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Efficiency of *Acalypha wilkesiana* (Muell Arg.) leaf oil extract applied as a biocide against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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Abstract. The ethanolic oil extracts of *Acalypha wilkesiana* was assayed for its biotoxicity against the survival of *Callosobruchus maculatus*, immature stages, damage and seed weight-loss. The result was dosage and time of exposure dependent. The contact treatment revealed *A. wilkesiana* oil as an applicable biocide as it significantly ($p < 0.05$) affect the survival rate, suppressed oviposition and adult emergence of *C. maculatus*, hence reduced seed damage and weight-loss in grains below economic injury level. Contact treatment were the most toxic with LD_{50} values of 3.97 and 5.85 at 72 h, significantly lower ($p < 0.05$) than fumigant values 4.16 and 6.04, suggesting its isolation for future biocide study to establish the dose and mode of action for commercialization in crop protection.

Keywords: *Callosobruchus maculates*; *Acalypha wilkesiana*; Biotoxicity; Biocide; Oviposition; Mortality.

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Introduction

In spite of introduction of many agricultural control programs by government of countries all over the world to improve agriculture, the post harvest losses of agricultural produce is still putting the food security of many of the developing countries in jeopardy. Food security is an integral role of an effective post-harvest system to ensure that the harvested food reaches the consumer while fulfilling consumer satisfaction.

Cowpea *Vigna unguiculata* is an important grain legume widely grown in many West African countries including Nigeria. This crop has played important function in the food security of the world at large but the post harvest losses of this crop both on the field and in storage is alarming.

Insect pests play an integral role in frustrating the food security of countries because their activities in storage are more pronounced than on the field and for any country to achieve food security it must be able to keep sizeable amount of agricultural produce in storage (Adamu, 2015).

In fact, *Callosobruchus maculatus* which is the most popular insect pest of cowpea has been noted of causing more than 50%-90% post harvest loss of cowpea (IITA, 1989; Oni, 2014; Ogungbite et al., 2014; Ajayi et al., 2015).

In decades, the use of conventional insecticides that pose threats to humans and environment; flora and faunas inclusive, was advocated as an effective means of insect control but recently their uses has dropped tremendously because of the downsides associated with them. In fact, their use in many developed countries has been banned (Isman, 2000). European countries recently barred the importation of many agricultural produce from Nigeria because of the DDVP (Dichlorovos) the country is using for the protection of their exported commodities (The Nigeria Punch July 31, 2015). Therefore, to comb for other methods of insect control becomes a subject of interest among the world food store managers. As alternatives, the use of natural pesticides becomes imperative in various forms as dusts, extracts, oils , indirect contact and fumes with little or no cost (Ukeh, 2009), have achieved striking results as crop protectants (Keita et al., 2001; Isman, 2008; Parugrug and Roxas, 2008).

With a view to ensure global food security, this study geared towards contributing to efforts of researchers to suppressing damage to food crops, through the discovery of essential components in *Acalypha wilkesiana* oil as sustainable biopesticides to investigate its contact and fumigant bioactivity against *Callosobruchus maculatus* in stored cowpea in the laboratory.

Materials and methods

Cowpea seeds preparation

The cowpeas seeds (ife-brown) cultivar used were sourced from the National Seed Service, Ibadan, Oyo State, Nigeria. The cowpea seeds were sorted and disinfested in freezer at -18 ^{o}C for two weeks Koehler (2003) and air dried in the open laboratory to prevent mouldiness and kept in dry polytheme bags prior bioassay.

Insect rearing

The culturing population of *Callosobruchus maculatus* adults used for this study were obtained from an existing infested cowpea in the Post Graduate Entomology Laboratory of the Department of Crop, Soil and Pest Management Federal University of Technology, Akure Nigeria at temperature 28± 2^{o}C and 75± 5 %relative humidity. The insects were reared in a perforated lid jar containing 200g of uninfected cowpea seeds, to allow for oviposition and to obtain teneral adults *C. maculatus* for the assay.

Plant materials and oil extraction

The leaves of *A. wilkesiena* used were obtained fresh from the open field around sports complex, Federal University of Technology, Akure. The leaves were air dried for 2 weeks in the open laboratory before milling into fine powder with binatone blender.

The oils were obtained by cold extraction, the powders were soaked in ethanol for 72 h, in a glass jar. The mixture was rigorously mixed together and extract obtained were filtered with muslin cloth on the fourth day. The solvent was removed from the oil using a rotary evaporator. The oil was left opened to allow the traces of the solvent used to escape.

Contact with *A. wilkesiana* oil

The contact toxicity of *A wilkesiana* oil at 1%, 2%, 3%, 4% and 5%

concentrations/20g of cowpea seeds was investigated by exposing ten pairs of 0 h-24 h old adult male and female *C. maculatus* to the treated cowpea seeds in Petri dishes. Cowpea without plant oil treatment served as the control. The experiment was replicated three times, data on adult morality was assessed at 24 h, 48 h, 72 h and 96 hof application of oil.

Mortality was corrected using Abbott's formula (Abbott, 1925) and was subjected to probit analysis to estimate LD_{50} and LD_{95} with 95% confidence limit (Finney, 1971).

Data on the number of eggs laid was taken after 7 days of oviposition. The set up was left in the rearing chamber undisturbed for four weeks after which they were examined for adult emergence daily for 10 days. Percentage seed damage and weight loss was taken immediately after adult emergence data collection.

Fumigation with *A. wilkesiana* oil

The fumigant toxicity of the oil extract was investigated against *C. maculatus* using the method of Zhou et al. (2012) as described by Yang et al. (2014) with little modifications. A cotton wool each fumigated with 1%, 2%, 3%, 4%, and 5% concentration of was hung on the cover of a 1L wide-mouth plastic containers, containing 20 g pairs of cowpea seeds. Ten pairs of one day old male and female *C. maculatus* were exposed to vapours of the oil for 4 days, while in the control set up, there was no plant material added. The experiment was replicated three times. The fumigation lasted for 96 h. Data on adult mortality was recorded and corrected, using Abbott (1925) formula LD_{50} and LD_{95} were calculated using probit analysis (Finney, 1971). Number of eggs laid, percentage emerged adult, seeds damage and weight loss in seeds were calculated as described above and at the same mentioned environmental conditions.

Repellency test

The repellency test of the oil against *C. maculatus* was assayed using the method of preferential zone on filter paper by Yang et al. (2014) with some minor modifications. Petri-dishes, 9cm each was lined with whatman filter paper and divided into half, different dosages of the oil were applied separately to one-half of the filter paper uniformly with a micropipette. The other half (control) was treated with 1ml of absolute ethanol. Both the treated half and the control half were air-dried to evaporate the solvent completely (30 s). A full disk was carefully remade by attaching the treated half to the negative control half with tape. Each reassembled filter paper after treatment with solid glue was placed in a Petri-dish with the same oriented in one of four different randomly selected directions to avoid any insecticidal stimuli affecting the distribution of insects. 20 starved adult cowpea weevils were placed at the center of the central zone of the divide and a cover was placed over the petri-dish. Insect count on each strip was recorded after 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 14 h. The experiment was conducted in triplicate. The percentage repellency (PR) of each dose was calculated using the formula:

$$PR\ (\%) = \frac{Nc - Nt}{Nc + Nt} \times 100$$

Where: Nc - Number of insects in the controlled zone (no – plant oil)
Nt - Number of insects in the treated zone (Plant oils available)
Pr - Percent repellency.

Statistical analysis

Abbott (1925) formula was used to correct data on mortality counts using control mortality. All data were subjected to analysis of variance (ANOVA) at $p < 0.05$. Means were separated with Duncan's Multiple Range Test. Mortality data and doses of *A. wilkesiana* were subjected to probit and log transformation respectively to determine the dose lethal to 50 and 95% of the insects (LD_{50} and LD_{95}) (Finney, 1971). All analysis were carried out using SPSS 20.0 software package.

Results

Contact toxicity to adults

The effect of test plant oil on the survival of adult *C. maculatus* was concentration and period of exposure dependent. At 72h post treatment 2-5% concentrations achieved above 50% insect mortality, likewise all tested concentrations achieved above 60% adult mortality, while 90% mortality was achieved at significantly greater doses (5%) Table 1. The number of eggs laid and the percentage adult emerged decreased with increasing concentration of *A. wilkesiana* oils. At all tested doses, the oil significantly (P<0.05) reduced seed damage and weight loss by *C. maculatus* and significantly different (P<0.05) from the controls Table 2.

Table 1. Effects of different concentrations of oil extracts of *A. wilkesiana* on mortality (%) of *C. maculatus* infesting cowpea seeds.

Concentration (%)	% mortality in hours			
	24	48	72	96
1	3.33±3.33^{ab}	13.33±3.33^{b}	30.00±5.77^{b}	60.00±5.77^{b}
2	6.67±3.33^{ab}	16.67±3.33^{b}	56.67±3.33^{c}	66.67±3.33^{bc}
3	6.67±3.33^{ab}	23.33±3.33^{bc}	63.33±3.33^{cd}	73.33±5.77^{c}
4	10.00±0.00^{b}	30.00±5.77^{cd}	70.00±5.77^{cd}	76.67±3.33^{cd}
5	13.33±3.33^{b}	36.67±3.33^{d}	73.33±6.67^{d}	90.00±5.77^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	3.33±3.33^{a}	3.33±3.33^{a}

Each data is mean ± standard error of three replicates. Values followed by the same letter in the same column are not significantly (p < 0.05) different from each other.

Table 2. Effects of *A. wilkesiana* oil on No. of eggs laid and adult emergence of *C. maculatus* and its ability to cause seed damage and weight loss.

Concentration (%)	No. of eggs laid	Parameters in percentages (%)		
		Adult emergence	Damage	Weight loss
1	44.33±2.33^{e}	27.87±1.77^{b}	11.53±0.82^{c}	1.40±0.32^{c}
2	37.33±2.60^{d}	18.35±3.57^{a}	5.92±1.12^{b}	0.97±0.19^{bc}
3	25.00±1.15^{c}	16.18±2.89^{a}	3.74±0.54^{ab}	0.73±0.04^{abc}
4	18.33±0.88^{b}	12.62±1.20^{a}	2.80±0.54^{ab}	0.32±0.04^{ab}
5	10.00±1.15^{a}	8.89±4.84^{a}	1.87±0.93^{a}	0.17±0.04^{a}
Control	89.00±1.73^{f}	45.77±2.83^{c}	37.69±1.64^{c}	26.40±0.40^{d}

Each data is mean ± standard error of three replicates. Values followed by the same letter in the same column are not significantly (p<0.05) different from each.

Fumigant toxicity to adults

Regardless of the concentration of the oil insect mortality was not up to 50% at 48 h post treatment. Within 96 h of application. The 5% *A. wilkesiana* oil recorded the highest insect mortality (83.33%), this effect was significantly (P < 0.05) different from other tested dosages/exposure time. The mortality of the insect is concentration and period of exposure dependent. Fumigant effect of plant oils at 1% to 5% dosage on survival of *C. maculatus* was presented in Table 3.

Table 4 shows the effect of *A. milkesiana* oil on oviposition, adult emergence of *C. maculatus* and the ability to cause seed damage and weight loss of cowpea seeds. Oviposition and adult emergence of the insect reduced with increase in the oil concentration. At all tested doses, the oil significantly (P < 0.05) reduced number of egges laid, percentage adult emerged, seed damage and weight loss by *C. maculatus* when compared to the control having highest values on the four parameters observed. However the treatments were significantly different (P < 0.05) from the control.

Table 3. Effects of fumigant toxicity of different Concentrations of oil extracts of *A. wilkesiana* on the survival (% mortality) of *C. maculatus* infesting cowpea seeds.

Concentration (%)	% mortality in hours			
	24	48	72	96
1	0.00±0.00^{a}	20.00±5.78^{b}	23.33±3.33^{b}	56.67±3.33^{b}
2	3.33±3.33^{a}	26.67±3.33^{b}	33.33±3.33^{bc}	66.67±3.33^{bc}
3	6.67±3.33^{a}	30.00±0.00^{bc}	46.67±3.33^{cd}	70.00±5.77^{c}
4	10.00±5.77^{a}	40.00±5.77^{cd}	53.33±6.67^{d}	73.33±3.33^{cd}
5	13.33±8.82^{a}	43.33±3.33^{d}	70.00±5.77^{e}	83.33±3.33^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	3.33±3.33^{a}	3.33±3.33^{a}

Each data is mean ± standard error of three replicates. Values followed by the same letter in the same column are not significantly (p<0.05) different from each other.

Table 4. Effects of *A. wilkesiana* oil fume on No. of eggs laid and adult emergence of *C. maculatus* and its ability to cause seed damage and weight loss.

Concentration (%)	Parameters are in percentages (%)			
	No. of eggs laid	Adult emergence	Damage	Weight loss
1	68.00±2.65^{d}	37.81±1.45^{c}	26.19±0.70^{d}	5.23±0.073^{d}
2	58.33±1.45^{c}	25.25±2.12^{b}	14.97±1.19^{c}	3.78±0.09^{c}
3	53.33±1.45^{c}	22.42±1.56^{ab}	12.27±1.30^{bc}	3.38±0.09^{bc}
4	45.00±1.53^{b}	20.78±2.11^{ab}	9.20±0.97^{ab}	2.97±0.12^{b}
5	39.00±1.53^{a}	15.65±3.49^{a}	6.14±1.13^{a}	2.13±0.12^{a}
Control	89.00±1.73^{e}	45.77±2.83^{d}	42.18±2.07^{e}	26.40±0.40^{e}

Each data is mean ± standard error of three replicates. Values followed by the same letter in the same column are not significantly (p < 0.05) different from each other.

Lethal dosage (LD_{50}) and (LD_{95}) of oil in C. maculatus at 72h

The LD_{50} and$_{95}$ values obtained in this study (Table 5) were very little. Contact treatment of the oil appeared most effective against the adult insect than the fumigant treatment of the oil, as it recorded only 3.97% and 5.85% oil dosage to achieve 50% and 95% adult mortality within 72 h of application, compared to 4.16% and 6.04% required for fumigant toxicity. The chi-square value of the treatments also reflected the level of their effectiveness as they recorded values above 1.98. Furthermore, their effects were significant as reflected in the Table 5. The slope and intercept of the treatments showed that the treatments are very effective as their values are very low.

Table 5. Lethal doses of *A. wilkesiana* oil on *C. maculatus* at 72 h of application.

Treatment	Slope (±SE)	Intercept (±SE)	X^2 (df)	LD_{50} (95% FL)	LD_{95} (95% FL)	Significant
Contact	0.42±0.12	-0.50±0.06	482.885(16)	3.97 (3.88-4.23)	5.85 (4.93-6.05)	0.0001
Fumigant	0.12±0.11	-0.07±0.06	361.347(16)	4.16 (3.98-4.75)	6.04(5.89-7.74)	0.0001

Note: LD: lethal dosage; SE: standard error; x^2: Chi-square; df: degree of freedom; FL: Fiducial limits.

Repellent action of *A. wilkesiana* oil to *C. maculatus*

The various concentration of *A. wilkesiana* oil showed different levels of repellence to *C. maculatus* at different periods of exposure. The 5% dose recorded the highest repellence of 83.33% at 14h after application while 1% dose recorded the lowest repellence of 16.67% at 2h after application as shown in Table 6 and each was significantly different from others.

Table 6. Repellent activity (%) of *A. wilkesiana* oil against adult *C. maculates.*

Conc. (%)	% repellent (h)						
	2	4	6	8	10	12	14
1	16.67±3.33^{a}	23.33±3.33^{a}	30.00±5.77^{a}	33.33±3.33^{a}	53.33±3.33^{a}	56.67±3.33^{a}	56.67±3.33^{a}
2	20.00±0.00^{a}	30.00±5.77^{ab}	36.67±3.33^{ab}	40.00±5.77^{ab}	46.67±3.33^{ab}	56.67±3.33^{a}	63.33±3.33^{ab}
3	23.33±6.67^{ab}	33.33±3.33^{ab}	40.00±0.00^{ab}	43.33±3.33^{ab}	56.67±3.33^{bc}	60.00±5.77^{ab}	70.00±5.77^{ab}
4	26.67±3.33^{ab}	36.67±3.33^{ab}	43.33±8.82^{ab}	50.00±5.77^{bc}	63.33±3.33^{cd}	63.33±3.33^{ab}	73.33±3.33^{bc}
5	33.33±3.33^{b}	43.33±6.67^{c}	46.67±3.33^{c}	60.00±0.00^{c}	66.67±3.33^{d}	70.00±0.00^{b}	83.33±3.33^{c}

Each data is mean ± standard error of three replicates. Values followed by the same letter in the same column are not significantly ($p < 0.05$) different from each other.

Discussion

Contact and fumigant bioactivity of *A. wilkesiana* oil extracts on survival and immature stages of *C. maculatus* as well as damage to seeds and weight loss was investigated. In a bid to identify the chemical components of biotoxic properties was characterized.

The result obtained in this study showed that *A. wilkesiana* oil extract have effective insecticidal properties as it significantly affected the survival rate, suppressed oviposition and percentage adult emergence of *C. maculatus* and as well affected the ability of the insect to cause damage and weight loss of the protected cowpea seeds. The ability of the oil to effect high mortality of the insect, low oviposition rate and low adult emergence varied with the examined concentration of the oil extract. Likewise in a study conducted by Ajayi et al. (2018) investigated combined toxicity of chemical compounds of *M. oleifera* and *Z. officinale* oils were identified in cowpea seeds against *C. chinensis*. She reported that both oils to a moderate extent was effective at suppressing the number of eggs, number of adults emerged, giving suggestive clues on their usage to suppress bruchid population below economic injury level should the need arise.

The high mortality of *C. maculatus* recorded by the oil of this plant could be due to inability of the insect to feed on the protected cowpea seed. Despite adult *C. maculatus* does not eat they can live for longer period if provided with honey or sugary materials. Therefore, insect mortality recorded by the oil indicated that, the oil had no sugary substance that can serve as food for the insect and thereby lead to the starvation of the insect. Also, botanical based insecticides have been noted to have negative effect on respiratory organ of insects leading to hyperactivity and convulsion and total knockdown of insects (Schmutter, 2002; Zibaee, 2011; Rajashekar et al., 2014).

Therefore, the effect of the oil on adult survival could be due to blockage of spiracles of the insect which resulted to asphyxiation and thus causing respiratory impairment which probably affected metabolism and consequently other systems of the body of the bruchids, Osisogwu and Agbakwu, 1987: Onolemhem and Ogiangbe, 1991: Lale and Abdulrahaman, 1999). In a similar vein, Leatemia and Isman (2004), Adebiyi and Tedela (2012) as well as Ogungbite et al., (2014) reported that oils of botanicals were found to effect high mortality of *C. maculatus* and other stored product insect pest. The peculiarity of this study showed that oil extract of *A. wilkesiana* exhibited considerable degree of toxicity on number of eggs laid by *C. maculatus,* indicated that the oil had significant effect on the oviposition rate and adult emergence of the insect. The degree of toxicity varied between oil contact and fumigant tests as shown on oviposition and adult emergence of the insect, when compared to the controls. The low rate of oviposition of the insect indicated that the oil extract must have caused low mating communication between the male and female *C. maculates* (Oni, 2009).

As revealed by LD_{50} and LD_{95} values, contact treatment were more toxic to *C. maculatus* than fumigant treatment. This may be attributed to the direct contac.t of all chemical components of insecticidal properties to the body wall of the weevils. Also, the low oviposition rate could be due to high mortality of the insect which resulted in low mating period that subsequently lead to low number of egg laid (Yusuf, 2009; Ashamo et al., 2013). Zibaee (2011) and Isman (2006) stated that botanical insecticides apart from having antifeedant effect on insects could also cause incomplete ecdysis in young insects and sterility in adult female insects. Therefore, the low oviposition rate implies that the oil must have caused sterility of the adult female *C. maculatus*.

Furthermore, the low rate adult emergence could be due to inability of egg laid by the insect to hatch into the larvae stage. The few larvae of the insect emerged may be unable to drop off their exoskelecton that remained connected to the posterior part of their abdomen (Oigiangbe et al., 2010; Adeyemo et al., 2013). In addition, the low oviposition rate recorded by the insect could also have caused the low rate of adult emergence. Obembe and Ogungbite (2016) suggested that botanical oils do block the chorion of insect’s egg and thereby prevents the emergence of adult insects or lead to deformation of the insect larvae. The result of this study showed that the oviposition rate of the insect was dependent on mortality rate of the insect while weight loss of the seed was also dependent on adult emergence of the beetle. The result of this research acquiesced with the findings of Yusuf (2009), Ileke and Olotuah (2012), Ashamo et al. (2013), Oni (2014), Oni et al. (2016) in which botanical oils and powders were found to significantly reduced or prevented the emergence of adult *C. maculatus*. Low grain damage, weight loss and weevil perforation index was recorded in this work. This implies low feeding habit of the insect larvae which could have caused high seed damage, weight loss and subsequent high weevil perforation index. This agreed with the report of Akinneye and Oyeniyi (2016) in which the powder of some botanicals was found to affect the ability of *Sitotroga cerealella* to cause damage and weight loss of paddy rice.

Ikewuchi et al. (2009) reported the presence of flavonoids, alkaloids and tannins in the extract of *A. wilkesiana*. These phytochemicals have been reported of displaying different insecticidal efficacy against wide range of insect pests. They are being reported of disrupting life cycle of insects by causing high mortality rate of insect (Adonu et al., 2013; Yang et al., 2006). Therefore, the high mortality rate, low oviposition rate, low adult emergence, low seed damage and seed weight loss could be due to the presence of the phytochemicals present in the oil of *A. wilkesiana.*

Active compounds found present in the leaf extract of *A. wilkesiana* reported to be insecticidal in nature and are all alkaloids (Yang et al., 2006).

Conclusion

In conclusion, the oil of *A. wilkesiana* used in this work has proven insecticidal against cowpea beetle, *C. maculatus*. Nevertheless, the insecticidal potential of this plant oil depended on the concentration of the oil and the period of application. The result showed that the oil had contact toxicity effects on the oviposition and adult emergence of the insect as well as the ability of the insect to cause seed damage and weight loss compared to its fumigant toxicity. In addition, different active compounds were found in the oil extract of the plant. This could be synthesized for commercialization of the oil extract as crop protectants. In this respect, additional studies are needed to determine role of all active oil components in the immunity of the insects and possible effects on mammals.

Conflict of interests

The author declares that there are no conflicts of interest.

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