SOLAR HEAT EFFECTS ON THE DIFFERENT STAGES OF COWPEA BEETLE *CALLOSOBRUCHUS MACULATUS* (F.)

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Abstract

Cowpea seeds are one of the main sources of human dietary proteins, planted and harvested in summer and vulnerable to storage infestation by *Callosobruchus maculatus* (F.). Sunning was tried as possible and safe control method of stored cowpea seeds after harvest from *C. maculatus* infestation. The biological effects of direct solar heat on the different stages (eggs, first-instar larvae, two-week old larvae, newly formed pupae and adults) were studied when exposed these stages for 6 hours daily along 4-5 consecutive days in cowpea seeds which the day temperature ranged from 32-38 degree C with a mean of 35 degree C. Effectiveness was variable with insect stage and duration of sun exposure. Eggs were the most susceptible stage to solar heat followed by first instar larvae while last instar larval instar and the pupal stages were the most resistant stage. A significant reduction of both eggs hatch and its penetration ratio (14.4%) and adult emergence when eggs (1-2 days old) exposed six hours for four consecutive days. Exposing newly hatched larvae, also leads to a reduction in adult emergence as compared to control. Adult oviposition, mortality and progeny produced also decreased with increase of the exposure period. Percentage of reduction in adult emergence (Inhibition rate, IR %) increased with increasing of the exposure period. Sunning thus gave good effects by reducing infestation rates and could suppress insect attack by killing eggs and newly formed stages.

INTRODUCTION

Cowpea seeds are considered one of the most important source of dietary proteins for low-income people and widely distributed in many tropical and subtropical regions. Seeds are infested by *C. maculatus* (F.) which make the seeds unsuitable for human consumption. The amount of annual loss reached 24 % of stored pulses from *C. maculatus* and *C. chinensis* infestation (Caswell, 1968, and Lale, 1992) within a very short period and complete infestation occur within 3-5 months under traditional storage conditions (Caswell and Akibu, 1980). Reduction of these bruchids by chemicals arisen many undesirable effects as danger of misuse by illiterated farmers, residue hazards in treated seeds as well as development of resistant strains to insecticides. The previous problems led to search for other safe cheap, locally available alternatives. Solarization method is one of the great potential methods for insect management in the Nigerian Savana and other tropical countries where there is abundant sunshine throughout the year. The solar heating was investigated for disinfesting of cowpea seeds infested by *C. maculatus* and *C. chinensis* in different countries as Nigeria (Lale and Vidal, 2003). The heat from the sun tends to drive out adult insects, kills the eggs and possibly the early stage larvae depending on the available temperature and periods of sun exposure (Chinwad and Giga, 1996). Utilization of solar heat for bruchid control is based on that insects die when exposed to high temperatures because of their limited physiological capacity to thermoregulate (Murdock and Shade, 1991, Kitch *et al.*, 1992, Murdocks *et al.*, 1997 and Lale, 1998). This study investigates sunning heat effects under Egyptian conditions for possible disinfesting of cowpea seeds from artificial infestation by *C. maculatus* by determining some biological characters of the different stages of this insect when exposed to sun for various periods.

MATERIALS AND METHODS

C. maculatus was reared for several generations at Stored Grain Insect's Laboratory, Plant Protection Research Institute, on cowpea seeds at 28±1 C and 65±5 % RH. Seeds were purchased from the local markets and sterilized by deep freezing, to free the seeds from any possible hidden infestation before use (Giga and Smith, 1987).

Insect stages were prepared as follows, about one kilogram of cowpea seeds were infested with about 500 adults of newly emerged for one day and then removed. Many replicates of Petri dishes, each contained about 25 seeds infested with eggs, and exposed to the sun for various exposure periods (3h, 1, 2, 3, 4 and 5 days).

Effects of solar heat were tested on 2-days old eggs as a mid dormant developmental stage which exposed to the sun in replicates of 25 seeds within clear Petri dishes. All the dishes were exposed to the sun in one time, and five dishes were withdraw daily and incubated for two weeks until determining the eggs state and re-incubated again until adult emergence start to appear, separated and counted as well as mean developmental period was recorded. The percentage of adult emergence (%) was determined from the total number of emerged adults compared with the total number of hatched eggs and penetrated larvae, as follows:

$$\text{Adult emergence (\%)} = \frac{\text{Total emerged adults}}{\text{Total hatched and penetrated eggs}} \times 100$$

Also, the reduction of the adult emergence (%) as the inhibition rate (IR %) was also calculated as an indication of the amount of the protection afforded compared to number of newly emerged adults in control and number of newly emerged adults in treatment:

To study sun heat effects on bruchid larvae (newly hatched larvae and two weeks old larvae) were selected to be exposed to direct sunlight. Infested seeds by both tested instar larvae were assumed to contain newly hatched larvae after four days of seed infestation while after two weeks were presumed to contain two- week's old larvae which the latter was considered as the most active feeding stage. The larvae-containing seeds were exposed to direct sunlight in replicates of 25 seeds for 1, 2, 3, and 4 and 5 successive days. Control dishes were prepared in the same period and left under open summer conditions without sun exposure. The emerged population were also counted and removed. After 2 days of appearing the future emergence windows seeds were considered to contain the bruchid pupae and at this stage, seeds were exposed to the sunlight for a similar exposure periods as before. The parameters measured were progeny number, adult emergence and reduction (%) compared to control.

To assay efficacy of solar heat on female oviposition by confining two newly emerged adult pairs in 9.0 cm Petri dishes with 25 seeds of cowpea and soon covered. Many similar units were done in one time as replicates. All the replicates were exposed to direct sun 6 hours daily within the daytime period of 10.0 am to 4.0 pm for 4- 5 consecutive days which the day temperature ranged from 32-38 degree C with a mean of 35 degree C . Three dishes were withdrawn each day at the end of the exposure period and placed under shade in open condition room. The parent mortality (%) was recorded after 5 days and the left live adults were removed after that. After two weeks, all the replicates were examined for counting number of hatched and unhatched eggs (oviposition). 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, while the unhatched eggs remains transparent and glossy (Giga and Smith, 1987). Eggs with clear hatched larvae and seen died within its shells were considered as unhatched eggs. Thus the total numbers of white and translucent eggs on the seeds indicate bruchid oviposition while numbers of white eggs indicate the number of larvae entering the seed (Dharmasena *et al.,* 2001). The obtained data were statistically analyzed using the analysis of variance (ANOVA). Means separation was conducted using Duncan multiple range test (Duncan, 1956) at 0.05 % probability level using a computer program. Standard error was also calculated.

RESULTS AND DISCUSSION

The effects of solar heat on *C. maculatus* stages were shown on Tables 1-5. Results showed that the efficacy was depended on the insect stage, and periods of sun exposure, where the efficacy increased with the increase among eggs hatch and larval penetration (%), adult emergence (%), reduction of adult rate. In respect to effects on *C. maculatus* eggs (Table 1), great significant differences were observed on the adult emergence, while the other characters as mean developmental period (MDP, days) was not differed significantly. The percentage of larval penetration reduced to 14.4% for eggs exposed to 4- day's period compared to 62.2 % of eggs exposed to only one day. Those larvae that hatched and died within egg shell was considered as killed. Progeny number and adult emergence were decreased by increasing periods of exposure to the sun. Reduction of adult emergence (%) reached 72% after 4 days. The latter character represents the amount of protection afforded by sunning and increased by increasing the exposure period. It was evident that eggs were the most sensitive to solar heat which kill even the newly hatched larvae within its shells and before its penetration inside the seed. Data in Table 2 showed sunning effects on the newly hatched larvae which showed significant differences were found in adult emergence (%) which was 41.8 compared to 54.4%. The reduction of adult emergence was also reduced to 22.0% compared to the control for 4-days exposed newly hatched larvae.

Table 1. Effects of solar heat on 1- 2 days old eggs of *Callosobruchus maculatus,* exposed 6 hours daily on cowpea seeds.

Time (days)	HP Eggs	UH Eggs	Total	Larval Penetration (%)	MDP (days)	Progeny No.	Adult Emrg (%)	Reduction (%)
3 Hours	36.3±3.5a	21.3±2.5d	57.5±5.3b	63.25±2.2a	22.0±0.0c	25.3±3.4ab	44.2±5.3ab	18.3±2.6c
1	33.8±3.4ab	26.5±2.7d	60.3±4.1b	56±3.9a	22.8±0.3b	28.8±3.3a	47.8±4.6a	12.3+5.9c
2	25.8±0.8b	35.8±2.3c	61.5±2.5b	41.3±5.0b	23.3±.3b	18.8±5.2bc	29.7±7.3bc	44.5±5.6b
3	14.5±1.3c	45.0±3.1b	59.5±4.2b	24.3±1.2c	24.8±0.3a	14±1.8c	23.4±1.8c	56.3±3.3ab
4	11.3±1.1c	67.3±4.3a	78.5±4.9a	14.35±1.2c	25.0±0.0a	11.5±1.3c	15.0±1.6c	72.0±30a
Control	37.5±0.8a	23.2±2.9d	60.7±3.5b	62.2±3.8a	22.0±0.3c	31.5±2.0a	53.4±6.0a	11.05±4.3c

HP=Hatched and penetrated eggs, UH=Unhatched and killed eggs, MDP=Mean growth period (in days).

Table 2. Effect of solar heat on newly hatched larvae of C.maculatus infesting cowpea seeds (exposed 6 hours daily).

Exposure time (days)	HP Eggs	UH eggs	Total	Larval Penetration (%)	MDP (days)	Progeny No.	Adult Emrg(%)	Reduction (%)
1	33.4±3.5a	24.4±1.6ab	57.8±3.7a	57.3±3.1a	21.8±0.2a	33.6±3.3a	57.6±3.1a	2.9±0.8a
2	42.4±6.9a	23.4±1.8ab	65.8±6.7a	63.1±3.9a	22.0±0.0a	28.4±4.6a	43.8±5.7ab	20.0±5.8a
3	34.4±1.8a	21.6±2.8b	56.0±2.8a	61.8±3.6a	22.0±0.0a	30.0±1.7a	54.2±1.1ab	22.0±0.4a
4	31.8±2.8a	30.0±3.0a	61.8±3.6a	51.6±3.4a	22.0±0.0a	25.6±3.3a	41.8±5.1b	21.9±9.5a
Control	37.5±0.8a	23.2±3.0ab	60.7±3.5aa	62.2±3.8a	22±0.0a	31.5±3.0a	53.4±6.0ab	11.1±4.3a

H& P=hatched and penetrated eggs, UH=Unhatched and killed eggs, MDP=Mean developmental period (in days).

Data in Table 3 showed sunning effects on the 2-weeks old larvae which no significant differences were found among the tested biological characters compared to the control. This indicated that this stage was much resistant to solar heat. Data in Table 4 showed sunning effects 1-2days old pupae which significant differences were found only in adult emergence (%) and reduction of adult emergence compared to control. it was 53.4% in control compared to 50 % for treatment of four days of sunning. The reduction of adult emergence was also reduced to 26.7% compared to the control (11.1%) for treated pupae.

Table 3. Effects of solar heat on two- week's old larvae of C.maculatus infesting cowpea seeds when exposed 6 hours daily.

Exposure time (days)	HP Eggs	UH Eggs	Total	Larval Penetration (%)	MDP (days)	Progeny No.	%Adult Emrg	Reduction (%)
1	35.2±0.8a	24.2±3.9a	59.4±3.1a	59.7±5.2a	22.0±0.0b	26.8±2.3a	45.1±3.1a	15.6±5.8a
2	33.2±3.0a	23.6±1.7a	56.8±2.5a	58.1±3.8a	22.8±0.2a	29.4±2.5a	51.5±3.3a	6.7±02.9a
3	35.8±1.7a	21.0±4.4a	56.8±4.8a	64.0±4.6a	23.0±0.0a	29.8±1.8a	49.8±5.4a	11.2±7.8a
4	38.5±2.7a	19.8±2.0a	58.3±4.7a	66.3±0.9a	22.5±0.3ab	31.0±1.2a	54.3±2.7a	2.7±0.04a
5	32.7±1.2a	18.3±0.9a	52.3±2.0a	62.6±3.0a	22.3±0.3ab	26.0±2.0a	49.7±2.3a	7.3±0.9a
Control	37.5±2.7a	23.2±3.0a	60.7±3.5a	62.2±3.8a	22.0±0.3b	31.5±2.0a	53.4±6.0a	11.1±4.3a

HP=hatched and penetrated eggs, UH=Unhatched and killed eggs, MDP=Mean developmental period (in days).

Table 4. Effect of solar heat on pupae of C.maculatus infesting cowpea seeds, exposed 6 hours daily.

Exposure time (days)	HP eggs	UH Eggs	Total	Larval Penetration (%)	MDP (days)	Progeny No.	Adult Emrg (%)	Reduction (%)
1	40.8±1.5a	17.0±1.8ab	57.8±3.0a	69.1±1.3a	22.0±0.0a	30.4±0.7a	52.9±1.5a	3.2±0.2b
2	39.6±2.9a	17.8±1.3ab	57.4±3.8a	68.9±1.5a	22.4±1.9a	22.6±1.9b	39.4±7.3a	26.7±2.2a
3	34.0±2.8a	15.8±1.4b	49.8±2.6a	68.0±3.2a	22.6±0.2a	27.0±3.2ab	54.2±5.7a	7.2±4.2b
4	35.2±3.6a	17.7±1.2ab	52.3±3.9a	66.6±2.7a	22.3±0.2a	26.0±1.6ab	50.1±2.1a	8.1±3.2b
Control	37.5±2.7a	23.2±3.0a	60.6±3.5a	62.2±3.8a	22.6±0.3a	31.5±2.0a	53.4±6.0a	11.1±4.3b

HP=hatched and penetrated eggs, UH=Unhatched and killed eggs, MDP=Mean developmental period (in days).

Data in Table 5 showed sunning effects on the adult mortality (%) and subsequent biological characters as oviposition, larval growth, progeny number and adult emergence (%). oviposition or the number of laid eggs/ 5 pairs on sun- exposed seeds was reduced from 317.0 eggs in control to 69.3 eggs in treatment. The eggs hatch and larval penetration (%) were also significantly affected compared to the control. It was also observed that the mean duration of development (MDP, days) was not affected compared to control i.e. it was 18.0 days. Percentages of adult emergence and its reduction showed significant differences. Progeny was much reduced to 8.0 adults emerged from the infested seeds compared to 180.4 adult in control. Also adult mortality was much affected (77.5%) after five days of exposure.

Table 5. Effect of solar heat on oviposition of Callosobruchus maculates infesting cowpea seeds exposed 6 hours daily.

Exposure time (days)	HP Eggs	Uh Eggs	Total Eggs	Larval Penetration (%)	MDP (days)	Progeny No.	Adult Emrg(%)	Reduction (%)	%Adult mort/w
1	149.7±22.3b	15.3±4.3b	165.0±26.5b	91.1±1.2a	19.7±0.9a	64.3±6.7b	41.7±9.8a	29.4±5.0bc	60.0±5.7b
2	156.7±11.7b	27.3±7.3b	184±7.3b	87.3±6.7a	22.7±0.7a	55.0±4.7b	37.1±5.3a	35.4±9.2bc	70.0±11.5ab
3	66.0±6.5c	41.0±3.5b	107.0±9.3bc	63.6±4.7b	21.0±0.6a	45.0±6.4b	47.1±9.4a	23.0±1.5bc	90.0±5.8a
4	29.7±4.2cd	99.7±7.2a	129.3±2.1bc	21.5±9.9c	21.7±0.3a	17.7±6.1c	12.9±3.8b	77.5±11.5a	80.0±11.5ab
5	12.0±0.9d	57.3±5.2ab	69.3±3.8c	13.8±7.0c	15.3±3.7a	8.0±2.6c	9.2±4.7b	51.0±5.1ab	90.0±10.0a
Control	282.6±7.5a	34.4±5.2b	317.0±12.4a	89.3±1.2a	17.8±0.2a	18.0±10.3a	57.5±5.1a	0.0c	8.0±5.1c

HP=Hatched and penetrated eggs, UH=Unhatched and killed eggs, MDP=Mean developmental period (in days).

Some workers have investigated the effect of solar heating of stored pigeonpea seeds as a low cost control method (Chauhan and Ghaffan, 2002) and recommended its use on other legume crops as cowpea while Ntoukam *et al.*, 1997, developed a successful heater technique with a 50-kg capacity to eradicate and kill all developmental insect stages with of *C. maculatus* infested cowpea seeds. Solar heating method eliminated *C. maculatus* infestations and had no significant effect on cowpea germination rates (Paddock and Reinhard, 1999, Murdock and Shade, 1991) even temperature raised to 89 C. The solar heat could be a practical and useful method particularly for researchers in developing countries for conserving cowpea germplasm and breeding stocks as well as for farmers holding their small scale productions. The seed moisture content of Sun-dried cowpea seeds is reduced to 1.5 % after a 2-h solar heater treatment and this makes the seed storage more successful. Lale and Maina(2002) reported that no adult progeny of *Caryedon serratus developed* in groundnut seeds when infested with the different larval instars and exposed to direct solar heat for 6-h. As well, Lale and Vidal (2003) reported no *C. maculatus* and *C. subinnotatus* adults developed after exposure to 50 C in seeds harboring first instar larvae when exposed to 2, 4 and 6 hours. These workers found that solarization is cheap, safe and effective method for managing bruchids and weevil populations in seeds and cereals in parts of the tropical Africa and Asia (Kitch *et al.,* 1992, Chinwada and Giga, 1996, More *et al.,* 1996, Murdock *et al.*, 1997 and Lale and Ajyayi, 2001). The reduction of oviposition and adult emergence afforded by solarization might due to its effects on both adults and eggs, thus rendering the adults prefer to escape from heated seeds and lay fewer eggs as well as render most of the eggs unviable or dead and so no emerged adults compared to control.

From the present work, varying effects have been found on oviposition, larval growth and adult emergence. Solarization thus could offers a great prospect for successful protection of seed legumes against *C. maculatus* infestations and does not need any extra financial investment in equipment or technical know –how or part of subsistence farmers has been used it successfully. Thus sunning could be easily available in the Egypt as well as easily collected. It had been used by the Egyptian farmers in groundnut pods after harvest to reduce its moisture content and to be safe in storage and thus could be used as protection method of cowpea seeds to reduce this bruchid infestation and avoid the risks associated with the insecticides use.

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تاثير حرارة الشمس علي الأطوار الحشرية المختلفة لخنفساء بذور اللوبيا

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بذور اللوبيا تمثل أحد المصادر الأساسية للبروتين الغذائي النباتي للإنسان ، ويزرع المحصول ويحصد صيفا ، وتتعرض البذور بعد الحصاد وخلال تخزينها للإصابة بخنفساء اللوبيا *Callosobruchus maculatus* (F.). وقد أجريت دراسات أولية تحت الظروف الجوية المفتوحة بمصر خلال صيف ٢٠٠٨ لمعرفة التأثير البيولوجي لحرارة أشعة الشمس ضد الأطوار الحشرية المختلفة لحشرة خنفساء اللوبيا وذلك بتعريض البذور المصابة بأطوار الحشرة الأربعة(البيضة– يرقات حديثة الفقس – يرقات عمر أسبوعين– وعذاري حديثة التكوين) لأشعة الشمس المباشرة في أطباق بتري لمدة ٦ ساعات يومياعلي مدي ٤– ٥ أيام متتالية، حيث تراوحت درجات الحرارة اليومية بين ٣٢–٣٨ درجة مئوية بمتوسط ٣٧ درجة.

عند تعريض الحشرات الكاملة لأشعة الشمس المباشرة حدث زيادة في نسبة الموت وإنخفاض عدد البيض ونسبة الفقس والنمو اليرقى وخروج الحشرات الكاملة ومعدل الإنخفاض في عدد الذرية الخارجة (نسبة الحماية الناتجة وصلت ٧٧%) مقارنة بالكونترول.

وجد أيضا أن التأثير يعتمد علي الأطوار الحشرية وفترة التعريض للشمس. وكان طور البيض من أكثر الأطوارحساسية مقارنة بالأطوار الحشرية الأخري. حيث حدث إنخفاض معنوي فى كل من نسبة الفقس وإختراق اليرقات للبذور (وصلت ١٤.٤%) وعدد الحشرات الخارجة من معاملة بيض عمر ١– ٢ يوم بعد أربعة أيام من التعريض.

وعند معاملة اليرقات حديثة الفقس حدث إنخفاض معنوي في نسبة خروج الحشرات الكاملة مقارنة بالكونترول. أما اليرقات عمر أسبوعين والعذاري كانت أكثر مقاومة ولم تتأثر بزيادة فترة التعريض. وعند تعريض الحشرات الكاملة زادت نسبة موت الحشرات الكاملة (٩٠%) بعد خمسة ايام من التعريض ، بجانب إنخفاض معنوي في كل من عدد البيض الموضوع (١٢.٠ بيضة) مقارنة بحوالي ٢٨٣.٠ بالكونترول لكل زوجين بعد خمسة أيام من التعريض .كما أدت المعاملة إلي إنخفاض معنوي في نسبة خروج الحشرات الكاملة (٩.٢ %) مقارنة ب ٥٧.٥% بالكونترول، كما حدث إنخفاض في الذرية الناتجة (نسبةالحماية). ويمكن القول أن تعريض بذور اللوبيا لحرارة الشمس توفر حماية نسبية جيدة لبذور اللوبيا وتقلل من درجات الإصابة الحشرية، مقارنة بالكونترول الغير المعامل ولكنها لاتمنع الإصابة نهائيا.