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FUMIGANT TOXICITY OF CRUSHED BULBS OF TWO *ALLIUM* SPECIES TO *Callosobruchus maculatus* (Fabricius) (COLEOPTERA: BRUCHIDAE)

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ABSTRACT

Fumigant toxicity of crushed fresh bulbs of *Allium sativum* L. and *A. cepa* L. to the *Callosobruchus maculatus* (Fabricius), a major pest of stored cowpea (*Vigna unguiculata* (L.) Walp.) seeds was assessed under laboratory conditions in Akure, Nigeria. In the tests, 20 g of infested cowpea seeds were suspended in a piece of muslin cloth, over an amount of crushed bulb in a container with a tightly fitted lid. Adult emergence was completely prevented from freshly laid eggs of *C. maculatus* on cowpea seeds that was fumigated with 6.0 g or more of crushed bulbs of *A. sativum*. Such fumigated seeds were not holed at all. Other amounts of *A. sativum* tested (1.0, 2.0, 3.0, 4.0 and 5.0 g) significantly reduced *C. maculatus* adult emergence from fumigated eggs and seed holing in comparison with the control. Crushed *A. sativum* was ineffective in preventing adult emergence from fumigated *C. maculatus* larvae in seeds. The fumigant effect of crushed *A. cepa* did not kill all *C. maculatus* eggs. An amount of 7.0 g significantly reduced *C. maculatus* adult emergence from fumigated eggs and seed holing in comparison with the control. There is good prospect in using crushed bulbs of *A. sativum* as fumigant in *C. maculatus* control in stored cowpea seeds.

Key words: Infestation, seed holing, pesticide, volatiles, essential oils.

INTRODUCTION

The cowpea storage beetle, *Callosobruchus maculatus* (Fabricius), is perhaps the most damaging insect that attacks stored cowpea (*Vigna unguiculata* (L.) Walp.) seeds in the tropical and subtropical regions of the world (Ofuya, 2001; 2003). One or more larvae may feed exclusively inside individual seeds and adult beetles emerge through circular exit holes. Severely damaged cowpea seeds are therefore riddled with adult exit holes and have reduced weight, poor food value and low viability. Cowpea seeds that are damaged by *C. maculatus* also command poor market prices. Often, after 6 mo storage, 100% seed infestation may be recorded. Cowpea seeds constitute the major source of protein in human nutrition in many African countries, and have sometimes been described as "poor man's meal" (Aykroyd *et al*., 1982). The optimal utilization of this protein-rich staple needs adequate protection from the cowpea storage beetle. Fumigation by application of synthetic chemical fumigants such as methyl bromide and aluminium phosphate is perhaps the most effective method of stored products protection against insect depredation (Lale, 2002). However, use of chemical fumigants in stored products protection is being phased out worldwide because of their adverse effects to the environment which includes ozone depletion (WMO, 1995) and the development of insect pest resistance (Zettler *et al*., 1989). Thus, there is an urgent need to develop new fumigants for post-harvest pest control that are safe, of low cost, convenient to use and environment friendly (Zettler *et al*., 1997; Papachristos and Stamopolos, 2002). Essential or volatile oils extracted from plants have been shown to possess good potential for use as fumigants against stored product insects including storage bruchids (Don-Pedro, 1996; Shaaya *et al*., 1997; Papachristos and Stamopolos, 2002; Tapondjou *et al*., 2002). Less refined plant materials such as crushed plant materials, if found to be effective, may be more affordable by poor farmers in developing countries. This study investigated fumigant toxicity of crushed fresh bulbs of *Allium sativum* L. and *A. cepa* L. to *C. maculatus*.

MATERIALS AND METHODS

C. maculatus culture and experimental conditions. The *C. maculatus* used was derived from a colony originated

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from infested cowpea seeds collected from a local market in Akure, Ondo State, Nigeria. The colony was maintained in Kilner jars of 250 mL capacity in an open laboratory at ambient temperature of 28 ± 3 °C and 70 ± 5% relative humidity for more than 60 generations, using Ife Brown cowpea as substrate. All experiments were carried out in the laboratory.

Preparation of crushed *Allium* species. The species used were fresh *A. sativum* and *A. cepa* chopped into various sizes to obtained different weights. Bulbs were purchased in local herbal stores in markets in Akure, Ondo State, Nigeria. Their identities were confirmed at The Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The bulbs were washed, chopped and crushed in a kitchen blender. The crushed bulbs of each species were kept in a plastic container with tightly fitted lid and kept in a freezer maintained at 0 ºC. They were used within 1 mo of preparation.

Fumigant toxicity of crushed *Allium* species to *C. maculatus*. Fumigant effect of *A. sativum* and *A. cepa* on freshly laid eggs (< 24 h), 3-d old eggs and larvae of *C. maculatus* was determined. Each species was tested at 7.0 g against 400 eggs or larvae of the beetle contained in 20 g of seed of Ife Brown cowpea at 70 ± 5% relative humidity. The selected rate of application has been found to be effective for reducing hatchability of eggs of some cotton insect pests (Gurusubramanian and Krishna, 1996). Infested cowpea seeds were suspended in a piece of muslin cloth, over the quantity of crushed bulbs in a plastic container. The container lid was properly screwed-up to hold the muslin cloth and the cowpea seeds in space, and also make the set up as airtight as possible. There was a control with no crushed bulbs in the container. All treatments were replicated three times during each experiment. Number of adults that emerged from treated eggs and larvae was counted. Number of seeds bearing adult exit holes was also counted. In another experiment, the same crushed bulbs of *A. sativum*, which had been used to fumigate *C. maculatus* eggs, were immediately reused to fumigate 20 g of seed bearing 400 *C. maculatus* freshly laid eggs as described previously. Number of adults that emerged from the second set of treated eggs was similarly counted. Number of seeds bearing adult exit holes was also counted.

Fumigant toxicity of different rates of crushed *A. sativum* to *C. maculatus*. Fumigant effect of different rates of application of crushed bulbs of *A. sativum* on freshly laid eggs (< 24 h) was determined. The different rates were 1.0, 2.0, 3.0, 4.0, 5.0, and. 6.0 g against 400 eggs contained in 20 g of seed. The experimental set up for each rate of application and data collected were as described previously.

Data analysis. Data collected were subjected to ANOVA. Percentages were arcsine transformed before analysis at $P < 0.05$. The standard error (SE) of means which determines the level of accuracy of the means and the Least Significant Difference (LSD) that separates the means to determine if there were significant differences were also determined.

RESULTS

Adults of *C. maculatus* did not emerge from freshly laid eggs fumigated with 7.0 g of crushed bulbs of *A. sativum* (Table 1). Percentage *C. maculatus* adult emergence was significantly lower from freshly laid eggs fumigated with 7.0 g of crushed bulbs of *A. cepa* than the control treatment. Seeds bearing freshly laid eggs and fumigated with 7.0 g of crushed bulbs of *A. sativum* were not holed at all after the period for normal adult emergence. Percentage of seeds holed by *C. maculatus* was significantly lower in those fumigated with 7.0 g of crushed bulbs of *A. cepa* than the control. Percentage *C. maculatus* adult emergence from freshly laid eggs on seeds in the treatment involving the immediate reuse of the crushed bulbs of *A. sativum* was significantly lower ($P < 0.05$) than in the control treatment (Table 2). Percentage of seeds with holes was

Table 1. Parameters measured in 20 g cowpea seeds bearing 400 freshly laid eggs of *Callosobruchus maculatus* fumigated with 7.0 g of crushed bulbs of two *Allium* species.

Fumigant	Adult emergence from fumigated *C. maculatus* eggs ± SE	Seeds with holes ± SE
	%	%
Control (no fumigant)	52.1 ± 1.11	55.9 ± 4.03
A. cepa	39.3 ± 1.02	39.2 ± 3.49
A. sativum	0.0 ± 0.00	0.0 ± 0.00
LSD (0.05)	2.38	8.45

SE: standard error; LSD: Least significant difference.

Table 2. Parameters measured in 20 g cowpea seeds bearing 400 freshly laid eggs of *Callosobruchus maculatus* fumigated with 7.0 g of previously used crushed bulbs of *Allium sativum*.

Fumigant	Adult emergence from fumigated *C. maculatus* eggs ± SE	Seeds with holes ± SE
	%	
Reused *A. sativum*	40.5 ± 2.51	56.9 ± 1.22
Control (without fumigant)	52.8 ± 1.03	55.0 ± 1.48
P*	< 0.05	> 0.05

*By ANOVA; SE: standard error.

not significantly different (P > 0.05) between treatment involving the immediate reuse of the crushed bulbs of *A. sativum* and the control. Percentage *C. maculatus* adult emergence was significantly lower from 3-d old eggs that were fumigated with 7.0 g of crushed bulbs of *A. sativum* than the control treatment (Table 3). Seeds bearing 3-d old eggs and fumigated with 7.0 g of crushed bulbs of *A. sativum* were not holed at all after the period for normal adult emergence, during which time 50.6% of the seeds in the control were holed.

Percentage *C. maculatus* adult emergence and percentage of seeds with holes were not significantly different between seeds bearing 10-d old larvae that were fumigated with 7.0 g of crushed bulbs of *A. sativum* and those in the control treatment (Table 4).

Irrespective of rate of application, percentage *C. maculatus* adult emergence and percentage of seeds with holes were significantly lower in seeds bearing freshly laid eggs that were fumigated with crushed bulbs of *A. sativum* than in the control treatment (Table 5). No adults of *C. maculatus* emerged from, nor exit holes in seeds bearing freshly laid eggs that were fumigated with 6.0 g of crushed bulbs of *A. sativum*. Percentage *C. maculatus* adult emergence and percentage of seeds with holes were significantly lower in seeds bearing freshly laid eggs fumigated with 4.0 and 5.0 g of crushed bulbs of *A. sativum* than in the treatments involving fumigation with lower amounts of the spice.

DISCUSSION

The findings in this study show clearly that bulbs of *A. cepa* and *A. sativum* exhibit insect controlling properties. Ofuya (1986) reported that crushed *A. cepa* bulb scale leaves reduced cowpea seed damage by *C. maculatus* through inhibition of oviposition and adult emergence, which is largely consistent with findings in this study. *A. sativum* was more effective in the control of *C. maculatus*

Table 3. Parameters measured in 20 g cowpea seeds bearing 400 3-d old eggs of *Callosobruchus maculatus* fumigated with 7.0 g of crushed bulbs of *Allium sativum*.

Fumigant	Adult emergence from fumigated *C. maculatus* eggs ± SE	Seeds with holes ± SE
	%	
A. sativum	0.0 ± 0.00	0.0 ± 0.00
Control (without fumigant)	49.5 ± 0.91	50.6 ± 1.39
P*	< 0.05	< 0.05

*By ANOVA; SE: standard error.

Table 4. Parameters measured in 20 g of cowpea seeds bearing 400 10-d old larvae of *Callosobruchus maculatus* fumigated with 7.0 g of crushed bulbs of *Allium sativum*.

Fumigant	Adult emergence from fumigated *C. maculatus* eggs ± SE	Seeds with holes ± SE
	%	
A. sativum	48.1 ± 1.07	58.8 ± 2.53
Control (without fumigant)	50.6 ± 2.36	60.6 ± 2.54
P*	> 0.05	> 0.05

*By ANOVA; SE: standard error.

Table 5. Parameters measured in 20 g cowpea seeds bearing 400 freshly laid eggs of *Callosobruchus maculatus* fumigated with different amounts of crushed *Allium sativum*.

Amount of *A. sativum* per cowpea seed	Adult emergence from fumigated *C. maculatus* eggs ± SE	Seeds with holes ± SE
g per 20 g	%	%
1.0	33.7 ± 0.79	51.8 ± 2.23
2.0	30.5 ± 0.66	49.9 ± 1.45
3.0	14.2 ± 1.05	21.6 ± 0.98
4.0	8.4 ± 1.26	11.9 ± 1.67
5.0	8.6 ± 0.80	13.1 ± 1.85
6.0	0.0 ± 0.00	0.0 ± 0.00
Control (without fumigant)	53.0 ± 2.05	58.0 ± 1.36
LSD (0.05)	2.75	3.77

SE: standard error; LSD: Least significant difference.

than *A. cepa*. Many chemical pesticide components which have been unravelled among *Allium* species include allicin, hydrocyanic acid, oxalic acid, pyrogallol, quercitin and saponin (Dales, 1996). Some of the volatile chemical ingredients present in volatiles of *A. sativum* are allicin, thioacrolein, ajoene, 2-propene sulfenic acid, 2-propene thiol and propylene (Jain and Apitz-Castro, 1993; Gurusubramanian and Krishna, 1996). Since there is no contact between the crushed bulbs and the insect pests, the mortality and subsequent reduction in percentage adult emergence could be presumably due to the diffusion of the volatile chemicals contained in the bulbs which might have affected vital physiological and biochemical processes associated with embryonic development, which were consequently disrupted. Gurusubramanian and Krishna (1996) have also reported that exposure of freshly laid eggs (< 24 h old) of *Earis vitella* Fabricius and *Dysdercus koenigii* (Fabricius) to volatiles from bulbs of *A. sativum* significantly reduced their hatchability.

The observed effective application rate of crushed bulbs of *A. sativum* (30% or more of protected seed weight) for fumigation against *C. maculatus*, is certainly on the high side, and may not be economical. Shaaya *et al*. (1997) observed that fumigant activity of the toxic vapour from Labiatae species, oil against *Sitophilus oryzae* L. and *Tribolium castaneum* (Herbst) remained high irrespective of volume of fumigation chamber and amount of substrate. A similar trend has been observed for powder of dry flower buds of *Eugenia aromatica* (L.) Baill. in the family Myrtaceae, as a fumigant for *C. maculatus* control in infested cowpea seeds (Longe, 2004). It will be interesting to find out if fumigant activity of crushed bulbs of *A. sativum* against *C. maculatus* is dependent on volume of fumigation chamber and amount of substrate to be protected. This will help to clearly elucidate the economics of using *A. sativum* as a fumigant in stored products protection.

RESUMEN

Toxicidad fumigante para *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) de bulbos trozados de especies *Allium.* Se evaluó la toxicidad fumigante de bulbos frescos trozados de *Allium sativum* L. y *A. cepa* L. sobre *Callosobruchus maculatus* (Fabricius), una importante plaga de semilla almacenada de caupí *Vigna unguiculata* (L.) Walp. bajo condiciones de laboratorio en Akure, Nigeria. En las pruebas, 20 g de semillas infestadas se suspendieron en un trozo de tela sobre cierta cantidad de bulbos trozados en un contenedor con una tapa ajustada. Se previno completamente la emergencia de adultos desde huevos recién puestos de *C. maculatus* en semillas de caupí que se fumigaron con 6,0 g o más de bulbos de *A. sativum*. Estas semillas fumigadas no estaban ahuecadas. Otras cantidades de *A. sativum* probadas (1.0, 2.0, 3.0, 4.0 y 5.0 g) redujeron significativamente la emergencia de adultos de *C. maculatus* desde huevos fumigados y el perforamiento de la semilla en comparación con el control. *A. sativum* trozado fue inefectivo en la prevención de emergencia de adultos desde larvas fumigadas de *C. maculatus* en semillas. El efecto fumigante *A. cepa* trozada no mató los huevos de *C. maculatus*. Una cantidad de 7,0 g redujo significativamente la emergencia de *C. maculatus* adultos desde huevos fumigados y el perforamiento de semilla en comparación con el control. Los bulbos trozados de *A. sativum* se presentan como una buena alternativa como fumigante en el control de *C. maculatus* en semillas almacenadas de caupí.

Palabras clave: infestación, perforamiento de semilla, pesticida, volátiles, aceites esenciales.

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