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EXPERIMENTAL PAPER

Eugenia aromatica O. Berg and *Afromomum melegueta* K. Schum botanical entomocides as possible synergetic protectant against *Callosobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae) infestation on stored cowpea (*Vigna unguiculata* (L.) Walp.)

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Summary

Introduction: Post-harvest protection of food grains against stored products insects' infestation is a complementary means towards ensuring food security.

Objective: Powders and extracts from *Eugenia aromatica* O. Berg and *Aframomom melegueta* K. Schum are to be evaluated for their synergetic effects on *Callosobruchus maculatus* Fabricus under laboratory conditions.

Methods: The products were applied at rates of 1 g/20 g and 1% conc./20 g cowpea seeds respectively.

Results: Findings show that *E. aromatica* products was more effective by evoking 100% mortality within 24 and 48 h, while synergetic effects of the two products evoked 100% mortality at 72 and 96 h. The survival of the bruchids treated with plant powder from eggs to adults indicated that, control had significant ($p<0.05$) progeny development (75.50%) in comparison with sole application of *E. aromatica* and combination with

A. melegueta which recorded no progeny development.

Conclusion: The study revealed that the plant products exerted toxicity effect against cowpea bruchid. Thus, they can be used to prevent egg hatching and thereby helping in the management of cowpea seeds.

Key words: ***Eugenia aromatica; Afromonum melegueta; egg hatching; mortality; post-harvest protection, plant products; progeny development; toxicity***

Słowa kluczowe: ***Eugenia aromatica, Afromonum melegueta, rozwój jaj; śmiertelność; ochrona po zbiorach, produkty roślinne; rozwój potomstwa; toksyczność***

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a prominent and essential grain legume crop widely cultivated in the developing countries for its inexpensive source of dietary protein which substitutes for the highly expensive animal source protein. Its production is limited by *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae: Bruchinae) infestation causing serious post-harvest losses and quality deterioration to the cowpea grains. The larvae feed on undamaged grains and reduce them to powdery form, causing severe loss by reducing the weight of agricultural products and consequently their commercial and nutritional values [1]. The infested seeds lost germination potentials and also became unfit for human consumption [2]. Infestation is high at farmers and traders level, where storage conditions are usually inadequate to prevent insect attack [3]. The rate of damage under normal condition increases with storage time [4].

To address the problem of food security and protein deficiency prevalent in developing countries, apart from increase of production, efforts should be concerted on the reduction of qualitative and quantitative losses caused by stored products insect infestation. The magnitude of competition between *C. maculutus* and human beings for this important crop necessitates its control to avoid food shortage, promote self–sufficiency and enhance food security. Management of stored products' insects on stored food grains in Nigeria and other tropical and subtropical nations over the years has been dependent on synthetic insecticides and fumigants which their indiscriminate applications led to evolution of resistance and reappearance of pest populations, human and environment health risks as a result of food and water contamination, non-availability at critical period of need, adulteration and high cost of procurement [5-8]. Owing to the various shortcomings associated with use of conventional insecticides, it becomes imperative to seek for alternative eco-friendly stored products' insect pest management techniques; among which is the use of plant products.

Natural products such as botanical insecticides may provide suitable alternatives and offer the possibility as a solution to control insect pests due to their bioactive chemicals that displayed strong biological activities that are eco-friendly and biodegradable [9-11].

Aframomum melegueta K. Schum., well-known as Alligator pepper (family *Zingberaceae*) is a very common spice which imparts a hot peppery flavour with hints of citrus and used mainly as food, in brewing, and in both veterinary and traditional medicine [12]. It is assumed to have laxative and haemostatic properties and also to be effective against schistomiasis [13].

Eugenia aromatica O. Berg, popularly known as Clove, is an aromatic tree in the *Myrtaceae* family. Clove buds possess intense fragrance and burning taste. They have deep brown colour, powerful fragrant odour which is warm pungent, strongly sweet and slightly astringent yet can be used to make table drawers and closets smell nice [14]. The clove (the flower bud) is used as a spice in the cuisine of Asia and Africa. In traditional medicine, it has been used to treat varieties of health-related problems, such as gastrointestinal, indigestion, parasitic infestation, cough, toothaches, headaches and blood impurities [15]. Phenol eugenol is a major component of its oil which is used in soaps, detergents, toothpaste and pharmaceutical products [16].

Several researchers have reported varying successful levels of grain protectant potential of *E. aromatica* and *A. melegueta* products admixed singly with various food grains [17-23]. However, insufficient literature data is available on the potential synergetic use of *E. aromatica* and *A. melegueta* for the suppression of stored products insect pest population and damage. Though, various formulations of phyto-insecticide product mixtures offered considerable levels of food grains protection by evoking numerous degrees of target insect pest population reduction. Burkill [24] reported the repellent properties of mixtures of pulverized dried fruit of *Xylopia aethiopica* and *Capsicum annum* against kola weevils. Therefore, as addendum to the knowledge of the utilization of botanicals as an eco-friendly insect pest control agents and to enhance food security, an investigation was conducted to ascertain the synergetic potential of botanical entomocides' properties of *E. aromatica* and *A. melegueta* products as protectant of cowpea seeds against *C. maculatus*.

MATERIALS AND METHODS

Insect rearing and experimental conditions

The insects population used to establish the stock culture was obtained from infested cowpea seeds, *V. unguiculata* bought from Ibaka market, Akungba Akoko, Ondo State, (5°44' E and 7°28' N) Nigeria. Fifty adults of mixed sex of *C. maculatus* were obtained from the seeds', sub cultured by being reared on 100 g of Sokoto white (susceptible local variety) by removal of infested and holed seeds and addition of fresh uninfested cowpea seeds in a glass jar covered with muslin cloth to allow for aeration till adult emergence. F1 progenies (1–3 days old adults) from the cultures were used for the toxicity tests. Insect culturing and other experiments were carried out under ambient temperature of 30±2°C, 75±5% relative humidity and 12L:12D photoperiod.

Collection and preparation of plant material

Seeds of *E. aromatica* and *A. melegueta* were bought from traditional herbal stall at Erekesan market, Akure, Ondo State, (5°12' E and 7°15' N), Nigeria. The identity of collected plant seeds were confirmed at the Department of Forestry and Wood Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The plant seeds were air-dried, pulverised into fine powder and sieved, using 1 mm^2 sieve. Prior to use, the powders were packed in tight lids plastic containers and stored at 4°C in a refrigerator.

One hundred and fifty grammes (150 g) of *E. aromatica* and *A. melegueta* powders were soaked for 72 h individually in a 1 l specimen bottle containing methanol using cold extraction method and stirred at intervals with a glass rod. The resulting mixture was filtered using a double layer Whatman No. 1 filter paper and the extract was recovered using rotary evaporator. The resulting materials were air-dried to eliminate traces of solvents. The crude extracts were kept in a dark bottle labeled and preserved in the refrigerator till further use. From this stock solution, 1% concentration, each was prepared by diluting 0.1 ml crude extract in 9.9 ml of methanol [25].

Collection and disinfection of cowpea seeds

Clean seeds of Ife brown variety were obtained from a newly stocked grains free of insecticides at Agricultural Development Programme (ADP), Akure, Ondo State, Nigeria. The seeds were disinfested by keeping in a freezer at -5°C for 7 days to get rid of any hidden infestations, since all the insect life stages, inparticular the eggs are very sensitive to cold [26]. Thereafter, the cowpea seeds were placed inside Gallenkamp oven (model 250) at 40°C for 4 hours [27] and later air dried to prevent mouldiness [17] before being stored in tight lid plastic container.

Insect bioassay

Toxicity of *E. aromatica* and *A. melegueta* products

One gram (1 g) of *E. aromatica* and *A. melegueta* powders were solely admixed with 20 g cowpea seeds in 250 ml plastic containers, while 5 g of each plant powder were weighed into a small plastic container and thoroughly mixed together before being applied at rate 2.0 g/20 g cowpea seeds. One percent (1%) concentration of *E. aromatica* and *A. melegueta* extracts were prepared from the stock extracts solution and mixed singly with 20 g of uninfested cowpea seeds in 250 ml plastic dishes. In another experimental set up, 5 ml of each plant extract (1:1) were measured into a 15 ml culture vials and thoroughly mixed together before being applied at rate of 1%/20 g. The contents of the dishes were stirred using a glass rod to ensure uniform seed coating and were left open for 20 minutes for traces of solvent to evaporate.

In all the experiments, ten pairs of 2–3 days old *C. maculatus* adults; sexed using the following morphological features of the insect abdomen, dorsal side of the terminal segment and dark noticeable spots on their elytra were introduced to each container and covered in four replicates laid out in Complete Randomized Design inside insect rearing cage in the laboratory [28-30]. Control experiment was also set up for each protocols as follows; control dishes for plant powder contains neither of the plant powder while that of extract was treated with methanol.

The toxicity of the plant products was evaluated at 24 hrs interval for 4 days, adult insects were considered dead after failure to responded to probing with safety pin [23]. Percentage adult mortality was calculated using Abbott [31] formula. After 96 h, all live insects were removed and oviposition (number of eggs laid) was recorded. The experimental set up was maintained in the laboratory till the emergence of first filial (F_1) progeny. At 30 days after treatment or infestation, emerged adults were sieved out, counted and recorded. This was used to determine the percentage adult emergence following the procedure adopted by Odeyemi and Daramola [30]:

$$\text{\% Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs laid}} \times \frac{100}{1}$$

Quantitative loss was determined by re-weighing the cowpea seeds and the number of damaged cowpea seeds (seeds with adult exit holes) was counted at the end of the experiment and percentage weight loss and seed damage were calculated, respectively:

$$\text{\% Weight loss} = \frac{\text{Change in weight}}{\text{Initial weight}} \times \frac{100}{1}$$

$$\text{\% Seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times \frac{100}{1}$$

Beetle Perforation Index (BPI) of treated cowpea seeds was determined by Fatope *et al.* [32] method where value higher than 50% indicated susceptibility to insect infestation or zero potentiality of the plant products evaluated

$$\text{BPI} = \frac{\text{\% treated cowpea seeds perforated}}{\text{\% control cowpea seeds perforated}} \times \frac{100}{1}$$

Phytochemical screening of *E. aromatica* and *A. melegueta*

Chemical tests were carried out on the methanolic extracts and powders for the qualitative determination of phytochemical constituents using standard procedures [33-35].

Statistical analysis

Data on percentages and oviposition were subjected to square root transformation and arcsine transformed respectively prior analysis. All data were subjected to analysis of variance (ANOVA) and treatment means were separated using Tukey's Test, with SPSS 16.0 software [36].

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

Toxicity of *E. aromatica* and *A. melegueta* seed products applied solely and in combination

Toxicological effect of *E. aromatica* and *A. melegueta* products on adult *C. maculatus* survival at different exposure periods is presented in Table 1. The results revealed that survival of adult *C. maculatus* decreased gradually with period of exposure. Sole application of *E. aromatica* powder resulted into 100% mortality of adult *C. maculatus* within 48 h exposure (tab. 1). *A. melegueta* sole seed powder and mixture of both plant powders evoked 100% mortality of adult *C. maculatus* 96 h after exposure. However, mixture of two plant products progressively increased adult mortality with increasing period of exposure and the adult mortality effect was significantly higher as compared to that obtained from sole application of *A. melegueta* products at 24, 48 and 72 h for plant powder and 24 and 48 h for the extract treatments, respectively. All the treatments showed significant difference with its control.

Effect of *E. aromatica* and *A. melegueta* products applied solely and combination on *C. maculatus* fecundity

Activities of the plant products applied solely and mixture on *C. maculatus* fecundity was presented in table 2. As a result, plant products effectively reduced or completely inhibited oviposition (tab. 2). Number of eggs laid on cowpea seeds admixed with plant powder was significantly (p>0.05) lower than those laid on untreated cowpea seeds as the dishes treated with *E. aromatica* recorded the minimum number of eggs laid (10.50 eggs). Followed by mixture of both plant powders (12.75 eggs) which was not significantly different (p<0.05) when compared, but was significantly different when compared to the untreated cowpea seed that recorded the maximum number of eggs (84.75 eggs) and cowpea seeds

Table 1

Percentage mortality of adult *Callosobruchus maculatus* treated with 1.0 g plant powders and 1% concentration plant extracts/20 g of cowpea seeds for contact toxicity

Treatments	% Mortality ± SE mean after Powders [1.0 g/20 g cowpea]				Plant extracts [% conc./20g cowpea]			
Treatments	24h	48h	72h	96h	24h	48h	72h	96h
E. aromatica	80.00±1.25d	100.00±0.0d	100.00±0.00c	100.00±0.00b	100.0±0.00d	100.0±0.00c	100.00±0.00b	100.00±0.00b
A. melegueta	45.00±2.04b	70.00±1.25b	85.00±2.04b	100.00±0.00b	70.00±1.25b	85.0±2.04b	100.00± 0.00b	100.00±0.00b
E. aromatica + *A. melegueta*	60.00±1.25c	85.00±2.04c	97.25±1.44bc	100.00±0.00b	85.00±2.04c	95.0±2.04bc	100.00± 0.00b	100.00±0.00b
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00b

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p>0.05$ using Tukey's Test.

Table 2

Effect of plant powders applied at rate of 1.0 g and 1% concentration plant extracts/20 g of cowpea on oviposition and progeny development of *Callosobruchus maculatus*

	Plant powders [1.0 g/20 g cowpea]		Plant extracts [1% conc./20g cowpeaN]	
Treatments	Oviposition	% Number of progeny development.	Oviposition	% Number of progeny development.
E. aromatica	10.50 ± 0.32a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
A. melegueta	22.25 ± 1.07b	8.99 ± 0.64b	8.00± 0.25b	0.00 ± 0.00a
E. aromatica + *A. melegueta*	12.75 ±0.02a	0.00 ±0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Control	84.75 ± 1.45c	75.50 ±1.24c	84.75±1.45c	75.50 ± 1.24b

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p > 0.05$ using Tukey's Test.

treated with sole application of *A. melegueta* powder (22.25 eggs). Application of sole *E. aromatica* extract and in combination with *A. melegueta* extract completely prevented the insects from laying eggs on the treated cowpea seeds. While, *C. maculatus* were able to lay significantly minimum number of eggs on cowpea seeds treated with sole application of *A. melegueta* extract (8.0 eggs), maximum eggs were laid on the untreated cowpea seeds (84.75 eggs).

Adult emergence percentage recorded from the control was significantly different ($p>0.05$) compared to emergence obtained in the treated cowpea seeds (tab. 2). From result, no progeny development or emergence took place in cowpea seeds treated with various plant extracts either in sole or combined application, as the eggs laid were prevented from hatching. Thus, adult *C. maculatus* emergence was completely prevented as compared to the control treatment where 75.50% adult emergence was recorded. Same scenario was found in the treatments that involved the plants powder except for sole application of *A. melegueta* seed powder which recorded 8.99% adult emergence.

Protectant effects of *E. aromatica* and *A. melegueta* products

All the plant extracts, *E. aromatica* and mixture of both plant powders completely prevented treated cowpea seeds from infestation and damage (tab. 3). The results showed that seeds treated with extracts recorded neither seed damage nor weight loss and Beetle Perforation Index (BPI) was zero except *A. melegueta* powder treated seeds that recorded 2.25 and 6.50% seed damage and weight loss respectively. However, the BPI of 3.32 obtained for cowpea seeds admixed with *A. melegueta* powder was significantly different from BPI of the untreated seeds. Owing to the feeding activity of *C. maculatus* larvae, 67.75% seed damage was recorded from the control dishes. Also the weight of untreated cowpea seeds

Table 3

Percentage seed damaged, weight loss, Beetle Perforation Index (BPI) caused by *Callosobruchus maculatus* on cowpea seeds treated with 1.0 g of two plants powders

	Plant powders applied at 1.0 g/20 g cowpea					Plant extracts applied at 1.0 g/20 g cowpea				
Treatment	Mean total number of cowpea seeds	Mean number of damaged cowpea seeds	Mean percentage cowpea seeds damaged	Mean percentage weight loss	Beetle Perforation Index (BPI)	Mean total number of cowpea seeds	Mean number of damaged cowpea seeds	Mean percentage cowpea seeds damaged	Mean percentage weight loss	Beetle Perforation Index (BPI)
E. aromatica	94.00	0.00	0.00+0.00a	0.00±0.00a	0.00±0.00a	93.75	0.00	0.00+0.00a	0.00±0.00a	0.00±0.00a
A. melegueta	93.25	2.00	2.25 ±0.15a	6.50 ±0.07b	3.32±0.33a	95.00	0.00	0.00 ±0.00a	0.00 ±0.00a	0.00 ±0.00a
E. aromatica +A. melegueta	94.75	00.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	92.75	0.00	0.00±0.00a	0.00± 0.00a	0.00±0.00a
Control	95.00	64.25	93.75	68.25±0.25c	>50.00±0.00b	95.00	64.25	67.75±1.24b	68.25±0.25c	>50.00±0.00b

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p > 0.05$ using Tukey(s) Test.

Beetle perforation index (BPI); value lower than 50 is an index of possible protectant effect while BPI greater than 50 is an index of negative protectability.

was significantly ($p<0.05$) reduced compared with cowpea treated with the different plant products.

Phytochemicals screening of *E. aromatica* and *A. melegueta*

Table 4 presented the result of the phytochemical screening of the methanolic extracts and powders of *E. aromatica* and *A. melegueta* seeds. The phytochemicals present in the products of *E. aromatica* and *A. melegueta* were alkaloids, saponins, tannins, flavonoids and cardiac glycosides. Phlobatannin and anthraquinones were found absent in both plant products. The phytochemicals present in *E. aromatica* and *A. melegueta* show high insecticidal property.

DISCUSSION

The use of plant products for the management of food grain against stored product coleopterans (weevils and beetles) infestation is an eternal practice in many developing countries [37]. Due to shortcomings associated with the uses of conventional insecticides and fumigants, this practice has been advocated as one of potentials for controlling stored product insects [38]. As a result, several plants products have been evaluated and shown to possess insecticidal potentials against several stored product insects [37, 39-44]. The literaturę data show that plant materials were found individually effective for the management of storage insect pest's control, although, this is the first report on the efficacy of the plant products mixture utilized to suppress the infestation of insect pests on stored cowpea in Nigeria.

High *C. maculatus* mortality recorded by plant products indicates the efficacy of plant as a promising tool for controlling *C. maculatus* infesting cowpea grains in storage. The level of mortality observed in the cowpea seeds treated with the plant powders was lower than what was observed in extracts. This suggests that the use of extracting solvent to extract plants make their active principles more readily available for the insects to pick up lethal doses during the course of their feeding and movement within the stored food material. Furthermore, the effectiveness of the extracts of *E. aromatica* and *A. melegueta* could be due to the effect of the extracts on the respiratory organ of the insects which later leads to suffocation and subsequent death.

The remarkable effects of the plant powders could be ascribed to the manifestation of their toxic constituents and irritating odour which prevented physical contact of adult weevils with grains or some of the powder came into contact with the insects' spiracles contributing to further mortality by suffocation thus significantly ($p< 0.05$) reduced the rate of the insect survival. Also, during insects crawling over the grains, the chemical constituents of plant powder could lodge between cuticular segments and

Table 4

Phytochemical composition of the methanol and aqueous extracts of *Eugenia aromatica* and *Afromonum melegueta* seeds

Phytochemicals	Methanol extract of *E. aromatica*	Powder of *E. aromatica*	Methanol extract of *A. melegueta*	Powder of *A. melegueta*
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phlobatannin	–	–	–	–
Anthraquinones	–	–	–	–
Flavonoids	+	+	+	+
Cardiac glycosides	+	+	+	+

Key: - Absent; +present

increase water loss through abrasion of the cuticle. Belmain and Stevenson [45] postulated that plant powders are coarse in nature and sometimes stick to grains depending on its constituent part which results in water loss and ultimately the insect's death.

This study clearly revealed that the plant products hindered the developmental process of the insect by decreasing the number of eggs laid or completely inhibiting the females from laying eggs on the treated cowpea seeds and exerting ovicidal effects on the insect. Eggs laid by female adults *C. maculatus* were completely reduced and inhibited when exposed to the plant extracts compared to plant powders, and comparable effects were noted for successive egg hatching and larval survival. This is due to the fact that plant extracts could diffuse inside the stored seeds whereas plant powders cannot. This is in agreement with Adedire and Lajide [19] findings, which stated that *E. aromatica* flowers and *A. melegueta* seeds were effective against *C. maculatus* as ovipositional deterrent as they exerted higher ovicidal effects which unfavourably affected survival and egg laying capability and reduced oviposition rates. Juneja and Patel [46] reported a complete inhibition of egg laid by *C. analis* in cowpea grains treated with different plant products. Raja *et al.* [47] also established that egg laid by *C. maculatus* were considerably influenced by volatile oils derived from *Mentha* species. The current results also get support from the previous works of Kamakshi *et al.* [48], which reported that *Mentha arvensis* and *Ocimum sanctum* caused decline of weight in the number of eggs laid by *C. maculatus* as compared to control.

The deterrent properties of the plant products nature which unfavourably affect the egg laying ability might be attributed to the changes in the behaviour and physiology of the insects after the treatment due its chemical constituents [23]. Besides, the pungency or characteristic odour exhibited by the plant products may also significantly contributed to the reduction in the egg laying capability of the insects. Adesina and Ofuya [49] reported that admixing cowpea seeds with plant powders cause physical impediment to beetle movement, sexual communication thereby preventing mating and oviposition.

Effectiveness of the plant products treatment against *C. maculatus* in inhibiting adult emergence revealed the insecticidal properties of the plants. All the cowpea-treated plant products either killed exposed *C. maculatus* before they could lay eggs onto the seed coats, or one way or another contaminated eggs during oviposition process such that the eggs did not hatch or the larvae died before they could metamorphose to adult stage. Although, *C. maculatus* survived exposure to *A. melegueta* powder and extract treatments, while no F1 adults emerged in cowpea seeds treated with sole application of *E. aromatica* powder and combination of both plant products, indicating suppression and elimination of the next generation. It is also possible that some of the plant products came into contact with the insects' larvae spiracles or by diffusion into the seeds contributing to further mortality by suffocation. Also since coating the grains with the plant products minimized contact between grains and weevils, some weevils might have died as a result of starvation and ultimately resulted in reduced or no emerged adults.

Adesina and Ofuya [49] raised the opinion that reduction in adult emergence might probably occur due to the behavioural tendency of the insects. Since *C. maculatus* eggs are laid on the seed coat, surface of storage materials and there is tendency that eggs were in closer contact with plant toxic secondary metabolites leading to higher egg mortalities, reduction of egg hatchability and in return suppress adult emergence. Adesina *et al.* [23] is of the opinion that changes in behaviour and physiology of insects following extract treatment due to its chemical nature might have also led to low egg laying capacity of *C. maculatus* on the grains and, subsequently, few hatch adults or no adult emergence. These factors collectively may have led to high inhibition rates of F1 adult emergence within the treated grains.

Results obtained from this study are in agreement with previous studies that showed various plant products as valuable source of potential grain protectant against development of all life stages of a number of postharvest grain pests [50]. Keita *et al.* [51] observed zero emergences of *C. maculatus* F1 progeny in cowpea treated with *Ocimum basilicum* extracts. Vanmathi *et al.* [52] also reported that aqueous extracts of *O. tenuiflorum* greatly reduced F1 adult emergence of coleopterons.

The reduction in percentage seed damage, weight loss and perforation index on treated grains as observed in the study can be ascribed to the low survival of adult beetles, reduced oviposition, eggs hatchability and low adult emergence where possible thereby reducing metabolic and feeding activities of insects. Also reduction in cowpea seed infestation maybe credited to the offensive and pepperish fragrance produced by the plant products which exerted toxic effect by disrupting normal respiratory activity of the insects. This is in alignment with Adesina *et al.* [53] and Adesina and Mobolade-Adesina [44] findings. The heavy infestation and attendant loss recorded from the untreated cowpea seeds clearly indicated that unprotected grains recorded high adult emergence and predisposed the cowpea seeds to increasing percentage seed damage owing to the unrestricted feeding, developmental and metabolic activities of the insects within the stored cowpea.

Insecticidal properties of any plant products relied on its bioactive chemical constituent. The active constituent present in these botanical products is responsible for their toxicity against *C. maculatus*. The ability of any material to act as insecticide is directly proportional to the inability of the insect to resist or tolerate the insecticidal property of such material. Yang *et al.* [54] reported that these allelochemicals have strong adverse effect on insect survival as they disrupt the normal developmental process of insects. The effectiveness of this plant products in causing mortality, inhibited or reduced oviposition, adult emergence and suppressed seed damage and weight loss could be due to the presence of active compounds such as terpenoids, alkaloids, saponins, glycosides and flavonoids that tested positive in the phytochemical analysis.

Ekeh *et al.* [21], Dushland [55], Iwuala *et al.* [56], Mbailao *et al.* [57], Ileke and Oni [58] postulated that aromatic compounds such as terpineol, glycosides, saponins, alkaloids and flavonoids possess ovicidal, toxic and deterrent activity effects on stored product coleopterans. Saponins for example, have been shown to impair ecdysteroid synthesis (Al-Rajhy *et al.* [59]). While, Karamanoli *et al.* [60] reported that alkaloids and tannins impart toxicity by iron chelation and enzyme inhibition. Shadia *et al.* [61] and Ekeh *et al.* [21] concluded that glycosides, terpenoids, tannins and certain monomeric flavonoids found among *Lamiaceae* are excellent feeding deterrents against insect pests. In addition, active phytochemicals such as alkaloids have been found to disrupt growth and reduce larval survival by hindering loss of exoskeleton during larval development [62]. Other active principles such as isoflavonoids, flavonoids and terpenoids have also been reported to inhibit reproduction and fertility among coleopterans [23, 63].

Different authors who carried out isolation and characterization on the two plant materials reported that, *E. aromatica* contains: eugenol, eugenyl acetate, cariofilen *β*-caryophyllene, 1,8-cineole and cadinene [64-54]. While *A. melegueta* contains the following bioactive molecules: *α*-caryophyllene, *β*-caryophyllene, E-nerolidol, linalool, gingerdione, gingerol, 2-heptanol, 2-heptyl acetate, paradol, shagaol and humulene [19, 66] which are accountable for the distinctive sharp, spicy flavour, fragrance and ultimately responsible for the insecticidal and biological activity exhibited by the plant [17, 19, 67-68].

Synergists are widely used also to overcome resistance and help control different species of pests on the farm and specially stored grains. The toxicity effects of the combination of two substances for mortality, oviposition, adult emergence and seed damage were all higher than sole application of *A. melegueta* products, suggesting a significant synergistic interaction. This confirmed previous investigation which showed that mixture of two or more botanical formulations is more effective than one-plant material [69-72]. One of the possible reasons for plant products efficacy could be the multiple mode of action arising from their mixture. Adesina *et al.* [23] opined that a complex mixture of the plant bioactive or secondary compounds also contributes to synergism of greater extent which could have promoted much activity against the tested pest and reduces the rate of resistance development. The synergetic activity of *E. aromatica* and *A. melegueta* products reported in this trial is in tandem with that of Musa *et al.* [73] and Akunne *et al.* [74] which confirmed the efficacy of mixed leaf powders of *Vernonia amygdalina* and *Ocimum gratissimum, V. amygdalina* and *Azadirachta indica* against *C. maculatus.*

CONCLUSION

Outcomes of this study revealed the bioactivity of *E. aromatica* products sole application and its synergetic potential with *A. melegueta* in conferring grains protectant ability on stored cowpea. In light of the foregoing, sole utilization of *E. aromatica* products and its mixture with *A. melegueta* products as biopesticides against cowpea bruchid should be encouraged as feasible alternative to conventional insecticides and fumigants in ensuring a steady supply of *C. maculatus* infestation free cowpea grains.

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