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Bioefficacy of *Allium sativum* (L.) Oil and *Capsicum annum* Miller (Chili Pepper) Fruit Powder Against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) Infestation in Stored Cowpea Grains

M.D. Mailafiya, M.M. Degri, Y.T. Maina and H.A. Sharah

Department of Crop Protection, Faculty of Agriculture,
University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

Abstract: The oil and fruit powder of *Allium sativum* Linn and *Capsicum annum* Miller (chili pepper) respectively and the combination of both were evaluated in the laboratory (30 - 35°C and 60 - 65% RH) against the pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in cowpea grains. The bioactivity of these plant materials on *C. maculatus* oviposition, adult (F_1) emergence, developmental period, percentage grain damage and percentage adult mortality were assessed on two cowpea grain cultivars (Borno Brown and Borno White) admixed (singly and in combination) at the rates of 0.0% (control) 0.4, 0.8, 1.2 and 1.6% and 0.0% (control), 0.5, 1, 1.5 and 2% (wt/wt) concentration respectively for the latter and other parameters. Except for toxicity test, where 35 adult beetles (zero to three days old) were used, ten pairs of beetles were introduced in each experimental jar for all the parameters tested. All tests were carried out in a complete randomized design (CRD) replicated four times and data generated were subjected to analysis of variance (ANOVA). The number of eggs laid, number of adult beetles (F_1) emerged and percentage grain damage significantly ($P<0.05$) reduced, whilst percentage adult mortality increased on treated cowpea grains of both Borno Brown and Borno White compared to the untreated control. The mean values of all the parameters were generally lower or higher at the highest test concentration i.e., 2 or 1.6% (wt/wt), respectively. Progeny development of *C. maculatus* was significantly ($P<0.05$) longer on both cowpea grain cultivars treated with *A. sativum* oil and *A. sativum* oil plus *C. annum* powder to those treated with *C. annum* powder as well as untreated control. In summary, these results indicate the potential of *A. sativum* oil and *A. sativum* oil plus as cowpea grain protectants against *C. maculatus* infestation especially at higher rates over *C. annum* powder that proved less effective.

Key words: *Callosobruchus maculatus* · *Allium sativum* oil · *Capsicum annum* fruit powder · Insecticidal activity · Biopesticide · Cowpea grains

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) food grain legume, is a good source of energy (337.57 - 360.67 kcal /100g), crude protein (25.79 - 29.25%), carbohydrate (53.56 - 57.36%), fat (0.79 - 3.18%), ash (2.72 - 3.73%) and crude fiber (1.92 - 3.37%), as well as small amounts of essential micronutrients including calcium, iron, magnesium and copper [1, 2]. The high protein and amino acids (lysine and tryptophan) contents of cowpea [3], particularly make it a natural supplement to the staple diets of mainly roots or tubers, cereals and vegetables to millions of people in sub-Saharan Africa. Being much less expensive than other sources of animal protein (i.e., meat, fish, milk and eggs), cowpea grain is highly esteemed and readily consumed as bean porridge or thick soup cooked together with vegetables and pieces of staple food such as plantain or yam. Also, when soaked and milled, the cowpea grain is further prepared and eaten as akara (in Yoruba) / kosai (in Hausa) (deep-fried cowpea cake), moin-moin (steamed cowpea pudding) or dan-wake (in Hausa) (cowpea dumplings).

Corresponding Author: M.D. Mailafiya, Department of Crop Protection, Faculty of Agriculture, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria.

Nigeria, with an average annual production of above 2 million metric tons, is a major world producer of cowpea [4, 5]. Cowpea grain production and marketing serve as a valuable source of income, which in turn, contributes greatly to food security as well as poverty alleviation. However, irrespective of the purpose of storage, i.e., domestic consumption or sales, cowpea grains especially under poor storage conditions often suffer severe attack by some insect pests. The pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is a principal pest reported to cause up to 100% loss of stored cowpea grains within a few months of storage [6-8] and by extension, can incur monetary losses to the tune of millions of United States dollar [9, 10]. One to two percent initial field infestation by *C. maculatus* may result in 80% of the pods attacked after six - eight months in storage [11]. The development of a single *C. maculatus* larva per individual cowpea grain can lead to weight losses of between eight percent and 22% [12]. This in consequence, drastically reduces both grain quantity and quality, making stored lots unfit for neither human consumption nor marketing purposes. Although some synthetic insecticides such as Actellic 25 E.C., Actellic 2% dust, Pirimiphos methyl and Phostoxin tablet(s) are toxic to *C. maculatus* and can reduce or eliminate beetles infestation of cowpea grains per given time period(s) in storage [13, 14], their usage have often been associated with harmful residual effects to consumers, health hazards to insecticide or grain handlers and the development of resistance in insect pests. Moreover, in addition to their high cost, synthetic insecticides are more often not readily available when crucially needed for application. These drawbacks taken together necessitate the development of alternative, effective, affordable, safe or biodegradable and eco-friendly control measures. Some food-based spices or edible plant materials including *Afromomum meleguata* Schumm, *Allium sativum* Linn, *Capsicum* species, *Piper guineense* Schum and Thonn, *Syzgium aromaticum* (L.) Merril and Percy, *Tetrapleura tetraptera* Schumach and Thonn and *Xylopia aethiopica* Dunal A. have been evaluated against storage insect pests by various researchers and found to posses insecticidal properties [15-24]. In addition to being affordable, these plant materials are safe for human consumption and / or have medicinal value. The above studies however, were strictly limited to either plant powders, oils or certain insect pest species (i.e., the maize weevil / *Sitophilus zeamais* Motsch., the rice weevil / *Sitophilus oryzae* (L.) and the lesser grain borer, *Rhizopertha dominica* (Fabricius)) other than *C. maculatus*. The present study therefore investigated the insecticidal potentials of two botanicals or food-based spices, *A. sativum* and *Capsicum annum* Miller (chili pepper), as either oil or powder and the mixture of both against *C. maculatus* infestation in stored cowpea grains.

MATERIALS AND METHODS

Insect Culture, Cowpea Cultivars and Plant Materials Preparation: *Callosobruchus maculatus* stock culture was reared on Banjara seeds under prevailing laboratory conditions (30 - 35°C and 60 - 65% RH). Adult pulse beetles used to set up the culture were from infested cowpea stocks sourced from Monday market, Maiduguri-Borno state. All introduced adult beetles were removed after one week so as to obtain a synchronized first filial generation (F_1).

The grains of two common local cowpea cultivars, Borno Brown and Borno White, were obtained from Monday market. The grain lot of each cowpea cultivar was thermally sterilized at 50°C over 3 hours in an air-oven and afterwards, conditioned for 14 days in the laboratory.

Freshly dried fruits of *C. annum* were procured from Monday market and afterwards, cleaned by sieving off and picking out all debris. The fruits were then ground into powder and safely kept in bottles within the laboratory. Freshly processed *A. sativum* oil was also purchased from Monday market and safely kept in the laboratory.

Bioactivity Tests: Ten pairs of zero to three days old adult *C. maculatus* were introduced to 20 gram (g) cowpea grains admixed with 0.1, 0.2, 0.3 and 0.4 milliliters (ml) of *A. sativum* oil, 0.1, 0.2, 0.3 and 0.4 g of *C. annum* powder and the mixture (half the concentration of each given dose) of both *A. sativum* oil and *C. annum* powder in 100 ml glass jars covered with muslin cloth. Each treatment along with the control (or non-admixed grains) was replicated three times per cowpea cultivar. Prior to infestation, the glass jars were manually agitated to ensure effective coating of the grains with the plant materials. Seven days following infestation, all introduced adult beetles were removed and the number of eggs laid per grain counted. The glass jars were then left undisturbed till the emergence of F_1 adults. Daily count of emerged beetles was performed until emergence was completed. Damaged grains, bearing adult emergence holes, were then counted and expressed as a percentage of the total number of grains in each replicate. In a separate set-up, 35 adult pulse beetles were introduced unto 25 g cowpea grains admixed with the same doses (0.1, 0.2, 0.3 and 0.4 ml / g) of *A. sativum* oil, *C. annum* powder and the mixture of both *A. sativum* oil and *C. annum* powder in 100 ml glass jars covered with muslin cloth. All four replicates of each treatment along with the control per cultivar were inspected at 24, 48, 72 and 96 hours after infestation and the number of dead beetles recorded.

Data Analysis: Data on the number of *C. maculatus* eggs laid, adults (F_1) emerged, developmental period, percentage grain damage and percentage adult mortality were subjected to one-way analysis of variance (ANOVA) following log [log (x + 1)] transformation of count data and arcsine square root transformation of percentage data [25]. Significantly different means at 5% level of probability were separated using the Least Significant Difference (LSD) [26].

RESULTS

The numbers of pulse beetle eggs laid were 20 (Borno Brown) to 185 (Borno White) times lower on cowpea grains treated with *A. sativum* oil than untreated ones (Table 1). Similarly, the numbers of beetle eggs laid on cowpea grains treated with *C. annum* powder were three (Borno Brown) to nine (Borno White) times lower than on untreated grains. Additionally, when treated with *A. sativum* oil plus *C. annum* powder, the numbers of beetle eggs laid were eight (Borno Brown) to 72 (Borno White) times lower than on untreated cowpea grains. On Borno Brown and Borno White grains treated with *A. sativum* oil, *C. annum* powder and *A. sativum* oil plus *C. annum* powder, the numbers of beetle eggs laid were generally lower at the highest test dose (0.4 ml/g). The numbers of F_1 adults obtained from cowpea grains treated with *A. sativum* oil were much lower (141 (Borno Brown) - 210 (Borno White)) than on untreated grains (Table 2). When treated with *C. annum* powder, the numbers of F_1 adults from these grains were also lower [four (Borno Brown) - 36 (Borno White) times] than from untreated grains. Likewise, when treated with *A. sativum* oil plus *C. annum* powder, the numbers of F_1 adults from these grains were much lower [11 (Borno Brown) - 29 (Borno White) times] than from untreated ones. On both Borno Brown and Borno White, the developmental period of *C. maculatus* was significantly higher (24 – 27 days) on cowpea grains treated with *A. sativum* oil and *A. sativum* oil plus *C. annum* powder than the untreated ones (23 - 24 days) (Table 3). By contrast, however, the developmental period of *C. maculatus* was not significantly different between untreated cowpea grains and those treated with *C. annum* powder (23 - 24 days). Percentage grain damage following

Table 1: Effects of *A. sativum* oil and *C. annum* powder on the number of *C. maculatus* eggs laid on the grains of two different cowpea cultivars

	Borno White			Borno Brown		
Dose (ml/g)	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder
0.1	48.25	115.53	108.05	150.23	181.52	141.22
0.2	62.54	84.00	99.76	104.31	149.58	81.15
0.3	29.33	140.00	15.55	82.00	114.51	127.28
0.4	1.00	23.51	2.13	9.25	60.53	22.24
Control	185.43	205.26	200.55	187.15	207.25	167.50
SEM	15.10	20.10	13.90	9.09	18.91	15.59
LSD	46.68	62.11	42.74	28.04	58.18	48.03

Table 2: Effects of *A. sativum* oil and *C. annum* powder on the number of adult *C. maculatus* (F_1) emerged from the grains of two different cowpea cultivars

	Borno White			Borno Brown		
Dose (ml/g)	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder
0.1	17.52	30.15	12.75	24.75	82.52	61.08
0.2	4.00	25.31	8.36	2.92	60.11	43.75
0.3	1.77	10.57	4.48	1.32	57.25	24.32
0.4	0.75	5.00	4.27	1.79	30.17	11.83
Control	158.01	182.20	123.35	141.09	127.22	125.25
SEM	4.18	9.15	7.66	9.33	6.57	7.21
LSD	12.29	28.21	22.04	27.83	20.26	21.13

Table 3: Effects of *A. sativum* oil and *C. annum* powder on the developmental period of *C. maculatus* in the grains of two different cowpea cultivars

	Borno White			Borno Brown		
Dose (ml/g)	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder
0.1	24.25	24.22	25.01	25.51	23.21	25.33
0.2	24.41	24.17	25.25	24.38	23.55	25.42
0.3	25.12	24.26	27.71	24.61	23.43	26.99
0.4	26.50	24.44	27.04	24.17	23.31	25.74
Control	24.53	24.31	23.51	23.12	23.00	23.50
SEM	0.25	1.04	0.95	0.54	0.13	1.11
LSD	1.37	0.67	3.09	1.63	0.39	3.42

Table 4: Effects of *A. sativum* oil and *C. annum* powder on percentage grain damage following adult (F_1) emergence of *C. maculatus* from the grains of two different cowpea cultivars

	Borno White			Borno Brown		
Dose (ml/g)	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder	*A. sativum* oil	*C. annum* powder	*A. sativum* oil + *C. annum* powder
0.1	1.75	27.50	1.25	17.21	47.22	33.21
0.2	6.53	46.28	9.53	3.53	36.13	34.74
0.3	0.50	22.24	1.52	2.12	37.50	18.53
0.4	0.54	22.37	3.28	1.31	37.28	16.42
Control	54.25	63.31	65.51	50.47	52.71	56.75
SEM	4.87	9.12	5.76	2.62	5.68	5.85
LSD	13.02	28.13	17.77	7.45	17.52	16.02

Table 5: Effects of *A. sativum* oil and *C. annum* powder on percentage mortality of adult *C. maculatus* on the grains of two different cowpea cultivars

	A. sativum oil				*C. annum* powder				*A. sativum* oil + *C. annum* powder			
	Hours				Hours				Hours			
Dose (ml/g)	24	48	72	96	24	48	72	96	24	48	72	96
	Borno White											
0.1	22.90	23.75	6.25	0.00	19.52	11.12	18.13	3.45	16.75	11.25	14.53	17.51
0.2	32.13	11.24	17.55	2.04	22.71	12.15	15.20	9.63	18.11	9.17	32.05	0.00
0.3	33.01	20.05	8.85	1.10	26.11	10.45	8.77	5.72	29.47	16.20	11.25	4.12
0.4	41.28	27.10	1.25	0.00	17.27	22.14	8.41	5.00	31.35	13.65	19.28	7.91
Control	6.25	8.63	3.75	8.12	1.70	8.10	4.37	11.32	2.05	8.31	12.13	9.15
SEM	1.15	7.97	1.95	0.15	0.59	1.34	1.17	0.32	0.58	1.05	1.61	0.28
LSD	1.63	11.28	2.75	0.64	0.84	1.89	1.63	0.45	0.82	1.49	2.27	0.32
	Borno Brown											
0.1	19.45	21.20	10.70	5.65	13.65	22.05	12.25	5.05	24.40	12.17	1.18	16.25
0.2	22.16	6.55	11.25	10.56	24.45	9.65	15.25	10.65	8.05	38.15	15.25	1.55
0.3	21.65	15.14	10.05	5.20	11.97	15.72	18.25	7.95	18.72	23.03	8.25	0.00
0.4	23.18	17.55	14.25	19.22	26.55	7.25	17.25	4.95	31.75	19.00	21.25	5.16
Control	4.60	5.32	10.25	2.73	4.83	13.55	9.26	1.65	8.42	6.95	3.42	2.21
SEM	1.44	1.02	1.39	0.63	1.97	0.94	0.77	0.47	1.77	1.18	1.08	0.38
LSD	2.03	1.44	1.97	0.98	2.78	1.33	1.09	0.67	2.52	1.66	1.53	0.54

F_1 adult emergence was lower on cowpea grains treated with *A. sativum* oil [50 (Borno Brown) – 109 (Borno White) times], *C. annum* powder [one (Borno Brown) - three (Borno White) times] and *A. sativum* oil plus *C. annum* powder [three (Borno Brown) - 19 (Borno White) times] than on those untreated (Table 4). Percentage mortality of adult *C. maculatus* after 96 hours exposure to cowpea grains treated with *A. sativum* oil, *C. annum* powder and *A. sativum* oil plus *C. annum* powder ranged between 50 – 77% (Table 5). Also, percentage mortality was mainly higher on cowpea grains treated with higher dosage (0.3 or 0.4 ml / g) of the applied plant materials.

DISCUSSION

Significantly lower number of eggs laid, F_1 adults emerged, percentage grain damage and higher mortality rates of adult *C. maculatus* from cowpea grains treated with *A. sativum* oil, *C. annum* powder and *A. sativum* oil plus *C. annum* powder than those untreated, highlights the protectant potentials of these materials against *C. maculatus* infestation on stored cowpea grains. Longer developmental period of *C. maculatus* on cowpea grains treated with *A. sativum* oil and *A. sativum* oil plus *C. annum* powder than untreated ones, further suggests the importance of *A. sativum* oil in suppressing the development or population build up of *C. maculatus*. Studies have identified methyl allyl disulfide and diallyl trisulfide, as the two major constituents of the essential oil of *A. sativum*, with potent toxicant and fumigant actions against *S. zeamais* and *Tribolium castaneum* Herbst [27], as well as the Japanese termite, *R*eticulitermes speratus Kolbe [28]. The powder and solution of *A. sativum*, have also been reported to have insecticidal properties. Akpabot *et al.* [21], for instance, in a laboratory study found the mixture of the fruit powder of *A. sativum* and seed powder of *P. guineense* applied at both 1 and 5% (wt/wt) to completely inhibit the survival *S. zeamais* and as a result, reduced the damage levels caused. Additionally, *A. sativum* powder, alongside those of *T. tetraptera* and *X. aethiopica* caused 100% repellent effect against the adult weevils in stored maize. Compared to untreated grains, Danjuma *et al*. [20] reported that the powders of *A. sativum*, *Azadiracta indica* A. Juss, *Nicotiana tabacum* L., *Ocimum basilicum* L. and *Zingiber officinale* Rosc. applied at 2% (wt/wt) adequately protected the nutritional composition of treated maize grains for three months. In particular, the percentage total protein (total nitrogen content) remained intact. Field application of half the recommended dose of Dimethoate 30 EC and Kartodim 315 EC each in combination with 5% of 15 days incubated *A. sativum* solution on water melon also found to be effective in reducing thrips infestation, with the consequent increase in crop yield [29]. Plant oils, moreover, have been shown to penetrate the chorion of bruchid eggs and asphyxiate developing embryos [30, 31] and also inhibit the development of immature stages of some insects or evoke mortality in the adults through the blockage of respiration on coated grains [22, 32, 33]. In addition to being repulsive to *S. zeamais*, the essential oil of *Ocimum grattissimum* (Labiatae) at very low rates (0.012, 0.06 and 0.3% (wt/wt)), for instance, significantly reduced the number of progeny surviving to adults and also induced high mortality of adult weevils in maize grains [34]. Similarly, groundnut oil applied at 20 and 10 ml respectively to 50 kg of stored cowpea grains significantly inhibited progeny emergence and caused high adult mortality (mainly within 24 hours) of *C. maculatus* [33]. Doharey *et al.* [35], further found that coconut oil applied at 1% (wt/wt) to green gram and rice against infestation by *C. maculatus*, *Callosobruchus chinensis* (L.), *R. dominica* or *Sitotroga cerealella* (Olivier) protected the grains in storage for up to six months.

Capsicum spp. contains capsicin (8-methyl-*N*-vanillyl-6-nonenamide), which through its hot sensation is believed to exert detrimental effects towards insect pests [17, 18, 36]. *Piper guineense*, another pepper (the West African Black Pepper) plant species, with biopesticidal activity attributed to its alkaloid constituents including Piperine, Piperidine and Chavicine [24], has been reported to elicit 100% mortality of *R. dominica* on wheat grains [24], *S. zeamais* on maize grains [37] and *Tribolium castaneum* (Herbst) on pearl millet [22] and bambara nuts [38] after 72 – 96 hours exposure to grains treated at 1 - 5% (wt/wt). The powder or essential oils of *P. guineense* have also been reported to greatly repel the larvae or adults of *T. castaneum* [22] and *S. zeamais* [37, 39], as well as impair progeny development and reduce adult emergence in *C. maculatus* [24], *S. zeamais* [21, 37] and *T. castaneum* [22]. In spite of all the above facts, *C. annuum* fruit powder generally performed less than *A. sativum* oil against *C. maculatus* infestation in this study. The generally lower performance of *C. annuum* fruit powder in this study aligns with the results of [18], who found the seed compared to the fruit powder of *C. annuum* and *Capsicum frutscens* L. to be more effective in protecting stored cowpea and maize grains against *C. maculatus* and *S. zeamais* infestation after evoking greater adult mortality within 96 hours of exposure to treated grains at the rates of 10% - 15% (wt/wt). Considering the higher concentration of plant materials tested in the above work than ours (<2% wt/wt), the low performance of *C. annum* fruit powder in this study was very likely not due to the concentration utilized.

In conclusion, although *A. sativum* oil and *A. sativum* oil plus *C. annum* powder rather than *C. annum* powder were more effective in protecting stored cowpea grains against *C. maculatus* infestation. Being readily available, as well as easy to prepare and apply these edible and nutritive plant materials can very well be adopted and incorporated into traditional cowpea storage systems.

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