

INFLUENCE OF SOME PLANT POWDERS AS NATURAL PESTICIDES ON THE OVARIES STRUCTURE OF PULSE BEETLE *Callosobruchus maculatus* (F.)

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ABSTRACT

Studies on the effect of black pepper, onion and garlic powders on reproduction of *Callosobruchus maculatus* and the correlation of this effect to the sterility, morphological and histological changes in the ovaries were carried out.

Results showed that there were progressive increase in sterility as the concentration of plant powders increased. The tested materials reduced the size of the ovarioles of females resulted from treated seeds. The decrease in measures of ovarioles and Spermatheca were connected with the increasing of plant powders concentration.

Some damaged were observed in ovarioles of females resulted from treated seeds with Lc₂₅ and Lc₅₀ of plant powders. Oocyte increase in size and shape changed from spherical in primary oocyte to rectangle shape in case of black pepper and onion whereas, the shape becomes oval in garlic case. In the germarium the nuclei lose their normal shape in the nuclear membrane is irregular the follicle cells and the follicular epithelium become thinner, loses its contact with the oocyte and becomes the vaculated.

INTRODUCTION

The pulse beetle *Callosobruchus maculatus* is consider cosmopolitan pest which attack pulses in store and cause serious damage (Bohoduri *et al.*, 1990 and Khaire *et al.*, 1992). Because of the negative aspects in chemical control, intensive study has been carried out to find safe alternatives. Some natural products are considered one of the most promising control agents. Such agents proved to be highly effective against pests (Mostafa, 1993, Ismail *et al.*, 1995, El-Degwi and El-Orabi, 1996, El-Degwi, 1997, El-Degwi, *et al.*, 2001/2002 and Rizk *et al.*, 2002).

The present work is aimed to study the damaged degree due to Lc₂₅ and Lc₅₀ of black pepper, Onion and garlic powders on reproductive system and the correlation of this effect to the sterility, morphological and histological changes in the ovaries.

MATERIALS AND METHODS

The pulse beetle adults were obtained from stock cultures reared in Insect and Pest control Lab (NCRRT). Adults were reared on cowpea *vigna unguiculata* in an incubator maintained at 30 ± 2 °C and 70% ± 5% R.H. All experiments were performed at this conditions.

Dry seeds of black pepper (*Piper nigrum*), bulbs of onion (*Allium cepa*) and cloves of garlic (*Allium sativum*) powders were mixed at (0.125,

0.250, 0.500, 1.00 and 1.500 gm) thoroughly with 25 gm seeds (w/w) for each concentration in Small jar. Three replicates for each concentration and untreated seeds were used. Ten newly emerged pairs of adults were released in each jar. The number of eggs laid on infested seeds, hatched eggs and sterility were calculated, according to Chamberlain's formula as mentioned by Guirguis (1979):

$$\% \text{ Sterility} = 100 - \frac{a \times b}{A \times B} \times 100$$

Where

a = Number of eggs / female in treated.

b = % hatch in treatment.

A = Number of eggs / female in untreated.

B = % hatch in untreated.

Powders were mixed at Lc_{25} (0.0083, 0.15, 0.15gm) and Lc_{50} (0.022, 1.0, 2.5gm) El-Degwi *et al.* (2001/2002) and Rizk *et al.* (2003), thoroughly with seed, resulted females from treated and untreated seeds were collected.

For morphological description of ovaries, the ovaries of newly emerged one-day old adults were removed in Sodium chloride Saline solution 0.9% and fixed for 5 min. in aqueous Bouin's, washed and mounted in saline solution on glass slides and examined by binocular with magnification of 160X, also spermatheca were examined.

For histological studies the abdomens of treated and untreated females were fixed for 24-48 hr. in aqueous Bouin's, dehydrated in ethanol series then cleared in xylene. After being impregnated in paraffin wax (M.P.55-60°C), they were sectioned at thicknesses of 4-6 μ and mounted in Canada balsam on glass slides, stained with Cason's stain (Gray, 1973). Abdomen sections were examined with light microscope and photographed.

RESULTS AND DISCUSSION

It is necessary to determine the plant powder concentration required to obtain sterility of the adult stage. Results in Table (1) show that all tested materials had effect on sterility percentage of *C. maculatus*. Black pepper powder showed the highest effect (80.72%) at 1.5gm, garlic gave (78.76%) while, onion caused (66.89%). Results showed that there were progressive increase in sterility as the concentration of plant powders increased. These results are in agreement with the finding of Ismail *et al.* (1995) who found that fecundity of *C. quadrimaculatus* was decreased when seeds of mungbean treated with eucalyptus and guava leaves powder at 5gm. Also, El-Degwi and El-Orabi (1996) reported that soybean, kidneybean and lupin powders at 12% reduced the number of eggs laid by *C. maculatus*. Rizk (1998) mentioned that the Lc_{10} of *Thevclia nerifolia* or *Azadirachta indica* were highly effective in increasing the sterility percentage of *Corcyra Cephalonica* resulted adults. El-Degwi and Rizk (2001/2002) observed that black pepper powder showed adverse effects on fecundity of *C. maculatus*, garlic and onion powders caused high decrease in the number of eggs as concentration increased.

Reproductive system of *C. maculatus* female has a pair of ovaries each ovary is composed of 6 ovarioles or egg tubes, the ovarioles of each ovary are loosely bounded together by a network of connective tissues and tracheoles, the whole ovary is surrounded by fatty tissue. The ovarioles have a milky white colour. The ovarioles opens into an egg calyx. The egg calyx of all ovarioles open into an oviduct, the paired oviducts, fuse posteriorly to form the vagina. The bursa copulatrix arises as sac like from the vagina and lies dorsally to the oviducts, there are also two spherical and cup like shaped bursal gland is approximately equal in size to spermatheca. The spermatheca is a brown well sclerotized hook shaped structure which is blunt at one end and tapers to a point at the other. The similar morphology was described by (Ahmed *et al.*, 1976 and Mohammed, 1990).

Table (2 and Fig 1) shows the changes in the length of ovarioles and spermatheca of females resulted from treated and untreated seeds with LC_{25} and LC_{50} of tested plant powders. The length of the untreated ovarioles was 2.4 mm, compared with those of treated which were 2.2, 2.1 mm, 2.1, 1.6 mm and 1.8, 2.3 mm at LC_{25} and LC_{50} of black pepper, onion and garlic, respectively. It is very important to note that the ovarioles of resulted females from treated seeds is shorter than those of untreated ones. The diameter and length of the Spermatheca of untreated female were 1.3 and 1.2 mm. These measures decreased in females resulted from treated seeds except in case of onion powder at LC_{25} where as the measures were 1.6 and 1.5 mm. The decrease in measures of ovarioles and spermatheca were connected with the increasing of plant powders concentration. Obtained results are in agreement with those of Paul and Mc Caffery (1990) who stated that azadirachtin in applied topically to final instar larvae of *Spodoptera exempta* adversely affected oogenesis and reproductive maturation in subsequent female moths. Rizk (1998) found that the length and width of *C. Cephalonic* moths ovarioles treated with LC_{10} of *Thevctia nerifolia* or *Azadirachta indica* were greatly reduced as compared with control.

The ovarioles of *C. maculatus* female are telotrophic type, each ovarioles is divided into 4 regions, the terminal filament, the germarium, the vitellarium and the ovariole stalk or pedicel. The whole ovariole is surrounded by an epithelial sheath and inner membrane, the tunica propria, the epithelial sheath contain small flattened nuclei embedded in a thin layer of cytoplasm with no distinct boundaries between cells.

The terminal filament distally is produced a long thread like filament which appears as a continuation of the epithelial sheath and the tunica propria enclosing the whole ovariole.

The germarium is the anterior part of the egg tube and contains trophocytes, young oocytes and prefollicular cells, and it is easily distinguished into the apical trophic zone and basal zone of oocyte differentiation.

The vitellarium lying beyond the germarium and it consists of series of chambers or follicles each contain an oocyte enveloped by epithelial cells.

Table (1): Effect of some plant powders as protectant materials against *C. maculatus* on sterility.

Plant powder conc.	Black pepper						Onion						Garlic					
	Mean no. of eggs / Female	% Hatchability	% observed sterility	% calculated sterility	Mean no. of eggs / Female	% Hatchability	% observed sterility	% calculated sterility	Mean no. of eggs / Female	% Hatchability	% observed sterility	% calculated sterility	Mean no. of eggs / Female	% Hatchability	% observed sterility	% calculated sterility		
Cont.	312.8	86.05	13.95	0	385.6	87.24	14.65	0	398.6	85.35	12.76	0	398.6	85.35	12.76	0		
0.125	252.6	84.00	16.00	21.17	336.2	85.73	15.32	16.62	335.0	84.68	14.27	14.32	335.0	84.68	14.27	14.32		
0.250	223.4	80.16	19.84	33.47	255.6	83.42	18.39	26.40	306.8	81.61	16.58	32.05	306.8	81.61	16.58	32.05		
0.500	205.8	76.01	23.99	41.88	235.6	80.56	21.54	36.95	273.4	78.46	19.44	43.57	273.4	78.46	19.44	43.57		
1.00	152.6	70.85	29.15	59.83	198.2	73.71	27.07	46.36	250.2	72.93	26.29	56.57	250.2	72.93	26.29	56.57		
1.500	81.2	63.89	36.72	80.72	102.6	69.65	33.05	66.89	168.2	66.95	30.35	78.76	168.2	66.95	30.35	78.76		

Table (2): Effect of LC₂₅ and LC₅₀ of some plant powders as protectant materials against *C. maculatus* on biometrics of the ovaries and spermatheca.

Concentration	Black pepper						Onion						Garlic					
	Ovariole		Spermatheca		Ovariole		Spermatheca		Ovariole		Spermatheca		Ovariole		Spermatheca			
	Av. Length ± S.E	Av. length ± S.E	Av. Diam. ± S.E	Av. length ± S.E	Av. length ± S.E	Av. length ± S.E	Av. length ± S.E	Av. Diam. ± S.E	Av. length ± S.E	Av. length ± S.E	Av. length ± S.E	Av. length ± S.E	Av. length ± S.E	Av. length ± S.E	Av. Diam. ± S.E			
0	2.4 ± 0.2345	1.3 ± 0.1732	1.2 ± 0.2309	2.4 ± 0.2345	1.3 ± 0.1732	1.2 ± 0.2309	2.4 ± 0.2345	1.3 ± 0.1732	1.2 ± 0.2309	2.4 ± 0.2345	1.3 ± 0.1732	1.2 ± 0.2309	2.4 ± 0.2345	1.3 ± 0.1732	1.2 ± 0.2309			
LC ₂₅	2.2 ± 0.2041	1.0 ± 0.0334	0.8 ± 0.2005	2.1 ± 0.7321	1.6 ± 0.0577	1.5 ± 0.0577	1.8 ± 0.1472	1.5 ± 0.0577	1.5 ± 0.0577	1.8 ± 0.1472	0.9 ± 0.0408	0.7 ± 0.2886	1.8 ± 0.1472	0.9 ± 0.0408	0.7 ± 0.2886			
LC ₅₀	2.1 ± 0.0707	0.8 ± 0.0577	0.5 ± 0.1155	1.6 ± 0.2972	0.8 ± 0.1732	0.6 ± 0.0577	2.3 ± 0.1155	0.8 ± 0.1732	0.6 ± 0.0577	2.3 ± 0.1155	1.0 ± 0.0577	0.9 ± 0.4041	2.3 ± 0.1155	1.0 ± 0.0577	0.9 ± 0.4041			



Fig (1) : Ovariole of newly emerged showing. (A) untreated ovariole. (B) ovariole in female resulted from seeds treated with Lc_{50} of black pepper powder. (C) in female resulted from seeds treated with onion powder. (D) in female resulted from seed treated with garlic powder . (50X)

The oocytes are arranged in a single row and grow successively bigger towards the posterior end of each vitellarium.

The pedicel is narrow short tubular portion that joins the ovariole to the oviduct.

In the present study, it could be seen from (Fig. 2) that the germarium of untreated newly emerged female are filled with similar cells. The germarium cells have voluminous, spherical nuclei with a regular nuclear membrane, this cells are probably resulting from mitotic division. The follicular epithelium are round the oocyte.

(Fig. 3 A, B) shows the changes in the ovarioles of females resulted from treated seeds with LC_{25} and LC_{50} of black pepper powder. The follicular epithelium become thinner, loses its contact with the oocyte because of the rapid increase of oocyte volume. Vacuoles are observed as a result of degeneration of yolk. Oocyte changing in shape from spherical to rectangle.

The female resulted in case of treated seeds with onion powder have rectangular oocyte and destruction epithelial sheath. Many cells are observed to be degenerated leaving cavities or vacuoles (Fig4 A, B).

LC_{25} and LC_{50} of garlic powder induces damage in oocytes (Fig 5 A,B). The oocyte increase in size and shape changing from spherical shape in the primary oocyte to an oval shape. Many of follicular cells are completely degenerated, nuclei loss their normal shape and the nuclear membrane is irregular. In the germarium the cells are separated by large cavities. Similar results were found by Ludlum and Seiber (1988) who observed that azadirachtin reduced oocyte growth when add to blood fed of *Aedes aegyptii* female flies. Rizk (1998) on *C. cephalonic*. Paul and Mc Coffary (1990) on *Spodoptera exempta*.

The results presented here lead to the conclusion that LC_{25} and LC_{50} of tested plant powders induces damage to the ovarian structure and inhibition of egg development so no fertile eggs can be produced.



Fig (2): Longitudinal Section in untreated ovariole of newly emerged showing spherical nucleuse (NC), follicular epithelium (FE) and oocyte (OOC). (400X)



Fig (3): Longitudinal Section in Females resulted from seeds treated with black pepper powder (A). Treated with I.e₂₅ (B). treated with Lc₅₀ showing vacuole (V), rectangular destroyed oocyte (RDO) (400X)



Fig (4): Longitudinal Section in Females resulted from seeds treated with onion powder (A) treated with Lc_{25} (B) with Lc_{50} showing rectangular oocyte (RO) and destroyed follicular epithelial (DFE).(400X)

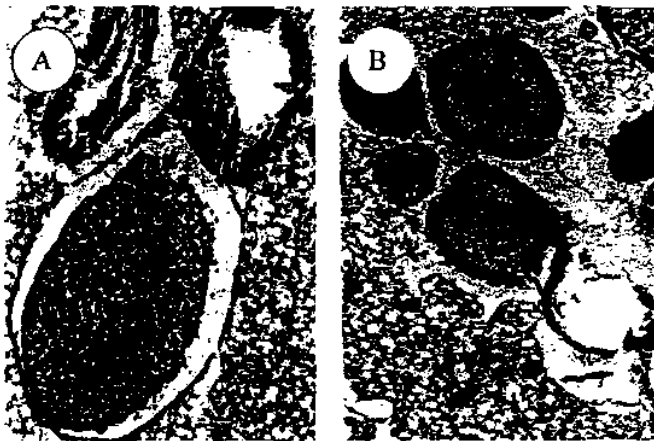


Fig (5): Longitudinal section in females resulted from seeds treated with garlic powder (A). treated with Lc_{25} (B) treated with Lc_{50} showing vacuoles (V), oval oocytes. (400 X)

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تأثير مساحيق بعض النباتات كمبيدات طبيعية على تركيب المبايض في حشرة خنفساء اللوبيا.

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المركز القومي لبحوث وتكنولوجيا الإشعاع.

أجريت دراسة على تأثير كل من مسحوق الفلفل الأسود ، والبصل ، والثوم بتركيزات مختلفة (١٢٥ ، ٥٠٠ ، ١٠٠٠ ، ١٥٠٠ جرام) على نسبة العقم لخنفساء اللوبيا. أشارت النتائج المحصل عليها أن هناك علاقة بين زيادة نسبة العقم وزيادة التركيز. تأثر طول المبيض في الإناث الناتجة من بذور اللوبيا المعاملة بمساحيق النباتات المختبرة وبالتركيز المعيت لنسبة ٢٥% ، ٥٠% حيث أصبح أقصر منه في حالة الإناث الغير معاملة ، وكذلك تأثر حجم القابلة المنوية بزيادة تركيز المسحوق المستخدم . أظهرت الدراسة الهستولوجية تغيير في شكل البويضة من الشكل المستدير إلى الشكل المسطيل في حالة مسحوق الفلفل والبصل وإلى الشكل البيضاوي في حالة مسحوق الثوم. وحدثت زيادة في الحجم ، كما فقدت الأنوية شكلها الطبيعي وأصبحت متعرجة الغلاف ، وتحلت الخلايا الحويصلية وأصبحت عديدة الفجوات. من الدراسة السابقة يتضح أن مساحيق النباتات المستخدمة تسببت في تنوؤ المبايض وتحلل نسي المحتوى الداخلي لها وإعاقة الخلايا المختلفة للقيام بنشاطها المعتاد مما يترتب عليه عدم تكوين بويضة منضوية.