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COMPARATIVE STUDY OF THE EFFECTS OF STEAM AND SOLAR HEAT TREATED COWPEA SEED ON THE DEVELOPMENT AND CONTROL OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

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ABSTRACT

Africa produces more cowpea (*Vigna unguiculata*) than any other continent but utilization in many countries is reduced due to seed destruction by the larvae of bruchids. The dried edible seeds of legumes are frequently attacked by beetles of the family Bruchidae. There are several genera of stored-product bruchids associated with a range of host plants. *Callosobruchus* species are the major bruchid pests of cowpea in Africa. *Callosobruchus maculatus* is the major pest of stored cowpea in Africa. Damage to cowpea seeds by *C. maculatus* during storage is widespread in Africa and constitutes a major constraint to food availability. Cowpeas damaged by *C. maculatus* have reduced weight, poor germinating ability and are unfit for human consumption, due to loss of vital nutrients such as vitamins and thiamine. It is during storage that cowpeas suffer heavy quantitative and qualitative losses from the attack by *C. maculatus*. Even though there are various methods of control of *C. maculatus*, some of the effective methods such as chemical insecticides pose environmental, social, financial and safety considerations in the tropics. There is need for alternative and less hazardous methods of control. Solar heating of cowpeas to control *C. maculatus* is one of the safe methods. However, steaming is thought to cause some physical modifications such as starch gelatinization and protein denaturation leading to a case hardening "effect" on the surface cell layers of the cotyledons and could therefore be used as alternative method of control. In the present study, the effects of steam treated, solar heat treated and untreated cowpea seeds on the development and control of *C. maculatus* were studied under ambient laboratory conditions (temperature range 28.0 - 30^{o}C and 62 - 74% RH). There was no significant difference (P> 0.05) in the number of eggs laid by *C. maculatus* under conditions 1 male: 1 female, 5 males: 5 females,12 males: 12 females on treated and untreated cowpea seeds. *C. maculatus* developed successfully in untreated and solar heat treated cowpea seeds, but could not develop in steam treated cowpea seeds. Thus, the novel method of steam treatment of cowpea seeds is a useful pest management strategy that can be used to prevent *C. maculatus* infestation of cowpea seeds meant for long-term storage and consumption since the cooking properties and processing qualities of the cowpea were not affected.

Key words: Bruchid, treatment, pest, *Callosobruchus*, biology

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INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. serves as an important dietary protein for many people in the tropics, especially in West Africa [1, 2, 3]. West Africa is the major producer of cowpeas in Africa [4]. In the field and during storage, cowpeas are attacked by *Callosobruchus maculatus* (F.), which is a major storage pest of legumes in the tropics and subtropics [5, 6, 7, 8]. Severe infestation of cowpeas by *C. maculatus* can result in losses in storage, ranging from 50 to 90 % annually throughout tropical Africa [9]. Approximately 50% loss of cowpeas while in storage for 3 or 4 months by *C. maculatus* has been reported [10]. The progeny of 8 mated females of *C. maculatus* caused 51% weight loss to 990 g of cowpeas during 3 months [11]. The control of *C. maculatus* by synthetic chemical insecticides is effective [1,12], but is limited in developing countries by environmental, social, financial and safety considerations [13]. This situation calls for alternative control methods [14, 15, 16].

The use of solar heating to control *C. maculatus* infestation has been reported [17, 18, 19], and as a result attention has been focused on different applications of heat to control *C. maculatus.* Steaming reduces the seed water absorption capacity. The microstructure of steamed cowpeas has been examined using scanning electron microscopy (SEM). The SEM revealed expanded and fused cotyledon surface cells that probably acted as the barrier to water uptake by the seed [20]. After steaming, an examination of the surfaces of the cotyledons showed marked differences in the surface topology when the seed coat was removed [21]. Steaming of cowpeas (physical modification by steam treatment) has been compared with groundnut oil and insecticide (pirimiphos-methyl) application for the control of *C. maculatus* [22]. While steaming offered no permanent protection, groundnut oil appeared to be nearly as effective as pirimiphos-methyl treatment [22]. However, in preliminary work, part of the USAID-funded CRSP-cowpea project (pers. comm.), it was found that steaming of dry cowpeas before drying to acceptable moisture levels effectively controlled bruchid weevil infestation. The steaming is thought to cause some physical modifications by starch gelatinization and protein denaturation leading to a case hardening “effect" on the surface cell layers of the cotyledons. Cowpea seeds steamed for 5 and 10 minutes were observed to be resistant to attack by *C. maculatus* and, therefore, this novel method of steam treatment of cowpeas could be used to control *C. maculatus*.

The present study compares the effect of steam and solar heat treated cowpeas on oviposition, developmental period, sex ratio, food preference and control of *C. maculatus* under ambient laboratory conditions.

MATERIALS AND METHODS

Steam and solar heat treatment

Cowpea seeds (*Vigna unguiculata*) were obtained from the agricultural farm of the University of Ghana, Legon. The seeds were divided into eight lots of 1.5 kg each. One lot was untreated, three lots were steamed (98^{o}C) without pressure for different lengths of time namely 5, 10, and 15 min, and dried in a solar drier (35 - 45°C) for 14 hours. Four lots were placed in a solar drier (35 - 45°C) and dried for 7, 14, 21 and 28 hours. The solar drier was constructed of plywood with dimensions of 122 cm/63 cm/18 cm in length/width/height, respectively with 4 spaced circular vents each 7.0 cm in diameter at the end of the frame and 14 x 1.2 cm diameter holes drilled at the ends of the cover. The base had 48 x 2.0 cm holes, 6 across the width and 8 lengthwise. Polyethylene sheet (0.07 mm in thickness) strengthened by nailing to long pieces of wood served as the cover.

Rearing of *C. maculatus*

Cultures of *C. maculatus* were established in glass culture cabinets (45 x 45 x 60 cm) using cowpea seeds (*V. unguiculata*) obtained locally from the Madina market in Accra. The cowpea seeds were initially sterilised in a hot-box Gallenkamp oven (range 0 - 200°C) at 60°C for 3 hours before usage. The weevils were collected from infested cowpeas in the same market. Each culture contained 200 randomly selected adult weevils per 600 g of cowpea per cabinet. All cultures and experimental jars were maintained at room temperature (range 28 - 31°C) and 62 - 74 % RH with alternating 12-h light and 12-h dark cycle. The procedures for maintaining cultures were similar to those previously described [23]. All equipment used in handling insects was dry-heat treated at 100°C for at least 3 h as a routine measure to prevent disease or cross infestation [24, 25].

Effect of cowpea seed treatments on oviposition, development and emergence of *C. maculatus*

Newly emerged and unmated *C. maculatus* adults (less than 24 hours old) from rearing cultures were paired and introduced into glass jars (6 cm diameter x 12 cm deep) containing untreated and treated cowpea seeds (steamed: 5 min, 10 min, 15 min; solar dry: 7h, 14h, 28h) at a pair (1 ♂= male: 1 ♀ = female) per 30 seeds (~ 6.0 g) per jar. A pair was chosen to give a comparative record of the fecundity (i.e. number of eggs laid per female) of *C. maculatus* on the treated and untreated seeds. Each jar was covered with a muslin cloth held in place by a rubber band. Five replicates were set-up for each treatment and control [14]. The jars were placed on a bench with supports immersed in engine oil to prevent insects from crawling into them [26, 27]. After 14 days, dead adults were removed with forceps and the eggs laid on the seeds in each jar were counted. The number of hatched eggs was also recorded. The set-ups were thereafter left until the emergence of new adults. The adults that emerged were removed and counted. The sex ratio of emerged adults was also determined. Male can be recognised from female by its antenna which is more serrate and last segment of pygidium. The pygidium of the male appears straight downwards from above. In the female, the pygidium protrudes more outwardly and has a narrow white vertical patch of hairs in the middle of fine black hairs in the flightless female forms and only fine white hairs in the flight forms.The procedure as outlined above was also followed using five paired *C. maculatus* adults (5 ♂♂: 5 ♀♀) per 40 g of cowpea seeds per replicate. Conditions of multiple infestations of cowpeas in markets and in stores are quite common in the tropics. Thus, this aspect of the study compared the effect of increased number of paired adults on a higher quantity of treated and untreated seeds. There were four replicates per treatment and control due to the

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number of adults introduced per replicate. Treatments were replicated as given earlier and arranged in a completely Randomised Design (CRD) and left on the laboratory bench undisturbed for daily observation until emergence of new adults. The number of emerged adults and daily mortality were recorded. Results were analysed using analysis of variance (ANOVA) and Student-Newman-Keuls method for pairwise comparison.

Oviposition preference studies

Glass cabinets (45 x 45 x 60 cm) with wooden base were used for the oviposition preference studies. Each cabinet contained 8 plastic Petri-dishes (9 cm dia.) arranged in a circle, with side separation of 3.5 cm and a radius of 10 cm from the centre of the base. One hundred of either steam or solar heat-treated cowpea seeds per Petri-dish together with a control were randomly arranged in the cabinet, based on the design explained earlier. Twelve pairs (12 ♂♂: 12 ♀♀) of newly emerged *C. maculatus* adults (less than 24 hours old) were released from the centre of the base of the cabinet and allowed to oviposit freely in the food of choice (preferred food). For the oviposition preference studies, four replicates were utilized due to the number of adults introduced. After 14 days, dead adults were removed with forceps and the number of eggs laid on the cowpeas was counted under a stereomicroscope (Wild M5). Females laid eggs on the cowpeas by gluing the eggs on to the outside of the cowpeas. The first instar larvae bored into the seeds and developed inside. Fourteen days was chosen for the counting of the eggs, based on the life span of the adult beetle, which is normally between one to two weeks; to avoid interference with the oviposition of the females and to simulate as far as possible the field situation where the insect can choose freely the preferred food commodity. The Petri dishes containing cowpeas were returned to their original positions after counting the eggs. The set-up was then left undisturbed until emergence of new adults. The number of newly emerged adults and the developmental period from egg to adult were determined from the data collected. Complete randomised design was used in all the experiments. The results were subjected to analysis of variance.

RESULTS

Table 1 shows adult weevil emergence and sex-ratios from untreated and treated (steam and solar) cowpea seeds initially infested (1♂: 1♀ per 30 seeds) with *C. maculatus*. The mean number of eggs laid by *C. maculatus* (1 ♂: 1 ♀) on steam/solar treated cowpea.The mean number of eggs laid by *C. maculatus* (1 ♂: 1 ♀) on steam/solar treated cowpea seeds is shown in Table 2. The mean number of eggs laid by *C. maculatus* (1 ♂: 1 ♀) was 46.4 ± 3.59 on untreated cowpea seeds, and ranged from 48.6 ± 2.38 to 59.0 ± 4.40 and from 47.8 ± 4.78 to 61.4 ± 9.57 on steam treated and solar heat treated cowpea seeds respectively (Table 2). There was no significant difference in the number of eggs laid on treated (steam and solar) and untreated cowpea seeds ($F_{(7, 32)} = 1.08$, $P > 0.05$, Table 2).

The results in Table 1 clearly demonstrated that *C. maculatus* could not develop in steam treated cowpea seeds as compared with solar heat treated and untreated cowpea seeds. Similar results (*C. maculatus* could not develop in steam treated cowpea seeds)

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were obtained from using 5 male: 5 female adult *C. maculatus* on treated (steam and solar) and untreated cowpea seeds. The sex ratio of emerged weevils was 1:1.6 on untreated cowpea and ranged from 1:1.1to 1:1.3 on solar treated cowpea seeds (Table 1).

The analysis of variance for the number of eggs laid by *C. maculatus* (5 ♂♂: 5 ♀♀) on treated (steam and solar) and untreated cowpea seeds did not show significant ($F_{(7, 24)}$=2.38, P> 0.05, Table 2) differences. The mean number of eggs laid by *C. maculatus* (5 ♂♂: 5 ♀♀) on untreated cowpea seeds was 258.5 ± 18.71, and ranged from 230.3 ± 11.96 to 248.8 ± 21.92 on steam treated cowpea seeds and from 243.8 ± 3.97 to 293.8 ± 15.88 on solar treated cowpea seeds (Table 2). Figure 1 shows the developmental period of *C. maculatus* (5 ♂♂: 5 ♀♀) on solar treated cowpea seeds. Similar observation was madeunder a paired condition (1 ♂: 1 ♀). The developmental period ranged from 20 to 34 days.The longevity of adult *C. maculatus* ranged from 3 – 12 days.

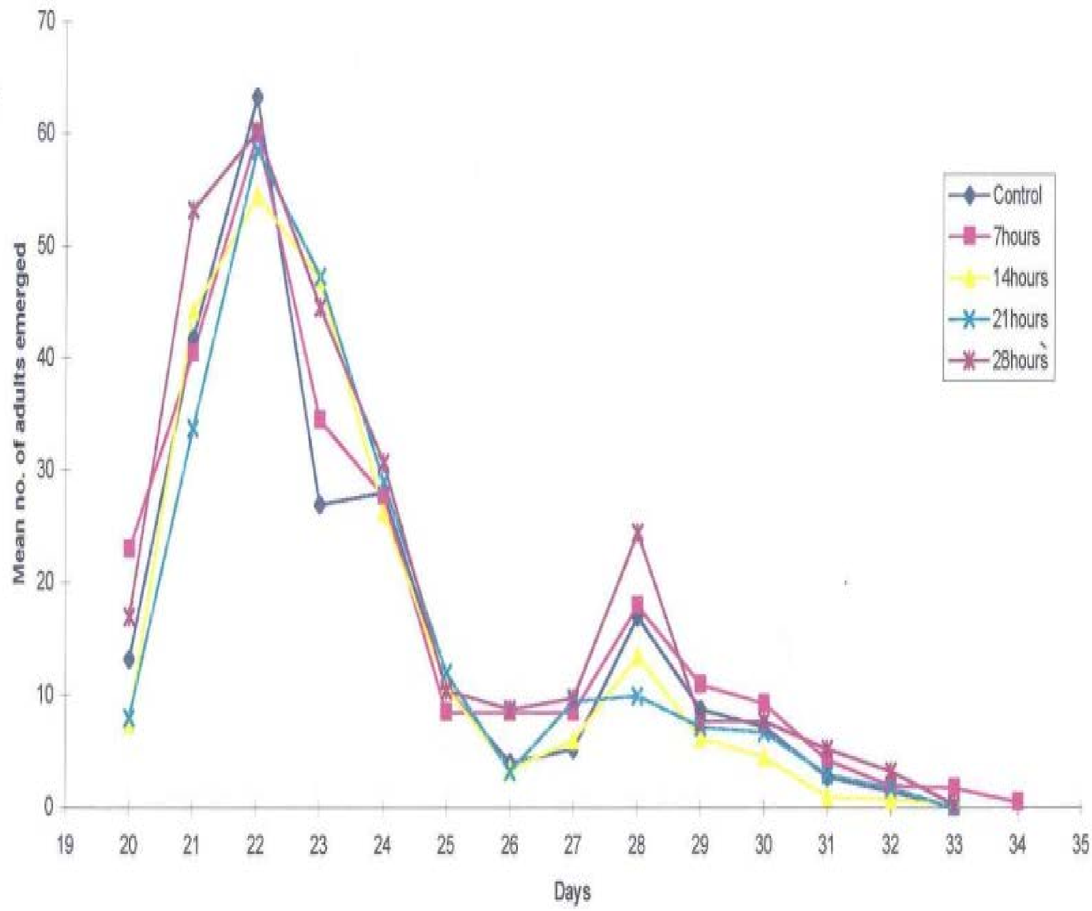

Figure 1: Dev. Period of *C. maculatus* (5 males: 5 females per 40g seeds) on solar treated cowpea

In the oviposition preference investigation using the glass cabinets, the outcome in terms of the number of eggs laid on treated and untreated cowpea seeds was similar to the results of the experiments given earlier. The analysis of variance for the number of eggs laid by *C. maculatus* (12 ♂♂: 12 ♀♀) on treated (steam and solar) and untreated cowpea seeds showed no significant difference ($F_{(7, 24)} = 1.0$, $P > 0.05$, Table 2). The mean number of eggs laid by *C. maculatus* (12 ♂♂: 12 ♀♀ in oviposition preference chamber) on untreated cowpea seeds was 99.8 ± 18.16 to 115.5 ± 23.04 on solar treated cowpea seeds. The developmental period ranged from 20 to 34 days as observed in the studies mentioned earlier (1 ♂: 1 ♀ and 5 ♂♂: 5 ♀♀). The emergence pattern of *C. maculatus* from untreated and solar heat treated cowpea seeds was similar. Figure 2 shows the emergence pattern of *C. maculatus* for solar treated cowpea from the oviposition preference studies. There was no emergence from the steam treated cowpea seeds. The untreated and treated (solar and steam) cowpea seeds from the experiment using 5 ♂♂: 5 ♀♀ weevils was placed under further observation for six months, yet there was no *C. maculatus* emergence from the steam treated seeds. The seeds of the untreated and solar heat treated cowpea were completely destroyed by the cowpea weevil, *C. maculatus* during this period.

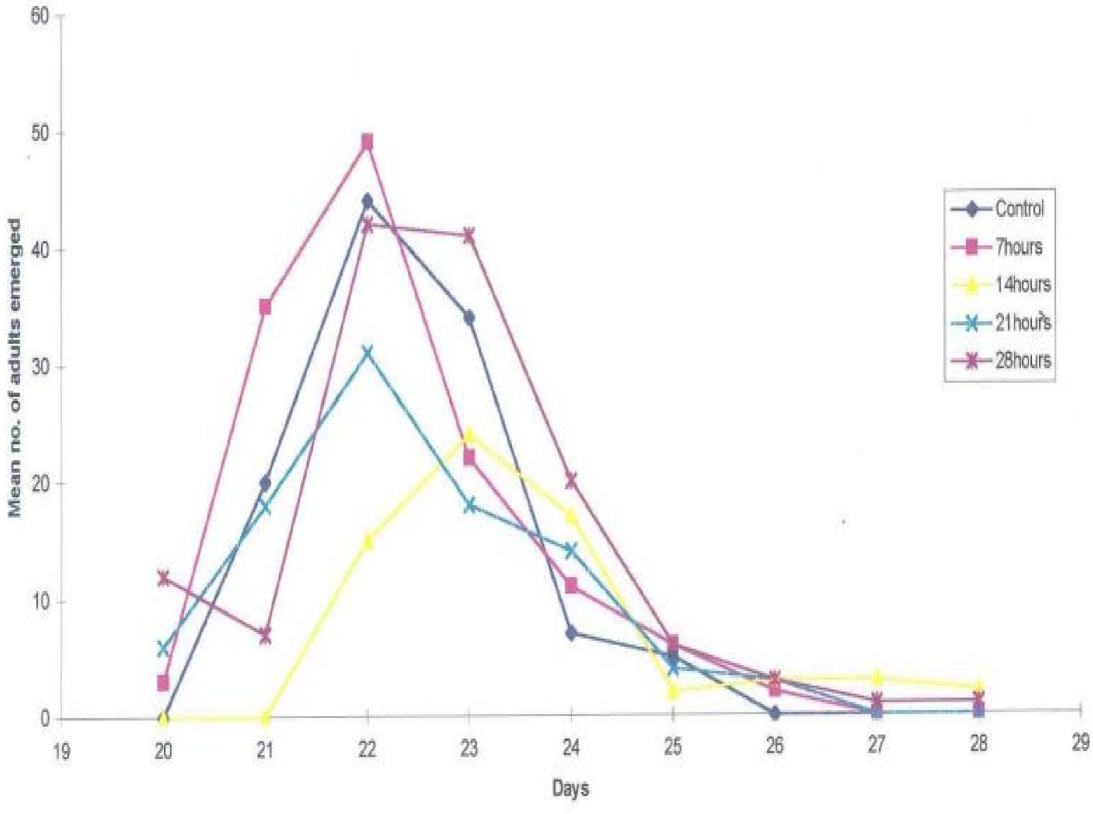

Figure 2: Emergence of *C. maculatus* from solar treated cowpea from oviposition studies

DISCUSSION

The present study showed that *C. maculatus* develops in untreated (control) and solar heat treated seeds, which leads to damage and loss of product but almost failed (only two emerged adults) to develop on steam treated seeds and hence causing less damage to the cowpeas. Thus, steam treatment of cowpeas is a valuable pest management strategy that can be used to prevent/reduce cowpea damage by *C. maculatus* during storage. The method would be most suitable for cowpeas intended for consumption and long storage and not necessary to be used as seeds.

Steaming of seeds reduces water absorption and therefore the cooking rate of cowpeas. The cooking properties and processing qualities of steamed cowpea seeds were not affected [28]. The effect of steaming of cowpea seeds on the functional properties of the flour has been reported [28]. Steaming increased flour water absorption capacity [28]. Dry cowpeas are processed into a wide variety of dishes, ranging from soups and snacks, to bean flour and baby foods [29]. These foods are of high protein value and a major post harvest constraint to cowpea utilisation is losses in storage due to infestation by *C. maculatus*. It is thought that the development of a hard shell as observed in SEM [28] provides a mechanism for the control of weevil infestation of stored cowpeas.

C. maculatus is an important pest of pulse crops in Africa and Asia under storage conditions. The larvae bore into the seed which become unsuitable, for human consumption, viability for replanting, or for production of sprouts [30]

Losses in weight due to infestation by one generation of *C. maculatus* on some pulses including cowpeas were found to be: 35% for lentils (*Lens esculenta* variety Giza 9), 20 % for black-eyed cowpea (*Vigna sinensis*), 8 % for mountain chick-peas (*Pisum sativum*, var. Little Marvel) and 4% for broad beans (*Vicia faba*, var. Giza 2) [31]. These losses can be prevented by using effective pest management strategies. There are many approaches to the control of *C. maculatus* in pulses, including chemical control, use of resistant varieties, plant materials [13, 32, 33, 34, 35].

In the present study, the adult *C. maculatus* emergence patterns from the untreated and solar heat treated cowpea seeds were similar and comparable to earlier studies [14]. However, even though C. *maculatus* oviposited comparatively equally on the untreated (control), solar heat and steam treated cowpeas, there was almost no adult emergence (1 emergence for 5 mins steam treated and 1 emergence for 15 mins team treated cowpeas) from the steam treated cowpeas. The developmental period ranged from 20 to 34 days. The sex ratio of emerged *C. maculatus* was 1:1.6 on untreated cowpea and ranged from 1:1.1 to 1:1.3 on solar treated cowpea seeds. The importance of the sex ratio can be explained in terms of the number of females which emerged from the untreated cowpeas. Females lay eggs on the cowpeas and hence if there are more females, the number of larvae will increase which will lead to more damage to the cowpeas as the larvae are the destructive stage. The adults of *C. maculatus* do not feed. The mean developmental period has been reported to be 23.7$\pm$ 10.02 (range 20-35 days) and the sex-ratio was 1.0:1.0. The number of eggs laid per female was

44.7±3.7 (range 7- 76). Egg hatchability was 96± 3.7. The mean adult longevity was 4.6 ± 0.1 days (range 1-12) [14]. Some workers have reported that oviposition by *Callosobruchus* spp. is affected by the plumpness or wrinkling of the seed-coat and perhaps by the size as well as its odour [36]. *C. maculatus* prefer cowpea varieties with smooth coated and well filled seeds to those with rough and wrinkled seeds [37, 38]. Many factors affect the number of eggs laid by females. For example, populations from different areas vary in their fecundity on the same number, species and cultivar host species [6], although others found minimal differences [7]. There are certainly major genetic differences among populations in the same environment [39]. In the present study, there was no significant difference in the oviposition of *C. maculatus* on untreated, steam treated and solar heat treated cowpeas. The treatments (steam, solar heat) did not affect the oviposition of *C. maculatus* on cowpea seeds. Steaming may not offer permanent protection for the farmer but may be an effective sanitation technique [22]. The present study has clearly demonstrated that steam treatment of cowpeas is a valuable pest management strategy that can be used against *C. maculatus*, to prevent/reduce damage to cowpeas meant for consumption and long storage.

CONCLUSION

Damage to cowpea seeds by *C. maculatus* during storage is widespread in Africa and constitutes a major constraint to food availability [40].Conservation of cowpea seeds to reduce losses due to bruchid infestation is very important in Africa. Thus, in order to provide and make available the important dieting protein in cowpea seeds to the people in the tropics who depend on it, the reduction or prevention of *C. maculatus* damage to cowpea seeds meant for consumption and long storage by steam treatment of cowpeas can be adapted.

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Table 1: Summary of adult *C. maculatus* emergence and sex- ratios from untreated (control) and treated (steam and solar heat) cowpeas (1 ♂: 1 ♀ per 30 seeds)

	Control		Steam treatment						Solar treatment							
			5 mins		10 mins		15 mins		7h		14h		21h		28h	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total (T)	81	131	0	1	0	0	1	0	126	157	116	122	97	110	105	136
Mean (x)	16.2	26.2							25.5	31.4	23.2	24.4	19.4	22	21	27.2
±S.E.	±2.2	±4.2							±4.6	±6.9	±2.6	±0.5	±3.1	±3.8	±4.4	±2.6
Sex ratio (♂: ♀) N=5	1:1.6		0:1		0:0		1:0		1:1.2		1:1.1		1:1.1		1:1.3	

Table 2: Mean no. of eggs laid by *C. maculatus* (1♂:1♀,5♂♂:5♀♀,12♂♂:12♀♀) on untreated and treated (steam and solar) cowpea seeds

Condition	*(1♂:1♀)* Mean no. of eggs*a ± S.E., N=5	*(5♂♂:5♀♀)* Mean no. of eggs*b ± S.E., N= 4	*(12♂♂:12♀♀)* Mean no. of eggs*c ± S.E., N= 4
Control	46.4± 3.59	258.5 ±18.71	99.8 ±12.45
Steam			
5 mins	52.4 ± 4.39	230.3 ± 11.96	146.8 ± 22.80
10 mins	59.0 ± 4.40	248.8 ± 21.92	123.0 ± 18.20
15 mins	48.6 ± 2.38	245.0 ± 15.61	151.8 ± 27.77
Solar			
7h	61.4 ± 9.57	293.8 ± 15.88	115 ± 23.04
14h	49.8 ± 3.01	239.8 ± 22.86	104.5 ± 4.57
21h	47.8 ± 4.87	243.8 ± 3.97	106.8 ± 30.21
28h	50.8 ± 5.96	303.5 ± 18.75	96.0 ± 18.16

*a Mean differences not significantly different ($F_{(7, 32)} = 1.08$, $P > 0.05$)
*b Mean differences not significantly different ($F_{(7, 24)} = 2.38$, $P > 0.05$)
*c Mean differences not significantly different ($F_{(7, 24)} = 1.0$, $P > 0.05$)

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