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USE OF DIFFERENT NON-CHEMICAL METHODS FOR THE MANAGEMENT OF ADULT *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE) IN STORED CHICKPEA

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Abstract

The efficiency of physical factors (dry heat, low temperature and UV-radiation), leaf powders of Neem (*Azadirachta indica* A. Juss.), Bichuti (*Flacourtia* indica Merr.) and Parthenium weed (*Parthenium hysterophorus* L.) and two insecticides (Salvo and Amithrin plus) was evaluated against *Callosobruchus maculatus* (F.). For dry heat treatment, the calculated LD_{50} values for *C. maculatus* were 56.92, 54.26, 52.27, 50.76, 49.09, 50.55 and 29.59°C after 50, 60, 70, and 80 minutes, 24, 36, 48 h of treatment respectively, and the LD_{50} values were 58.61 and 5.18°C at 1 and 2 h for low temperature treatment respectively. LT_{50} values of the treatment of UV-radiation were 48.63, 29.89, 17.54, 11.11, 5.93 and 1.35 °C for the exposure period of 24, 36, 48, 60, 72 and 48 h respectively. The calculated LD_{50} values of leaf powder were 3.38, 3.15, 2.88, 2.45 and 2.40 mg cm^{-2} for *A. indica*, 3.91, 3.80, 3.55, 3.30, and 3.08 for *F. indica*, 12.11, 4.35, 1.86, 1.49, and 1.36 for *P. hysterophorus* after 12, 24, 36, 48 and 60 h of treatment respectively. The calculated LD_{50} values were 0.64, 0.33, 0.23, 0.04 and 0.008 mg cm^{-2} for Salvo and 0.35, 0.23, 0.09, 0.08 and 0.01 mg cm^{-2} for Amithrin plus at 12, 24, 36, 48 and 60 h respectively. The order of effectiveness of physical factors was dry heat >low temperature >UV-radiation. On the other hand, the order of toxicity of plant powders was *F. indica >A. indica >P. hysterophorus*. In case of insecticides it was Amithrin plus >Salvo. The findings suggest that physical factors and plant leaf powders can be used in integration with other bio rational approaches.

Key words: *C. maculatus*, dry heat, low temperature, non-chemical methods, plant powders, toxicity, UV-radiation

Introduction

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) is a major pest of several stored pulses and usually found in Southeast Asia including Bangladesh, and the loss caused to stored grain was 55-69% weight loss and 45.6-66.3% loss in protein substance for chickpea (Alam 1971, Gugar and Yadav 1978). Pest management through temperature application (dry heat and low temperature) is receiving renewed interest as a non-chemical method with lack of residue problem (Hallman and Denlinger 1999). It is also one of the most promising bio-rational insect management tools for farm stored grain and grain processing industries (Fields 1992, Dosland et al. 2006, Phillips and Throne 2010). Low temperature control is also currently used along with other pest management techniques to kill insects within a store. Low temperatures have been used to successfully control insect populations in the fur and food industries for over a century. There are a few studies that have examined the management of bruchids by dry heat and low temperatures (Hallman and Denlinger 1999, Dosland et al. 2006).

The possible use UV-radiation as an alternative treatment method in storage premises was used in the laboratory. Experiments with UV-rays, for the management of coleopteran pests have shown to be very promising. Calderon et al. (1985) reported that the egg-hatching in *Tribolium castaneum* was negatively

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affected by UV-radiation, whereas Sharma and Dwevedi (1997) observed adverse influences of UV-rays on the egg-to-adult development of *C. chinensis* L. Many of the plants use as protectants have a strong smell whichrepels or kills insect. Plant powders reduce oviposition in bruchids under laboratory conditions (Iqbal and Poswal 1995). The plants, *A. indica* kernel powder, *Tridax procumbens* (L.) and *A. squamosa* seed powder control *C. chinensis* and *C. maculatus* (Ali et al. 1981, Sowunmi and Akinnusi 1983, Bhaduri et al. 1985). *A. indica* seed kernel applied to pea seeds reduced damage by *C. chinensis* over a three month storage period by reducing F1 adult progey (Kumari et al. 1990). The efficacy of diatomaceous earth in mixed formulation with other dusts and an insecticide against *C. chinensis* and *C. maculatus* was reported by Mahdi and Khalequzzaman (2012).

Different non chemical control methods against pulse beetle were thus evaluated. The present research was designed to find out the potentiality of some non-chemical methods *viz.*, dry heat, low (cold) temperature, UV-radiation, leaf powders of *Azadirachta indica* A. Juss., *Flacourtia indica* Merr. and *Parthenium hysterophorus* L. and two insecticides as reference for the control of *C. maculatus*.

Materials and Methods

Test insect: *C. maculatus* used in the experiments was collected from a private store house of Rajshahi, Bangladesh. The cultures were maintained in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi. For continuous and huge supply of the beetles, mass cultures were maintained in earthen pots (3000 ml) and sub-cultures in glass jars (500 ml) or beakers (500 ml) with the food medium.

Food medium: The chickpea, *Cicer arietinum* seeds were used as food medium for *C. maculatus* throughout the experiment. The seeds were kept in an oven and/or incubator for sterilization, about 24 h at 60°C to disinfest them. Then the seeds were thoroughly washed with tap water to remove dusts and carefully sun-dried having 13-14% moisture content. The sterile foods were then preserved in air tight glass jars (1000 ml) in order to impede further infestation.

Dry heat: One hundred chickpea seeds and 30 adults (1-2 days old) of *C. maculatus* were kept in a petri dishes (90 mm) in the dry air oven at 50, 55 and 60±1°C for exposure periods of 50, 60, 70 and 80 minutes, 24, 36 and 48 h for dry heat treatment. The treated and control batch at 29±1°C were maintained in 3 replications. Adult mortality was recorded after the exposure period of each treatment.

Low temperature: One hundred chickpea seeds and 30 adults of *C. maculatus* (1-2 days old) in a petri dish (90 mm) were exposed to low temperatures at 5, 1 and –4±1°C for 1 and 2 h of treatment while control was kept at 29±1°C. Three replicated trials were made, and adult mortality was recorded after each exposure period.

Ultra-violet radiation: A 15W germicidal lamp (GE1578) that emitted a wavelength of 254 nm (1 nmb = 1× 10-9 m) and installed at the Genetics and Molecular Biology Laboratory, Department of Zoology, Rajshahi University, was used as a source of UV radiation. Time-mortality response tests were conducted at a series of irradiation exposure periods *viz*., 5, 10, 15, 20 and 25 minutes. For irradiation, 1-2 day old 30 *C. maculatus* were kept in each 90 mm petri dishes, and placed on table surface 12 cm below the lamp at above time periods. Petri dishes were then exposed to UV-rays for estimating their respective mortalities at 24, 36, 48, 60, 72 and 84 h after post-irradiation. The same number of non-irradiated insects was maintained as controls in room temperature in three replications.

Plant leaf powders: Fresh leaves of Neem (*Azadirachta indica* A. Juss.), Bichuti (*Flacourtia indica* Merr.) and Parthenium weed (*Parthenium hysterophorus* L.) were collected from the surroundings of the campus of

University of Rajshahi, Bangladesh. Afterwards they were washed in running water. The plant materials were kept in shade for air-drying and then dried at room temperature (25 - 35°C) until they become crisp dry. Powdered samples were prepared by pulverizing the dried leaves using a blender. All the plant materials were sieved repeatedly to obtain the fine dust particles. The ground samples were passed through a 25 mesh sieve to obtain fine and uniform dust. The resulting dusts were used as direct admixture to the chickpea seeds at different doses. The dust was preserved in airtight condition in polythene bags till their use. Plant leaf powder was tested individually by mixing in w/w with 10 g of chickpea seed at the doses of 2.594, 2.672, 2.750, 2.830, and 2.908 gm cm^{-2} for Neem, 3.301, 3.379, 3.458, 3.537 and 3.615 gm cm^{-2} for Bichuti, and 1.415, 1.493, 1.572, 1.650 and 1.730 gm cm^{-2} for *Parthenium* weed. Three replications and a control (without insecticide) batches were made and 30 adult beetles were released in each petri dishes. The mortality of the beetles was recorded after 12, 24, 36, 48 and 60 h of treatment.

Dust formulation insecticides: The commercial dust formulation “Salvo 20 sp” and “Amithrin plus 3% WDG” of Bayer Crop Science, Germany was used. Insecticide was tested individually by mixing in w/w with 10 g of chickpea seed at the doses of 0.031, 0.049, 0.063, 0.079 and 0.094 gm cm^{-2}, 0.094, 0.129, 0.157, 0.289 and 0.220 gm cm^{-2} for Salvo and Amithrin plus respectively. Three replications and a control (without insecticide) batches were made and 30 adult beetles were released in each petri dishes. The mortality of the beetles was recorded after 12, 24, 36, 48, and 60 h of treatment.

Analysis of the data: The mortality percentage was corrected using Abbott’s formula (Abbott 1925, Busvine 1971): where, $Pt = [(Po - Pc) / (100 - Pc)] \times 100$, where Pt is the corrected mortality (%), Po is the observed mortality (%) and Pc is the control mortality (%). The observed data were then subjected to probit analysis according to Finney (1947) and Busvine (1971) LT_{50} for lethal temperature and lethal period, and LD_{50} for lethal dose were considered through the experiment. Heterogeneity is tested by chi-squared test.

Results

Effect of dry heat, low temperature and UV-radiation

The maximum LT_{50} was 56.95°C at 50 minutes while the minimum LT_{50} was 29.59°C at 48 h of treatment period for dry heat treatment to *C. maculatus* (Table 1). For low temperature treatment, the minimum LT_{50} was 5.18°C at 2 h; however, the maximum LT_{50} was not effective at 1 h treatment period (Table 1). For UV-radiation, the maximum LT_{50} was 48.63 minutes at 24 h while the minimum LT_{50} was 1.35 minutes at 84 h after post-irradiation to *C. maculatus* (Table 1). The result shows that the LT_{50} values of dry heat, low temperature and UV-radiation were decreased gradually with the increase of treatment period. Table 1 also shows the results of 95% confidence limits, regression equations (Y) and χ^2 of dry heat, low temperature treatment and UV-radiation on *C. maculatus*. The order of effectiveness of physical factors was dry heat > low temperature >UV-radiation.

Effect of plant leaf powders

The maximum LD_{50} was 3.38, 3.91and 12.11 mg cm^{-2} at 12 h while the minimum LD_{50} was 2.40, 3.08 and 1.36 mg cm^{-2} at 60 h of treatment period for leaf powders of *A. indica*, *F. indica* and *P. hysterophorus* respectively (Table 2). The result shows that these plant powders were effective against this test insect. With the increasing treatment period, the LD_{50} values of leaf powders were reduced remarkably. The results of LD_{50} (mg cm^{-2}), 95% confidence limits, regression equations (Y) and χ^2 of two insecticides against *C. maculatus* are presented in Table 2. The order of toxicity of plant powders was *F. indica >A. indica >P. hysterophorus*.

Table 1. LT_{50}, 95% confidence limits and regression equations of physical factors (dry heat, low temperature and UV-radiation) to adult *C. maculates.*

Treatment period	LT_{50} (°C)	95% confidence limits		Regression equation	χ^2 (df)
		Lower	Upper		
Dry heat					
50 min	56.92	55.29	58.59	Y = –39.45881 + 25.33171X	0.33 (1)
60 min	54.26	52.66	55.90	Y = –32.38392 + 21.53968X	2.57 (1)
70 min	52.27	50.64	53.95	Y = –34.25336 + 22.85801X	0.93 (1)
80 min	50.76	49.21	52.36	Y = –41.04586 + 27.00517X	0.57 (1)
24 h	49.09	42.38	56.87	Y = –11.82663 + 9.939746X	2.70 (1)
36 h	50.55	35.96	71.05	Y = –2.861599 + 4.614365X	1.15 (1)
48 h	29.59	18.87	46.40	Y = –1.1125 + 4.154708X	0.42 (1)
Low temperature					
1 h	58.61[ne]	5.02	6.84	Y = 3.778285 + 0.6910058X	31.39 (1)
2 h	5.18	2.53	10	Y = 4.630652 + 0.5164818X	67.26 (1)
UV-radiation					
24 h	48.63	16.17	146.22	Y = 3.40967 + 1.800528X	0.12 (2)
36 h	29.89	16.09	55.51	Y = 3.842401 + 1.722514X	4.69 (2)
48 h	17.54	13.79	22.30	Y = 1.841425 + 2.192907X	1.87 (3)
60 h	11.11	7.99	15.44	Y = 3.121617 + 1.511519X	4.78 (3)
72 h	5.93	3.27	10.78	Y = 3.694252 + 1.343494X	1.49 (3)
84 h	1.35	1.38	8.17	Y = 3.775727 + 1.686104X	1.29 (1)

ne: not effective

Effect of two insecticides

The maximum LD_{50} was 0.64 and 0.35 mg cm^{-2} at 12 h while the minimum LD_{50} was 0.008 and 0.01 mg cm^{-2} at 60 h of treatment period for insecticide Salvo and Amithrin plus respectively (Table 3). The result shows that the LD_{50} values of insecticides were decreased progressively with the increase of treatment period. Table 3 also shows the results of 95% confidence limits, regression equations (Y) and χ^2 of two insecticides against *C. maculatus*. In case of insecticides, the order of toxicity was Amithrin plus >Salvo.

Table 2. LD_{50}, 95% confidence limits and regression equations of three leaf powders to adult *C. maculates.*

Leaf powders	Treatment period (h)	LD_{50} (mg cm^{-2})	95% confidence limits		Regression equation	χ^2 (df)
			Lower	Upper		
A. indica	12	3.38	16.17	146.22	Y = 3.40967 + 1.800528X	0.12 (2)
	24	3.15	16.09	55.51	Y = 3.842401 + 1.722514X	4.69 (2)
	36	2.88	13.79	22.30	Y = 1.841425 + 2.192907X	1.87 (3)
	48	2.45	7.99	15.44	Y = 3.121617 + 1.511519X	4.78 (3)
	60	2.40	3.27	10.78	Y = 3.694252 + 1.343494X	1.49 (3)
F. indica	12	3.91	4.38	146.22	Y = -7.750948 + 21.49975X	1.58 (3)
	24	3.80	4.21	55.51	Y = -4.006943 + 15.50508X	7.21 (3)
	36	3.55	3.72	22.30	Y = -2.303605 + 13.25912X	4.97 (3)
	48	3.30	3.49	15.44	Y = -2.328491 + 14.10567X	0.26 (3)
	60	3.08	3.56	10.78	Y = -1.06144 + 12.38746 X	0.52 (3)
P. hysterophorus	12	12.11	2.66	55	Y = 3.338045 + 1.534327 X	2.47 (3)
	24	4.35	3.26	57	Y = 4.037036 + 1.507658 X	4.51 (3)
	36	1.86	0.90	3.86	Y = 4.538531 + 1.70042 X	1.77 (3)
	48	1.49	1.35	1.65	Y = 4.024939 + 5.600217 X	1.05 (3)
	60	1.36	1.20	1.55	Y = 3.897927 + 8.102146 X	2.63 (3)

Table 3. LD_{50}, 95% confidence limits and regression equations of two insecticides to adult *C. maculates.*

Insecticides	Treatment period (h)	LD_{50} (mg cm^{-2})	95% confidence limits		Regression equation	χ^2 (df)
			Lower	Upper		
Salvo	12	0.64	0.01	29.96	Y = 3.372366 + .8990858 X	1.77
	24	0.33	0.02	4.54	Y = 3.635021 + .894236 X	0.35
	36	0.23	0.01	4.53	Y = 4.173747 + .6000106 X	3.60
	48	0.04	0.02	0.07	Y = 4.315999 + 1.023755 X	1.27
	60	0.008	0.0004	0.18	Y = 5.04595 + .7742266 X	0.41
Amithrin plus	12	0.35	0.15	0.77	Y = 2.024741 + 1.925898 X	0.72
	24	0.23	0.15	0.35	Y = 2.24921 + 2.007077 X	0.52
	36	0.09	0.04	0.20	Y = 3.927226 + 1.079516 X	0.36
	48	0.08	0.04	0.15	Y = 3.483278 + 1.667075 X	1.61
	60	0.01	0.0006	0.55	Y = 4.686913 + 1.087835 X	0.01

Discussion

Temperature is one of the principal factors delimitating survival and reproduction of insects. The application of hot air is an easy, simple and environment friendly method in grain processing industries. Pest management through temperature manipulation is receiving renewed interest as a non-chemical method with lack of residue problem (Hallman and Denlinger 1999). The dry heating of chickpea for 10 minutes at 120°C is used as one of the methods for reducing the anti-nutritional factors and reduced 46% of α-galactosides and 27% of tripsin inhibitor activity (Frias et al. 2000). Adult mortality of *C. maculatus* increased with increased duration of solarisation (exposure period to sun) in Nigeria (Lale 1998). The maximum LT_{50} rate was 56.92°C at 50 minutes and minimum was 29.59°C at 48 h in this experiment agrees with the report of the previous works.

Mullen and Arbogast (1979) investigated the time-mortality relationships for eggs of five species of store-product insects, and found that the *C. maculatus* eggs to be among the most cold tolerant, with LD_{50} values of 2.7, 1.3 and 0.3 h at -10, -15 and -20°C, respectively. The efficacy of microwave radiation and cold storage on *T. castaneum* Herbst and *Sitophilus oryzae* L. was also examined by Gasemzadeh et al. (2010). There was complete egg mortality after 14 days of cold storage (-18°C) and highest survival of eggs located at the centre of the bin. The present finding indicates that LT_{50} value of low temperature was 5.18°C at 2 h. The present study also suggested the temperature-exposure time combinations required for the control of *C. maculatus* in chickpea, which are sometimes different than previous studies.

Faruki et al. (2007) noted that UV treatment on the *T. castaneum*, *T. confusum* and the almond moth *Cadra cautella* decreased egg-hatching and reduced adult emergence. The growth and development in the lesser mealworm *Alphitobius diaperinus* has been shown to be manipulated by UV treatments on eggs, larvae, pupae and adults by a number of workers (Parween et al. 2004, Faruki et al. 2005, Begum et al. 2007). These results nicely corroborate with the findings of the present study. Adult mortality and reduced longevity in the UV irradiated insects might result from structural changes in the haemolymph as well as reduction in the total haemolymph count as demonstrated in the flesh fly *Parasarcophaga ruficornis* by Krishna and Srivastava (1991). The most likely explanation of the UV irradiation effects on the adult insects is that UV-C at 254 nm causes adjacent thymine (T) molecules of the DNA strands to dimerize, and further accumulation of these defects inhibits DNA replication, thereby rendering its harmful impacts on the exposed organism (Allen 2001). The present study thus clearly demonstrated the UV irradiated adult time-mortality response.

Pandey and Singh (1995) found that seeds of black gram could be effectively protected from damage by *C. chinensis*, by mixing the seed with dried powder of *A. indica* leaves at a rate of 100 - 400 mg $^{-50\ gm}$ seed. Rajapakse et al. (1998) observed that *A. indica* gave significant reduction of oviposition and adult emergence of *C. maculatus*. The leaf powder of *A. indica, V. negundo* and *P. hydropiper* and their combinations were tested against *C. chinensis* on *Lens esculenta* seeds by Rouf et al. (1996) who reported that *P. hydropiper* leaf powder at $4g^{-50g}$ lentil seeds was the most effective in reducing ovipositiotion and adult emergence of *C. chinensis*. Lakshmi and Venugopal (2000) tested six plant products *viz.*, *V. negundo, A. squamosa* (leaf and seed). *Annona calamus, Curcuma longa* L., *A. indica* seed kernel dusts for their effectiveness against *C. maculatus* in chickpea seeds. It has also been reported that powders of clove and black pepper were most effective within eleven spices powders on *C. maculatus* in black gram seeds (Mahdi and Rahman 2008).

Pulse beetles are not advisable to mix insecticides with food grains. Nevertheless, the potential hazards for mammals from synthetic insecticides and the increase of insect resistance to pesticides has led to explore for new classes of insecticides with lower mammalian toxicity and a lower persistence in the environment (Roger and Hamraoui, 1993). In this experiment, the maximum LT_{50} was 3.38, 3.91 and 12.11 mg cm^{-2} and minimum LT_{50} was 2.40, 3.08 and 1.36 mg cm^{-2} for Neem (*A. indica*), Bichuti (*F. indica*) and Parthenium weed (*P. hysterophorus*) respectively. The findings of the present study also confirmed the toxic effects of leaves powders of *A. indica*, *F. indica* and *P. hysterophorus* and two insecticides Salvo and Amithrin plus (used as reference) on adult mortality of *C. maculatus* as admixtures in pest management strategies, especially by small scale farmers who store small amounts of pulses for consumption and planting.

These results concluded that LT_{50}/ LD_{50} values were dose and exposure time dependent. Mortality of pulse beetle was increased with the increase in exposure period which may clear the dose-time-mortality relationship in different treatment methods. These data also indicate the potential use of different non-chemical methods for the management of *C. maculatus* in stored chickpea. These physical factors and plant products can be used in integration with other bio-rational approaches. However, further research may be required on these aspects.

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