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Effect of katel-sous dust and clove powder and their mixtures on the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: bruchidae)

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ABSTRACT

The present work was carried out to evaluate the toxicity of katel-sous dust and clove powder and its combinations. Evaluation has been conducted on *Callosobruchus maculatus* (F.) adults to determine the toxicological effect and the changes in insect biology caused by these products. The results referred to the availability to use these products to control cowpea seed beetle and consider clove powder as spiracle-blocking insecticides. Clove powder and katel-sous dust and its combinations made a sharp reduction in the number of eggs laid and the number of adults emerged. Mixing clove powder with katel-sous dust was helping in using katel-sous dust in pest management without any problems such as; its high concentration which caused suffering from the expensive costs and hardly remove the dust.

Key words: Katel-sous, Clove powder, Control, Bruchids, Coleoptera, *Callosobruchus maculatus*, Cowpea seed beetle and Scanning.

INTRODUCTION

Pulses are important sources of proteins, fats, carbohydrates, sugars, vitamin B and minerals. The infestation of leguminous seeds with any kind of bruchid species caused an increase in both weight loss and moisture content of the infested seeds (El- Bandy *et al*. 1985).

The pulse beetle, *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae), is a major pest of economically important leguminous seeds, such as cowpeas, lentils, green gram, and black gram (Park *et al.* 2003).

The amount of annual loss reached 24% of stored pulses due to infestation of *Callosobruchus maculatus* (Caswell, 1968). During the storage, the cowpea weevil causes heavy qualitative and quantitative losses. Caswell (1981).

Using plants with insecticidal properties is therefore an attractive to the more expensive and unpleasant pesticides. Various plant products have been used recently with a good degree of success as protectants against a number of stored grain insect pests (Dixit and Saxena 1990).

Inert materials are the oldest and most widely used method of protecting grains in many countries (e.g. Egypt and India). Their mode of action on various stored grain insects was studied by (Alexander *et al*., 1944 and Wigglesworth, 1944) who showed that inert dusts make insects lose their body water and thus die by desiccation.

The objective of the present study is to minimize the usage of chemical control of stored legume insect pests by evaluating the efficacy of some safety control measures against one of serious stored grain pest, *Callosobruchus maculatus*, by using clove (*Eugenia aromatica*) powder and katel-sous dust as grain protectants and try to use their combinations.

MATERIALS AND METHODS

Insect Culture:

Adults (1-2 days old) of cowpea beetle, *Callosobruchus maculatus* (F.) used in this study were obtained from 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.

Plant Materials:

Two plant materials clove powder obtained from the market and Katel-sous dust (contains 84% calcium triphosphate plus 16% sulpher, produced by Kafr El-Zayat Company for Pesticides- Egypt).

Toxicological evaluation of katel-sous dust and clove powder and its mixtures on *Callosobruchus maculatus* (F.) adults:

Evaluation the toxicity of katel-sous dust and clove powder:

To estimate the percentage mortality which is caused by using the tested materials, five different concentrations from katel-sous dust and clove powder were evaluated to determine the efficiency of these compounds. Every concentration for each treatment was added to 10 grams of cowpea seeds in 1 × 7.5 cm glass tube and was infested by 25 adults of the tested insect. Three replicates were made for each concentration and kept at 27± ºC and 65±5 % RH. Mortality counts of *Callosobruchus maculatus* adults were recorded in all experimental treatments after 1 day, 2 days, 3 days and 5 days from exposure. Percentage mortality were taken after 48 hours and were calculated and corrected according to Abbott's formula (1925) before probit statistical analysis.

Experimental design and analysis of data:

The experiments in each set were designed to provide concentration mortality regression lines for tested products against *Callosobruchus maculatus* adults according to the method described by Finney (1971).

Concentration mortality relationships were plotted on probit logarithmic transformation for each case studied. The slope values of established lines, LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} and LC_{99} (for *Callosobruchus maculatus*) were estimated after 48 hrs from insect exposure (Bliss, 1935).

Combination between Katel- sous dust & clove powder:-

Katel-sous dust and clove powder were tested separately and in the following combinations Table (1).

Table 1: Mixture percentages between Katel- sous dust and the clove powder:

No. of the mixtures	The percentages of the materials in each mixture
Mixture No. (1)	LC_{50} Katel- sous + LC_{50} Clove powder
Mixture No. (2)	LC_{25} Katel- sous + LC_{75} Clove powder
Mixture No. (3)	LC_{75} Katel- sous + LC_{25} Clove powder
Mixture No. (4)	LC_{25} Katel- sous + LC_{25} Clove powder

Evaluation the toxicity of the tested mixtures:

Evaluate toxicity of prepared mixtures against *Callosobruchus maculatus* adults by adding each of the forementioned mixtures to ten grams of cowpea seeds in 1 × 7.5 cm glass tube kept at 27± ºC and 65±5 % RH. Batches of 25 adults of the tested insect were introduced into each tube, also untreated control was included. Three replicates were used for each mixture. Tubes were covered with plastic cover fixed with rubber band. Mortality was recorded after 2, 3 and 5 days. Percent insect mortality was corrected using Abbott's formula (1925), and computed according to Finney (1971).

Biological aspects of *Callosobruchus maculatus* after treatment with the tested natural products:

Insects for the following experiments were maintained under constant conditions in an incubator set as previously mentioned.

Number of deposited eggs laid by *Callosobruchus maculatus* adults & percentage hatchability:

To determine the number of eggs laid by mated female adults of *Callosobruchus maculatus* exposed to each of the tested products, 5 gm of cowpea seeds treated with LC_{50} and LC_{95} (for insects) of each product were placed in glass tube measuring 1 × 7.5 cm (each treatment had three replicates). Two pairs of newly emerged adults (18 hrs old) were placed in each tube and covered with plastic cover and kept in the incubator till death. After 10 days the insects were removed and the numbers of hatched and non hatched eggs were counted and were recorded and percent hatchability was determined and similar the reduction in hatchability was determined. Hatched eggs were defined by the presence of the larval frass, which causes the egg to turn milky white as neonate larvae bore into the seed or black color which was defined as the head capsule of the larva, while the non hatched eggs remain transparent and glossy (Giga and Smith, 1987). Thus the total numbers of white (or black) and translucent eggs on the seeds indicate bruchid oviposition and numbers of white eggs indicate the number of larvae entering the seed (Dharmasena *et al*., 2001).

To determine the effect of the mixtures on the number of deposited eggs, hatchability and F_1 progeny of *Callosobruchus maculatus* adults, five grams of cleaned cowpea seeds were mixed with the tested mixtures at the percentage values which mentioned before in Table (1) and then were placed in clean glass tubes measuring 1 × 7.5 cm. Two couples of newly emerged adults were placed in each tube which was covered with plastic cover. After ten days, dead insects were disregarded and the seeds were examined for the number of eggs laid. Hatchability of eggs were indicated when they turned white showing that eggs had hatched and larvae had penetrated the seeds (Salwa *et al.*, 2001).

The above collected cowpea seeds bearing deposited eggs were placed in clean glass tubes and kept in an incubator under the aforementioned constant conditions till adult emergence.

Percentage adult emergence:

The infested treated seeds with eggs were incubated and observed regularly until adult emergence. Emerging adults were counted and discarded for a period of 10 days. The adult emergence (%) was calculated from the number of hatched eggs and F_1 adult progeny and the reduction in F_1 progeny was also calculated.

Infested seeds were maintained in glass tubes until offspring emerged, which were then counted and then the adult emergence percentage and the reduction in F_1 progeny emergence were calculated.

Three replicates were made for each mixture, also for control test three replicates of untreated seeds made.

Effect of clove powder on spiracles of *Callosobruchus maculatus* adults using scanning electron microscope:

Sample Collection and Preparation:

Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples. Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold, or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired. In our study we used gold as a conducting material by

a machine called a sputter coater. Metal coating are most effective for high resolution electron imaging applications. Then the sample is placed inside the microscope's vacuum column through an air-tight door.

RESULTS AND DISCUSSION

Toxicological evaluation of katel-sous dust and clove powder and its mixtures on *Callosobruchus maculatus* (F.) adults:

Evaluation the toxicity of katel-sous dust and clove powder:

The results of the susceptibility tests of *callosobruchus maculatus* adults to clove powder and katel-sous dust, inert material (84% calcium triphosphate + 16% sulpher) are presented in Table (2). The estimated toxicity values of LC_{25}, LC_{50}, LC_{75}, LC_{95} and LC_{99} for katel-sous dust and clove powder are presented in Table (2), LC_{95} of katel-sous dust for cowpea seeds was 1.76 while LC_{95} of clove powder was 0.28**.** The percentage mortality increase with the increase of concentration of each tested compound, i.e. a potential linear relationship between the concentrations and mortality percentages exists.

Katel-sous dust was a good grain protectant against *Callosobrochus maculatus* adults as the result evaluated by Abdel-Latif, (1999).

The highest concentration of clove gave 100% mortality with three days of exposure. Rajapakse, *et al.,* (1998) found that clove powder gave the highest mean number of adult mortality of *C. maculatus*. Abdellah-Kellouche and Noureddine-Soltane, (2004) reported that among the tested botanicals, the clove powder was the most toxic to *C. maculatus* as adult survival in treated seeds, even at the lowest dosage of 1 %. Ntonifor, *et al.,* (2010) found that clove powder treated grains caused 100 % adult mortality of *Callosobruchus maculatus*.

Table 2: Corrected percentage mortality of *Callosobruchus maculatus* adults& Collective toxicity data caused by katel-sous dust and clove powder after two days of exposure:

Treatments	Conc. (%) W/W	% adult mortality after indicated periods (days)				LC_{25}	LC_{50}	LC_{75}	LC_{95}	LC_{99}	Slope of Regression line
		1st	2nd	3rd	5th						
Katel- sous dust	0.1	12	22.6	49.3	100	0.14	0.3	0.62	1.76	3.7	2.1189 ± 0.2266
	0.3	14.6	32	64	100						
	0.4	20	60	89.3	100						
	0.5	28	73.3	96	100						
	0.7	46.7	85.3	97.3	100						
Eugenia aromatica (Clove powder)	0.03	12	20	36	58.7	0.04	0.06	0.09	0.18	0.28	3.4172 ± 0.2938
	0.05	18.7	33.3	42.7	68						
	0.07	26.6	54.6	64	84						
	0.1	41.3	82.6	93.3	100						
	0.2	54.6	97.3	100	—						

The slope of regression lines obtained after 2 days of treatment indicate that clove powder at both LC_{50} and LC_{95} levels have the steeper toxicity lines. The slope of toxicity lines indicated that, the highest insect sensitivity to gradual increased concentrations was exhibited by clove powder. This result is agreed with Salwa,

(1992), Zidan *et al.,* (1993) & Rohan, *et al.,* (2002).

Evaluation the toxicity of the tested mixtures:

Mortality values (%) of the cowpea beetle, *Callosobruchus maculatus* adults exposed to the tested mixtures of clove powder with katel-sous are given in Table (3).

By making comparison between the mixtures it showed that mixture 3 showed 100 % mortality from the second day of exposure. Mixtures 1, 2 and 4 gave 98.7%, 96% and 60% respectively.

All the tested mixtures were also very effective in suppressing the survival of *C. maculatus* adults when applied at the concentrations used.

Table 3: Corrected percentage mortality of *Callosobruchus maculatus* adults treated with different mixtures of natural powders with Katel-sous at different concentrations:

	Mixtures	% Adults mortality after indicated period (days)		
		2 days	3 days	5 days
The combination of katel-sous dust with the clove powder	Mix. No. 1 LC_{50} katel- sous + LC_{50} clove	98.7	100	—
	Mix. No. 2 LC_{25} katel- sous + LC_{75} clove	96	100	—
	Mix. No. 3 LC_{75} katel- sous + LC_{25} clove	100	—	—
	Mix. No. 4 LC_{25} katel- sous + LC_{25} clove	60	72	92

In the present investigation the tested mixtures as compared with the clove powder has demonstrated that the mixtures caused significant mortalities better than clove alone. So, it can be said that the combination between two products gave better results. This was in agreement with (Govindan and Nelson, (2008), Ntonifor *et al.,* (2010) & Durga Singh and Srivastava, (2011).

On the other hand, the using of katel-sous alone gave high effect better than the mixtures but it is not preferred to use it because of its high concentration and its high weight. So the present study is a trial to mix katel-sous dust with clove powder to get best results Abdel-Latif, (1999).

Mixture 3 which have 75% katel-sous gave 100% mortality of the adults of the tested insects from the second day.

The order of the other mixtures according to the mortality caused by them is mix. 1 > mix. 2 > mix. 4.

Biological aspects of *Callosobruchus maculatus* after treatment with the tested natural products:

Number of deposited eggs laid by *Callosobruchus maculatus* adults & percentage hatchability:

Katel-sous dust significantly reduced the oviposition and the number of eggs laid by *C. maculatus* female adults (Table 4 & Fig. 1).

In case of treatment with clove powder, the insect did not lay any egg on the surface of cowpea seeds. However, with katel-sous dust the mean number of hatched eggs was 41 eggs/ female. The mean number of eggs laid by one female in untreated control sample was 92 eggs/ female, 91 from them were hatched.

98.9% hatchability observed with the untreated control, while 90, 87.2, 0, 0% for katel-sous LC_{50}, katel-sous LC_{95}, clove LC_{50} and clove LC_{95} respictively.

Table 4: Efficacy of the katel-sous dust and clove powder on the oviposition and hatchability of eggs and on adult emergence % of *Callosobruchus maculatus*.

Tested powder	Conc.	Mean no. of eggs / female									Progeny emergence				
		hatched eggs		unhatched eggs		Total no. of eggs	Hatchability		Reduction		Female No.	Male No.	Total No. (F_1 emerged adults)	Adult emergence %	Red. %
		No.	S.D	No.	S.D		%	S.D	%	S.D					
Katel-sous	LC_{50} LC_{95}	36 41	3.5	4 6	1.4	40 47	90 87.2	1.97	59.3 54.95	3.1	15 13	10 9	25 22	69.4 53.7	69.13 72.8
Eugenia aromatica	LC_{50} LC_{95}	0 0	0	0 0	0	0 0	0 0	0	100 100	0	0 0	0 0	0 0	0 0	100 100
Control		91	0	1	0	92	98.9	0	0	0	46.7	34.7	81.3	89.34	0

The mixtures 1 and 2 totally prevented the females to lay eggs. The mixture 3 showed highly reduction in total number of eggs/ female. This reduction was 78.92% Table 5 & Fig. 1.

Table 5: Efficacy of the tested mixtures on oviposition and hatchability percentage of *Callosobruchus maculatus* adults.

Treatments	Mean No. of eggs / female					Progeny emergence				
	No. of hatched eggs	No. of unhatch. eggs	Total no. of eggs	Hatch.%	Red. %	Female no.	Male no.	Total no. (F_1 emerged adults)	Adult emergence %	Red. %
Mix. No. 1 LC_{50} katel-sous + LC_{50} clove	0	0	0	0	100	0	0	0	0	100
Mix. No. 2 LC_{25} katel-sous + LC_{75} clove	0	0	0	0	100	0	0	0	0	100
Mix. No. 3 LC_{75} katel-sous + LC_{25} clove	18.7	18.7	37.3	50.13	78.92	5	9	14	74.86	76.9
Control	88.7	21	109.7	80.86	0	53	29.7	82.7	93.2	0

There was a great reduction in egg laying (zero eggs were laid) in case of clove powder treatment. This was in agreement with Adebayo and Gbolade, 1994; Lawal, (2001); Salwa, (2002); Ajayi and Wintola, (2006) & Javaid and Poswal, (1995) reported that clove powder gave results which were not significantly different from those produced by malathion.

In case of the mixtures of katel-sous dust and clove powder, some eggs were recorded with mixture 3 and no eggs for mixtures 1 & 2. This result may be due to the high concentration of katel-sous in the mixture 3 (75%).

Percentage adult emergence:

The highest concentration (LC_{95}) of katel-sous dust significantly reduced the number of F_1 emerged adults to 22 and 25 in lowest concentration (LC_{50}) compared with 81.3 in the untreated seeds. There is no adults emerged with clove powder due to preventing the adults to lay eggs on the seeds.

There was significant difference in number of *C. maculatus* emerged adults when treated with different mixtures (Table 5& Fig. 1). The highest number of

total emerged adults was observed in mixture 3, 75% katel-sous + 25% clove, (37.3), while in control 109.7 eggs were observed. Zero emerged adults were observed in mixture 1 (25% katel-sous + 75% clove) and mixture 2 (50% katel-sous + 50% clove).

The F_1 emerged adults of the tested insect *C. maculatus* decreased significantly in the treated seeds with mixture no. 3 (Table 5 & Fig. 1), the percentage adult emergence in this mixture was 74.86%. The reduction in the adult emergence in the mixtures no. 1 and 2were 100% due to there is no eggs laid by the tested insect adults with the pervious mixtures.

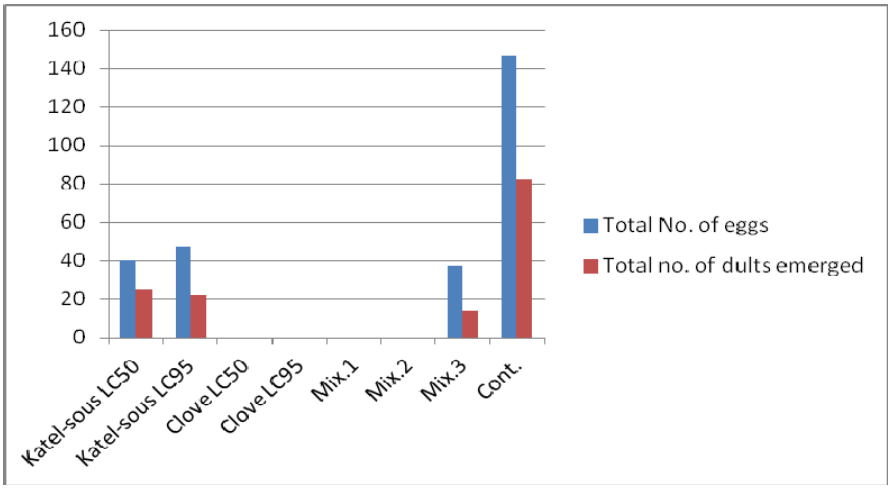

Fig. 1: Total number of eggs and adults emerged after treatment with the katel-sous dust, clove powder and their mixtures.

Results showed a negative correlation between the numbers of F_1 emerged adults and the concentration of katel-sous dust (increasing the concentration reduces the number of F_1 emerged adults).

As a result of preventing egg laying by clove there was no adults emerged with this treatments. There was a significantly reduction in hatchability and adult emergence with the natural products and this is in agreement with Taheya *et al.*, (1996), Halawa *et al.*, (1998) & Ajayi and Wintola, (2006).

Mixture 1, mixture 2 showed 100% reduction in the adult emergence as well as in egg laying. Mixture 3 showed high significant decrease in the adult emergence compared with control Fig. (1).

Similar results obtained with Govindan and Nelson, (2008) who reported that the number of eggs laid by female, hatchability per cent and adult emergence of *Callosobruchus maculatus* of the black gram seeds treated with mixtures of plant powder were significantly difference from each other.

Effect of clove powder on spiracles of *Callosobruchus maculatus* adults using scanning electron microscope:

Spiracles are external openings found in some animal species, such as insects, spiders and some species of fish and whales. The function of spiracles is linked to respiration, helping oxygen to reach internal respiratory organs, tracheae in insects. In *Callosobruchus maculatus* adults these organs are located in the abdomen.

Untreated Sample:

The spiracles of the *C. maculatus* adults appeared on the side of the abdomen as shown in Fig. (2) (The SEM shows very detailed 3-dimensional images at much higher magnifications

than is possible with a light microscope). The opened spiracles (breathing opens) represented in Fig. (3).

Treated Sample:

Spiracles were closed in Fig. (4) after treatment with clove powder.

Treatment with clove powder leads to the closure of spiracles of the *Callosobruchus maculatus* adults and this leads to suffocation of the insect and thus causing death. While in the untreated sample the spiracles were clearly opened.

So it can be concluded that clove powder as spiracle-blocking insecticides which are highly effective against major pests, such as thrips, aphids, whiteflies and spider mites (Matsuda *et al*., 1995; Motegi, 2001; Hondo *et al*., 2001; Ota, 2008).

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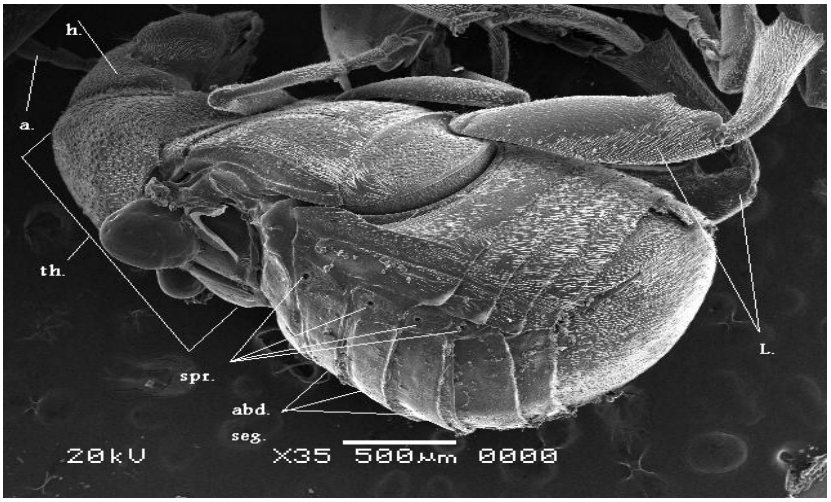

Fig. 2: The Scanning Electron Microscope (SEM) shows very detailed 3-dimensional images showing the normal spiracles on the abdomen of the untreated *Callosobruchus maculatus* adults.
Antenna (a.), head (h.), thorax (th.), spiracles (spr.), abdominal segments (abd. seg.) & legs (L.).

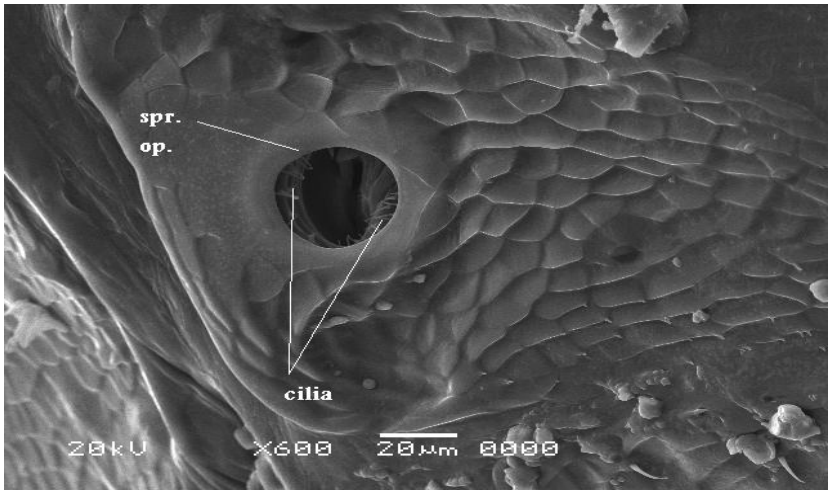

Fig. 3: Spiracle openings on the abdomen and the cilia which surround the spiracle openings of the untreated *Callosobruchus maculatus* adults. Spiracle openings (spr. op.).

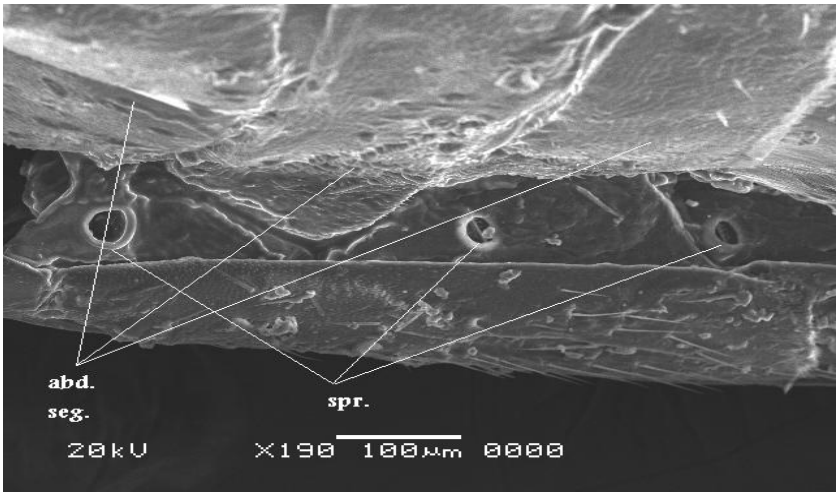

Fig. 4: The closed spiracles on the abdomen of *Callosobruchus maculatus* adults after treatment with clove powder. Abdominal segments (abd. seg.) and spiracles (spr.).

ARABIC SUMMARY

تأثير قاتل السوس ومسحوق القرنفل ومخاليطهما على خنفساء اللوبيا (كالوزوبروكاس ماكيولاتس)

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اشتملت الدراسة الحاليه على :

تقييم سمية كل من قاتل السوس و مسحوق القرنفل والخلط بينهما بنسب مختلفة. وقد أجريت هذة الدراسة على الطور البالغ لخنفساء اللوبيا (كالوزوبروكاس ماكيولاتس) لتحديد تأثير السمية، التغيرات البيولوجية للحشرات التي سببتها هذة المنتجات و محاولة معرفة تأثير القرنفل على فتحات التنفس للحشرة. النتائج تشير لاستخدام هذة المنتجات لمكافحة خنفساء اللوبيا (كالوزوبروكاس ماكيولاتس) كما يمكن إعتبار مسحوق القرنفل كمبيد يسبب غلق الفتحات التنفسية في جسم الحشرة البالغة وحدوث إختناق للحشرة. خلط مسحوق القرنفل بقاتل السوس يساعد على إعادة استخدام قاتل السوس بدون مشاكل ولا عيوب قاتل السوس التي تتمثل في وزنه العالي وتركيزة العالي والتي تجعلنا نعاني من تكاليف باهظة الثمن ويصعب إزالته من على البذور ويصعب استعماله على الشون لكميته الكبيرة. كما أن خلط االقرنفل بقاتل السوس يزيد من فترة بقاء المادة على البذور لتميز قاتل السوس بفترة بقاء عالية على البذور.