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TOXICITY OF SHIH (*Artemisia annua*) EXTRACTS TO COWPEA BEETLE; *Callosobruchus maculatus* FABRICIUS

Amira Sh.M. Ibrahim
Economic Entomology Dept., Fac. Agric., Kafrelsheikh Univ.

ABSTRACT

Cowpea is an important source of nutrients, particularly of plant proteins. Unfortunately, this crop is infested by several insects in both field and storage. The most important insect pest in the storage is the cowpea beetle, *Callosobruchus maculatus* Fabricius. The current study investigated the control of this insect pest using plant essential oils extracted from Shih, *Artemisia annua*. The highest accumulative mortality of *C. maculatus* adults was achieved by ethanol extract followed by Acetone and Acetone Ethanol extract were 80.00, 66.67 and 66.00%, respectively giving high significant differences. While the numbers of egg laying were the lowest in ethanol and acetone extracts (11.00 and 12.00 eggs/ 10g, respectively) compared to acetone ethanol extract (48 eggs/10 g). On the contrary, acetone extract showed the best repellent impact of the cowpea beetles in egg numbers (6.50 eggs/10 g). However, ethanol extract treatment recorded the highest mortality (76.67%).The reducing damage test in cowpea seeds (damage reduction and weight loss %), either by filter paper technique or cowpea seed technique, was high in ethanol extract treatment . Ethanol extract caused 97.83 and 93.71% damage reduction without any weight loss in both cases.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L) Walp is one of the most widely cultivated grain legumes. It has been consumed by humans since the earliest practice of agriculture in developing countries where it is a valuable source of proteins, vitamins and salts (Singh *et. al*., 2003). This crop is liable to infestation with several insect pests, in both field and storage. The main storage pests are the bruchids, particularly the cowpea beetle, *Callosobruchus maculatus* (F.). Over 90% of the insect damage to cowpea is caused by this pest, which is the main constraint to increased cowpea production (Singh and Luse, 1978 & Joana and Daniel, 2010). The infestation of cowpea by *C. maculatus* may be undetectable at harvest, but it multiplies very fast in storage giving rise to a new generation every month (Ouedrago *et. al.,* 1996). The weight loss of cowpea seeds may reach to about 30% due to this insect pest if no control measures were applied (Badi *et. al.,* 2013).

Chemical control of stored product pests with current chemical pesticides may cause special problems on stored products (Collins *et. al.,* 2002). Hence, the researches have been concentrated on plants for solutions leading to the production of a multitude of secondary compounds that can have toxic, growth reducing, and antifeedant properties against insects. Prior to the development and commercial success of synthetic insecticides in the beginning of 1940s, botanical insecticides were major weapons against crop pests (Isman, 2008). Four major types of botanical insecticides are being used for insect control, including pyrethrum, rotenone, neem and essential oils.

The genus *Artemisia*, included in the family Asteraceae, comprises a variable number of species (from 200 to over 400, depending on the authors) found throughout the northern half of the world (Marco and Barbera, 1990). *Artemisia herba-alba* is a shrub usually found in Northern Africa and the Middle East. The above ground parts of the plant are usually used as medicine. Many species of *Artemisia* have been used as medicinal materials. *Artemisia anomala* S. Moore (Nan-Liu-Ji-Nu) has been used for centuries to treat fever, empyrosis, inflammation, and dissipated liver function caused by hepatitis in China (Xi Tan *et. al*., 2014). The essential oil extracted from the plant's aerial parts showed antibacterial activity (Yashphe *et. al*., 1979) as well as, antispasmodic activity on rabbits (Yashphe *et. al*., 1987 & Aziz *et. al*., 2012). An aqueous extract of aerial parts of the plant has shown a hypoglycemic effect in alloxan-induced diabetic rabbits and mice (Al-Khazraji *et. al*., 1993 & Al-Shamaony *et. al*., 1994 & Marrifa *et. al*., 1995)

Aerial parts of *A. herba-alba* Asso may have insecticidal or biological activity against insects and insect antifeedant (Regnault Roger & Hamraoui, 1994 ; El-Baroty & Abdel-Lattif, 1997 and Iman *et. al*., 2012). Both *A. herba-alba* and *A. monosperma* oils were more toxic on *Thrips tabaci* Gennadius*, Aphis gossypii* Glover and *Bemisia tabaci* Lindman in the laboratory test (Soliman, 2005). In such concern, chilies, garlic and peppermint products have negative effects on cowpea beetle, *C. maculates* biology, the results indicated that these plant products have the potential to protect cowpea from this insect damage (Trioesele, 2015).

With this outlook, the present study was conducted at the laboratory of Entomology Department, Faculty of Agriculture, Kafrelsheikh University to investigate the role of shih extracts against the cowpea beetle; *C. maculatus* , concerning the extract inhibitory effect on egg laying, repellent impact and reducing damage to cowpea seeds, resulting from the attack of this insect pest.

MATERIALS AND METHODS

1. Extraction techniques of Artemisia essential oils:

Artemisia extracts were prepared according to Ekanem and Brisibe (2010). The aerial parts of Artemisia were dried in the shade at room temperature and the dried foliage was ground to powder. Three amounts of the powder, each of 20 g, was added to 100 ml of acetone, 100 ml of ethanol and 100 ml of acetone ethanol to obtain three extracts due to solvents. The extracts were stirred for 24 hours using a magnetic stirrer, and then filtered through filter paper three times. Using water bath, the solvents were evaporated to get concentrated extracts. The extracts were diluted with petroleum ether and kept in sealed bottles in the refrigerator at 4°C until use.

2. The cowpea beetle; *Callosobruchus maculatus* culture:

A culture of *C. maculatus* was obtained from Stored Product Pest Research Department, Sakha Agricultural Research Station. The cowpea beetles were reared in glass jars on cowpea seeds for several generations under constant temperature of 30± 2° C. The cowpea seeds used in rearing were disinfested by picking those without damage holes and heating in the oven at 50° C for five minutes. After seven days, the culture was cleaned from dead and old cowpea beetles for getting newly hatched adults (El-Degwi and El-Orabi, 1996).

3. The inhibitory effect on the egg-laying:

Filter paper impregnation method (FPIM) was used according to Morallo Rejesus *et al*. (1990). Filter papers were moistened with the prepared extracts and air-dried under room temperature. Treated filter papers were transferred individually to Petri-dishes 9cm in diameter. Then, forty grams of non-infested cowpea seeds were put on the papers. Five pairs of *C. maculatus* adults (24-48 hrs-old) were provided to each treatment (3 replicates/ extract). Results were compared to the control Petri-dishes containing treated filter papers with acetone as follows:

- The number of dead adults after two, four, six and seven days.
- The accumulative mortality and death percentages.
- The egg numbers In a random sample of cowpea seeds weigh 10 g after seven days after treatments.

4. The repellent impact for shih extracts:

Forty grams of cowpea seeds were dipped into each extract for 30 seconds. After air-dried, seeds were removed to Petri-dishes. Ten newly hatched *C. maculates* adults (5 females + 5 males) were introduced. The number of dead adults after two, four, six and seven days from treatment, eggs in a random sample of cowpea seeds after seven days and the accumulative mortality and death percentages were counted.

5. Reducing damage test:

Uninfested cowpea seeds and filter papers were dunked in the three shih extracts and put separately in jars. The treated cowpea seeds were divided into three parts, 200 g each. Three jars containing treated filter papers were supplied with 200 g cowpea seeds. Fifty pairs of newly emerging *C. maculatus* adults were released in each jar. In the control, cowpea seeds and filter papers were treated with acetone and replicated individually three times. All treatments were incubated for one month.

The number of first generation of adults, eggs, damaged cowpea seeds; seed weight and the rate of loss in seed weight were recorded.

The reductions in damaged seeds were calculated as:

$$\text{Damage reduction \%} = \frac{\text{damaged seeds in control} - \text{damaged seeds in treatment}}{\text{damaged seeds in control}} \times 100$$

To determine the weight loss, the following equation was used:

$$\text{Weight loss \%} = \frac{\text{seeds weight before treatment} - \text{seeds weight after treatment}}{\text{seeds weight before treatment}} \times 100$$

Significance of inhibitor, repellent impact and reducing damage for shih extracts were analyzed using Duncan's Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Inhibitory effect of Artemisia extracts on *Callosobruchus maculatus* adult mortality and egg-laying:

Data presented in Table (1) and illustrated in Fig. (1) show the effect of different Artemisia extracts on *C. maculatus* adults mortality and egg- laying, throughout seven days after treatments using filter paper technique. The accumulative mortality increased gradually from the second up to the seventh day after treatment. Two days after treatments, the accumulated adult mortality were 5.50, 10.00, 3.00 and 1.80 out of 30 adults due to acetone, ethanol, acetone ethanol and control, respectively. The highest accumulative adult mortality were found seven days after treatments with corresponding values of 20.00, 24.00, 18.00 and 16.33. Also, the highest percentages of adult death were obtained seven days after treatments; however acetone and ethanol extracts were more effective with 66.67 and 80.00%, respectively. The death percentage in the control was 54.43%. Statistical analysis revealed highly significant differences in the death percentages due to different solvents.

Number of laid eggs by *C. maculatus* adults took the same trend with lowest numbers (12.00 & 11.00 eggs / 10g) due to acetone and ethanol extracts, respectively, compared to 48.00 eggs in acetone ethanol treatments and 66.17 eggs/ 10 g in the control. Also, the differences were highly significant.

Table (1): Accumulative mortality and egg numbers of *C. maculatus* adults during seven days after filter papers treatment.

Shih extract	Mortality / 30 adults								No. of eggs/10g
	2nd day		4th day		6th day		7th day		
	Acc.	%	Acc.	%	Acc.	%	Acc.	%	
Acetone	5.50	18.00	14.00	46.66	17.00	56.67	20.00	66.67b	**12.00c**
Ethanol	10.00	33.33	20.00	66.67	23.00	76.67	24.00	80.00a	**11.00c**
Acetone Ethanol	3.00	10.00	7.00	23.33	16.00	53.33	18.00	60.00b	**48.00b**
Control	**1.80**	**6.00**	**5.50**	**18.33**	**14.33**	**47.77**	**16.33**	**54.43c**	**66.17a**

P<0.01.Means of each factor designated by the same letter are not significantly different at 1% level using Duncan's multiple test.

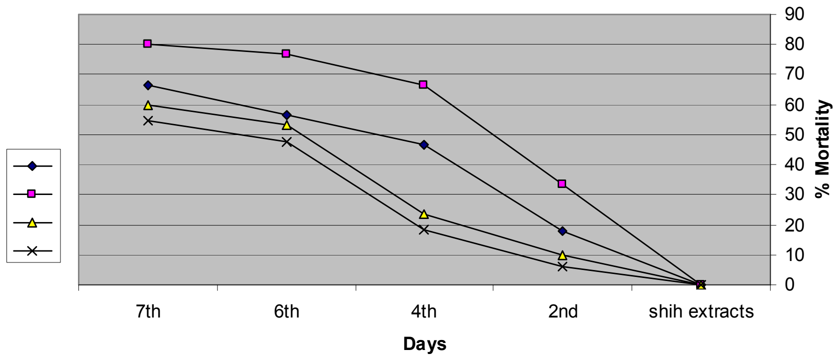

Fig. (1):Accumulative mortality of *C. maculatus* during seven days after filter papers treatment

2. Repellent impact for shih extracts:

Data presented in Table (2) and illustrated in Fig.(2) show the effect of extracts of shih foliage, using variable solvents, on *C. maculates* adult mortality and egg-laying, when treating the cowpea seeds. Throughout seven days after treatment, the accumulative adult mortality due to acetone solvent were 5.50, 11.50, 19.00 and 20.00 adults out of 30 ones, 2, 4, 6 and seven days, respectively, with 66.67% adult mortality at the end of the experimental period. However, ethanol solvent gave Shih extract more effectiveness (76.67% death) against insect adults, while the control treatment had only 51.67% adult death.

As for egg laying, the untreated cowpea seeds had 62.00 eggs/10 g, while acetone treatment induced the best repellent effect against *C. maculates* adults with 6.50 eggs/10 g.

Table (2): Accumulative mortality and egg numbers of *C. maculatus* adults during seven days after cowpea seeds treatment.

Shih extract	Mortality/30 adults								No. of eggs/10g
	2nd day		4th day		6th day		7th day		
	Acc.	%	Acc.	%	Acc.	%	Acc.	%	
Acetone	5.50	18.00	11.50	38.33	19.00	63.33	20.00	66.67b	6.50d
Ethanol	6.00	20.00	12.50	41.67	20.50	68.33	23.00	76.67a	22.00c
Acetone Ethanol	2.50	8.33	7.50	25.00	18.00	60.00	19.00	63.33b	33.50b
Control	1.75	5.83	7.75	25.83	13.50	45.00	15.50	51.67c	62.00a

P<0.01.Means of each factor designated by the same letter are not significantly different at 1% level using Duncan's multiple test

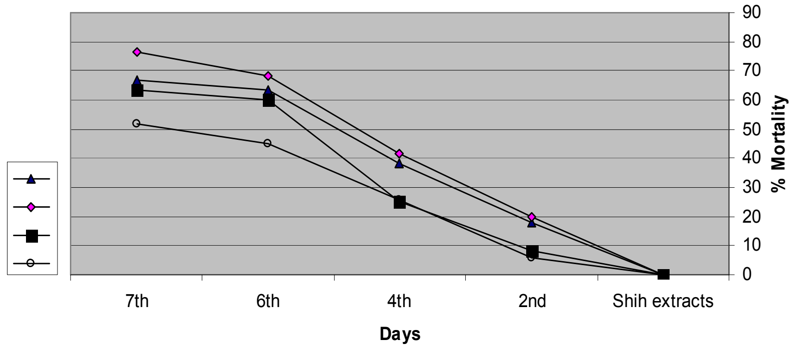

Fig. (2): Accumulative mortality of *C. maculatus* during seven days after cowpea seeds treatment.

3. Reducing damage test:

Data in Tables (3 and 4) show the effectiveness of different Artemisia extracts either by filter paper or cowpea seed treatment after a month of storage. There were high significant differences among the three Artemisia extracts in damage reduction and weight loss %. The lowest number of eggs, adult emergence and damaged seeds resulted from ethanol extract (12.50, 25.00 eggs; 3.50, 18.33 adults and 3.13, 9.50 damaged seeds, respectively) followed by acetone and acetone ethanol extracts.

Thus, ethanol extract achieved the maximum damage reduction (97.83 and 93.71%) compared to acetone and acetone ethanol extracts. Treatment of ethanol did not result in any losses in cowpea seed weight

Table (3): Effect of filter paper treatments by Shih extracts in reducing cowpea seed damage due to *C. maculatus*

Shih extract	No. of			%damage reduction	Seeds weight	%weight loss
	Eggs	Adult emergence	Damaged seeds/50g			
Acetone	75.00	35.67	18.00	87.53b	198.00a	1.00c
Ethanol	12.50	3.50	3.13	97.83a	200.00a	0.00c
Acetone Ethanol	532.00	100.00	68.00	52.88c	161.10b	19.45b
Control	832.50	222.88	144.30	-	132.23c	33.89a

P<0.01.Means of each factor designated by the same letter are not significantly different at 1% level using Duncan's multiple test.

Table (4):Effect of seed treatments by Shih extracts in reducing cowpea seed damage due to *C. maculatus*.

Shih extract	No. of			%damage reduction	Seeds weight	%weight loss
	Eggs	Adult emergence	Damaged seeds/50g			
Acetone	124.50	105.00	32.50	78.48b	192.00a	4.00c
Ethanol	25.00	18.33	9.50	93.71a	200.00a	0.00c
Acetone Ethanol	646.00	140.00	95.00	37.09c	161.10b	19.45b
Control	862.50	221.83	151.00	-	150.90c	24.55a

P<0.01.Means of each factor designated by the same letter are not significantly different at 1% level using Duncan's multiple test.

These findings revealed that, ethanol extract was the most effective Artemisia extracts on cowpea beetle; *C. maculatus* mortality, egg laying, adult emergence, damage reduction and weight loss. However, its impact was higher against insect pest in filter paper treatment than cowpea seed treatment, followed by acetone extract while acetone ethanol extract was the lowest.

From the previously results, it can be concluded that plant oils were potent in protecting stored grains of cowpea against infestation and damage by *C. maculatus* as pure neem and moringa seed oils and their mixtures. These have repellent, antifeedant, insect growth regulatory and pesticidal properties (Joana and Daniel, 2010). Also, extract of cashew kernelis proved to be effective in controlling cowpea storage bruchid, *C. maculatus* and could serve as an alternation to synthetic insecticides for the protection of stored cowpeas against bruchids (Adedire *et. al*., 2011)

The essential oil of Artemisia proved to be most effective as a biocide against *Callosobruchus chinensis* L. and achieving a mortality rate of 100% (Kada *et. al*., 2014). In India, Tripathi *et., al.* (2000) tested toxic repellent and development inhibitory activities of the essential oil of *Artemisia annua* L. against two economically important stored product insects: *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (L.). They found that oil of *A. annua* was largely responsible for both repellent (behavioral) and toxic (physiological) actions on both insect species.

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سمية مستخلصات الشيح على خنفساء اللوبيا *(Callosobruchus maculatus* Fabricius*)*

أميرة شوقي محمد ابراهيم
قسم الحشرات الاقتصادية – كلية الزراعة – جامعة كفرالشيخ

اللوبيا مصدر غذائي هام خاصة البروتين النباتي , يصاب هذا المحصول بحشرات عديدة في كل من الحقل و المخزن . خنفساء اللوبيا *Callosobruchus maculatus* Fabricius هي الآفة الحشرية الأهم التي تصيب في المخزن. أجريت هذه الدراسة لاختبار مكافحة تلك الآفة الحشرية بمستخلص الشيح *Artemisia annua* . أشارت النتائج إلى أن أعلى نسبة موت تراكمي للأطوار الكاملة للخنفساء تحققت عند المعاملة بمستخلص الإيثانول ثم مستخلص الأسيتون ومستخلص الأسيتون و الإيثانول فكانت ٨٠ ، ٦٦,٦٧ و ٦٦ % على الترتيب معطية فروقا معنوية عالية. بينما كانت أعداد البيض على البذور أقل عند المعاملة بمستخلص الايثانول ومستخلص الأسيتون (١١,٠٠ و ١٢,٠٠ بيضة /١٠ جم على الترتيب) مقارنة بمستخلص الأسيتون الإيثانول (٤٨,٠٠ بيضة /١٠جم). وعلى العكس، كان مستخلص الأسيتون الأكثر طردا لخنافس اللوبيا من حيث أعداد البيض على البذور (٦,٥٠ بيضة /١٠جم) لكن مستخلص الإيثانول سجل أعلى نسبة موت (٦٧,٦٧%).

تقليل الضرر في بذور اللوبيا (خفض الضررو فقد الوزن) سواء باستخدام أوراق الترشيح او طريقة بذور اللوبيا كان عاليا عند المعاملة بمستخلص الإيثانول حيث بلغ خفض الضرر نتيجة معاملة الايثانول ٩٣,٤٢%، ولم يحدث اي فقد في الوزن في كل من طريقتي الاستخلاص .