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. Oosyte increase in size and shape changed from spherical in primary oosyte to rectangle shape in case of black pepper and onion whereas, the shape becomes oval in garlic case. In the germanium 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 oosyte 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,
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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 Guirgis (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 LC15 (0.0083, 0.15, 0.15gm) and LC50 (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 (30.72%) at 1.5gm garlic gave (78.76%) while, onion caused (86.88%). 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. quadrinaculatus was decreased when seeds of mungbean treated with eucalyptus and guava leaves powder at 5gm. Also, El-Degwi and El-Oraibi (1995) reported that soybean, kidneybean and lupin powders at 12% reduced the number of eggs laid by C. maculatus. Rizk (1998) mentioned that the LC10 of Thevetia 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.

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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 ovariole 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 a sac like from the vagina and lies dorsally to the oviducts, there are also two spherical 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 LC25 and LC50 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 LC25 and LC50 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 case of onion powder at LC25 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. Risik (1998) found that the length and width of *C. cephalonica* moths ovarioles treated with LC10 of *Thevetia nerifolia* or *Azadirachta indica* were greatly reduced as compared with control.

The ovarioles of *C. maculatus* female are telegynous type, each ovariole is divided into 4 regions, the terminal filament, the germanium, the vitellarian 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 germanium is the anterior part of the egg tube and contains trophocytes, yung oocytes and prefollicular cells, and it is easily distinguished into the apical trophic zone and basal zone of oocyte differentiation.

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

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

<table>
<thead>
<tr>
<th>Plant powder conc.</th>
<th>Black pepper</th>
<th></th>
<th></th>
<th></th>
<th>Onion</th>
<th></th>
<th></th>
<th>Garlic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean no. of eggs / Female</td>
<td>% Hatchability</td>
<td>% observed sterility</td>
<td>% calculated sterility</td>
<td>Mean no. of eggs / Female</td>
<td>% Hatchability</td>
<td>% observed sterility</td>
<td>% calculated sterility</td>
<td>Mean no. of eggs / Female</td>
<td>% Hatchability</td>
</tr>
<tr>
<td>Cont.</td>
<td>312.8</td>
<td>86.05</td>
<td>13.95</td>
<td>0</td>
<td>395.6</td>
<td>87.24</td>
<td>14.65</td>
<td>0</td>
<td>396.6</td>
<td>85.35</td>
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<tr>
<td>0.125</td>
<td>252.6</td>
<td>84.00</td>
<td>16.00</td>
<td>21.17</td>
<td>336.3</td>
<td>85.73</td>
<td>15.32</td>
<td>16.62</td>
<td>335.0</td>
<td>84.68</td>
</tr>
<tr>
<td>0.250</td>
<td>223.4</td>
<td>80.16</td>
<td>19.84</td>
<td>33.47</td>
<td>256.3</td>
<td>83.42</td>
<td>18.39</td>
<td>26.40</td>
<td>306.8</td>
<td>81.61</td>
</tr>
<tr>
<td>0.500</td>
<td>205.8</td>
<td>76.01</td>
<td>23.99</td>
<td>41.88</td>
<td>235.6</td>
<td>80.56</td>
<td>21.54</td>
<td>36.95</td>
<td>273.4</td>
<td>78.48</td>
</tr>
<tr>
<td>1.00</td>
<td>152.6</td>
<td>70.85</td>
<td>29.15</td>
<td>59.83</td>
<td>198.2</td>
<td>73.71</td>
<td>27.07</td>
<td>46.36</td>
<td>250.2</td>
<td>72.93</td>
</tr>
<tr>
<td>1.500</td>
<td>81.2</td>
<td>63.89</td>
<td>36.72</td>
<td>80.72</td>
<td>102.6</td>
<td>69.65</td>
<td>33.05</td>
<td>65.89</td>
<td>182.2</td>
<td>66.95</td>
</tr>
</tbody>
</table>

### Table (2): Effect of LC$_{25}$ and LC$_{50}$ of some plant powders as protectant materials against *C. maculatus* on biometrics of the ovariole and spermatheca.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Black pepper</th>
<th></th>
<th></th>
<th></th>
<th>Onion</th>
<th></th>
<th></th>
<th>Garlic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovariole</td>
<td></td>
<td></td>
<td></td>
<td>Spermatheca</td>
<td></td>
<td></td>
<td>Ovariole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.4 ± 0.2345</td>
<td>1.3 ± 0.1732</td>
<td>1.2 ± 0.2309</td>
<td>2.4 ± 0.2345</td>
<td>1.3 ± 0.1732</td>
<td>1.2 ± 0.2309</td>
<td>2.4 ± 0.2345</td>
<td>1.3 ± 0.1732</td>
<td>1.2 ± 0.2309</td>
<td></td>
</tr>
<tr>
<td>LC$_{25}$</td>
<td>2.2 ± 0.2041</td>
<td>1.0 ± 0.0334</td>
<td>0.8 ± 0.2005</td>
<td>2.1 ± 0.7321</td>
<td>1.6 ± 0.0577</td>
<td>1.5 ± 0.0577</td>
<td>1.8 ± 0.1472</td>
<td>0.9 ± 0.0408</td>
<td>0.7 ± 0.2866</td>
<td></td>
</tr>
<tr>
<td>LC$_{50}$</td>
<td>2.1 ± 0.0707</td>
<td>0.8 ± 0.0577</td>
<td>0.6 ± 0.1155</td>
<td>1.6 ± 0.2972</td>
<td>0.8 ± 0.1732</td>
<td>0.6 ± 0.0577</td>
<td>2.3 ± 0.1155</td>
<td>1.0 ± 0.0577</td>
<td>0.9 ± 0.4041</td>
<td></td>
</tr>
</tbody>
</table>
Fig (1): Ovariole of newly emerged showing. (A) untreated ovariole. (B) ovariole in female resulted from seeds treated with Lc0 of black pepper powder. (C) ovariole in female resulted from seeds treated with onion powder. (D) in female resulted from seeds treated with garlic powder. (50X)
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The oocytes are arranged in a single row and grow successively bigger towards the posterior end of each vitellarium.

The pedicle 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 form 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 (Fig. 4 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 Ludum and Seiber (1988) who observed that azadirachtin reduced oocyte growth when add to blood fed of Aedes aegypti 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 nucleus (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 LE25 (B), treated with LE50 showing vacuole (V), rectangular destroyed oocyte (RDO) (400X)
Fig (4): Longitudinal Section in Females resulted from seeds treated with onion powder (A) treated with Lc25 (B) with Lc30 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 Lc25 (B) treated with Lc30 showing vacuoles (V), oval oocytes. (400X)
REFERENCES


تأثير مساحيق بعض النباتات كبرادات طبيعية على تركيب الميتابوم في حشرة خنفساء اللوبيا

أجريت دراسة على تأثير كل من مساحيق القلق الأسود، والرمل، والكركم، والتمور نباتات مختلفة (في 121...120000 جرام) على نسبة الميتابوم خنفساء اللوبيا.

وشهدت النتائج المحصلة أنها هناك علاقة بين زيادة نسبة الميتابوم وزيادة التركيز.

تأثر طول الميتابوم في اللوبيا الناتجة من بذور اللوبيا النباتية بمساحيق النباتات المختلفة، وبالتركيز السمت نسبة 100%، حيث أضحى أقصر منها في حالة اللوبيا غير معالمة، وكذلك تأثر حجم القابلة المنوية بزيادة تركيز المخزون المستخدم.

لقد أظهرت تأثيرات الاستخدام تغيير في شكل البويضة من الشكل المستدير إلى الشكل المستدير، في حالة مساحيق القلق والرمل والكركم البويضوي في حالة مساحيق اللوبيا وجدت زيادة في المجم، كما قلت الأوزان شكلها الطبيعى وأصبحت مضرحة الشكل، وتحتال الاعتقاد البسيطة وأصبحت عديدة القفوات.

من النتائج السابقة يضمن أن مساحيق النباتات المستخدمة ت الصحيح في تكوين النبات، وتحلل في الشكل الداخلي لها وإحالة العلاج الشامل للقيام بنشاطها المحدد، مما يرتبط عليه عدم تكوين بويضة مخصصة.