Adult emergence %**	**Reduction %**
Fenugreek	LC_{50}	3.50	0.50	4.00	10.67	94.81
	LC_{95}	2.00	1.00	3.00	5.88	96.10
Ginseng	LC_{50}	4.00	2.00	6.00	85.71	92.21
	LC_{95}	0.00	0.00	0.00	0.00	100.00
Abamectin	LC_{50}	1.00	0.00	1.00	40.00	98.70
	LC_{95}	00.00	00.00	00.00	00.00	100.00
Mix.1	00.00	00.00	00.00	00.00	00.00	100.00
Mix.2	00.00	00.00	00.00	00.00	00.00	100.00
Control	00.00	48.00	29.00	77.00	91.67	00.00

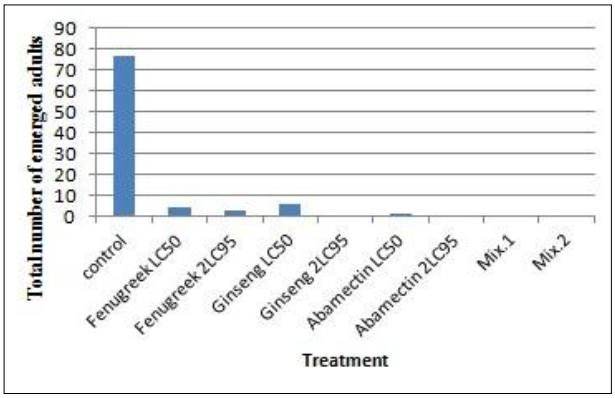

Fig. 2: Effect of tested materials on adult of *C. maculatus*.

Activity of the tested materials on *C. maculatus* at storage period.

A sharp decline in the efficiency of oils occurred after seven days; recorded 43.43% for mix.1. at 7th day, while for mix.2. it was 51.70%. Ketoh *et al.* (2005) stated that after a 72hr exposure, mortality percentage of *C. maculatus* adult was lower than 50% in the presence of treated seeds. Hundred percent mortality of *C. maculatus* adults was recorded at initial treatment up to three months for high treatment (LC_{95}) of abamectin. This means that there was a high residual effect of Abamectin against *C. maculatus* Table (4).

Table 4: Mortality percentage of *C. maculatus* treated with LC_{95} and $2LC_{95}$ of tested materials and their mixtures at storage periods.

No.	**Treatments**	**Mortality % at storage periods**				
		Initial	3 **days**	5 **days**	7 **days**	3 months
1	LC_{95} fenugreek oil (8.48 ml/Kg)	100	20	16.7	10.3	---
2	LC_{95} ginseng oil (9.89 ml/Kg)	100	85	36.7	24.19	----
3	LC_{95} abamectin	100	100	100	100	100 till 3 month
4	Mix 1 ($2LC_{95}$ fenugreek + LC_{20} abamectin)	100	100	63.3	43.43	----
5	Mix 2 ($2LC_{95}$ ginseng + LC_{20} abamectin)	100	90	86.7	51.70	---

Effect of $2LC_{95}$ tested oils and LC_{95} abamectin on *C. maculatus* F_1 (progeny) at storage periods.

Table (5) showed that $2Lc_{95}$ of fenugreek and ginseng oil have a reduction effect on F_1 emerged adults; which decreased gradually from 100% at initial time till reached 21.26 and 51.97 respectively at the 6th week. On the other hand, Lc_{95} of abamectin reduces the number of F_1 emerged adults to zero in all periods of treatments until storage periods (3 months).

Table 5: Percentage of *C. maculatus* F_1 (progeny) in Cowpea seeds treated with $2LC_{95}$ of tested oils and LC_{20} Abametin

Treatment		Storage Time (weeks)						
		Initial	1st week	2nd week	3rd week	4th week	5th week	6th week
Fenugreek $2LC_{95}$	Adult emergence %	___	7.63	10.9	20.52	45.59	62.71	78.74
	Reduction %	100	92.83	89.07	79.48	54.37	37.29	21.26
Ginseng $2LC_{95}$	Adult emergence %	___	___	0.9	3.28	7.35	27.09	48.03
	Reduction %	100	100	99.09	96.72	92.65	72.90	51.97
Abamectin LC_{95}	Adult emergence %	—	—	—	—	—	—	—
	Reduction %	100	100	100	100	100	100	100 till 3 month
Control	Mean number of emerged Adult	43.67	45.66	36.60	40.6	45.33	39.34	42.33

Mix.1 has a high reduction effect on the progeny of *C. maculatus*. This reduction recorded 100% at the initial time, and slowly decreases till reached 85.22% at the12thweek. On the other hand, mix.2 showed that reduction percentage of emerged *C. maculatus* recorded 100% from the initial time up to 12th week without any decrease in the percentage all over the period of storage Table (6).

Table 6: Percentage of *C. maculatus* F_1 (progeny) in Cowpea seeds treated with $2LC_{95}$ of tested oils and LC_{20} Abametin

Treatment		Storage periods (weeks)						
		Initial	2nd week	4th week	6th week	8th week	10th week	12th week
Mix.1 (2 LC_{95} fenugreek + LC_{20} abamectin)	**Adult emergence %**	0.00	0.00	2.48	3.76	8.12	8.59	14.78
	Reduction %	100	100	97.52	96.24	91.88	91.43	85.22
Mix.2 (2 LC_{95} ginseng oil + LC_{20} abamectin)	**Adult emergence %**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Reduction %	100	100	100	100	100	100	100
Control	**Mean number of adult emerged**	47	56	67.33	62	41	62	46

Germination test

Germination of cowpea seeds treated with Abamectin insecticide at the tested rate remained almost equal to the control at the initial and after storage period (6 weeks). Fenugreek and ginseng indicate 2% reduction in germination at initial time only. The percentages germination has non-significant effect with control on germination of Cowpea seeds. Slight reduction in germination was observed with the tested mixtures at the initial and after storage (3 monthes) treatment for Mix.1. ($2LC_{95}$ fenugreek + LC_{20} abamectin) the reduction slightly decreased from 6% at the

initial time to 5.2% at the after storage time(Table 7). While reduction did not change from the initial and after storage time for Mix.2. ($2LC_{95}$ Ginseng + LC_{20} abamectin).

These results agree with Ketoh *et al.* (2005), (Udo, 2011), Fawzia and El-Ashry (2012), and disagree with Bamalyl *et al*. (2006) who reported that that *Khaya senegalensis* seed oil reduced germination of infested cowpea seeds and it appears to affect the viability of seed.

Table 7: Effect of the three tested oils and abamectin on cowpea seeds germination at initial and after storage treatment

Treatment ml/kg		**Control**	**$2LC_{95}$ Fenugreek**	**$2LC_{95}$ Ginseng**	**LC_{95} Abamectin**	**Mix.1**	**Mix.2**
Germination %	**Initial**	100	98	98	100	94	98
	After storage	100	100	100	100	94.8	98

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ARABIC SUMMERY

كفاءة إستخدام بعض الزيوت النباتية والأبامكتين كمبيد حيوي ومخلوط كل منهم على حماية بذور اللوبيا من الإصابة بحشرة خنفساء اللوبيا أثناء فترة التخزين.

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أجريت هذه الدراسة لتقييم تأثير كل من زيت الحلبة والجينسينج ومبيد الأبامكتين والذي تم فصله من *Streptomyce avermilite* ومخلوط كلا من زيت الحلبة والجنسينج مع الأبامكتين على خنفساء اللوبيا. والذي شمل التأثير السمي على الحشرات البالغة والتأثير البيولوجي بالإضافة إلى قدرة كل مادة على الحفاظ على بذور اللوبيا من خطر الإصابة بخنفساء اللوبيا طوال فترة التخزين (٣ أشهر) . أظهرت النتائج ان مبيد الأبامكتين كان الأقوي حيث ظل محتفظا بقدرته على قتل الحشرات البالغة ومنع وضع البيض او ظهور أيا من حشرات الجيل الأول للحشرة حتى نهاية فترة التخزين (٣ أشهر) عند تركيز Lc_{95}. تراوحت القدرة التخزينية لكل من زيت الحلبة والجنسيج على تقليل نسبة خروج حشرة خنفساء اللوبيا في الأسبوع السادس بنسبة 21.26% و51.97% على الترتيب عن تركيز $2Lc_{95}$. أدى خلط المبيد مع كلا من الزيتين على حدى إلي استخدامه بشكل فعال حتى نهاية فترة التخزين مع تجنب الأثر الضار لاستخدامه بتركيزات عالية. لم يكن هناك تأثير ضار لجميع المواد المستخدمة على معدل إنبات بذور اللوبيا المخزنة.