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Toxic potential of wild mustard fruit essential oil against maize weevil (*Sitophilus zeamais* Motschulsky)

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Abstract
Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is associated with substantial losses of cereals at storage. Therefore, it is essential to adapt effective and ecofriendly strategies for protecting stored cereals. This study evaluates the toxic effect of *Cleome viscosa* L. fruit essential oil (EO) against *S. zeamais*. The EO was applied at 4.5 – 22.5 µl/ml air in the fumigant toxicity bioassay, while 5 – 40 µl/10 g grains were used to determine the toxicity of the EO against parental (P_1) and first filial (F_1) weevils. The bioassays were laid out in Completely Randomized Design and each treatment was replicated five times. Data on fumigant toxicity bioassay were collected at 1.5, 3, 6, 12 and 24 Hours After Treatment (HAT). Data on toxicity of the EO on P_1 and F_1 generations were collected at 7 and 50 - 57 Days After Treatment (DAT), respectively. All data were subjected to analysis of variance. Fumigant toxicity of the EO was dose-dependent. At 24 HAT, application of the EO at 22.5 µl/ml air evoked 79.40% mortality which was significantly higher than 8.80 – 61.33% mortality recorded in lower EO doses. The median lethal dose of the EO at 24 HAT was 12.19 (11.03 – 13.44) µl/ml air. Mortality of the P_1 *S. zeamais* at 7 DAT was also dose-dependent; while 87.50% F_1 progeny mortality observed in grains treated with 40 µl EO per 10 g grains was significantly higher than 33.33% observed in the grains treated with 5 µl EO per 10 g grains.

Keywords: *Cleome viscosa*, *Sitophilus zeamais*, toxicity, essential oil

Introduction

Maize is a member of grass tribe Maydeae under the family, Poaceae. The dispersal of its seed by man aids its propagation and survival abilities as a vigorous, monoecious annual plant. In many developing countries, maize is a dietary staple (Lewis *et al*., 2005) [25]. It is consumed mainly as food in Africa (Erenstein *et al.*, 2012) [17]. In many developed countries, maize is used to feed animals, directly in the form of grain and forage or sold to the feed industry. It can also be used as raw material for industries that expertise in extract resources, such as being transformed into fabrics and plastics. It can be hydrolysed, and treated with enzymes to produce pharmaceutical syrups, a sweetener, and can also be fermented and distilled to produce grain alcohol.

Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is an insect pest associated with the substantial risks and losses of maize at storage (Babarinde *et al*., 2008; Tefera *et al*., 2012; Baoua *et al*., 2014) [11, 14, 34]. It is the most common *Sitophilus* species found in granaries and its larvae feed on cereal grains and pose more havoc at postharvest level (Haines, 1991; Babarinde *et al*., 2008) [11]. It can develop on all cereals and solid processed cereal products such as pasta, and processed tuber product (Babarinde *et al.*, 2013a, b) [5, 7, 18].

In some parts of Africa, small-scale farmers have been using plant products for generations in the protection of stored products from insect infestation (Poswal and Akpa, 2008) [32]. An effective way to control *S. zeamais* is the use of synthetic chemical insecticides. However, associated with this method are several side effects such as environmental damage, lethal effects on non-target organisms, pest resistance, resurgence, and high-cost implications (Özkara *et al.*, 2016) [30]. Also, synthetic chemical insecticides are often not compatible with biological control method in Integrated Pest Management Scheme because the insecticides are lethal to biological control agent. Therefore, ecofriendly and cost-effective alternatives have been the resolve of many scientists and farmers globally.

Cleome viscosa (Wild mustard) belongs to the Cleomaceae family, order Brassicales, a relatively small group of flowering plants. Its vegetative parts are commonly used in traditional medicine to treat a variety of ailments, such as worms, scurvy, infections, heart problems, gas, fever, seizures, diarrhoea, and skin conditions (Ahmed *et al*., 2011) [2]. It is known to have insecticidal properties, which include contact and stomach toxicity against the early instar stages of *Clavigralla tomentosicollis* (Ba *et al*., 2009) [4], contact toxicity on adult *Cyclas formicarius elegantulus* (Williams *et al*., 2003) [36], and *Spodoptera litura* (Phowichit *et al*., 2008) [31]. Oviposition is significantly inhibited in *Callosobruchus chinensis* by different extracts of *C. visocsa*, with a percentage oviposition deterrence index of 56.31–85.51% (Upadhyay and Yadav, 2012) [35]. Moreover, extracts from the seeds of *C. viscosa* have been shown to have larvicidal potential against *Culex quinquefasciatus* (Say), *A. aegypti* (Linnaeus) and *Anopheles stephensi* (Liston) and in different organic solvents (Bansal *et al*., 2014) [13].

To meet the rising demand for food of the global population, it is essential to come up with new strategies for protecting crops from pests besides the use of environmentally aggressive chemicals. Essential oils (EOs) are highly complex mixtures of often scores of individual aroma compounds and can be used as an alternative to traditional pest control agents due to their specificity towards certain insect pests, bio-degradability, efficacy at small doses even without the physical contact with the target organism. Moreso, EOs have high commercial application feasibility. Therefore, this present study was designed to evaluate the effect of *C. vicosa* fruit EOs on the mortality of *S. zeamais and* assess the effect of the EOs on the mortality of *S. zeamais* F_1 progenies.

Materials and Methods

Insect Culture

Sitophilus zeamais adults were obtained from heavily infested maize kept in the Department of Crop and Environmental Protection Laboratory, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. The insects (30 mixed sex) were introduced into a sterile transparent jar containing un-infested pesticide-free grains of Pajo white local maize variety. The jar was kept under fluctuating ambient humidity (75 ± 3%) and temperature (29 ± 3ºC). The dead insects were removed from culture and the setup was managed under the laboratory conditions until the emergence of F1 progeny which were used for subsequent re-infestations.

Source of Maize Seeds

Local maize variety (Pajo white) used for the experiment was obtained from Sabo Market Ogbomoso. The maize was not exposed to any pesticide treatment.

Plant Materials and Essential Oils Extraction

Cleome viscosa plants were obtained from LAUTECH Teaching and Research Farm, Ogbomoso. Authentication was done using the WFO Plant List (wfoplantlist.org) in the Botany Unit, Department of Pure and Applied Biology, LAUTECH in accordance with Onifade *et al.* (2022) [29]. The fresh fruits were plucked from the branches and pounded with metallic mortar and pestle. Thereafter, a portion (1 kg) was weighed into 5-litre capacity round bottom-flask with 3 litres of distilled water. It was hydro-distilled for 3 hours with Clevenger type apparatus, and the EO was collected and stored in a glass vial and put in a refrigerator before use (Babarinde *et al.*, 2023) [8].

Fumigant Bioassay of *Cleome viscosa* Essential Oil Against Adult *Sitophilus zeamais*

The experiment was carried out in fumigation chambers following the method of Babarinde *et al*. (2017) [9]. Each fumigation chamber was a 33 ml capacity plastic hermetic vial with what-man No.1 filter paper measuring appropriately 4 m^2 area (6 g) firmly fixed to the inner surface of its lid using super glue. *Cleome viscosa* EO was applied on the filter paper using a Hamilton syringe at the rate of 0, 4.5, 9.0, 13.5, 18.0, and 22.5 µl/ml per fumigation chamber. The control set-up consisted of 10 adults of the same age used in the EO-treated fumigation chamber but without the EO application. After 5 minutes of application of the EO, ten 1-to-3-day old adult insects were introduced into the fumigation chamber and were firmly closed. The experiment was set up in five replicates.

Data were collected on mortality at 1.5, 3, 6, 12 and 24 HAT. Insects were adjudged dead when they did not respond to a pin probe. Mortality was recorded as a percentage of the total number of insects introduced into the jars using the formula:

$$Percentage\ mortality = \frac{number\ of\ dead\ insects}{total\ number\ of\ insects\ introduced} \times 100$$

Toxicity of *Cleome viscosa* Essential Oil on Parental and First Filial Adult Emergence of *Sitophilus zeamais*

Essential oil of *C. viscosa* (5 - 40 µl) was dissolved in 1.0 ml of n-hexane and mixed with 10 g of un-infested maize grain in 150 ml capacity glass jar. One control consisting of 1.0 ml of N-Hexane and another control with no solvent were included. Ten (10) *S. zeamais* adults similar to those used for the fumigation bioassay were introduced into the setup. Mortality of the parental *S. zeamais* was recorded. The setup was kept until emergence of F_1 progeny at 49 DAT. Data on percentage mortality of F_1 progeny was recorded at 50 – 57 DAT. There were five replicates for each treatment.

Experimental Design and Data Analysis

The experiment was laid out in a completely randomized design (CRD), while data were subjected to angular transformation to normalize for the variance. The variation among the replicates were shown by the standard errors. The means were separated using Student-Newman-Keuls at 5% significance levels after the data have been subjected to analysis of variance. Probit analysis was used to determine the median lethal dose (LD_{50}).

Results

Fumigant Toxicity *Cleome viscosa* Essential Oil Against Adult *Sitophilus zeamais*

Table 1 shows the fumigant toxicity of *C. viscosa* EO against *S. zeamis* adults. At 1.5 HAT, there was no mortality in the fumigation chamber with the low EO doses (0, 4.5, and 9.0 µl/ml air). However, when 18.0 and 22.5 µl/ml air were applied, the mortality in the fumigation chamber was significantly higher than the values observed at other lower EO doses. The same trend was observed at 3 HAT. At 6 HAT, 57.20 and 59.40% mortality observed when 13.5 and 22.5 µl/ml air were applied, respectively were significantly higher than 0.00 – 21.60% mortality observed when the lower EO doses were applied. At 12 HAT, mortality was equally dose-dependent. At 24 HAT, 79.40% mortality observed when the EO was applied at 22.5 µl/ml air was significantly higher than 8.80 – 61.33% mortality observed in lower EO doses.

The median lethal dose (LD_{50}) decreased with the exposure period and when the insects were exposed for 24 hours, the LD_{50} of 12.19(11.03 – 13.44) µl/ml air, was significantly lower than the LD_{50} values obtained when the weevils were exposed to the EO for 1.5, 3.0 and 6.0 hours (Table 2).

Table 1: Fumigant toxicity of *Cleome viscosa* essential oils against adult *Sitophilus zeamais*

	Hours after treatment				
Treatment (µl/ml air)	**1.5 hours**	**3 hours**	**6 hours**	**12 hours**	**24 hours**
0	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
4.5	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	8.80 ± 5.54 a

9.0	0.00 ± 0.00 a	12.40 ± 5.27 a	13.80 ± 6.26 ab	34.20 ± 4.51 b	39.80 ± 4.44 b
13.5	10.20 ± 6.68 a	15.00 ± 7.23 a	21.60 ± 6.80 b	43.40 ± 4.18 c	58.40 ± 9.33 c
18.0	40.40 ± 11.92 b	45.20 ± 8.48 b	57.20 ± 6.50 c	53.40 ± 4.84 c	61.33 ± 4.72 c
22.5	30.60 ± 5.40 b	50.20 ± 7.23 b	59.40 ± 4.11 c	68.60 ± 5.85 d	79.40 ± 6.91 d

Means with same superscript(s) along the column are not significantly different at 5% level of probability

Table 2: Median Lethal Dose (LD_{50}) of *Cleome viscosa* Essential Oil against *Sitophilus zeamais* Fumigant Toxicity Bioassay

Duration (h)	LD_{50} (LFL– UFL) (µl/ml air)	Regression equation	Chi Square	df	P	Slope ± SE
1.5	24.90 (19.37 – 39.67)	Y=4.63+6.87X	18.72	3	0.000	6.87±4.63
3.0	21.65 (17.02 – 42.61)	Y=3.63+8.36X	8.39	3	0.039	8.36±3.63
6.0	18.47 (15.01 – 27.06)	Y=4.04+9.45X	8.64	3	0.034	9.45±4.04
12.0	15.41 (11.01 – 26.00)	Y=3.16+9.80X	12.93	3	0.005	9.80±3.16
24.0	12.19 (11.03 – 13.44)	Y=2.84+10.24X	4.34	3	0.227	10.24±2.84

LFL = Lower Fiducial Level
UFL = Upper Fiducial Level

Effect of *Cleome viscosa* Essential Oil on Parental *Sitophilus zeamais*

The percentage mortality of the parental *S. zeamais* introduced into maize grains was dependent on EO doses. At higher doses of 20 and 40 µl/10 g grains, the morality values (15.6 and 17.3%) were significantly higher than 6.1 and 10.1% mortality observed in the grains with 5 and 10 µl/10 g grains. There was no mortality in untreated grains and the grains treated with N-hexane. (Figure 1)

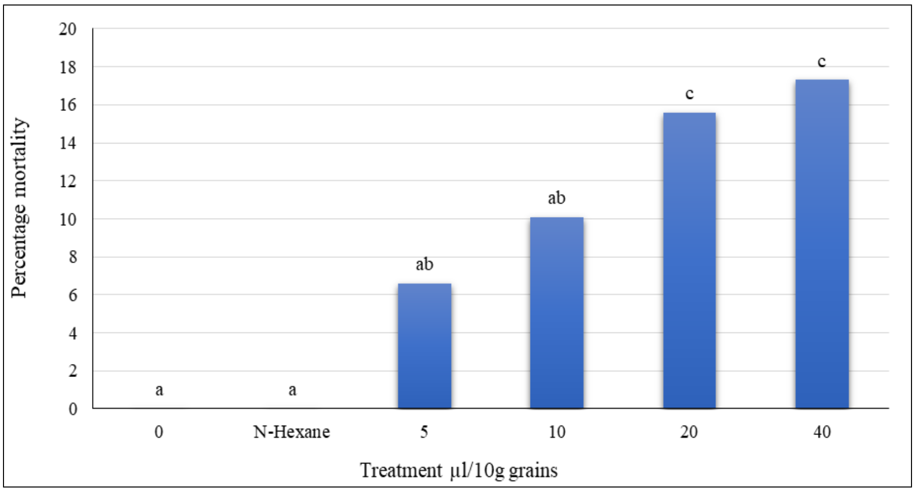

Fig 1: Effect of *Cleome viscosa* essential oil on parental generation of *Sitophilus zeamais* in essential oil-treated maize grains
ANOVA Result F=12.50; df=5,23 P<0.0001

Mortality of F1 Progeny of *Sitophilus zeamais* in Maize Seed with *Cleome viscosa* Essential Oils at 7 Weeks After Infestation

There was no mortality in the First filial (F_1) progeny in the untreated and maize grains treated N-Hexane. However, the percentage of dead F_1 progeny was dependent on the dosage of the EO applied on the 10 g maize grains. The mortality (87.50%) observed in the grains treated with 40 µl/10 g treatment was significantly higher than the mortality (33.33 – 62.50%) observed when the EO was applied at 5 – 10 µl/10 g maize grains. (Figure 2)

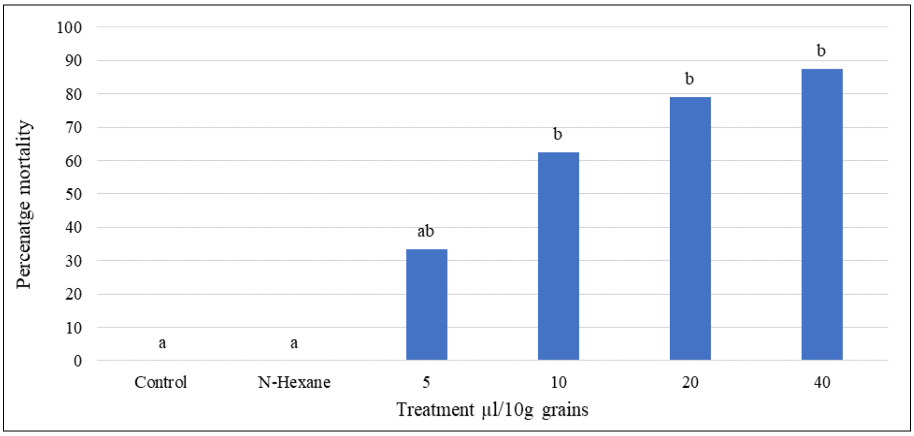

Fig 2: Mortality of F1 progeny of *Sitophilus zeamais* in maize grains treated with *Cleome viscosa* essential oils at 50 – 57 Days after Infestation
ANOVA Result F=6.21; df=5, 23 P=0.002

Plate 1: *Sitophilus zeamais* infestation of maize see

Plate 2: *Sitophilus zeamais*

Plate 3: Maize seeds destroyed by *Sitophilus zeamais* infestation

Plate 4: Dorsal views of *Sitophilus zeamais*

Plate 5: Dorsal views of Sitophilus zeamais

Plate 6: Maize seeds with varying degrees of damage caused by Sitophilus zeamais

Discussion

Many studies on ecofriendly control of insect pests have focused on the use of plants products which are cheaper, safer and eco-friendly (Nicoletti *et al.,* 2016; Babarinde *et al.,* 2017; 2019; Ajiboye et al., 2023) [3, 6, 9, 27]. Botanical EOs have been reported to be effective against storage pests (Mssillou *et al.*, 2022; Babarinde *et al.*, 2017; 2018) [9, 12, 26]. *Cleome viscosa* EO bears a wide range of compounds that are toxic and have oviposition inhibitory and insecticidal properties (Kaul and Saroop, 2021) [21]; particularly, there have been reports on the insecticidal activities of *C. viscosa* on *Callosobruchus chinensis* and *Sitophilus oryzae* (Somboon and Pimsamarn, 2006; Dabire *et al.*, 2008; Kaul and Saroop, 2021) [16, 21, 33].

In this study, the result of the fumigant toxicity bioassay was dose-dependent and the values progressed with the exposure periods. This is in agreement with previous studies on botanicals against maize weevil. For instance, *Jatropha curcas* seed oil was toxic against *S. zeamais* in a dose-dependent pattern (Babarinde *et al.*, 2019) [6]. The toxicity of *C. ciliata* leaf extract against *S. zeamais* also been reported to be dependent on the dose and exposure period (Oyegoke *et al*., 2010). Also, the EO of *Syzygium aromaticum* (L.) was reported to be toxic against the weevil (Jairoce *et al.*, 2016) [19]. The pathway of the EO into the body of an insects includes the respiratory system via the spiracles. Neurotoxicity has been identified as a mode of action of the EOs of monoterpenoid and phenylpropanoid classes (Kanda *et al.*, 2017) [20].

The EO was toxic to the parental generation of *S. zeamais.* The EO doses were applied on the grains and the portions of EO were absorbed by the grains. It implies that *S. zemais* adults could only pick the toxic EO when they fed on the treated grains. Although the level of toxicity (< 20% at the highest applied dose of 40 µl/10 g grains) was low, the results show that the EO had detrimental effects on the survival of *S. zeamais.* Therefore, the impact of increased dose of the EO should be investigated because conclusion can only be made based on empirical studies.

In the study of Adeyemo *et al.* (2019) [1], EO of *Hyptis suaveolen*, Thuja orientalis, Cymbopogon citratus, Eucalyptus camaldunensis applied on the maize grains at 0.1 - 1.0 ml/20 g grains evoked 100% adult mortality at 72 hours post treatment. Also, Babarinde *et al.* (2018) [12] reported that EO of *Citrus jambhiri* (27- 107 µL/L air) evoked 46 – 100% *S. zeamais* adult mortality. The mortality of the first filial *S. zeamais* emerging from the grains treated with *C. viscosa* EO was significantly higher in the grains treated with higher dose than what was observed in the untreated grains and grains treated with lower EO doses. That implies that the EO was absorbed by the grains and the emerging *S. zeamais* picked lethal dose from the grains when they fed on them. It further indicates that the EO that was absorbed by the grains had comparative stability and reduced volatility. In addition, the emerging F_1 progeny could have packed toxic doses of the EO prior to emergence which then weakened them and affected their post embryonic longevity.

Although the chemical composition of the EO was not investigated in the present study, earlier work of Olatunji *et al.* (2005) [28] reported the presence of Oct-1-ene, Non-1-ene, Dehydrosabinene, *p*-Cymene, Limonene, E-Ocimene, Undecane, Limonene oxide, Benzoic acid, a-Terpineol, Decan-2-ol, Undeca-10-en-1-al, Decan-2, 4-dien-1-al, and Coumarin in *Cleome viscosa* seed EO. Therefore, our opinion is that Limonene oxide, *p*-Cymene, Limonene, a-Terpineol, Coumarin identified in *C. viscosa* seed EO were contributory to the observed bioactivity. Since the fruits used in the present study contained seeds. Limonene Oxide showed significant insecticidal potential against adult *S. zeamais* and *S. granarius* through fumigant toxicity (Yıldırım *et al.*, 2013; Kordali *et al.*, 2017) [23, 38], *p*-Cymene exhibited insecticidal potential on adult housefly through injection bioassay (Yoon and Tak, 2023) [39]. Also, Limonene showed insecticidal actions through contact toxicity against Coffee borer, and fumigant toxicity against *Sitophilus species* (Brito *et al.* 2021; Kordali *et al.*, 2017) [15, 23], while a-Terpineol and Coumarin were reported to have insecticidal actions against different species of insects (Khaleel *et al.*, 2018; Xia *et al.*, 2023) [22, 37].

In conclusion, the results of the present study indicates that *Cleome viscosa* EO can control *S. zeamais* via fumigant, stomach, and contact toxicity. It implies that the EO can control the resident population of *S. zeamais* and also reduce the survival of the first filial generation. The findings of this study establish that *C. viscosa* EO can be a viable option for controlling *S. zeamais* in maize grains.

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