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Callosobruchus maculatus (Fab.) (Coleoptera: Chrysomelidae) infestation and tolerance on stored cowpea seeds protected with *Anchomanes difformis* (Blume) Engl. extracts

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ABSTRACT

Purpose: The study was conducted to evaluate the infestation and tolerance of Callosobruchus maculatus to stored cowpea treated with Anchomanes difformis extracts. Research Method: Different concentrations of A. difformis extracts were admixed with 20g of uninfested cowpea seeds in 250ml plastic dishes. Ten unsexed adult C. maculatus were released into each treatment and toxicity was assessed at 24 hrs interval days after infestation, number of eggs laid on cowpea seeds was counted. At the end of the experiment, seeds were reweighed, number of emerged adult and damaged seeds were recorded. Findings: All extracts were significantly toxic to C. maculatus as concentration increased. Ethyl acetate (EA) extract exerted highest mortality ranged 90-100%, while methanol recorded highest longevity. Maximum reduction in egg laid was observed with EA extract (51.44-74.45%), followed by methanol extract at 100 (58.69%) and 150 µl (69.56%). While maximum adult emergence inhibition was observed at 150 µl (93.33%) with EA. Cowpea seeds in control dishes suffered the heaviest infestation (31.76%) compared to EA and acetone extracts that recorded zero infestation at 100 and 150 μl concentration respectively. Susceptibility of cowpea seeds treated with extracts was significantly lower compared to control with 100% infestation. Limitations: No hindrances was encountered during the study. Originality/Value: The study revealed that A. difformis possesses oviposition deterrent and adult emergence inhibition properties against C. maculatus that can be utilize for the management of C. maculatus in stored cowpea seeds. Further studies are recommended for exploring the active compound responsible for its insecticidal activities and toxicological effect using albino rats as a model.



INTRODUCTION

Cowpea production and storage is severely hampered by *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) infestation leading to enormous nutritional and economic loss. Infestation of the bruchids starts in the field but it is difficult to detect it at the time of harvest, but its infestation is generally manifested in storage where substantial loss occurs when seeds are stored for longer period.

Cowpea bruchid, *C. maculatus* as an economically important insect pest of stored cowpea causes 20-50% losses in storage (Gosh & Durbey, 2003). Astronomical losses attributable to the insect during post-harvest storage is the possible reasons for importing cowpea from neighbouring West African countries to compliment local production in spite of Nigeria been the largest producer of cowpea.

Successful management of stored grain insects is the final component of the struggle to limit postharvest losses of agricultural produce due to insect infestation and diverse measures have been employed to control these species. The use of synthetic insecticides and fumigants remains the main and most effective means of protecting and controlling stored food grains against insect infestation and grains/seeds damage (Adesina et al., 2012). Their continuous and indiscriminate use has over the years been associated with numerous problems that include environmental pollution (Assad et al., 2006), development of resistant strain (Nisha et al., 2018), toxicity to non-target organisms (Dennis, 1981) and insecticide residues in food (Shazali et al., 2003). The various problems caused by the persistent use of pesticides have gradually led to an increasing interest in the development of alternative pest control methods, such as the use of biopesticides.

Plants with insecticidal properties could be regarded as potential alternatives to chemical pesticides. Indeed, various plants or plant extracts are used to control agriculturally important insect pests. A wide range of medicinal plants are toxic, repellent, ovicidal or antioviposition, while some have antifeedant properties, insect growth and development regulators several of which were regarded as insecticides (Khoshnoud et al., 2008; Boulogne et al., 2012; Addisu et al., 2014; Adesina et al., 2015). The insecticidal proprieties of plants lie mainly on their secondary metabolites or bioactive compounds (Pettersen, 1984).

Anchomanes difformis (Blume) Engl. (Family Araceae) commonly known as forest Anchomanes, children's umbrella and God's umbrella is native to tropical Africa (Burkill, 1985). In Nigeria, the plant is known as Chakara by the Hausa, Olumahi by the Igbo and Abirisoko or ogirisako by the Yoruba (Egwurugwu et al., 2016). Anchomanes difformis is a perennial, herbaceous and deciduous plant with stout prickly stem growing about 2metres high and whitish horizontal tuber/rhizome that measures 50-80 cm long, and 10-20 cm in diameter.

Anchomanes difformis is used in African traditional medicine in the treatment of various ailments among which are dysentery, diabetes, gonorrhoea, oedema, jaundice, scabies, hypertension, respiratory diseases and as poison antidote, diuretic, laxative and ease child birth (Burkill, 1985; Oyetayo, 2007; NsondeNtandou et al., 2017; Ahmed, 2018). Studies also indicates that *A. difformis* leaves, tuber and roots extracts possessed analgesic, antibacterial, antimalarial, antioxidant, anti-inflammatory, and antipyretic properties (Oyetayo, 2007; Adeleke & Adetunji, 2010; Eke et al., 2013; Abubakar et al., 2013; Abiodun et al., 2014; NsondeNtandou et al., 2017; Ahmed, 2018).

There appears to be a scarcity of empirical information on the utilization of *A. difformis* extracts for their insecticidal activities. However, Akinkurolere (2007) and Adebo et al. (2018) reported the efficacy of *A. difformis* powder for the management of stored product insects. The present work is carried out to determine the toxicity of *A. difformis* extracts against pulse beetle, *C. maculatus*.



MATERIALS AND METHODS

Experimental location

The study was conducted under ambient laboratory conditions $(30\pm2 \text{ °C} \text{ temperature}, 65\pm5\%$ relative humidity and 12L:12D photo regime) in the Department of Crop, Soil and Pest Management Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria (Latitude 5° 12' N and Longitude 5° 36' E).

Cowpea seeds

Drum variety of cowpea used for the study was procured from Oja Ulede, Owo, Ondo State, Nigeria. On receipt, the seeds without no visible signs of beetle eggs and presence of adults or exit holes were handpicked and sterilized in an oven at 100 °C for an hour (Adesina & Idoko, 2013). This was done to terminate any developmental stages of *C. maculatus* that might be in the seeds (Idoko & Adesina, 2013). Thereafter, the seeds were allowed to cool to avoid mouldiness (Olotuah et al., 2007).

Insect culture

The initial culture of *C. maculatus* used in this study were obtained from infested stock purchased from Oja Ulede market, Owo, Ondo State, Nigeria. From this initial culture, new cultures of the insect were reared on Sokoto white variety (a local susceptible variety) of cowpea in one liter plastic jars covered with muslin cloth to allow for aeration and oviposition in the laboratory under ambient conditions (25-30 °C, 70-75% relative humidity). First generation of *C. maculatus* adults that emerged from these were used in the experiment.

Plant collection and extraction

Tubers of *A. difformis* were harvested from abandoned farmland within Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The tubers were cut into small pieces, air dried at room temperature. Thereafter, the dried piece of tubers was pounded in a mortal with pestle and passed through a uniform size sieve. The powder was stored in airtight container till need for extraction. About 250 g of *A. difformis* powder was subjected to cold extraction by soaking in 400 ml of hexane, ethyl acetate, acetone and methanol respectively for 48 hrs. Then the extracts were decanted and concentrated in a rotary evaporator to make it solvent free. The residues were stored in vial bottles and tested for insecticidal activities by contact and fumigant toxicity.

Effect of A. difformis extracts on C. maculatus adult mortality and longevity

Different concentrations (50, 100 and 150 μ l) of the extracts were mixed with 20 g of uninfested and wholesome susceptible drum cowpea seeds in 250 ml plastic dishes with the aids of micro pipette. The treated cowpea seeds were stirred using a glass rod to ensure proper coating of the seeds with the extract. The seeds were then air-dried for some minutes to evaporate the solvent (Talukder & Howse, 1994). There was also control experiment with no addition of plant extract and each treatment was replicated three times. Ten unsexed newly emerged 2-3 days old *C. maculatus* from the culture were released into each treatment and toxicity of the extracts was assessed at 24 hrs interval for 4 days, adult insects were considered dead after failure to respond to probing with sharp safety pin. Percentage adult survival was calculated using Abbott (1925) formula.

Effect of A. difformis extracts on C. maculatus oviposition and fecundity

After 7 days of infestation, the number of eggs laid by female beetles on the cowpea seeds in each treatment were counted and recorded separately. All the eggs laid in different Petri



dishes were examined and the viable eggs were identified. Viable eggs were recognized by their morphological feature (Lima et al., 2004), since they become opaque as a function of their residue discharged by the larvae during penetration. This was used to calculate the percentage reduction of egg laid (1) and hatched egg (2) as follows:

% Reduction of egg =
$$\frac{\text{No of laid in control-no of egg laid in treated dish}}{\text{no of eggs laid in control dish}} \times \frac{100}{1}$$
 (1)

% Egg hatchability = $\frac{\text{mean no of hatched eggs}}{\text{mean no of eggs laid}} \times \frac{100}{1}$ (2)

The experiment was kept undisturbed on the laboratory workbench for 30 days to allow for the emergence of the first filial (F_1) offspring. The number of emerged adult from each treatment was used to calculate the percentage reduction of adult emergence (3):

% Reduction in adult emergence =
$$\frac{\text{no of emerge adult from control dish-no of emerge adult from treated dish}}{\text{no of emerge adult from control dish}} \times \frac{100}{1}$$
 (3)

Effect of A. difformis extracts on C. maculatus infestation and tolerance on stored cowpea

At the end of the experiment, the seeds were sieved and reweighed to get the final weight which was used to determined percentage weight loss. Thereafter, numbers of seeds with adult exit hole(s) were sorted, counted and recorded. This was used to calculated percentages infestation (4) and tolerance (5).

$$Percentage infestation = \frac{number of seeds with emergent/adult exit or eggs (or both)}{Total number of seed observed} \times 100$$
(4)

Where: Nh = number of seeds with emergent/adult exit or eggs (or both) and No = Total number of seed observed.

% Pest tolerance=
$$\frac{\text{number of undamaged seeds - number of damaged seeds}}{\text{Total number of undamaged}} \times 100$$
 (5)

Statistical analysis

Each treatment was replicated three times and arranged in a Completely Randomized Design (CRD). Data obtained were subjected to analysis of variance (ANOVA); where significant differences were obtained (P \leq 0.05), means were separated with Duncan New Multiple Range Test (DNMRT). Data in percentages were arcsine transformed prior to analysis (Sokal & Rohlf, 1981).

RESULTS

Effect of A. difformis extracts on C. maculatus adult mortality and longevity

All extracts were toxic to *C. maculatus* and there was significant difference in the mortality recorded as concentration increased over exposure period and in relation to the solvents used for the extraction (Table 1). At 24-96 hrs exposure periods, ethyl acetate (EA) extract at all concentrations exerted the highest mortality ranged 90-100% mortality, closely followed by hexane (45-96.67% and acetone extracts (39.25-96.67%). Among the extracts, *A. difformis* extracted with methanol recorded the least *C. maculatus* mortality and highest longevity (18.43-86.67%).

| Exposure Time | C a lava mé | Concentration (µl) | | | | |
|---------------|---------------|----------------------------|-----------------------------|---------------------------|--|--|
| (hrs) | Solvent | 50 | 100 | 150 | | |
| 24 | Hexane | 45.00 ±2.89 ^c † | 45.67 ± 0.67^{d} | 71.56 ± 0.00^{d} | | |
| | Methanol | 18.43 ± 0.01^{b} | 33.17 ± 0.34^{b} | $56.83 \pm 0.04^{\circ}$ | | |
| | Acetone | $39.25 \pm 0.02^{\circ}$ | $39.32 \pm 0.09^{\circ}$ | 50.75 ± 0.02^{b} | | |
| | Ethyl Acetate | 90.00 ± 5.77^{d} | 90.00 ± 0.00^{e} | 96.67 ±3.33 ^e | | |
| | Control | 0.00 ± 0.00^{a} | $0.00\pm\!0.00^{\rm a}$ | 0.00 ± 0.00^{a} | | |
| 48 | Hexane | 63.43 ± 0.01^{d} | 71.67 ±0.11 ^d | 74.89 ±3.33 ^b | | |
| | Methanol | 19.77 ± 0.67^{b} | $39.22 \pm 0.01^{\text{b}}$ | 71.23 ±0.33 ^b | | |
| | Acetone | $50.70 \pm 0.07^{\circ}$ | $56.53 \pm 0.26^{\circ}$ | 71.57 ± 0.01^{b} | | |
| | Ethyl Acetate | 93.33 ± 3.33^{e} | 96.67 ±3.33 ^e | $96.67 \pm 3.33^{\circ}$ | | |
| | Control | 3.33 ± 3.33^{a} | 3.33 ± 3.33^{a} | 3.33 ± 3.33^{a} | | |
| 72 | Hexane | 71.04 ± 0.52^{d} | $71.57 \pm 0.01^{\circ}$ | $93.33 \pm 3.33^{\circ}$ | | |
| | Methanol | 30.15 ± 0.15^{b} | 63.43 ± 0.01^{b} | 77.37 ±2.91 ^b | | |
| | Acetone | $62.44 \pm 1.00^{\circ}$ | 93.33 ± 3.33^{d} | $96.67 \pm 3.33^{\circ}$ | | |
| | Ethyl Acetate | 93.33 ±3.33e | 96.67 ± 3.33^{d} | $100.00 \pm 0.00^{\circ}$ | | |
| | Control | 13.33 ± 3.33^{a} | 13.33 ± 3.33^{a} | 13.33 ± 3.33^{a} | | |
| 96 | Hexane | 90.00 ± 0.00^{d} | 93.33 ±3.33 ^{bc} | 96.67 ±3.33 ^{bc} | | |
| | Methanol | 43.33 ± 3.33^{b} | 83.33 ± 3.33^{b} | 86.67 ±3.33 ^b | | |
| | Acetone | $71.54 \pm 0.02^{\circ}$ | 96.67 ±3.33 ^c | 96.67 ±3.33 ^{bc} | | |
| | Ethyl Acetate | 93.33 ± 3.33^{d} | $100.00 \pm 0.00^{\circ}$ | $100.00 \pm 0.00^{\circ}$ | | |
| | Control | 20.00 ± 5.77^{a} | 20.00 ± 5.77^{a} | 20.00 ± 5.77^{a} | | |

| Table 1 Effect of A | difformis extracts on | C maculatus adult | t mortality and longevity |
|-----------------------|------------------------|-------------------|---------------------------|
| Table 1. Effect of A. | ujjornus extracts on v | c. macaiaias adun | i mortanty and longevity |

[†]Mean with the same alphabet down the column are not significantly different using Duncan New Multiple Range Test (DNMRT) at p > 0.05.

Effect of A. difformis extracts on C. maculatus oviposition and fecundity

Anchomanes difformis extracts significantly reduced the number of egg laid by *C. maculatus* on the treated cowpea seeds. The numbers of egg laid were significantly reduced with increased in the extracts concentrations (Table 2). Maximum reduction in egg laid was observed in cowpea seeds treated with ethyl acetate extract (51.44-74.45%), followed by methanol extract (ME) at 100 (58.69%) and 150 μ l (69.56%). Percentage reduction of egg laid between hexane and methanol were not significantly different at 50 and 150 μ l, while cowpea seeds in control dishes recorded zero percent egg laid reduction (Table 2).

Percentage egg hatchability

Percentage egg hatchability significantly decreased with increased in the extracts concentration. Also the various extracts significantly suppressed egg hatching (Table 2). Ethyl acetate extract significantly recorded the lowest percentage egg hatched at 50 and 100 μ l (26.58 and 16.67%) respectively. While at 150 μ l no egg was hatched from both ethyl acetate and acetone extracts (Table 2). Meanwhile, 62.32% of the eggs laid in control were hatched.

Percentage adult emergence reduction

Cowpea seeds treated with the various extracts significantly inhibited adult emergence, reduction in adult emergence increases as the concentration of the extracts increased (Table 2). Maximum adult emergence inhibition was observed at 150 μ l (93.33%) with ethyl acetate and minimum inhibition at the same concentration was observed in hexane extract (63.43%). However, non-significant difference was observed in the reduction of adult emergence at 50 μ l between methanol, acetone and ethyl acetate extracts, at 100 μ l between acetone and ethyl



acetate extracts and at 150 μ l between hexane and methanol extracts respectively. Adult emergence in control dish does not experience any inhibition as they recorded zero percentage adult emergence inhibition (Table 2).

Effect of A. difformis extracts on C. maculatus infestation and tolerance on stored cowpea

Percentage infestation

Result in Table 3 shows that percentage *C. maculatus* infestation on treated cowpea seeds was extracts and concentration dependent. The various extracts significantly suppressed *C. maculatus* infestation as their concentration increases. Cowpea seeds in control dishes suffered the heaviest infestation (31.76%) compared to those treated with ethyl acetate extract that suffered 5.63% infestation at 50 μ l concentration and recorded zero infestation at 100 and 150 μ l concentration respectively. While acetone extract completely suppressed infestation at 150 μ l concentration.

Percentage pest tolerance

Susceptibility of the cowpea seeds treated with *A. difformis* extracts was significantly lower compared to those in control that were more susceptible (100%) to *C. maculatus* infestation followed by hexane extract. Ethyl acetate treated seeds had the lowest significant percentage tolerance to *C. maculatus* infestation followed by acetone extract. While hexane and methanol extracts exhibited no significant percentage tolerance at 50 and 150 µl, respectively (Table 3).

Percentage weight loss

The percentage weight loss caused by *C. maculatus* during storage was significantly reduced (p<0.05) in seeds treated with extracts from *A. difformis*. The results indicated that control had the highest significant weight loss (12.61%) and ethyl acetate had the least weight loss (2.33, 1.67 and 0.67%) while hexane, methanol and acetone extracts were not significantly different (p>0.05) and had similar effect on weight loss.

| | Solvent | Concentration (µl) | | |
|-----------------------------|---------------|--|--------------------------|--------------------------|
| | | 50 | 100 | 150 |
| % Reduction of egg laid | Hexane | 30.05 ± 0.02^{b} † | 32.71 ± 0.01^{b} | 67.53 ± 0.01^{b} |
| | Methanol | 28.27 ± 1.33^{b} | 58.69 ± 0.00^d | 69.56 ± 2.96^{b} |
| | Acetone | $44.08 \pm 3.35^{\circ}$ | $55.73 \pm 0.00^{\circ}$ | 67.77 ± 0.09^{b} |
| | Ethyl Acetate | 51.44 ± 0.67^{d} | 58.93 ± 0.33^d | $74.45 \pm 0.67^{\circ}$ |
| | Control | $0.00\pm\!0.00^{\rm a}$ | $0.00\pm\!\!0.00^a$ | $0.00\pm\!0.00^a$ |
| % Egg hatchability | Hexane | $43.36 \pm 0.02b^{c}$ | $42.81 \pm 0.01^{\circ}$ | $42.23 \pm 0.33^{\circ}$ |
| | Methanol | 40.27 ± 0.13^{b} | $38.40\pm\!\!0.05^c$ | 15.00 ± 2.08^{b} |
| | Acetone | $47.55 \pm 0.08^{\circ}$ | 32.00 ± 1.15^{b} | $0.00\pm\!0.00^a$ |
| | Ethyl Acetate | $26.58 \pm \hspace{-0.5mm} \pm \hspace{-0.5mm} 4.30^a$ | 16.67 ± 3.33^{a} | $0.00\pm\!0.00^a$ |
| | Control | 62.32 ± 1.67^d | 62.32 ± 1.67^d | 62.32 ± 1.67^d |
| % Reduction adult emergence | Hexane | 33.17 ±0.04 ^b | 44.00 ± 1.00^{b} | 63.43 ± 0.01^{b} |
| | Methanol | $44.67 \pm 0.33^{\circ}$ | $58.67 \pm 1.33^{\circ}$ | 64.60 ± 0.30^{b} |
| | Acetone | $47.49 \pm 3.33^{\circ}$ | $62.00\pm\!\!1.00^d$ | $80.00 \pm 5.77^{\circ}$ |
| | Ethyl Acetate | $42.86 \pm 3.59^{\circ}$ | 61.89 ± 0.00^d | 93.33 ± 3.33^{d} |
| | Control | 0.00 ± 0.00^{a} | 0.00 ± 0.00^{a} | $0.00\pm\!0.00^a$ |
| | | | | |

 Table 2. Effect of A. difformis extracts on C. maculatus oviposition and fecundity

[†]Mean with the same alphabet down the column are not significantly different using Duncan New Multiple Range Test (DNMRT) at p > 0.05.

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| 11 | | u | 11 |

| | Solvent | | Concentration (µl) | |
|------------------------|---------------|----------------------------|--------------------------|--------------------------|
| | | 50 | 100 | 150 |
| Percentage infestation | Hexane | 15.57 ±0.01 ^b † | $10.31 \pm 0.01^{\circ}$ | 8.32 ±0.01 ^c |
| | Methanol | $21.19 \pm 0.06^{\circ}$ | 6.01 ± 0.01^{b} | 5.63 ± 0.11^{b} |
| | Acetone | 15.23 ± 0.09^{b} | $10.45 \pm 0.02^{\circ}$ | 0.00 ± 0.00^{a} |
| | Ethyl Acetate | 5.63 ± 0.11^{a} | $0.00\pm\!0.00^a$ | 0.00 ± 0.00^{a} |
| | Control | 31.67 ± 1.67^d | 31.67 ± 1.67^d | 31.67 ± 1.67^{d} |
| % Pest tolerance | Hexane | $81.81 \pm 0.06^{\circ}$ | 79.45 ± 0.08^{d} | $73.75 \pm 0.03^{\circ}$ |
| | Methanol | $83.51 \pm 0.20^{\circ}$ | $69.55 \pm 0.08^{\circ}$ | $67.17 \pm 0.04^{\circ}$ |
| | Acetone | 73.33 ± 0.67^{b} | 62.44 ± 1.00^{b} | 28.00 ± 4.16^{b} |
| | Ethyl Acetate | 48.33 ± 4.41^{a} | 36.67 ± 3.33^{a} | 13.33 ± 3.33^{a} |
| | Control | 100.00 ± 0.00^{d} | 100.00 ± 0.00^{e} | 100.00 ± 0.00^{d} |
| % Weight loss | Hexane | 7.04 ± 0.00^{b} | $6.29 \pm 0.00^{\circ}$ | 2.33 ± 1.20^{b} |
| | Methanol | 5.74 ± 0.00^{b} | $4.33 \pm 0.33b^{c}$ | 2.67 ± 0.33^{b} |
| | Acetone | 5.41 ± 0.33^{b} | 3.67 ± 0.33^{ab} | 1.33 ± 0.67^{b} |
| | Ethyl Acetate | 2.33 ± 0.33^{a} | 1.67 ± 0.33^{a} | 0.67 ± 0.67^{b} |
| | Control | $12.61 \pm 1.33^{\circ}$ | 12.61 ± 1.33^{d} | 12.61 ± 1.33^{a} |

Table 3. Effect of A. difformis extracts on C. maculatus infestation and tolerance on stored cowpea

[†]Mean with the same alphabet down the column are not significantly different using Duncan New Multiple Range Test (DNMRT) at p > 0.05.

DISCUSSION

The use of plant parts and products to control agriculturally important insect pest have been an age long practice among resource poor African farmers, this indicates that naturally occurring bioactive compounds extracted from locally available medicinal and aromatic plants have potentials for managing stored products insect infestation (Golob & Webley, 1980; Ileke & Ariyo, 2015).

Treatment of stored cowpea seeds with crude A. difformis extracts at various concentrations significantly reduced the adult insect longevity, egg production and hatchability, which in turn influenced the number of adults emerged and resulted in seed damage and loss in weight in relation to the increased in the extract concentrations. This indicated that A. difformis extracts were lethal to adult C. maculatus and could serve as a bioinsecticide which could be due to the presence of some bioactive compounds. The consequential high adult mortality observed could be due to high toxic effect of the plant extracts. Aniszewski (2007) postulated that toxic secondary metabolites found in botanicals can block ion channels, inhibit enzymes, or interfere with neurotransmission, loss of coordination, and death. The higher insect mortality caused by ethyl acetate extract was the maximum as compared to the mortality caused by acetone and hexane extracts while the least insect mortality in extract treated cowpea was observed in methanol extract. This shows that the effectiveness of the plant materials hinges on the plant active constituents; these might possibly get into the body system of the insect and interfere with the normal development causing mortality of the insect. The study equalled to the finding of Shabnam (2009); Achio et al. (2012) and (Ojianwuna et al., 2016) who reported that high mortality (40-100%) of Rhyzopertha Dominica (F) and Sitophilus granuriuss, Macrotermes bellicosus was evoked when exposed to different doses/concentrations of Capsicum annuum, Zingiber officinale, Dennettia tripetala and Allium sativum products respectively.

The extracts mode of action based on the bioassay might be: through contact of the various extract with the body wall of the insects causing irritation of the skin (Williams et al., 2004); inhalation resulting in inflammation of pulmonary tissue and damage to respiratory cells (Reilly et al., 2007) and metabolic disruption, membrane damage and nervous system dysfunction (Ojianwuna et al., 2016). The finding is in consonance with the finding of Shabnam (2009) who reported that *A. sativum* and *Curcuma longa* significantly reduced *Tribolium castaneum* larval and adult emergence as well as weight loss in infested stored grains.

Oviposition deterrent activity showed that female beetles preferred to lay eggs in the control dish or in the dish that contain extracts of lower concentration. The extracts of highest concentration were least preferred by the female beetles for oviposition. It is noteworthy that all the extracts showed more than 50% of deterrent activity even at 100µl with the exception of hexane extract (32.71%). This aligned with study of Elango et al. (2009), who reported the oviposition activity indices of acetone, ethyl acetate, and methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus* against *Anopheles subpictus*. The results from this study also agrees with that of Rao and Sharma (2007) and Adesina and Ofuya (2015) who observed significant ovicidal effect of ethyl acetate and hexane extracts of *custard apple seed* on rice moth and *Secamone afzelii* methanol and hexane extracts on *C. maculatus* in reducing the number of eggs laid per female beetles.

The potential of the extract to reduce the egg laying ability by the female beetles may be attributed to the presence of toxic bioactive chemicals present in the plant (Adesina & Ofuya, 2015) which prompted alterations in the physiology and behavior of the insect species reflected by their egg-laying capability (Prathibha et al., 2014). Mehra and Hiradhar (2002) and Rajkumar and Jebasan (2009) opined that plant extracts that demonstrated significant oviposition deterrent activity were insect repellent. The reduction in number of eggs laid at higher doses of *A. difformis* extracts can be attributed to the interruption of vitellogenesis and damage to the egg chambers during various life stages of *C. maculatus* (Pandey & Khan, 1998). Dhar et al. (1996) reported that oviposition was possibly regulated by the volatile compounds absorbed through cuticle

The percentage of adult emergence reduction increases with increased concentration of extract. In the present trial, it was observed that the number of emerged adult insects was directly proportional to the number of hatched eggs. Insecticidal activity of the plant extract might be due to the presence of various bioactive compounds which may jointly or independently contribute to inhibition of adult emergence (Arivoli & Tennyson, 2011). Plant extracts have the prospective to impede the growth of various developmental stages during insect life history such as interruption of larval development, extend pupal duration, inhibit moulting, cause morphological defects and mortality during moulting and melanization processes of insect (Shaalan et al., 2005; Arivoli & Tennyson, 2011)

The significant reduction in adult emergence from treated cowpea seeds could be ascribed to the ovicidal properties of the plant, which leads to egg mortality, reduction in number of hatched eggs or larval mortality which caused the larvae from maturing to adult. This shows that *A. difformis* extracts undoubtedly have oviposition deterrent, ovicidal, and lavicidal properties. Jayakumar (2003) reported that plant extracts have obvious effects on postembryonic survival of the insect and resulting reduction in adult emergence in all the concentrations of different plants. The worthy inhibitory effects *A. difformis* extracts on the procreative cycle in which the F₁ progeny was reduced by more than 50% give a glimmer of optimism for use as stored grains protectants.

The non-tolerance and low susceptibility of cowpea seeds treated with the extracts to C. *maculatus* infestation was concentration dependent. Seed treated with 100 and 150µl ethyl acetate recorded zero percent infestation. Result from this trial agrees with Adesina and



Mobolade-Adesina (2016) who reported significant protection of cowpea seeds treated with *S. afzelii leaf* extracts to insect infestation. He opined that the protection confers on the treated seeds might be due to the high insect mortality rate and inability of the eggs to hatch; thereby reducing metabolic activities of insects. The significant protection recorded may also be due to the repellent activity of the plant extracts.

Weight loss indicated the quantitative loss in stored grains due to larvae feeding showing a direct relationship between insect population and weight loss. In present findings, all the extracts provide a significant reduction in seed damage and weight loss compared with the untreated seeds. The significant reduction in seed damage and lower weight loss is due to reduced oviposition and number of hatched eggs; consequently, reduced larval feeding, thus lowered the percentages of seeds damaged and seed weight losses. This supports the findings of Wahedi et al. (2013); Adesina and Mobolade-Adesina (2016) where neem seed extract and *S. afzelii* leaves extract significantly prevented emergence of F1 adults of *C. maculatus* and subsequent weight loss done due to pest respectively.

CONCLUSION

The findings of the present research have lead credence to the use plant material as phytoinsecticide for the control of *C. macuatus* against stored cowpea seeds. The study revealed that *A. difformis* possesses oviposition deterrent and adult emergence inhibition properties against *C. maculatus*. Among the extracts tested, ethyl acetate extract exhibited best result as insecticidal product for the management of *C. maculatus* in stored cowpea. Further studies are recommended for exploring the active compound responsible for such activities and its toxicological effect on albino rats.

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Conflict of interest

The authors declare no conflict of interest to report.

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