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Biopesticidal Extractives and Compounds from *Warburgia Ugandensis* against Maize Weevil (*Sitophilus zeamais*)

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Abstract: Agricultural production is constrained by insect pests, which cause serious post-harvest losses of up to 43% in developing countries. Maize weevil (*Sitophilus zeamais* Motschulsky.) is one of the most destructive pests of maize. Synthetic chemicals have been used to protect stored grain from damage by insects. The increasing knowledge about the harm derived from the indiscriminate use of synthetic insecticides has prompted research aimed at finding safe methods of pest control. Efficacy of extractives and isolates from *Warburgia ugandensis* (Canellaceae) were evaluated for maize grain protection against *S. zeamais.* The oil extract was the most repellent ($P<0.05$) with repellence distance of 6.37 within 2-hour exposure duration followed by *n*-hexane extract (6.20 cm). The most repellent compounds were mukaadial (**6**) and polygodial (**1**) (5.43 and 4.83 cm, respectively). Essential oil was the most toxic ($P<0.05$) to the weevils and showed 100% mortality at 21 days. The toxicity levels of the organic extracts ranged from 18.3 to 78.0% with *n*-hexane exhibiting the highest toxicity followed by ethyl acetate extract. Polygodial (**1**) and warburganal (**2**) were the most toxic compounds (70.0 and 65.0% respectively). The oil extract was as active as the Actellic dust and completely inhibited the emergence of the insect adults. Polygodial (**1**), ugandensolide (**3**) and warbuganal (**2**) had the best growth inhibition activity. The results from the present study indicate that *W. ugandensis* could be a useful alternative in stored grain protection against maize weevil and the isolated compounds could be good candidates as phytoinsecticidal agents against insect pests.

Keywords: Biopesticidal agents, extracts, maize weevil, insect pests, isolates, *Warburgia ugandensis*.

1. INTRODUCTION

Agricultural production is constrained by more than 20,000 species of field and storage insect pests, which cause serious post-harvest losses from up to 9% in developed countries to 43% in developing African and Asian countries [1-3] resulting to loss of weight, seed viability, and nutritive quality of foodstuffs [4]. Maize weevil (*Sitophilus zeamais* Motschulsky.) is one of the most destructive insect pests of maize [5]. The chewing damage caused by the insect brings about increased respiration in the cereal (hot spots), which promotes evolution of heat and moisture and in turn provides favorable living condition for molds leading to production of aflatoxin. Subsequently, at very high moisture levels, bacterial growth is favored which ultimately gives rise to depreciation and finally total loss of grains [6].

Several methods are used in controlling damage by insect in stored grains, including smoking, sun-drying, heating, and use of synthetic chemical [7, 8]. Several workers have reported the successful wide scale use of synthetic organic insecticides, commencing with the organochlorines in the middle 1940s, followed by the later use of organophosphates, carbamates, pyrethroids, avermectins, and others [7-9]. However, the use of such chemicals has deleterious side-effects to non-target species including humans and the development of resistant strains of pests [10-12]. Furthermore, the high cost of synthetic insecticides limits their accessibility to peasant farmers [13, 14]. The increasing knowledge about the harm derived from the indiscriminate use of synthetic insecticides has encouraged research aimed at finding safe methods of pest control. Plants provide an alternative source of biodegradable insecticides that are safe to humans and the rest of the environment and are readily available and renewable [15-19]. *Warbugia. Ugandensis* (Canellaceae) is traditionally used as a remedy for stomachache, constipation, toothache, malaria, sexually transmitted diseases, diarrhoea, cough and internal wounds/ulcers [20]. *Warburgia* species are characterized by the presence of drimane sesquiterpenes some of which have been reported to exhibit antifungal, insect antifeedant, insecticidal and molluscicidal activities [21-23]. The present study was conducted to investigate the efficacy of essential oil, leaf powder, organic extracts and compounds of *W. ugandensis* in the control of *S. zeamais* infestation in stored maize grain.

2. MATERIALS AND METHODS

2.1. General

Melting points were determined on a Gallenkamp (Loughborough, UK) melting point apparatus and are

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uncorrected. The UV spectra were run on Pye Unicam SP8-150 UV–vis spectrophotometer (Cambridge, UK) using acetonitrile. IR data were recorded on a PerkinElmer FTIR 600 series spectrophotometer (Waltham, MA, USA) as KBr pellet. The ^{1}H and ^{13}C NMR data were measured in $CDCl_3$ and $CDCl_3$–DMSO-d_6 on a Bruker NMR Ultrashield TM (Darmstadt, Germany) operating at 500 and 125 MHz, respectively. The MS data were obtained on a Varian MAT 8200A instrument (Bremen, Germany).

2.2. Collection and Preparation of Plant Materials

Leaves of *W. ugandensis* were collected along Nakuru-Gilgil Highway near St. Mary's Hospital (latitude 0° 24' 42.49" S and longitude 36° 15' 10.59" E) in May 2014 and voucher specimen (2014/5/SAO/CHEMMK) was identified at the Kenya National Museum Herbarium after comparison with authentic samples. The plant materials were air dried at 24-28°C until crispy. The dried leaves were pulverized and sieved through a 0.5 mm size mesh to obtain uniform particle size for bioassays.

2.3. Extraction, Fractionation and Isolation

Two kg of powdered leaves of *W. ugandensis* was cold extracted with organic solvents of varying polarities (*n*-hexane, ethyl acetate and methanol) sequentially by soaking in the solvents for seven days with occasional shaking. The mixture was filtered and concentrated using a rotary evaporator at reduced pressure to yield 20.2, 58.6 and 97.8 g of *n*-hexane, ethyl acetate and methanol extracts, respectively. The resultant extracts were stored at 4°C for bioassays and phytochemical studies. *n*-Hexane and ethyl acetate extracts showed similar TLC profile and were combined for phytochemical isolation. The combined extract (50 g) was dissolved in a small amount of *n*-hexane - ethyl acetate mixture (1:1) and subjected to in silica gel for column chromatography using silica gel. Elution was done using *n*-hexane, *n*-hexane - ethyl acetate mixture, ethyl acetate and methanol to give 200 fractions (each 20 ml) whose compositions were monitored by TLC and those with similar profiles were combined to give seven pools labeled I -VII. Pool I, 3g, which was eluted with *n*-hexane did not show any major spot on TLC and was discarded. Pool II (7 g) was subjected to further column chromatography eluting with *n*-hexane: ethyl acetate (95:5, 9:1, 85:15 and 4:1) to give polygodial (**1**) 30 mg and warbuganal (**2**) 55 mg. Pool IV (5g) on further fractionation with gradient *n*-hexane-ethyl acetate mixture (85:15, 4:1 and 7:3) gave polygodial (**1**) 35 mg and ugandensolide (**3**) 38 mg. Pool V (8 g) on further fractionation with *n*-hexane: ethyl acetate (4:1, 7:3 and 65:35) gave ugandensolide (**3**) 24 mg, ugandensidial (**4**) 42 mg and muzigadial (**5**) 75 mg. Pool VI (9 g) gave ugandensidial (**4**) 43 mg wile Pool VII gave muzigadial (**5**) 15 mg and mukaadial (**6**) 72 mg.

Polygodial (**1**)**:** white needle crystals (n-hexane- ethyl acetate mixture), mp 58 -59 °C; IR ν_{max} (KBr) cm^{-1}: 1730, 1680, 1645; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.86 (1H, d, *J* = 4.5 Hz, H-11), 9.41 (1H, s, H-12), 7.17 (1H, dt, *J* = 6.0, 3.0 Hz, H-7), 3.25 (1H, ddd, *J* = 4.6, 2.4, 2.2Hz, H-9), 0.94, 0.97, 0.92 (9H, 3 s, 13, 14, 15-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c: 39.4 (C-1), 17.9 (C-2), 41.6 (C-3), 33.0 (C-4), 49.0 (C-5), 25.1 (C-6), 154.4 (C- 7), 138.1 (C-8), 60.2 (C-9), 36.6 (C-10), 201.9 (C-11), 193.2 (C-12), 33.0 (C-13), 21.9(C-14), 15.2 (C-15); EIMS *m/z* (rel. int.): 234 $[M]^+$ (1), 206 (55), 191 (25), 121 (65), 109 (60), 41 (100).

Warbuganal (**2**): White crystals (n-hexane- ethyl acetate mixture), mp 134 -135 °C IR ν_{max} (KBr) cm^{-1}: 3451, 2947, 2921, 2868, 2803, 1714, 1681, 1644; ^{1}H NMR (500 MHz, $CDCl_3$); 9.73 (1H, s, H-11), 9.41 (1H, s, H-12), 7.28 (1H, dd, *J* = 4.9, 2.8 Hz, H-7), 4.09 (1H, s, 9-OH), 1.09, 0.99, 0.95 (9H, 3 s, 13, 14, 15-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c: 41.3(C-1), 17.7 (C-2), 31.1 (C-3), 33.0 (C-4), 41.7 (C-5), 25.9 (C-6), 157.6 (C- 7), 140.3 (C-8), 77.7 (C-9), 41.4 (C-10), 202.3 (C-11), 192.7 (C-12), 33.0 (C-13), 22.1 (C-14), 15.2 (C-15); ESI-MS *m/z:* 273 $[M+Na]^+$.

Ugandensolide (**3**): White needle crystals (CH_2Cl_2-MeOH), mp 215-218 °C; IR ν_{max} (KBr) cm^{-1}: 3451, 29.31, 2887, 1733, 1672, 1463, 1370, 1342, 1251, 1203, 1142, 1063, 1027; ^{1}H NMR (500 MHz, $CDCl_3$); 5.34 (1H, s, H-6), 4.85 (1H, d, *J* = 17.2 Hz, H-11a), 4.61 (1H, dd, *J* = 17.2, 1.0 Hz, H-11b), 4.17 (1H, dd, *J* = 5.5, 1.0 Hz, H-7a), 3.57 (1H, d, *J* = 5.5Hz, H-7b), 1.43, 1.02, 1.00 (9H, 3 s, 13, 14, 15-Me), 2.03 s(3H, s, $\underline{C}H_3CO$); ^{13}C NMR(125 MHz, $CDCl_3$) δ_c: 43.0 (C-1), 18.3 (C-2), 36.3 (C-3), 35.3 (C-4), 49.2 (C-5), 73.8 (C-6), 66.0 (C- 7), 154.1 (C-8), 138.0 (C-9), 33.3 (C-10), 171.9 (C-11), 69.7 (C-12), 20.7 (C-13), 33.1 (C-14), 21.4 (C-15), 171.0 ($CH_3\underline{C}O$), 23.0 ($\underline{C}H_3CO$); EIMS *m/z* (rel. int.): 308 $[M]^+$ (10), 266 (96), 248 (96), 233 (28), 215 (20), 177 (28), 163 (35), 69 (28), 55 (26), 43 (100).

Ugandensidial (**4**) White needles (n-hexane- ethyl acetate), mp 136 - 137 °C; IR ν_{max} (KBr) cm^{-1}: 3428, 3007, 2945, 2848, 1743, 1781, 1692, 1462; ^{1}H NMR (500 MHz, $CDCl_3$); 9.77 (1H, s, H-11), 9.49 (1H, s, H-1), 7.01 (1H, d, *J* = 4.7 Hz, H-7), 5.90 (1H, t, *J* = 5.70 Hz, H-6), 4.10 (1H, t, *J* = 1.4 Hz, H-9), 1.17, 1.34, 1.03 (9H, 3 s, 13, 14, 15-Me), 2.15 s(3H, s, $\underline{C}H_3CO$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c: 44.0 (C-1), 32.6 (C-2), 17.6 (C-3), 33.9 (C-4), 44.9 (C-5), 66.9 (C-6), 148.5 (C- 7), 140.9 (C-8), 76.6 (C-9), 41.5 (C-10), 201.0 (C-11), 192.9 (C-12), 17.7 (C-13), 24.7 (C-14), 31.8 (C-15), 169.9 ($CH_3\underline{C}O$), 21.5 ($\underline{C}H_3CO$); EIMS *m/z* (rel. int.): 308 $[M]^+$ (5), 280 (34), 237 (12), 220 (50), 148 (56), 109 (60), 60 (80), 43 (100).

Muzigadial (**5**): White plate-like material (n-hexane: ethyl acetate mixture), mp 123 -125 °C; IR ν_{max} (KBr) cm^{-1}: 3455, 2966, 2921, 2870, 1731, 1671; ^{1}H NMR (500 MHz, $CDCl_3$); 9.64 (1H, s, H-11), 9.43 (1H, s, H-1), 7.24 (1H, t, *J* = 3.5 Hz, H-7), 4.93 (1H, s, H-13a), 4.75 (1H, s, H-13b), 4.05 (1H, s, 9-OH), 1.08, (3H, d, *J* = 6.6 Hz, 14-Me), 0.88 (3H, s, 15-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c: 31.7 (C-1), 30.8 (C-2), 38.2 (C-3), 151.6 (C-4), 40.2 (C-5), 27.6 (C-6), 155.6 (C- 7), 139.9 (C-8), 77.6 (C-9), 42.3 (C-10), 201.2 (C-11), 192.7 (C-12), 106.1 (C-13), 18.4 (C-14), 15.1 (C-15); EIMS *m/z* (rel. int.): 248 $[M]^+$ (10), 237 (100), 219 (19), 109 (43)69 (50), 41 (52).

Mukaadial (**6**): White needdle crystals (CH_2Cl_2-MeOH), mp 228-229 °C IR ν_{max} (KBr) cm^{-1}: 3350, 2990, 2923, 2798, 2701, 1720, 1696, 1466; ^{1}H NMR (500 MHz, $CDCl_3$); 9.64 (1H, s, H-11), 9.40 (1H, s, H-1), 7.03 (1H, s, H-7), 5.15 (1H, s, H-9), 5.04 (1H, s, *J* = 4.5 Hz H-6a), 4.41 (1H, ddd, *J* = 10.6, 9.0, 2.4 Hz H-6b), 1.03, 1.08, 1.15 (9H, 3 s, 13, 14, 15-

Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c: 42.8 (C-1), 32.8 (C-2), 17.8 (C-3), 36.2 (C-4), 47.4 (C-5), 67.0 (C-6), 158.9 (C- 7), 139.0 (C-8), 77.4 (C-9), 43.1 (C-10), 203.5 (C-11), 193.5 (C-12), 17.9 (C-13), 22.5 (C-14), 36.3 (C-15); EIMS *m/z* (rel. int.): 266 $[M]^+$ (5), 248 (100), 236 (15), 230 (46), 220 (23), 202 (21), 175 (27), 160 (6), 138 (8), 104 (10), 94 (4).

2.4. Essential Oil Extraction

Fresh leaves of *W. ugandensis* (2 kg) were cut into pieces and boiled with distilled using Clevenger-type apparatus for six hours. The superior phase was collected from the condenser, dried over anhydrous sodium sulfate and kept in a refrigerator (4°C) for further experiments.

2.5. Mass Rearing of *S. Zeamais*

Adult weevils (*S. zeamais*) were obtained from infested maize grains purchased from local market and from this stock, new generation was reared on dry pest susceptible maize grains [24]. Two hundred maize weevils of mixed sexes were introduced into a two liter glass jars containing 400 g weevil susceptible maize grains [25]. The mouths of the jars were then covered with nylon mesh held in place with rubber bands and the jars left undisturbed for 35 days for oviposition. Thereafter, all adults were removed through sieving and each jar was left undisturbed for another 35 days. Emerging adult insects were collected and kept in separate jars according to their age. Adults that emerged on same day were considered of the same age [26].

2.6. Repellency Test

The test was done according to [24] with some modifications. Transparent plastic tubings, 13 cm long x 1.3 cm diameter were used as test cylinders. Each test cylinder was plugged at one end with cotton ball containing powdered leaf from *W. ugandensis* while the other end was plugged with clean cotton ball which served as control. Actellic dust was used as a positive control. Ten-three-day old unsexed test insects were introduced at the middle of each test cylinder through a hole at the middle portion of the cylinder (0.0 cm) and let to move in any direction of their choice with scoring of distance moved measured in cm with using a ruler. The score time was 2, 5, 24 and 96 hrs after exposure. Each treatment was done in triplicates. The test was repeated with essential oil, organic extract and pure compound obtained from the plant.

2.7. Adult Mortality Test

Contact toxicity assay was done according to [27] with some modifications. Toxicity of the leaf powder, essential oil, organic extracts and pure compounds were tested against adult weevils. The test samples were mixed with talc thoroughly and the dust was admixed with 20 g of maize held in 12 cm high x 6.5 cm diameter glass jars covered with ventilated lids. To ensure a thorough admixture, the grain was put in 12 cm high x 6.5 cm diameter glass jars, dust applied and top lid replaced. The grain was then swirled within the jar until a proper admixture was realized [28]. Twenty-three-day old unsexed insect pairs were then introduced into each dish and exposed to treatments. Actellic dust was used as a positive control. The treatments were laid out in a completely randomized design with three replicates per treatment. Maize weevils were considered dead when probed with sharp objects and there were no responses [27]. The number of dead insects in each vial was counted after 1, 2, 7, 14 and 21 days after treatment to estimate maize weevil mortality as follows:

% Mortality = 100* (Number of dead insects) / (Total number of insect).

Data on percentage adult weevil mortality were corrected using Abbott's formula [30].

PT = (Po – Pc) / (100 - Pc)

Where PT = Corrected mortality (%); PO = Observed mortality (%); PC = Control mortality (%).

2.8. Growth Inhibition Assay

The test was done according to [27] with some modifications. 20 g of clean undamaged and uninfected corn grains were placed in 12 cm high x 6.5 cm diameter glass jars glass jars. Test material (powdered leaves or organic extracts or pure compounds) was thoroughly mixed with the grains in each jar. Crude extracts and pure compounds were mixed with talc thoroughly before being applied to the grains [28]. Three replicates of the treatments and untreated controls were laid out in Complete Randomized Design. A mixture of twenty -seven-day old unsexed maize weevils was introduced in each jar and covered with filter paper [26]. The female adults were allowed to oviposit on the seeds for 4 days. On day 5, all insects were removed from each container and the seeds returned to their respective containers. Progeny emergence (F1) was then recorded at six weeks (42 days). The containers were sieved out and newly emerged adult weevils were counted [27]. At week six, the grains were reweighed and the percentage loss in weight was determined as follow:

$$\% \text{ Weight loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Data obtained from the experiments were subjected to analysis using MSTAT C version 2.10 for analysis of variance (ANOVA); means were separated by least significant difference (LCD) at five percent significant level.

3. RESULTS AND DISCUSSION

3.1. Compounds Isolated from *W. ugandensis*

n-Hexane and ethyl acetate gave similar profile on TLC and were combined (some left for bioassays). The combined extract subjected to repeated chromatographic separations which lead to the isolation of six drimane sesquiterpenes: polygodial (**1**), warbuganal (**2**), ugandensolide (**3**), ugandensidial (**4**), muzigadial (**5**), and mukaadial (**6**) (Fig. **1**). The structures of the compounds were characterized and identified by their IR., ^{1}H NMR and ^{13}C NMR, and comparing with data of authentic samples [22, 23]. The six compounds were previously reported from the plant [22].

Compound **1** gave a molecular ion peak at *m/z* 234, corresponding to $C_{15}H_{22}O_2$. ^{13}C NMR showed 15 signals

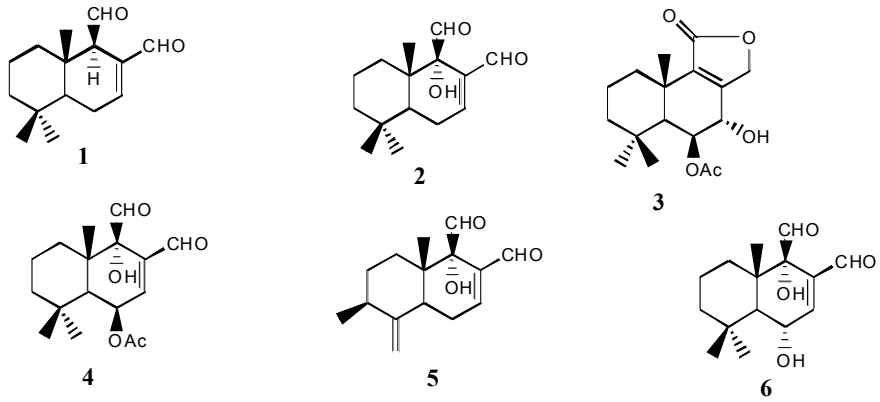

Fig. (1). The Structures of compounds isolated from *W. ugandensis*.

with diagnostic peaks at δ_C 201.9 and 193.2 for two aldehyde carbonyl carbons; δ_C 154.4 and 138.1 for the carbon – carbon double bond; δ_C 33.0, 21.7 and 15.2 for ther methyl groups at C-13, C-14 and C-15. ^{1}H NMR singlets at δ_H 9.53 and 9.47 were assigned to the aldehyde protons [21, 22]. Compound **2** gave a molecular ion peaks at *m/z* 273 $[M+Na]^+$, corresponding to $C_{15}H_{22}O_3$. Its NMR data were similar to those of **1** except for the presence of peaks at δ_H 4.09 and δ_c 77.65, for the OH group at C-9. The peaks at δ_c 202.27 and 192.70, δ_H 9.73 and 9.41 confirmed the presence of an isolated and α,β-unsaturated aldehyde groups [21, 22]. Compound **3** gave a molecular ion peak at *m/z* 308 corresponding to $C_{17}H_{24}O_4$. The ^{13}C NMR spectrum shows 17 peaks consisting of four methyl, four methylene, three methine and six quaternary carbons, suggesting an acetylated drimane sesquiterpene. NMR data showed signals at δ_H 2.03 and δ_C 171.0 for the acetate group; δ_C 171.9 for the lactone carbonyl; δ_C 154.1 and 138.0 for a tetra-substituted carbon-carbon double bond [21, 22]. Compound **4** gave NMR peaks at δ_C 201.0 and 192.9 suggested an isolated and a conjugated carbonyl carbons; δ_C 148.54 and 140.89 for a tri-substituted carbon-carbon double bond; δ_C 169.9, 21.5, and δ_H 2.15s the acetate group [21, 22]. Compound **5** gave a molecular ion peak at *m/z* 248 corresponding to $C_{15}H_{20}O_3$. NMR data showed four olefinic peaks at δ_C 155.7, 151.6, 139.9 and 106.1 for exocyclic and internal C-C double bonds; δ_C 201.2 and 192.7 for two aldehyde carbonyl groups at C-11 and C-12; δ_C 77.61 for oxygenated quaternary carbon C-9 [21, 22]. Compound **6** showed a molecular ion peak at *m/z* 266 corresponding to $C_{15}H_{22}O_4$. ^{13}C NMR data resembled closely those of **1**, with the major difference being the presence peaks at δ_C 77.4, 67.0, δ_H 5.15 and 5.04 for hydroxylated quaternary and methine carbons at C-9 and C-6, respectively. Peaks at δ_C 203.49, 193.47, δ_H 9.64 and 9.40 confirmed the presence of two alsehyde groups while peaks at δ_C 158.99 and 138.76 were attributed to the olefinic carbons [21, 22].

3.2. Repellent Activity

The repellence activity of leaf powder, extracts and compound from *W. ugandensis* against maize weevil was observed after 2, 5, 24, 48 and 96 hrs of exposure duration and the results are presented in Table **1**. The distance moved by the weevils varied significantly (P<0.05) after the 2, 5, 24, 48 and 96 hrs period across the test materials used. The oil extract was the most active (mean repellency = 4.44cm) with repellence distance of 6.37 and 6.17 cm) within 2- hour and 5-hours exposure durations, respectively. Among the organic extracts, *n*-hexane was the most reppellent (6.20 cm) within 2-hour exposure period while ethyl acetate was the most active (5.57 cm) after 5-hour. All the isolated compounds caused some repulsion against the weevils. However, all the compounds were less repellent compared to the essential oil, n-hexane and ethyl acetate extracts. Mukaadial (**6**) and Polygodial (**1**) were the most repellent compounds with the weevils moving 5.43 and 4.83 cm, respectively within 2-hour exposure duration. All the tested materials showed significantly (P<0.05) higher mean repellence activity over the test period than Actellic powder which was used as a positive control except methanol extract, ugandensolide (**3**) and ugandensidial (**4**) with means of 2,22, 2.36 and 2.23 cm, respectively.

3.3. Mortality Activity against Adult Maize Weevils

The mortality effects of theessential oil, leaf powder, organic extracts and isolated compounds from *W. ugandensis* against *S. zeamais* are presented in Table **2**. All the tested materials significantly (P<0.05) reduce the longevity of adults *S. zeamais* on treated maize grains. Mortality varied significantly (P<0.05) amongst the test materials and also increased significantly with duration of exposure. Essential oil was the most toxic to the weevils and showed 100% mortality at 21 days. The toxicity levels of the organic extracts ranged from 18.3 to 78.0% with *n*-hexane exhibiting the highest toxicity followed by ethyl acetate extract. All the six compounds isolated from the combined *n*-hexane and ethyl acetate extracts showed varied levels of toxicity to the weevils which were lower than those of the crude extracts. Polygodial (**1**) and warburganal (**2**) evoked significantly (P<0.05) high mortality (70.0 and 65.0% respectively) at 21 days after treatment compared to the other isolated compounds. No mortality was observed in the untreated control.

3.4. Growth Inhibition activity and Weight Loss in Maize Grains

Different treatments significantly (P<0.05) reduced the progeny of *S. zeamais* (Table **3**). The adult emergence in the untreated control was significantly higher than in the treated grains. In the untreated control the number of the insects

Table 1. Repellent activity of leaf powder, extracts and compounds from *W. ugandensis* against *Sitophilus zeamais*.

	Repellence*					
	Exposure Duration in Hours					
Repellent	2	5	24	48	96	Mean Repellency
Essential oil (0.2 ml)	6.37±0.12	6.17±0.15	5.57±0.21	3.30±0.17	1.33±0.15	4.55
Leaf powder (2 g)	5.33±0.47	4.63±0.15	3.20±0.20	1.87±0.21	1.70±0.10	3.35
n-Hexane extract (50 mg)	6.20±0.10	5.57±0.21	4.43±0.15	2.13±0.15	1.70±0.20	4.01
Ethyl acetate extract (50 mg)	4.77±0.15	5.17±0.15	5.50±0.20	3.33±0.31	1.27±0.12	4.01
Methanol extract (50 mg)	3.73±0.15	2.67±0.15	1.83±0.25	1.43±0.12	1.43±0.15	2.22
Polygodial (1) (2 mg)	4.83±0.06	5.17±0.15	4.43±0.21	2.17±0.06	1.50±0.20	3.62
Warbuganal (2) (2 mg)	3.7±0.10	4.47±0.15	3.97±0.12	1.73±0.06	1.53±0.15	3.08
Ugandensolide (3) (2 mg)	2.97±0.15	3.27±0.25	2.13±0.15	1.63±0.15	1.80±0.10	2.36
Ugandensidial (4) (2 mg)	3.27±0.25	2.57±0.25	2.70±0.20	1.50±0.10	1.13±0.15	2.23
Muzigadial (5) (2 mg)	4.03±0.06	4.03±0.12	3.37±0.21	2.03±0.15	1.33±0.25	2.96
Mukaadial (6) (2 mg)	5.43±0.12	5.40±0.10	4.33±0.25	2.63±0.38	1.60±0.10	3.88
Actellic dust (2 mg)	3.23±0.15	3.73±0.15	3.10±0.10	2.17±0.15	1.47±0.15	2.74
LSD, P ≤ 0.05	0.12					

*Mean (±SD) distance (in cm) values of weevil away from the tube centre (0.0 cm).

Table 2. Mortality effect of leaf powder, organic extracts and isolates from *W. ugandensis* against *S. zeamais.*

	% Mortality*					
	Exposure duration in days					
	1	2	7	14	21	Mean Mortality%
Essential oil (0.2 ml)	33.3±2.9	38.3±2.9	48.3±7.6	65.0±13.2	100.0±0.0	57.0
Leaf powder (2 g)	26.7±7.6	33.3±2.9	45.0±7.6	58.3±7.6	71.7±7.6	47.0
n-Hexane extract (50 mg)	28.3±7.6	40.0±5.0	56.7±7.6	70.0±5.0	78.3±7.6	54.7
Ethyl acetate extract (50 mg)	18.3±2.9	41.7±2.9	48.3±2.9	73.3±7.6	75.0±10.0	51.3
Methanol extract (50 mg)	20.0±5.0	21.7±2.9	28.3±2.9	31.7±7.6	40.0±8.7	28.3
Polygodial (**1**) (2 mg)	16.9±2.9	25.0±5.0	40.0±5.0	58.3±10.4	70.0±8.7	42.0
Warbuganal (**2**) (2 mg)	31.7±2.9	36.7±2.9	38.3±2.9	55.0±8.6	65.0±5.0	45.3
Ugandensolide (**3**) (2 mg)	20.0±5.0	23.3±5.8	35.0±5.0	36.7±2.9	43.3±2.9	31.7
Ugandensidial (**4**) (2 mg)	11.7±2.9	21.7±2.9	26.7±2.9	31.7±2.9	38.3±2.9	26.0
Muzigadial (**5**) (2 mg)	16.67±5.8	23.3±7.6	26.7±10.4	33.3±7.6	41.7±7.6	28.3
Mukaadial (**6**) (2 mg)	11.7±2.9	18.3±2.9	28.3±7.6	31.7±2.9	41.7±2.9	26.3
Actellic dust (2 mg)	71.7±12.6	88.3±7.6	100.0±0.0	100.0±0.0	100.0±0.0	92.0
Control (untreated)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0
LSD, P ≤ 0.05	3.81					

* Each value is a mean ± SD of three replicates.

Table3. Growth inhibition activity of leaf powder, extracts and isolates from *W. ugandensis* against *S. zeamais* and maize grain weight loss.

Treatments	Adult Emergence after 42 Days	Percentage Weight Loss after 42 Days
Essential oil (0.2 ml)	0.0±.0.0	0.0±0.0
Leaf powder (2 g)	4.7±0.6	4.2±0.8
n-Hexane extracts (50 mg)	4.0±1.0	2.8±0.8
Ethyl acetate extracts (50 mg)	9.3±0.3	6.7±1.3
Methanol extracts (50 mg)	21.7±1.5	17.5±1.4
Polygodial (1) (2 mg)	7.7±0.6	11.9±0.8
Warbuganal (2) (2 mg)	15.3±0.6	16.5±0.6
Ugandensolide (3) (2 mg)	11.7±1.5	16.5±0.5
Ugandensidial (4) (2 mg)	26.0±1.0	19.7±0.9
Muzigadial (5) (2 mg)	36.0±1.0	23.9±0.6
Mukaadial (6) (2 mg)	26.7±0.6	15.0±0.5
Actellic dust (2 mg)	0.0±0.0	0.0±0.0
Control (untreated)	48.0±1.0	33.1±1.1

Values are means ± SD of three replicates.

emerging was 48.0. The oil extract was as active as the standard insecticide, Actellic dust and completely inhibited the development of the insects. Adult emergence in the grains treated with the leaf powder, *n*-hexane, ethyl acetate and methanol extracts was 4.7, 4.0, 15.0 and 21.7, respectively. For the pure compound, polygodial (**1**), ugandensolide (**3**) and warbuganal (**2**) exhibited the highest emergence inhibition of 7.7, 11.7 and 15.3, respectively. Percent weight loss incurred in botanically treated maize seeds was significantly ($P<0.05$) lower than weight loss in the untreated control (33.1%). The best protection of the grains from weight loss was obtained from the oil extract (0.0 %). *n*-Hexane, ethyl acetate and methanol extracts recorded 2.8, 6.7 and 17.5% weight loss, respectively while the leaf powder had 4.4%. Polygodial (**1**) and Mukaadial (**6**) were the best in maize grain protection with 11.9 and 15.9% weight loss respectively.

The repellent, mortality and adult emergence inhibition tests have demonstrated that the oil extract is the most effective in controlling maize weevil (*S. zeamais*) which is among the most destructive insect pest of maize and other cereals in storage. The finding is in agreement with previous authors [31, 32, 33] who reported the activities of oil extracts from various plants. The leaf powder was also effective in controlling the weevil and this is in agreement with previous reports [34, 35, 36] indicating the use of various plant leaves to manage storage grain insect pests. All extracts from the plant (*n*-hexane, ethyl acetate and methanol) demonstrated repellent, mortality and adult emergence inhibition activities against maize weevil. From the isolated compounds, polygodial (**1**), warburganal (**2**), ugandensolide (**3**) and mukaadial (**6**) were the most potent for maize weevil control. Previous reports indicate that extract and compounds from *Warburgia* species have antifeedant and insecticidal activities [21, 37, 38]. Polygodial (**1**) has been used experimentally in the UK to protect barley from the bird-cherry-oat aphid (*Rhopalosiphum padi*) a transmitter of barley yellow dwarf virus [39, 40]. The compound caused reduced feeding in Colorado potato beetle (*Leptinotarsa decemlineata*) on potato leaf discs [41] and in New Zealand had antifeedant activity against the Australian carpet beetle (*Anthrenocerus australis*) at a concentration as low as 0.04% wool weight [42]. It had also both antifeedant and insecticidal activity against a lepidopteran, the webbing clothes moth (*Tineola bisselliella*) [43]. Ugandensidial showed antifeedant activity on *Spodoptera species* at 0.1 ppm [40] while Warburganal showed antifeedant activity on *Spodoptera exempta* [40, 43].

Findings from this study revealed that extracts of *W. ugandensis* have repellent, toxicity and growth inhibition activities against *S. zeamais* which destroy maize and other cereal grains both in the field and in storage. This suggests that insect pests can be managed using herbal extracts as had also been observed in other studies [8, 44]. Use of plant extracts as bio-pesticides is environmentally safe compared to the chemicals. From the results, the isolated compounds are less active compared to the essential oil and crude extracts in the repellence, adult mortality as well as in the growth inhibition tests suggesting possible synergistic effect in the extracts. Further research to investigate the synergism or antagonistic effects of the pure compounds is necessary to determine the combinations with best activities. It is also necessary to isolate and characterize the insecticidal compounds present in the essential oil from the plant.

Hexane and ethyl acetate extracts gave similar TLC profiles but exhibited different levels of activities in bioassays. This can be attributed to presence of different fatty acids and other minor components in the extracts.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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