The use of solar energy and citrus peel powder to control cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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Abstract

This work aimed to evaluate the efficiency of different natural promising techniques in controlling cowpea beetle *Callosobruchus maculatus* the main pest of different legume seeds. To achieve this, the potential of solar energy and citrus peel powder to suppress cowpea beetle population were investigated. First, effect of solar energy was evaluated using a metal-box heater at different exposure times (5- 20 min). The box heater resulted in 100% of adult beetles mortality within 15 min (54°C), and totally suppressed the beetle population when immature stages were exposed to sun for 20 min (58- 64°C). The adult beetle was the most sensitive stage where as the second larval instar was the most tolerant one. The pupal stage when exposed to sub-lethal dose of solar energy produced adults with some morphological abnormalities. Solar energy treatment had no negative effect on germination of cowpea seeds, but interestingly, the germination was even better. Secondly, different concentrations of lemon and orange peel powders were applied: 0.01, 0.03, 0.075 and 0.1 gm/gm of cowpea seeds. Lemon peel powder was more effective than orange peel powder and had higher repellency activity. The (LC_{50s}) were determined to be 0.025 and 0.086 gm/gm cowpea seeds for adult beetles after 3 d of treatment with lemon and orange peel powders, respectively. The effect of citrus powder on the adult insect cuticle and spiracles was also investigated using scanning electron microscopy.

Keywords: Bruchids, stored-product pests, natural control, sun heating, heat control, botanical powder

1. Introduction

Callosobruchus maculatus (F.) is one of the most destructive pest of legume seeds including cowpeas either on the field or in storage (Lale, 1998; Moravvej and Abbar, 2008). Several methods have been used to protect stored-grains from pest infestation including chemical substitutes, exploitation of controlled atmospheres, integration of physical methods such as heating (Mekasha et al., 2006) and cooling (Thorpe, 1998) and biological agents such as parasitoids (Velten et al., 2007).

In many developing areas, it is hard for local farmers to adopt the high technology strategies for pest control due to the high expenses needed. A very promising controlling method is utilization of solar energy. Heating produced from solar radiation has been demonstrated to have a potential role in disinfestations of stored insect pests (Mekasha et al., 2006). Many other studies investigated the utilization of solar heating in post-harvest cowpea beetle control (Ntoukam et al., 1997; Chauhan and Ghaffar, 2002; Lale and Vidal, 2003; Murdock et al., 2003).

On the other hand, other studies have reported the effectiveness of citrus peel products in controlling the stored grain pests (Allotey and Oyewo, 2004; Rajendran and Sriranjini, 2008). It has been shown that the mode of action of botanical powders based on their fumigant effect, mechanical and contact effect which eventually could affect the insect spiracles (Shukla et al., 2007). Therefore, studying insect body cuticle and spiracles using such technique like scanning electron microscopy would be very helpful.

The current study aimed to investigate the effectiveness of both utilization of solar energy and using citrus peel powder as a protectant of cowpeas against cowpea beetle attack by incorporating scanning electron microscopy as a diagnostic technique to study the powder mode of action. A main objective was to use effective safe controlling methods that are easy to be manipulated by local farmers rather than large industries.

2. Materials and Methods

2.1. Maintenance of insect culture in laboratory

The strain of cowpea beetle, *C. maculatus* was obtained from the Plant Protection Research Institute, Dokki, Giza, Egypt. Adult beetles were reared in glass jars provided with a suitable amount of cowpea seeds (*Vigna unguiculata* (L.) Walpers) that were obtained from a local market, washed, dried and disinfested by keeping them in a refrigerator at -20°C for 2-3 d before being used.

2.2. Effect of solar heat on cowpea beetle C. maculatus

2.2.1. Sun exposure technique

Exposure to solar heat was conducted using an obtuse-base-angle box heater described by Mekasha et al. (2006) with some modifications. The box was constructed with 1 mm thick galvanized metal sheet; the upper open side of the box was 51 x 20 cm, length by width and the perpendicular height was 23 cm with obtuse-base angle (about 120°). The box was covered with black polystyrene sheet to increase the capability of sun rays absorption. In the middle of the box, a glass plate was introduced to help in raising the inner temperature during the exposure (Fig. 1A). Black polystyrene sheet was held on the ground for about 10-15 min to collect the sun rays on the experimental spot and the box was put on it during the exposure time (Fig. 1B). Temperature and relative humidity inside and outside the box were recorded during the exposure times using thermometers and hygrometers. All the experiments were done on sunny clear-sky days between July and September of 2009 and 2010. The sun exposure conducted in the mid-day, between 12-3 pm, as the sun rays become perpendicular on earth and thus leads to maximum sun radiation and maximum heat degrees.

2.2.2. Effect of solar energy on survival, oviposition and development of C. maculatus

The effects of sun heat at different exposure times (5, 10 and 15 min) on adult survival were studied. Four replicates of three pairs of newly emerged adults (0-24 h old) were held in a transparent plastic vials (8.5 x 5 cm) supplied with 15 gm of cowpea seeds. The vials were covered with lids and put on the glass plate in the middle of metal heater previously described. Then, the metal heater was covered with transparent polyethylene sheet to insure no air exchange during the exposure. At the end of each exposure period, the vials were kept in the laboratory for about 24 h then adult mortality was recorded. Vials containing insects and seeds which were not exposed to sun heat and were kept in the laboratory for the same time and used as untreated controls (0 min exposure). All the vials were kept at controlled condition (29 ± 1°C), 35 ± 5% r.h. and a photoperiod of 16 L: 8 D.

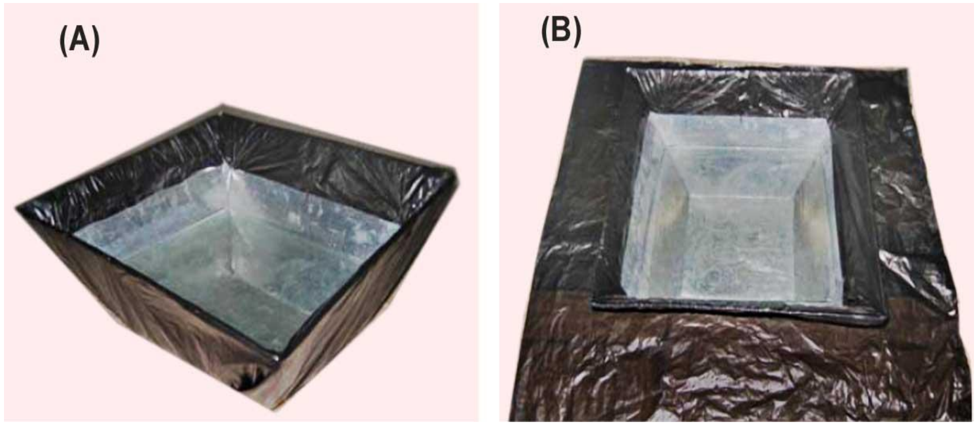

Figure 1 The metal box heater (A), Black polystyrene sheet under the box (B), to collect the sun rays on the experimental spot.

To study the effect of solar heat on different developmental stages of cowpea beetle, three pairs of newly emerged adults (0-24 h old) were introduced into vials supplied with 15 gm of cowpea seeds. The females were allowed to lay eggs for 24 h then all insects were removed from the seeds. The infested seeds were then held under the aforementioned conditions until sun treatment. The age of different stages of insects in the infested seeds was estimated according to Lale and Vidal (2003). Four replicated vials, each containing 15 gm of cowpea seeds infested with a single stage of cowpea beetle were exposed to sun heat in heater box under the same conditions as the aforementioned for adults. The five developmental stages (eggs, three larval instars and pupae) were exposed separately to sun heat for 10, 15 and 20 min. For each developmental stage a control group (four replicates) was run at the same time of the experiment with 0 min exposure. After exposure to sun heat all vials with various developmental stages were kept at the aforementioned conditions until adult emergence. Mortality of all developmental stages was based on those that failed to emerge to adults. Adults emerged from untreated controls were used as an estimate of the number of insects treated and to calculate treatment mortality.

2.2.3. Effect of solar energy on germination of cowpea seeds

Plastic vials supplied with 15 gm of seeds were added to the heater box. Samples for germination were drawn immediately after solar heating and up to 41 weeks of storage. Germination was determined in the laboratory at 28°C using Petri dishes lined with filter paper to which 10 ml distilled water were added. Ten seeds were placed in each Petri dish and germination was recorded after 3 d. Untreated seeds were used as controls (0 min exposure) and three replicated dished were used for each experiment and control.

2.3. Toxicity of some citrus peel powder on cowpea beetle C. maculatus

Efficiency was based on adult mortality and repellency test under controlled conditions of 29 ± 2°C, 35 ± 5% r.h. and ambient photoperiod of about 16 L: 8 D.

2.3.1. Citrus peel powder used

The peel powder of lemon (*Citrus aurantifolia* (Christm.) Swingle) and orange (*Citrus sinensis* (L.) Osbeck) were used during this experiment. The thin outer covering of the citrus were peeled and then air dried in shade in the laboratory for about 5 d until become crispy. The dried peels were milled and sieved through a 0.3 mm size mesh to obtain a fine powder with uniform particle size. The resulting powder was kept in a sterile glass bottles with screw cap and used within 24 h.

2.3.2. Susceptibility of adult cowpea beetles to citrus peel powders

Citrus peel powders were applied at rate of 0.01, 0.03, 0.075 and 0.1 gm to a gm of cowpea seeds in a plastic vials (8.5 x 5 cm). The vials were shaken vigorously for optimum coverage of seeds surface. Clean untreated cowpea seeds were used as control. Three pairs of newly emerged adults (less than 24 h old) were introduced into each vial and then covered tightly with the lids. Four replicates of each treatment and untreated seeds were set up. The cowpeas in each vial were sieved after 1, 2 and 3 d and percentage of adult mortality was calculated and corrected by using Abbott's formula (1925). The LD_{50}'s and LD_{95}'s values were obtained 3 d after the insect's release using probit analysis (Probitvb 6 varsion 4).

2.3.3. Repellent effect of citrus peel powders

The repellence effect of lemon and orange peel powders was tested according to a free choice bioassay method recorded by Parugrug and Roxas (2008) with some modifications. Transparent glass tubes, 13 cm long x 1 cm diameters as test cylinders were used in the experiment. There was a small hole, 4 mm diameter, at the middle portion of each tube. Both ends of each tube were supplied with a small glass vial, 6 cm long x 1 cm diameter, which was fixed to the tube with transparent adhesive tape. The vial at one end was supplied with 15 cowpea seeds and 0.3 gm of the citrus peel powder and the other end was supplied with 15 clean seeds only representing the control side. A group of ten adult cowpea beetles (males and females) were introduced at the middle of each tube through the hole in the middle portion of the tube. The hole was covered with a small piece of paper fixed with adhesive tape and tiny holes were made by a needle to ensure good aeration. Finally, two groups were set up; one for lemon and the other for orange peel powders. Each treatment consisted of five tubes and replicated twice. The cylinders were left undisturbed and the number of the beetles that moved towards the untreated and treated halves of the tubes were recorded after five hours. The degree of repellency of each citrus powder was based on the distribution of adult beetles between the two sides.

2.3.4. Scanning electron microscopy (SEM) for studying the effect of citrus powder on spiracles of cowpea beetle adults

Three pairs of adult beetles were added to 2 gm of citrus peel powder/ 20 gm cowpea seeds in a plastic vial with a lid. After 24 h the adult beetles were removed and freeze killed at -20°C for at least 2 h. Another group of normal adults of cowpea beetles were kept in deep freezer at -20°C. Both treated and normal adults of cowpea beetles were ready for scanning by SEM (Quantum 200, S.E.I. Company) at the Center of Research and Conservation of Antiquities. They were fried in the chamber of the SEM.

2.4. Data analysis

For data analysis, homogeneity of the variances was tested using Levene's test. When the assumption of homogeneity of the variances was fulfilled, one-way ANOVA was applied followed by pairwise comparisons using LSD-test. When no transformation was able to homogenizse the group variances, Welch ANOVA was applied. After that, pairwise comparisons were made using the Thamhane-test. The SPSS program package was used to conduct analyses.

3. Results and Discussion

3.1. Effect of solar heat on cowpea beetle

3.1.1. Effect of solar energy on survival, oviposition and development of C. maculatus

The current study demonstrated that the metal heating box was effective in gathering solar radiation and raising the temperature than ambient with up to 14°C within 15 – 20 min and caused the 100% mortality of cowpea beetle population. Adult mortality were significantly different between different exposure times (Table 1, $P = 0.004$). Temperature of 54°C for only 15 min was able to completely suppress the adult population of cowpea beetles (Table 1).

Table 1 Mortality of adult cowpea beetle *Callosobruchus maculatus* exposed to solar energy.

Exposure duration (minutes)	**Temperature (°C)**		**Mean no. of dead adults ± SE**	**% Adult Mortality**
	Inside the metal heater	**Outside the metal heater**		
0	-	28	0±0.0 **a**	0
5	42	36	0.25±0.25 **a**	4.17
10	52	38	2±1.41 **ab**	33.3
15	54	39	6±0.0 **b**	100
20	64	40	6±0.0 **b**	100

N.B. Different letters indicate significant differences between varieties at the 0.05 level.

There was also an overall significance difference between different treatments for each developmental stage ($P = 0.003$, $P = 0.0$, and $P = 0.002$ for egg, all larval, pupal stage, respectively). Complete inhibition of adult emergence was achieved at 64°C after only 20 min of solar energy exposure for all developmental stages (eggs, three larval instars, and pupae, Table 2). The effect of solar energy was always time and temperature dependent (Table 1, 2) as it has been found previously (Fields, 1992; Mahroof et al., 2003). The adult stage was the most susceptible stage as 100% mortality was achieved within 15 min at 54°C. These in agreement with earlier studies on adult of *C. maculatus* to be the least tolerant stage (Murdock and Shade, 1991; Johnson et al., 2010), while disagrees with others as adult of *C. maculatus* was found to be the most resistant stage (Loganathan et al., 2011; Purohit et al., 2013). Previous works also reported that elevated temperature > 55°C caused a rapid mortality to stored-product insects, and the entire population death occurred in minutes to seconds (Fields, 1992; Beckett et al., 2007).

Table 2 Survival of different developmental stages of cowpea beetle *Callosobruchus maculatus* exposed to solar energy.

Developmental stages	Exposure duration (minutes)	Temperature (°C)		Mean no. of emerged adults ± SE	% Adult Reduction
		Inside the metal heater	Outside the metal heater		
Eggs	0	-	28	64.75±3.98 **a**	0
	10	52	36	29.5±7.75 **ac**	54.44
	15	54	36	11±2.79 **bc**	83.01
	20	64	40	0±0.0 **c**	100
1st larvae	0	-	28	51.5 **a**	0
	10	46	37	19.25±2.68 **b**	62.62
	15	54	36	6±4.06 **c**	88.35
	20	58	36	0 **c**	100
2nd larvae	0	-	28	64.87 **a**	0
	10	50	38	48±7.22 **b**	26.01
	15	54	37	32.75±2.49 **c**	49.52
	20	58	36	1.25±1.25 **d**	98.07
3rd larvae	0	-	28	66.9 **a**	0
	10	43	30	38.5±3.57 **ac**	41.66
	15	54	37	16.33±4.40 **bc**	75.25
	20	52	34	0 **b**	100
Pupae	0	-	28	66.9 **a**	0
	10	57	38	44.5±8.43 **b**	21.93
	15	54	36	18.5±7.64 **c**	67.54
	20	59	38	0±0.0 **c**	100

N.B. Different letters indicate significant differences between varieties at the 0.05 level.

According to the sub-lethal dose of solar energy (15 min) on the percent of beetle reduction (Table 2), the most tolerant stage was the second larval instar followed by the pupal and third larval stages and finally the first larval and egg stages. Earlier studies found also that larval (Murdock and Shade, 199) and pupal (Loganathan et al., 2011; Johnson et al., 2010) stages are the most resistant stages. The present work also supported previous studies in that the high temperature has a great effect on reducing the insect fertility and progeny (Neven, 2000; Mahroof et al., 2005). A very interesting point is that most of the pupae survived from 15 min of solar energy produced malformed adults. These malformations varied between depigmentation of body cuticle and wings abnormalities. On the other hands, these malformations did not appear in adults produced from other cowpea beetle stages that exposed to solar radiation.

3.1.2. Effect of solar energy on the germination of cowpea seeds

There was no adverse effect of solar radiation on seeds germination. This agrees with previous studies (Murdock and Shade, 1991; Chauhan and Ghaffar, 2002; Murdock el al., 2003). Murdock et al., 2003 also reported that the cowpea grains when heated up to 80°C, had no significant effect on seed germination. Interestingly, the germination of treated seeds was even better than the control one. The germination of treated seeds of different solar energy (10, 15 and 20 min) was

100% compared to the control one that was 83.3%. In addition to that, the cowpea seed shoots of treated ones were in some cases longer than the control. Similarly, Buriro et al. (2011) demonstrated that the shoot dry weight of wheat seedling increased with increasing temperature. All seeds of the triplicates used for each solar energy group were completely germinated except one replicate of the control group had a 50% of seed germination. Another work obtained a similar result (Chauhan and Ghaffar, 2002).

3.2. Toxicity of some citrus peel powders on cowpea beetle C. maculatus

3.2.1. Susceptibility of adult cowpea beetles to citrus peel powders

The results indicated that lemon peel powder was more toxic to cowpea beetle than orange peel (Table 3).

Table 3 Mean percent mortality of cowpea beetle adults, LC_{50} and LC_{95}, their 95% confidence limits and slope of tested powders mixed with seeds to cowpea beetle adults.

Citrus peel powders	Concentrations (gm/gm seeds)	% Adult Mortality			LC_{50} (gm/gm seeds)	LC_{95} (gm/gm seeds)	Slope
		Day 1	Day 2	Day 3			
Orange	0.01	0	0	5.93	0.086	0.121	11.04
	0.03	0	0	9.63			
	0.075	3.33	10	25.93	(0.079- 0.094)	(0.098- 0.153)	
	0.1	3.33	58.33	79.85			
Lemon	0.01	0	3.33	8.15	0.025	0.068	3.82
	0.03	6.66	30	62.96			
	0.075	54.16	95.83	100	(0.019- 0.033)	(0.049- 0.109)	
	0.1	70	93.33	100			
Control	0	0	0	10			

The probit analysis for adult mortality was significantly different between different concentrations of lemon and orange peel powders (*P*= 0.27). The LC_{50s} were determined to be 0.025 and 0.086 gm/gm cowpea seeds after 3 d of treatment with lemon and orange peel powders, respectively. Generally, percentage adult mortality increased with increasing of citrus powders concentration and exposure time (Table 3). Comparable results have shown also that increasing of adult insect mortality depends on the increasing of concentrations and exposure time of citrus peel oils (Moravvej and Abbar, 2008; Zewde and Jembere, 2010).

The slopes of the probit mortality lines of lemon and orange peel powders were 3.82 and 11.04, respectively (Table 3), indicating that cowpea beetle population is more homogeneous for their response to lemon peel powder than that of orange peel powder. The steep slope value for orange powder indicates that the adult beetles' mortality increased with relatively small increases in powders quantities (Moravvej and Abbar, 2008). This was also the case with orange peel powder in the current study as the increasing of 0.025 gm orange peel powder from 0.075 to 0.1 gm/ gm seeds caused 25.93 to 79.85 of % adult mortality after 3 d.

3.2.2. Repellent effect of citrus peel powders

Lemon peel powder had a significantly high repellency activity over the control over time (Table 4, *P* = 0.0). Orange peel powder had also a significantly high repellency activity over the control over time (Table 4, *P* = 0.0). Generally, the lemon powder deterrent effect was higher than that of the orange powder as the overall high insect distribution at lemon was significantly different than that of the orange (Table 4, *P* = 0.0). Many studies have been shown that extracts of citrus peels were effective as oviposition deterrent against *C. maculatus* (Jayakumar, 2010; Zewde and Jembere, 2010; Rotimi and Evbuomwan, 2012).

Table 4 Mean distribution % of adult cowpea beetles on treated and untreated cowpea seeds with citrus peel powders in the repellency test over five hours.

Time (hours)	**% mean distribution of adults (Lemon)**		**% mean distribution of adults (Orange)**	
	Treated	**Control**	**Treated**	**Control**
1	4 **a**	93 **b**	31	62 *
2	3 **a**	91 **b**	31	64 *
3	4 **a**	91 **b**	25	68 *
4	4 **a**	91 **b**	20	70 *
5	5 **a**	89 **b**	20	70 *
Mean± SE	(4±0.32) **A**	(91±0.63) **B**	(25.4±2.47) **C**	(66.8±1.62) **D**

N.B. Different letters indicate significant differences between varieties at the 0.05 level.
*Indicates significant differences between the control group of orange group and treated side.

3.2.3. Scanning electron microscopy for studying the effect of citrus powder on spiracles of cowpea beetle adults

Observation of *C. maculatus* adult spiracles revealed that they are located laterally along the thorax and abdomen of the insect; one pair of spiracles per body segment. Spiracles are annular (simple), uniforous (one-opening) and atriate (spiracle chamber) with an internal closing apparatus (Fig. 2). The inner wall of the atrium bears numerous small cuticular hairs (Fig. 2).

Applying of lemon and orange peel powders made some morphological changes to spiracle shape. Concerning the response of adult *C. maculatus* to orange peel powder, it seems that the spiracle opening were contracted to prevent volatile oil entry. This appeared from decreasing of atrium size (depth) and well appearance of the inner valve parts (Fig. 2). The insect cuticle shows some sort of shrinkage due to the contraction of spiracle's closing muscles to prevent citrus powder particles and oil from entering, and thus preventing respiration via trachea (Shukla et al., 2007). The effectiveness of citrus peel powder is probably due to silica or silica like components, which are abrasive and the ability of the particles to adhere to the grain and insect cuticle that accelerating water loss by evaporation (De Sousa et al., 2005; Zewde and Jember, 2010). In some area of the insect cuticle, it seems that the surface folds and the wax layer have been solubilized. While, in the case of lemon peel powder the cuticle surrounding the spiracle was more sensitive and the edge of spiracle opening was swollen (Fig. 2). The reason could be the presence of d-limonene, which is the main component of the volatile oil of the peel powder. Limonene has been mentioned to destroy the wax coating of the insect's respiratory system. When applied directly, the insect suffocates (1).

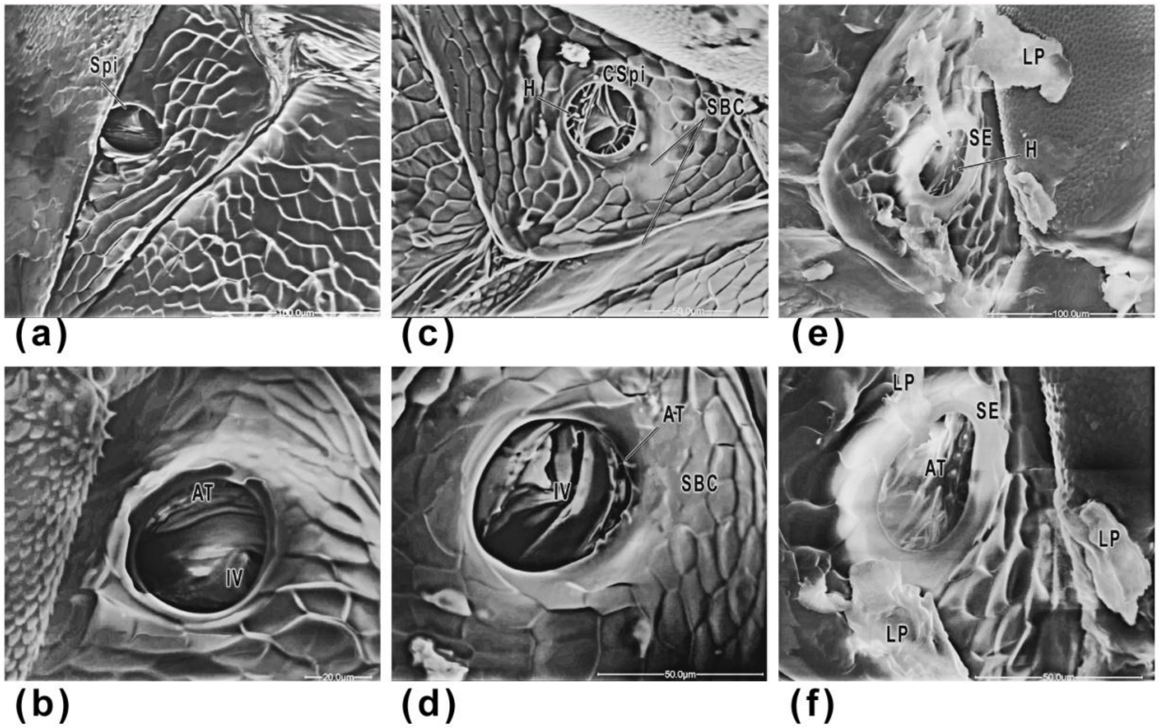

Figure 2 (a and b) A spiracle of *C. maculatus* normal female showing the first spiracle (Spi) (line), the atrium (AT) of the spiracle and the Inner valve (IV). Scale bar: 100 µm, 20 µm, for a and b, respectively. (c and d) A spiracle of *C. maculatus* female treated with orange peel powder showing contracted first spiracle (CSpi), spiracle hairs (H), closing of the inner valve (IV) and solubilization of body cuticle (SBC). Scale bar: 50 µm. (e and f) A spiracle of *C. maculatus* female treated with lemon peel powder showing the first spiracle, the spiracle hairs (H), atrium (AT) of the spiracle, Swollen edge of spiracle opening (SE) and Lemon particles (LP). Scale bar: 100 µm, 50 µm, for e and f, respectively.

4. Conclusions

Generally the present work demonstrated that the metal-box heater was very effective in suppression of *C. maculatus* population. The adult stage of *C. maculatus* was the most sensitive stage to solar energy exposure. Results of adult emergence indicated also that the second larval instar was the most tolerant stage to solar energy treatment, third and pupal stages had intermediate tolerance and the first larval instar and egg stage were the most susceptible immature stages.Solar energy treatment had no negative effect on germination of cowpea seeds, but even had an advantage.

This work also showed that utilization of lemon and orange peel powders is very promising as protectants of stored grain from the damage caused by bruchids and other stores-products pests.

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