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Control of all stages of the cowpea seed beetle *Callosobruchus maculates* (Coleoptera: Chrysomelidae) by low temperature inside different bags

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Abstract:

Cooling and freezing is one of the means of physical insect control. Low temperature was evaluated for disinfestations all stages of the cowpea seed beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) in cowpea seeds. Eggs, larvae, pupae and adults were exposed to temperature degrees; 5, 0, -5, and -10 ℃ kept inside different packages (plastic vial, plastic sack and glass jar) for various durations (1, 2, 4, 6, 8, 10, 12, 24 and 48 hrs.). Results showed that the adults were the most cold-tolerant while the eggs were lowest cold-tolerant. Packages were significantly different in adults stages and in larvae stages but in eggs and pupae not significantly different between packages. LT_{90} of adults were 313.18, 42.22, 13.82 and 5.75 hrs. For plastic sack at 5, 0, -5 and -10 ℃, respectively. For eggs LT_{90} recorded 19.64,10.55, 4.06 and 2.43 hrs. at 5, 0, -5 and -10 ℃, respectively, for plastic sack. Larvae and pupae also recorded.

Introduction

Cowpea, (*Vigna unguiculata* (L.) Walpers, Fabaceae), is an important edible legume crop in many parts of the world especially in tropical and subtropical regions. It is used as human food due to its high protein content and as livestock feed to make silage and hay (Diouf, 2011). One of the major destructive post- harvest pests of cowpea worldwide is the cowpea weevil *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae). In stored seeds, cowpea weevil causes irreparable damage to the tissue, one that can reduce nutritive value and quality seeds for planting in many areas of developing countries. The damage is caused by larvae feeding and development inside the seed, and when adults emerge, they leave circular exit holes (Davidson and Lyson, 1979). The damage reduces the weight and may render the seeds to be unfit for human consumption due to fungal growth associated with increased temperatures in storage. This insect also causes secondary infestation during pulse storage and may cause total loss within three months (Singh and Jackai, 1985). Being an internal feeder, it is very difficult to control the larval stage of *C. maculates* with insecticides. The management of this insect in storage using chemical insecticides leads to insecticide residues in grains and insecticide resistant populations. Chemicals, grain protectants and fumigants, are extensively used around the world to control insect pests in stored commodities. So, there is a need for the ecologically benign methods to control cowpea weevil. The use of cool or cold temperatures to manage stored grain can be an important component of insect pest management programs. Temperature treatment of stored grains is a best physical method which successfully kills several life stages of insects at a time. Most of the stored product insects cannot tolerate extreme temperature, heating and cooling and show heavy mortality (Upadhyay and Shoeb, 2011). For several years, the use of extreme temperatures, particularly low temperatures, has been extensively used to control stored product insects. The advantages of physical control methods are that: (1) There are no leave chemical residues on the product after treatment, (2) They are effective against insecticidal resistant strains, (3) There are few risks for operators, and (4) There are no negative effects on environment.

The purpose of this study was to evaluate the effectiveness of low temperature in control of cowpea beetle exposed inside three types of packages materials to prevent damage by direct insect pests.

Materials and methods

For starting a culture of tested insect, adults *C. maculatus* reared on cowpea seeds in a glass jars (each of approximately 500 ml) and each jar was covered with muslin cloths and fixed with rubber bands. Four temperatures were used as physical control methods against egg, larvae, pupae and adult of the cowpea seed beetle, *C. maculatus*. Tested temperatures degrees were 5, 0, -5, and -10 °C. for various exposed durations (1, 2, 4, 6, 8, 10, 12, 24 and 48 hrs.). Exposure times were chosen to cover the range of lethal times. Three different types of packages were tested for this study. The used packages were plastic vial, plastic sack and glass jar. To have an initial population of insect adults homogenous in age, about 500 adults were introduced into jars containing seeds for egg laying and then kept in an incubator at 28±2°C and 65±5 % RH. After three days, all insects were removed from the media and the jars were kept again at controlled conditions. The seeds were regularly observed for egg deposition and subsequent adult emergence. Hatched eggs were identified by the presence larval frass which causes egg to turn milky-white as larvae tunnel into the seeds for larvae and pupae age calculated. Five replicates of exposure and non-exposure (0 – 1 day-old) adults, (0 – 2 day-old) eggs, (8 – 10 day old) larvae and (2 – 3 day-old) pupae were used in this experiment. The temperature degrees used in this study were 5, 0, -5 and -10 ℃; the time exposure at each degree were 1, 2, 4, 6, 8, 10, 12, 24 and 48 hrs. Three different packages (plastic vial, plastic sack and glass jar) were used for each temperature degrees. Mortality ratios of exposure and non-exposure adults were observed after each time exposure. Reduction percentages of adults emerged from exposure and non-exposure eggs, larvae and pupae were calculated according to equation (Yamamoto and Casida, 1999). The study was terminated when no adult emergence was observed.

Data collected were analyzed using the ANOVA followed by the mean separation using the Duncan Multiple Range Test (DMRT). The differences were statistically significant at $p < 0.0001$.The results were statistically analysis based on statistically analyzed by Finney (1971) using thelog-probitsoftware programLdpLine®model"Ehabsoft, (Bakr, 2000).

Results and discussion

The data showed in Table (1) represented the mortality ratios of adult stage of *C. maculatus* insects exposed to different low temperature degrees (5, 0, -5 and -10 ℃) for (1, 2, 4, 6, 8, 12, 24 and 48 hrs.) and kept in various packages (plastic jar, plastic sack and glass jar). The data observed that in all temperature degrees and packages, as the time increased as the mortality % increased and as temperature decreased the mortality were faster in few hours. On the other wise, the glass jar package was less response for temperature, so the mortality of adult insects was less than plastic jar and plastic sack packages, but plastic sack package was more response for temperature. LT_{90} recorded 313.18, 42.22, 13.82 and 5.75 hrs. for plastic sack package at 0, 5, -5 and -10 ℃, respectively as showed in Table (5). The statistical analysis investigated that there are significant differences between four temperature degrees and there are significant differences between three packages as showed in Table (6).

The data showed in Tables (2, 3 and 4) represented the reduction in adult emergence % treated as eggs, larvae and pupae stages of *C. maculatus* insects exposed to different cold temperature degrees (5, 0, -5 and -10 ℃) for (2, 4, 6, 8, 12 and 24 hrs.) which kept in various packages (plastic jar, plastic sack and glass jar). The data observed that in all temperature degrees and packages as the time increased as the reduction % increased and as temperature decreased the mortality were faster in few hours so the reduction % increased. Also, the glass package was less response for temperature so that the mortality of eggs, larvae and pupae stages was less than plastic and sack packages. LT_{90} recorded 19.64, 10.55, 4.06 and 2.43 hrs. at 5, 0, -5 and -10 ℃ for plastic sack package, respectively for eggs as showed in Table (5). For larva was 38.4, 40.37, 5.83 and 2.95 hrs. at 5, 0, -5 and -10 ℃, respectively. Finally, LT_{90} for pupa was 20.14, 11.53, 4.36 and 3.73 hrs. at 5, 0, -5 and -10 ℃, respectively as showed in Table (5).

The statistical analysis investigated that there are significant differences between packages for eggs and larvae stages but for pupae there are no significant differences between three packages. Also, for temperature degrees there are significant differences between all temperature degrees in eggs and larvae treated while in pupae treated there are not significant differences between -5 and -10 ℃ as showed in Table (6).

In general, the adults were the most cold-tolerant while the eggs were lowest cold-tolerant, but larvae and pupae were equally cold-tolerant. Finally, the sack package was the better and more response in control of c. maculates insects in all different stages by freezing temperature (-5 and -10 ℃) for 12 hrs. exposure to obtained 100 % mortality and 100 % reduction of their offspring.

Our results stated that the adults were the most cold-tolerant while the eggs were lowest cold-tolerant. In contrast, Johnson and Valero (2003) they confirm that *C. maculatus* eggs are the most tolerant stage to freezing temperatures and that adults were highly susceptible. Finally, the sack package was the better and more response in control of *C. maculates* insects in all different stages by freezing temperature (-5 and -10 ℃) for 12 hours exposure to obtained 100 % mortality (100 % reduction) of their offspring.

Also, these results indicated generally that *C. maculatus* in glass jars were more tolerant than those in the other bags to freezing temperature; consequently, glass jars required longer exposure periods than plastic vial and plastic sack bags to give a complete control against tested insect. These findings are agreement with those obtained by Johnson and Valero (2003) indicated that mortality % eggs of *C. maculatus* was reached to more 98 % at – 18 ^{0}C after just 7 d of exposure. Complete mortality of *C. maculates* eggs occurred after 14 d of cold storage. Also, showed that the egg stage was most tolerant to -18 ^{0}C and that adult was most susceptible. A 2-wk treatment regimen may be enough for control of cowpea weevil in organic legumes.

Product storage at temperatures of -7 to 1 ^{0}C was recommended for control of *Callosobruchus* species early in the previous century (Duvel, 1905 and Larson and Simmons (1924). Mullen and Arbogast (1979) determined the LD95 of *C. maculatus* eggs after exposure to -15 ^{0}C to be 5 h, noting that this species was among the more cold-tolerant stored-product insects. Various Cooperative Extension sources recommend 4 d at -18 ^{0}C for control of cowpea weevil in home pantries (Sorensen, 1994 and Lyon, 1997). Larson and Simmons (1924) indicated that eggs were the most susceptible stage when exposed to -7 ^{0}C. In contrast, Mullen and Arbogast (1979) compared the effect of low temperatures on the eggs of five species of stored-product insects and found *C. maculatus* eggs to be among the most cold tolerant, with LD 50 values of 2.7, 1.3, and 0.3 h for -10, -15, and -20 ^{0}C, respectively. Obretenchev (1983) who found that adults of *O. surinmensis* were the most resistant stage to low temperature. At 0.0^{o}C, they died after 6 days, at -5^{o}C after 60 hrs., at -10 ^{o}C after 3 hrs. and 55 min. and at -15^{o}C after 47 min. Larvae were the most susceptible from the egg stage Donahaye *et al.* (1995) showed that the time required to produce 99.0 % kill for *O. surinamensis* larvae was 1.22 hrs. at -10 degrees ^{o}C and 0.32 hrs. at -18 degrees ^{o}C. While, the time required to produce 90.0 % kill for *O. surinamensis* adult was 1.49 hrs. at -10 degrees ^{o}C and 1.05 hrs. at -18 degrees ^{o}C., stated also , *O. surinamensis* the least sensitive stage at 0.0^{o}C was the larvae , at -5 and -18^{o}C was the adult. Stoyanova (1984) noticed that complete mortality of the-adult stages of *S. granarius* and *S. oryzae* occurred after 10 days at -16^{o}C. Mullen and Arbogast (1979) showed that at -5 ^{0}C 50% of *T. castaneum* eggs survived for 0.3 days. Also, Nagel and Shepard (1934) stated that 50 % of *T. confusum* eggs exposed to - 6 ^{0}C survived for 0.2 days. Flinn *et al.* (2015) they showed that treating flour pallets in commercial freezers is a feasible method to disinfest flour that may be infested with *T. castaneum* eggs. The fact that the treatment only required 5.5 days in the freezer makes it a practical method to disinfest pallets of flour, especially because the flour does not need to be removed from the shipping pallet. The fact that no chemicals are used would allow use of this treatment for organic or conventional flour. Dupuis *et al.* (2006) mentioned that the complete kill of all hidden stages of *Acanthoscelides obtectus* directly exposed at 0 °C and -10 °C was observed at 32 d and 24 hrs. exposure time, respectively.

Johnson and Valero (2003) stated that because the larval stages of the cowpea weevil feed and pupate within host seeds, larvae and pupae may experience some degree of insulation from cold temperatures when compared with free-living adults. This may account for some of the difference in cold tolerance between these stages. However, cowpea weevil eggs are laid on the seed surface and are not insulated from freezing temperatures, suggesting that cold tolerance of cowpea weevil eggs is due to a physiological mechanism and not placement within a protected microhabitat. Our results confirm that *C. maculatus* adults are the most tolerant stage to freezing temperatures and that eggs were highly susceptible.

It is concluded that there are many factors, such as freezing degree, exposure period and insect stage in addition to bag materials can be required to determine the time needed to kill all individuals.

Table (1): Effect of different cold temperature degrees and packages on mortality % of adult stage of *Callosobruchus maculatus.*

	Exposure time (h)	Mortality % of adult		
		Plastic jar	Plastic bag	Glass jar
5	1	0	3.33	0
	2	3.33	5	0
	4	6.67	8.33	1.67
	6	10	13.33	5
	8	15	21.67	8.33
	12	25	36.67	15
	24	35	43.33	23.33
	48	45	53.33	41.67
0	1	3.33	6.67	3.33
	2	10	11.67	10
	4	15	18.33	13.33
	6	28.33	40	15
	8	50	55	26.67
	12	55	68.33	43.33
	24	70	75	51.67
	48	83.33	100	100
-5	1	15	18.33	10
	2	35	43.33	31.67
	4	43.33	50	40
	6	58.33	66.67	56.67
	8	76.67	88.33	70
	12	95	100	91.67
	24	100	100	100
	48	100	100	100
-10	1	23.33	30	28.33
	2	46.67	58.33	43.33
	4	70	75	68.33
	6	86.67	95	85
	8	95	100	98.33
	12	100	100	100
	24	100	100	100
	48	100	100	100

Table (2): Effect of different cold temperature degrees and packages on reduction of emerged adults treated as eggs stage of *Callosobruchus maculatus.*

Degree	Exposure time (h)	Reduction of emerged adults %		
		Plastic jar	Plastic bag	Glass jar
5	2	6.93	16.79	5.95
	4	17.95	23.83	14.88
	6	29.83	34.88	29.50
	8	47.34	53.95	46.61
	10	61.07	62.44	54.55
	12	80.23	85.89	79.07
	24	91.02	97.88	89.51
0	2	16.28	14.33	18.83
	4	29.40	26.35	26.48
	6	55.55	60.16	48.28
	8	77.44	79.12	76.28
	10	94.26	95.66	89.37
	12	98.73	100	94.95
	24	100	100	98.90
-5	1	47.74	29.33	47.76
	2	86.24	89.07	88.69
	4	100	100	95.70
-10	1	63.14	83.33	42.64
	2	96.00	100	92.92
	4	100	100	100

Table (3): Effect of different cold temperature degrees and packages on reduction of emerged adults treated as larvae stage of *Callosobruchus maculatus.*

Degree	Exposure time (h)	Reduction of emerged adults %		
		Plastic jar	Plastic bag	Glass jar
5	**2**	**5.84**	**2.58**	**0.67**
	4	**12.57**	**11.16**	**4.29**
	6	**13.43**	**19.62**	**9.68**
	8	**26.50**	**28.43**	**24.50**
	10	**37.20**	**43.82**	**33.94**
	12	**55.28**	**65.76**	**53.51**
	24	**65.12**	**71.26**	**64.71**
0	**2**	**11.14**	**14.80**	**14.18**
	4	**21.12**	**20.31**	**18.48**
	6	**25.60**	**31.66**	**23.16**
	8	**32.72**	**43.26**	**29.85**
	10	**52.39**	**54.57**	**37.60**
	12	**65.40**	**67.28**	**61.04**
	24	**75.96**	**79.82**	**69.31**
-5	**2**	**11.60**	**11.58**	**15.04**
	4	**42.62**	**59.63**	**39.98**
	6	**84.55**	**92.07**	**80.64**
	8	**100**	**100**	**92.40**
-10	**2**	**67.02**	**75.53**	**45.41**
	4	**89.94**	**96.00**	**78.94**
	6	**100**	**100**	**100**

Table (4): Effect of different cold temperature degrees and packages on reduction of emerged adults treated as pupae stage of *Callosobruchus maculatus.*

Degree	Exposure time (h)	Reduction of emerged adults %		
		Plastic jar	Plastic bag	Glass jar
5	2	4.98	7.14	3.56
	4	12.16	22.42	11.74
	6	25.52	29.03	24.55
	8	43.34	46.69	41.21
	10	57.94	59.15	52.59
	12	77.73	80.24	77.31
	24	90.64	96.28	85.62
0	2	20.87	11.78	26.39
	4	26.92	24.73	27.17
	6	57.09	62.22	53.05
	8	75.93	75.45	75.41
	10	88.17	89.10	88.07
	12	95.57	100	91.00
	24	100	100	98.40
-5	2	38.23	39.33	39.38
	4	74.99	84.74	79.34
	6	96.57	98.21	92.07
	8	100	100	98.45
-10	2	50.73	63.38	45.89
	4	85.29	89.52	85.94
	6	100	100	100

Table (5): Lethal time and slope values of all stages of *Callosobruchus maculatus* in plastic sack package.

Temperature degree	Stage	LT50	LT90	Lower limit	Upper limit	Slope
5	Adult	33.60	313.18	178.12	721.56	1.32±0.13
	Egg	6.57	19.64	16.95	35.86	2.69±0.19
	Larva	11.86	38.4	30.97	72.09	2.51±0.20
	Pupa	7.63	20.14	16.97	28.60	3.04±0.22
0	Adult	8.36	42.22	33.54	56.35	1.822±0.012
	Egg	4.79	10.55	10.10	27.37	3.74±0.30
	Larva	8.89	40.37	30.80	58.89	1.95±0.17
	Pupa	5.07	11.53	9.84	20.37	3.55±0.30
-5	Adult	2.96	13.82	13.819	95.8	1.91±0.19
	Egg	2.47	4.06	3.7	4.7	5.94±0.58
	Larva	3.42	5.83	5.36	6.47	5.55±0.44
	Pupa	2.31	4.36	3.91	5.06	4.64±0.51
-10	Adult	1.69	5.75	4.721	7.65	2.409±0.25
	Egg	1.03	2.43	1.99	2.93	3.44±0.82
	Larva	1.26	2.95	2.55	3.56	3.47±0.66
	Pupa	1.63	3.73	3.27	4.5	3.57±0.54

The same letters are not significantly different. P>0.0001

Table (6): Statistical analysis and Duncan's multiple range test for various packages on different temperature of all stages of *Callosobruchus maculatus* insect.

		Adult	**Egg**	**Larva**	**Pupa**
Package	Plastic	48.97 B	63.87 AB	47.43 AB	62.98 A
	Sack	52.28 A	65.95 A	49.96 A	63.97 A
	Glass	43.33 C	61.27 B	44.32 B	62.83 A
	F values	67.01	3.48	10.38	1.10
Temperature	5	19.84 D	49.05 D	30.94 D	45.23 C
	0	44.36 C	63.93 C	40.46 C	64.36 B
	-5	65.42 B	76.06 B	62.71 B	79.47 A
	-10	78.9 A	85.82 A	82.05 A	80.08 A
	F values	1261.7	135	233.45	322.47

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