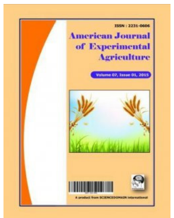

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Bioefficacy of Garlic, *Allium sativum* and Tobacco *Nicotiana tabacum* on Mortality, Ovipository Inhibition and Adult Emergence of the Cowpea Beetle *Callosobruchus maculatus* (Fab.) on Cowpea *Vigna unguiculata* (L.) (Walp.)

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Authors' contributions

This work was carried out by both authors. Authors PKB and MBM wrote the study protocol. Author MBM reviewed the experimental design and analyzed the data collected. Author PKB wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the efficacy of aqueous and ethanolic extracts of garlic and tobacco in the management of the cowpea beetle (*Callosobruchus maculatus*) and how these affect oviposition by adult females, adult emergence and sex ratio.
Study Design: The study was conducted in a randomised complete block design consisting of six treatments and three replications.
Place and Duration of Study: The experiment was carried out at the Biocontrol Section of Crops

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Research Institute, Kwadaso, Kumasi over a four month period.
Methodology: Aqueous and ethanolic extracts of garlic and tobacco leaves were prepared; these preparations were compared with a standard chemical insecticide, Betallic and a control. Four kilogrammes of untreated cowpea seeds were sorted to obtain whole uninfested grains. Six hundred of the sorted seeds were placed in 1L Kilner jars. Fifty millilitres of each extract was sprayed onto the seeds in their respective jars and allowed to dry. Ten pairs of adult *C. maculatus* were introduced into each jar. Similar set-ups were done with the insecticide and control seeds. Mortality was recorded every 6 hours after treatment for 24 hours. Data were also collected on oviposition, adult emergence and sex ratio.
Results: Garlic-treated seeds did not record any mortality within the first 18 hours; no mortality was recorded in the control seeds, whereas the insecticide-treated seeds recorded 100 % mortality. Significantly fewer eggs were laid on the treated seeds than the control seeds. Sex ratio of the emerged adults varied within the period of observation. Aqueous extracts of the botanicals performed better in terms of insect control and oviposition inhibition than the ethanolic extracts.
Conclusion: Aqueous extracts of both plants produced better results than the ethanolic extracts. Aqueous extract, being cheaper, easier and safer to prepare could be adopted by the average small-scale farmer to manage *C. maculatus*.

Keywords: Allicin; aqueous extract; insect culture; insecticide; oviposition; sex ratio.

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) (Walp.) is a leguminous crop and one of the most important crops cultivated in the tropics and subtropics. It is one of the most adapted and nutritious legumes [1] grown for human consumption and as feed for farm animals [2,3]. Being a leguminous crop, cultivation of cowpea enriches the nutrient content of the soil by fixing atmospheric nitrogen into nitrates and some vining varieties are excellent nitrates producers [4]. It can also be cultivated as a cover crop and as green manure to improve soil fertility and land conservation purposes [5]. The mature legume contains 23-25% protein, 30-67% carbohydrates, 1.9% fat, 6.35% fibre and small percentage of the B vitamins [6]. The crop is well adapted to the drier regions of West and Central Africa [7], from where it spread to other parts of the world. The nutritional qualities of cowpeas make them important component of the diets of many African countries where other protein sources such as fish and meat may not be readily available and affordable to the rural and urban poor due to their high cost.

In Africa, the yield of cowpea has been low due to a number of constraints such as low rainfall, poor seed quality and the incidence of diseases and pests. The incidence of pests on cowpea is due to its high nutritional qualities which make them attractive and highly susceptible to many insects which infest it, both on the field and during the storage period. In Ghana, the problem of poor seed quality has been addressed by the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) in collaboration with the International Institute of Tropical Agriculture (IITA). They have jointly released high-yielding cowpea varieties for cultivation by farmers. These varieties are high yielding and have varying degrees of resistance to some of the cowpea pests.

Attempts by African farmers to achieve higher yields have been foiled by field and storage pests [8]. The major storage pest of cowpea is *Callosobruchus maculatus* (Coleoptera: *Bruchidae*). This pest causes significant damage to the stored grains if not protected. Between 30 and 50% of cowpea grain can be lost during storage. Yield reductions caused by insect infestation can be as high as 95% [9]. In Northern Ghana, Golob, [10] observed that damage done by *C. maculatus* to stored cowpea varied from 15-94%. According to Brooker [11] a single *C. maculatus* can cause up to 3.5% weight loss in cowpea grains and up to 66% loss in protein content.

The larva of *C. maculatus* feeds on the seed of legumes, causing quantitative and qualitative losses to the farmer. The feeding activities by the larvae are manifested by seed perforation, reduction in weight and market value and viability of the seed to germinate when sown [12]. Infestation of cowpea seeds by *C. maculatus* occurs on the field, before harvest [13] and carried into storage. Females prefer laying eggs on mature green pods but will also oviposit on dry mature pods [14]. The length of time cowpea stays on the field before harvest can influence the level of infestation and subsequently the population of *C. maculatus* that will be carried into storage. According to Baidoo et al. [13], early harvested cowpea recorded lower infestation than late-harvested cowpea.

The destructive activities of *C. maculatus* require protection from this storage pest if the seeds are to be stored for longer periods. Synthetic insecticides have been used to control *C. maculatus* and other storage pests. There are a number of problems associated with the use of synthetic insecticides. Some of the insecticides are persistent [15] and each generation of insects become immune to the chemical insecticide leading to resistance in subsequent generations [16]. The most important negative effect to the use of chemical insecticides on storage products is food contamination as a result of indiscriminate use of these insecticides. Thus Mellor and Adams [17] stated that in many developing countries, improper use of pesticides by untrained workers often led to poisoning during application. They therefore concluded that human poisoning is the highest price paid for using pesticides.

Due to the negative effects of eating contaminated food on the health of humans, other more sustainable and healthy means of controlling storage pests must be adopted to reduce the possibility of food contamination. The use of botanical insecticides has been suggested as an alternative to chemical insecticides. Plants from many different families exhibit biological activities against insect pests [18,19]. These naturally-occurring compounds can affect the physiology of insects, kill insects or cause loss in fecundity and viability of eggs [20]. Botanical pesticides have low persistence, are less toxic to non-target organisms [21] and have low mammalian toxicity. Two of such plants with insecticidal properties are garlic, *Allium sativum* and tobacco, *Nicotiana tabacum*. Garlic produces various sulphur compounds that together with their breakdown products yield a characteristic pungent taste and odour. These compounds have antimicrobial and antifungal effects [22]. Allicin, which is derived from garlic combats fungal infections and parasites, lowers blood cholesterol and treats arteriosclerosis and promotes circulatory functions in humans [22]; however, its insecticidal and repellent properties have not been fully exploited.

Tobacco, *Nicotiana tabacum*, has been used as an important insecticide and insect repellent since the 17th century [23]. Tobacco contains many phytochemicals such as nicotine, nornicotine, anabasine, glucosides, acrolein and pyrene. Even though tobacco contains many chemicals, nicotine is the principal toxic component. It is a quick-acting insecticide which acts as stomach poison and is an important chemical defence mechanism of the plant [24]. In order to store cowpea for longer periods, they must be protected from *C. maculatus* infestation. The most widely used insecticide for protecting cowpea seeds are methyl bromide and phosphine; however, currently there is restriction on the use of phosphine because this chemical has ozone depletion potential [25] as well as reported cases of resistance in some stored products across the world [26]. Therefore there is the need to develop safe, environmentally friendly and sustainable means to protect stored products from pest infestation. The population of *C. maculatus* at any time depends on the number of eggs laid by the females of the previous generation. Therefore any control measure that will reduce oviposition by female *C. maculatus* will ultimately reduce the population of this pest. The current study evaluated the efficacy of aqueous and ethanolic extracts of garlic and tobacco in managing the cowpea beetle and how the botanicals affected oviposition by females, adult emergence and sex ratio.

2. MATERIALS AND METHODS

2.1 Culturing of *C. maculatus*

Five hundred grams of cowpea (*Vigna unguiculata* var. Asontem) seeds were each placed in 6 1L Kilner jars and covered with muslin cloth. Fifty adult *C. maculatus* were introduced into each jar, covered with muslin cloth and secured with a rubber band. The set-ups were allowed to stand for 48 hours to allow mating and oviposition, after which the insects were sieved out. The seeds were placed backed in the Kilner jars and left standing for 21 days to allow for hatching and adult emergence.

2.2 Preparation of Plant Extracts

Thirty (30) grams of garlic was weighed and blended with 200 ml distilled water. The resulting mixture was diluted with water to a volume of 500 ml and stored in a dark cup board for 24 hours. The same procedure was employed with the ethanolic extract, using ethanol as the solvent. After the storage period, the extracts were filtered using a fine muslin cloth and then through a Whatmann No. 1 filter paper.

Dried tobacco leaves were purchased from the local market. Fifty grams of it was weighed and 500 ml of distilled water was added. The same quantity of tobacco leaves was used for the ethanolic extract using the same quantity of ethanol. The set-ups were left for 24 hours after which they were filtered following the steps outlined above.

2.3 Experimental Set-up

Four kilogrammes of untreated cowpea seeds (var. Asontem) were sorted to obtain whole, uninfested seeds. The seeds were disinfested by keeping them in the freezer for 24 hours. The seeds were conditioned to room temperature before being used for the experiment. Six hundred of the sorted seeds were placed in 1L Kilner jar, covered with muslin cloth and secured with a rubber band. There were six treatments, each of which was replicated 3 times. The treatments were: garlic in water (GW), garlic in alcohol (GA), tobacco in water (TW) tobacco in alcohol (TA), Betallic insecticide (a.i Permethrin and Pirimiphos-methyl) and a control set-up in which there was no control measure. Fifty millilitres of each extracts were sprayed onto the seeds in their respective jars and allowed to dry before the insects were introduced into the set-ups. Betallic was applied at a rate of 5 ml/litre. In the control set-up, the seeds were sprayed with the same quantity of distilled water. Ten pairs of adult *C. maculatus* were introduced into each jar and left for 24 hours.

2.4 Data Collection

After 24 hours, the numbers of *C. maculatus* killed in each treatment were recorded after sieving the seeds to recover the beetles. The seeds were placed in their respective jars. The numbers of eggs deposited on 100 seeds were counted daily for 4 days and the means were calculated. Counting of the eggs was done with the aid of a powerful hand lens. The seeds were kept for 25 days after infestation; then the numbers of adults that emerged from each treatment were counted daily until no more adults emerged. Adults that emerged were separated into their various sexes to determine the sex ratio.

2.4.1 Data analysis

Data obtained were subjected to the General Linear Model (GLM) procedure of SAS [27]. Analysis of variance was done on the parameters studied. Where the difference was significant, the means were separated using the Student Neuman's Keul's (SNK) test. Chi square test was used to determine any significant difference in sex ratio. Significant difference was set at $P \leq 0.05$.

3. RESULTS

3.1 Mortality of *C. maculatus*

The percentage of adult *C. maculatus* that died 24 hours after the application of the control measures are shown in Table 1. With regards to garlic-treated seeds, no mortality was recorded within the first 18 hours after application. Mortality after 24 hours was 2.50% and 7.50% for ethanol and aqueous extracts respectively. Similarly tobacco extracts recorded low mortalities. In the case of Betallic treatment, 57.75% mortality occurred within the first 6 hours of application; all the beetles were dead after the 24 hour period. The control set-up did not record any mortality at all.

Table 1. Percent mortality and egg oviposition by *C. maculatus* on cowpea at 24 hours

Treatment	Mortality %	Oviposition
Betallic	100.00^{a}	$14.75^{a} \pm 4.82$
Garlic in water	7.50^{b}	$82.34^{c} \pm 34.50$
Garlic in alcohol	2.50^{c}	$41.25^{b} \pm 3.99$
Tobacco in water	12.50^{b}	$44.00^{b} \pm 8.22$
Tobacco in alcohol	1.50^{c}	$61.25^{b} \pm 19.88$
Control	0.00^{c}	$112.25^{c} \pm 24.24$

Within columns, means with same letter are not significantly different (P> 0.05)

3.2 Adult Emergence from Cowpea Seeds

Most of the adult beetles that emerged from the seeds were recorded on the first day. Very few adults emerged from day 2 to 4. The mean number of adults counted on day 1 ranged from 120.5 in the untreated (control) seeds to 18.25 in the seeds treated with aqueous tobacco extract. No adult beetles were recorded from the Betallic-treated seeds (Table 2). The differences in adult emergence on day 1 were significant (P= 0.0001). Adult emergence from the aqueous garlic and ethanolic extracts of garlic did not differ significantly. Similarly adult emergence from the two tobacco treatments was not significantly different; both however, differed significantly from the garlic–treated seeds. Apart from the 2nd day during which the untreated seeds recorded significantly larger number of emerged adults, the subsequent days recorded fewer adults, the numbers of which were not significantly different.

3.3 Egg Deposition on Cowpea Seeds

Egg deposition on the treated cowpea seeds varied with regards to the control agent used. The largest number of eggs was deposited on the untreated seeds, whilst the insecticide-treated seeds recorded the least egg deposition (Fig. 1). The numbers of eggs deposited on the different treated seeds differed significantly (P= 0.035). Further analysis of the numbers of eggs deposited on the seeds indicated no significant differences among all the seeds treated with the botanicals, but all differed from the untreated control seeds. Significantly fewer eggs were laid on the insecticide-treated seeds.

3.4 Sex Ratio of Emerged Adults

Mean distribution of males and females is represented in Table 3. Analysis of data using Kruskel-Wallis (Chi square) test showed that on day 1, male distribution recorded a P- value of 0.004, indicating a deviation from the expected 50%, while females recorded a P value of 0.095, which was not significantly different from the expected number. On day 2, the number males recorded varied significantly from the expected; females did not vary significantly and so were closer to the expected 50%. On day 3, the calculated P-values for both sexes did not deviate significantly from the expected 50%. However, the P-value recorded for the males indicated significant deviation from the expected 50%.

Table 2. Effects of treatment of cowpea seeds on adult emergence over a 4-day period

Days	Treatments					
	GA	GW	TW	TA	Betallic	Control
1	73.25^a	58.50^a	18.25^b	37.25^b	0.00	120.50^c
2	3.25^a	1.50^a	1.25^a	4.25^a	0.50^a	7.50^b
3	1.50^a	1.50^a	0.50^a	0.75^a	0.00	2.75^a
4	1.50^a	0.50^a	0.25^a	0.00^a	0.00	2.01^a

Within rows, means with the same letters are not significantly different (P> 0.05)

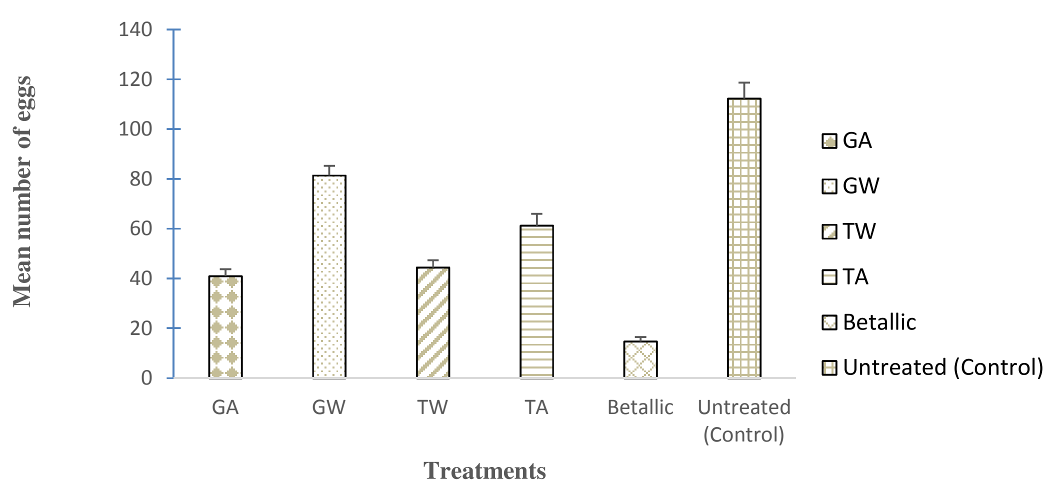

Fig. 1. Mean numbers of eggs counted per 100 cowpea seeds

Table 3. Mean sex ratio of adult *C. maculatus*

Treatments	Days							
	1		2		3		4	
	M	F	M	F	M	F	M	F
Garlic in water	9.25	12.00	1.25	2.00	0.50	1.00	1.00	0.75
Garlic in alcohol	8.75	10.75	0.50	1.00	0.75	0.75	0.00	0.50
Tobacco in water	2.75	8.50	0.75	0.50	0.25	0.25	0.00	0.00
Tobacco in alcohol	8.50	11.44	2.00	2.25	0.50	0.25	0.00	0.00
Betallic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	9.75	10.25	4.25	3.75	1.50	1.25	0.75	1.25
Total	39.00	52.94	8.75	9.50	3.50	3.50	1.75	2.50
P-value	0.00	0.10	0.05	0.11	0.63	0.36	0.04	0.20
Chi-square	17.19	9.35	11.18	8.90	3.50	5.44	11.73	7.32

**M= male, F= female*

4. DISCUSSION

In Ghana and other parts of Africa, harvested cowpea is attacked by the storage beetle, *Callosobruchus maculatus*. Infestation of cowpea seeds by this pest occurs on the field and carried into storage. Infestation of the seeds in subsequent generations depends on the population of the previous generation and the extent of egg deposition by adult females. During the study, it was observed that the use of the botanicals could not result in significant mortalities within the 24 hour period compared with the insecticide-treated seeds. Thus in terms of insecticidal activity both garlic and tobacco did not produce any significant effect against *C. maculatus*. The insecticidal property of any plant depends on the chemical component responsible for mortality. Garlic has been reported to contain several chemicals, including diallyl disulphide and diallyl trisulphide, which have been shown to cause mortality in mosquito larvae as reported by Olkowski et al. [28]. The killing effect of the botanicals was, however, not as quick as the chemical insecticide because in some of the plant preparations, mortality was observed only after 24 hours of exposure, whereas a very high mortality was recorded only after 6 hours in the Betallic-treated seeds. In a study by Wekesa et al. [29], they observed that the rate of mortality of *Sitophilus zeamais* increased with concentration and duration of exposure to essential oils of *Hyptis spicigera* and that total mortality was observed within 48 hours of exposure. The observed low action of the botanicals could be attributed to their inability to quickly enter the insect's internal tissue. These botanicals have to be ingested for effective action in susceptible insects. Death would therefore result only after several days of ingestion of these botanicals. There is therefore a delayed effect between ingestion and mortality. Report by Adedire and Ajayi [30] showed that garlic powder and oil may be toxic to *C. maculatus* due to the strong choking odour it produces which disrupts normal respiratory activities of susceptible insects leading to asphyxiation and death. Reports by other workers indicate similar effects of botanicals in the control of the maize weevil, *Sitophilus zeamais* [29,31].

Tobacco has been used as an insect control agent for many years. Even though the plant contains many chemicals, nicotine has been identified as the principal toxic component [24]. Tobacco possesses contact, stomach and respiratory poisoning properties which have been attributed to the presence of nicotine in the plant [32]. Toxicity of susceptible insects to nicotine is achieved through fumigation or direct contact [33]. Being a contact poison, it enters the body through areas such as the tracheal system.

With regard to ovipository inhibition by adult females, seeds treated with ethanolic extract of garlic recorded the least number of eggs among the different plant extracts. The untreated recorded by far the largest number of eggs because these had no control agents to hinder the activities of the insects. The fewer eggs laid on the treated seeds compared with the untreated control seeds was the results of relatively higher mortality of adult *C. maculatus* as well as the repellent properties of the plant materials that reduced sexual communication between the surviving adults [34]. A reduction in contact between the sexes led to reduced mating behaviour and consequently fewer eggs laid. Therefore, even though the use of these botanicals may not entirely reduce the population of insect pests below the economic damaging levels, surviving adults will produce fewer eggs which will ultimately reduce the population of insects of the next generation.

With respect to different treatments of the cowpea seeds on adult emergence, it was observed that the insecticide-treated seeds recorded the least number of emerged adults. In the case of the plant extracts, aqueous preparations of both plants performed better at reducing adult emergence than the ethanolic preparations. This could be attributed to a reduction in potency of the active ingredient when alcohol is used as the solvent for extraction. Earlier report by Abiodun [35] showed that aqueous extract of garlic was more toxic to *S. zeamais* than the ethanolic extract. This was attributed to the alkyl compounds in the Alliaceae family which are readily obtained by distillation with water than with ethanol. It appears that the effectiveness of garlic and tobacco as pest control agents increases with increasing polarity of the solvent used for extraction. Water, being more polar than ethanol was therefore able to extract more of the active ingredients, which accounted for the better results that were obtained. The effect of tobacco on susceptible insects are said to be ovicidal and inhibitory of larval instar development or both [36]. Boateng and Kusi [37] showed that jatropha seed oil was highly toxic to the eggs of *C. maculatus*, resulting in significant reduction in adult emergence. Ovicidal and larvicidal properties of the tested plant extracts probably killed some of the eggs and also prevented some of those that hatched into larvae from developing into pupae and subsequently adults. The principal component of tobacco is nicotine, and to some extent nornicotine and anabisine. These, according to Isman [21] are synaptic poisons that mimic the neurotransmitter acetylcholine and cause symptoms of poisoning similar to those produced by organophosphate and carbamate insecticides.

5. CONCLUSION

Plants with insecticidal properties have been used to manage both field and storage pests. The results of the study showed that botanicals have the potential to replace the use of synthetic insecticides in the management of pests of crops. Even though the use of garlic and tobacco could not completely eliminate *C. maculatus* from the cowpea seeds, surviving female adults produced fewer eggs. Aqueous extracts of both plants produced better results than the ethanolic extracts. Aqueous extract, being easier and safer to prepare could be adopted by the average small-scale farmer to manage pests. The use of botanicals on storage products could be adopted to reduce the incidence of food contamination and its negative effects on the health of man which are associated with the use of synthetic insecticides on stored products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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