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Efficacy of Botanical Extract Formulations of *Zanthroxylum usambarense* and *Warburgia ugandensis* on Post-Harvest Management of *Sitophilus zeamais* in Maize

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Abstract: *Sitophilus zeamais* causes significant losses to maize produce worldwide. The use of biodegradable and environmentally friendly botanicals as an alternative to synthetic pesticides is increasingly becoming important. Therefore, we sought to determine the use of plant extract formulations to manage *S. zeamais* during storage. Crude *Zanthroxylum usambarense* and *Warburgia ugandensis* stembark extracts were used for contact toxicity and repellent bioassays against *S. zeamais*. The formulations that exhibited the highest repellence and mortality were tested for insecticidal activity during storage for six months. Phytochemical profiles of the extracts were determined using GC-MS, and molecular docking of active compounds against insect target proteins was done. Mortality analyses revealed LD_{50} values of 114.89 µg/mL and 197.19 µg/mL for *Z. usambarense*'s hexane and methanol organic extracts, respectively. *Warburgia ugandensis* extracts had LD_{50} values of 69.25 µg/mL and 163.52 µg/mL, respectively. Extract formulations achieved weevil perforation index values of <50.00 in all treatments. The docking analysis showed the pesticidal potential of several compounds, and mortality could be attributed to Eugenol (19.28%), 1,8-cineole (5.78%) and Linalool (21.42%). The tested botanicals have demonstrated their ability to suppress *S. zeamais* development in stored maize and could be utilized to protect maize grains during storage.

Keywords: *Zanthroxylum usambarense*; *Warburgia ugandensis*; *Sitophilus zeamais*; GC-MS; repellence; molecular docking; long-term storage

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1. Introduction

Average maize production in Kenya is estimated at three million tons per annum, giving a national mean yield of two tons per hectare [1]. Maize grain dominates food security issues in the country, with about 90% of Kenya's population depending on it as a staple food [2]. Maize infestation during storage with *Sitophilus zeamais* (Motschulsky) larvae and adults is the major challenge for maize production in Kenya. While it decreases world grain yield generation by a huge margin, the stored maize loss to *S. zeamais* in Kenya is estimated at 0.7 million tons with an estimated Kenyan monetary value of over 13.5 billion Kenyan shillings per year [3]. The insect contaminates the attacked grains and flour with excretory wastes and frass [1]. Furthermore, *S. zeamais* infestation and damage during storage, increases respiration in the maize grain which leads to moisture and heat evolution, creating favorable conditions for growth and development of fungi [4]. If no prevention measures are taken, these spoliators can destroy a whole stock. For over forty years, the chief weapon in the fight against *S. zeamais* infestation in stored maize has been synthetic insecticide [3]. The current effective insecticide in the market is Actellic gold dust and DEET (N, N-diethyl-m-toluamide) [5]. Gold synthetic repellent DEET blocks the odorant receptors

neurons that detect plant odors in herbivorous insects' antennae [6]. Actellic gold dust has two active ingredients: Thiamethoxam, which inhibits the acetylcholinesterase enzyme that is critical in the hydrolysis of acetylcholine at the neuromuscular synapses and at cholinergic synapses preventing transmission of subsequent impulse between neurons [7]. This typically ends in twitching, knockdown, and death of insects. While pirimiphos-methyl inhibits GABA-gated chloride channel receptors dedicated to attraction and feeding in herbivorous insects [8]. However, the use of synthetic insecticides has received a jolt from different parts of the world due to the environmental and health damage they impose [9]. Indiscriminate use of chemical pesticides can give rise to problems of resistance, resurgence of insect pests and adverse effects on non-targets organisms, making their management a complicated issue for smallholder farmers [6]. Botanical insecticides, which are plant derivatives, may offer a suitable alternative strategy to synthetic insecticides [10] as they are environmentally friendly, often species-specific, cheap, biodegradable hence less susceptible to insect resistance and non-toxic to humans. They also exhibit various modes of action and thus have a quick knockdown effect and an overall impact in ameliorating storage losses [11]. Botanical pesticides that are already being commercialized have been sourced from the following plants: *Ryania speciosa*, *Schoenocaulon officinale*, *Nicotiana tabacum*, *Tanacetum cinerariifolium*, and *Azadirachta indica* [12]. Previous studies have indicated that stem barks of *Zanthroxylum usambarense* and *Warburgia ugandensis* extracts contain secondary metabolites with pesticidal activities [13]. The use of in silico studies to screen for the bioactivity of phytochemicals on pests of economic significance using "docking" and "scoring" techniques can lead to the discovery of new eco-friendly pesticides. These computer-aided techniques are faster, less risky, cheap, and ultimately can avoid the loss of traditional knowledge on botanical pesticides [14]. Understanding how these plant extracts and their formulations kill and repel insect pests is important because it will help determine whether they are toxic to non-target organisms [13]. This study, therefore, sought to investigate the insecticidal potential of bark extracts of the two plants and their formulation in control of maize grain damage caused by *S. zeamais* during storage. It is envisaged that preservation will be achieved only if initially stored products do not significantly differ in quantity and quality at the end of storage [15].

2. Materials and Methods

2.1. Identification of Sitophilus zeamais

The molecular identification of *S. zeamais* insects was done by extracting total genomic DNA from individual insects using Isolate II Genomic DNA Kit (Bioline, London, UK) per the manufacturer's instructions. A 450 bp region of the 28S ribosomal RNA gene was amplified using LepD2-Fw and LepD2-RV primers [16]. Amplified 28S regions were purified using Isolate II PCR and Gel Kit (Bioline) as per the manufacturer's instructions and sent to Macrogen (Amsterdam, The Netherlands) for bidirectional sequencing. Sequences obtained were assembled and edited using BioEdit software (Version 7.2). Sequence identities were determined using the Basic Local Alignment Search Tool (BLASTn) version 2.13.0+ in NCBI.

2.2. Preparation of Maize Grains, Sitophilus zeamais Colony and Organic Plant Extracts

Newly harvested and untreated maize grains were prepared for experimentation by a method described by Mutungi et al. [17]. Adult weevils (*S. zeamais*) were reared in a control room with ambient conditions that allowed the colony to grow exponentially for four months [18]. The stem barks of *Z. usambarense* and *W. ugandensis* were sampled at their secondary stage of growth. The two plants' stem bark materials were air-dried and pulverized into powder using an electric mill. Organic extraction by immersion and percolation methods [19] was done using 70% methanol and n-hexane separately for each plant. A rotary evaporator was used to concentrate extract filtrates at 69 °C and 65 °C for n-hexane and methanol, respectively, and the solvents recovered. Water remaining in the 70% methanol extracts was removed by freeze-drying the aqueous extracts.

2.3. Determination of Plant Extracts Lethal Dose and Repellent Activities

2.3.1. Bioassays with Solvent Extracts

A stock solution of (1 µg/µL *w*/*v*) was prepared for each of the four experimental setups using plant extracts from both plants with their respective 70% methanol and n-hexane extraction organic solvents separately, using WHO standard operating procedure for testing insecticide susceptibility [20]. Stock solutions for each of the two categories (n-hexane and methanol) plant extracts were then aliquoted to attain different concentration levels, namely, 25, 50, 75, and 100% (*w*/*v*), by diluting the stock solutions using their respective extraction solvents. A stock solution was used at 100% (*w*/*v*) concentration. Repellence bioassays for each extract treatment concentration of both *Z. usambarense* and *W. ugandensis* were performed in a Y-tube olfactometer separately, as described by Obeng-Ofori et al. [21] with some modifications. It involved the use of Whatman filter paper of 9 cm diameter, where each was cut into two equal halves. On the first half-filter paper, each solution (0.5 mL) of predetermined concentrations of 25, 50, 75, and 100% *w*/*v*, which represents 62.5, 125, 187.5 and 250 µg/mL, respectively, was applied uniformly on the surface of filter paper using a micropipette. On the second half of the filter paper, 200 µL of their respective extraction solvents (n-hexane and 70% methanol) were applied using a micropipette to serve as a negative control. Both halves with treatment and controls were allowed to air dry for 10 min before transferring into each of the olfactometer lateral arms. 0.0089 g of conventional pesticide (DEET) was used as the positive control. Thirty insects were tested for each of the four treatments, where only one *S. zeamais* was introduced at a time to the central arm of the Y-tube and observed for a maximum of ten minutes to make a choice to either of the lateral arms. Percent repellency (PR) and repellency index (RI) was determined using the repellence formula of Takakura et al. [22].

$$RI = 2T/(T + C)$$

where C is the percentage of insects attracted to the control and T is the percentage attracted to the treatment.

$$PR = \frac{\text{Number of insects in untreated half}}{\text{Total number of insects in troduced}} \times 100$$

A repellency index of less than one (<1) implied repellency, while that greater than one (>1) showed no repellency.

2.3.2. Contact Toxicity Bioassay of Plant Extracts

These tests were carried out using a randomized controlled study design as described by Don-Pedro et al. [23] with modifications. Previously determined concentrations of 25%, 50%, 75% and 100% (*w*/*v*) n-hexane and 70% methanol extracts from both plants were each administered separately on the posterior part of the thorax of each test insect using a 10-µL micropipette tip to dose each insect with 1 µL of plant extracts. The procedure was repeated using Actellic super^{TM} gold as a positive control in concentrations of 25%, 50%, 75% and 100% *w*/*v*, which were achieved by diluting 0.0047 g of Actellic dust with n-hexane and 70% methanol solvents for experiment uniformity. Insects were transferred to a jar with untreated maize after each treatment. The jars with treated insects were all covered with a muslin cloth to prevent insects from escaping and allow ventilation before placing them in a control room. Each jar had ten weevils with four replicates per treatment group. Mortality was assessed every 24 h for four days. The LD_{50} values for each of the four experimental setups were determined using R-software 4.2.1 version. The correct percentage of adult mortality was computed according to Abbott's formula:

$$\%\ \text{Mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

2.4. Phytochemicals Detection Using Gas Chromatography and Mass Spectrometry

Gas Chromatography coupled with Mass Spectrometry (GC/MS) analysis of the polar and non-polar stembark extracts of *Z. usambarense* and *W. ugandensis* was carried out using the method of Yakubu et al. [24]. Characterization and identification of the constituents of plant extracts were performed on a Thermo Finnigan Trace DSQ GC/MS instrument (Thermo Finnigan, Lutz, FL, USA) equipped with an ionization detector and a capillary column of HP-5MS (30 m × 0.25 mm × 0.25 µm).

2.5. Bioassays with Extract Formulation on Sitophilus zeamais

2.5.1. Plant Extract Formulation Repellent Activity

Repellence bioassays for prepared extract formulations by sorption method were performed in a Y-tube olfactometer as described by Obeng-Ofori et al. [21]. 8.5 g of fresh grains was thoroughly mixed with 0.12 g dust formulation (*w*/*w*) in a jar for 2 min before placing it in one odor source flask of olfactometer, while a glass vial with 8.5 g of untreated fresh maize grains was placed in the other odor source flask as a negative control. DEET (0.0089 g) treated maize was used as the positive control. The treatment and control were replicated four times each. Thirty *S. zeamais* were introduced one at a time at the release part of the olfactometer using a soft camel's hairbrush, and the time taken for each *S. zeamais* to make a choice was recorded. Percent repellency (PR) and repellency index (RI) was determined using the repellence formula of Takakura et al. [22].

2.5.2. Plant Extract Formulation Mortality Assessment under Laboratory Conditions

The method of Don-Pedro et al. [23] was adopted. The plant extracts of *Z. usambarense* and *W. ugandensis*, which exhibited the highest mortality activities, were mixed at appropriate ratios of (0.25:1) for *W. ugandensis* and *Z. usambarense* respectively, at different concentration levels together with talc carrier powder by sorption method to form plants extract formulation. In the first group, 8.5 g of fresh maize was weighed into four 50 mL jars. Prepared dust formulations (0.12 g/8.5 g) were added to each jar. To ensure uniform distribution of the extract formulation over the grain surface, jars with maize and extract formulation mixture was shaken gently for two minutes. In the second group, 8.5 g of fresh maize in jars were treated with the recommended dose of 50 g/90 kg Actellic super^{TM} gold dust as the standard pesticide in four replicates. In negative control groups, 8.5 g maize grains in each of the four replicates were mixed with 1 mL of the respective solvent. The method was repeated using maize only (with no extraction solvent nor insecticide Actellic super^{TM} gold dust) in four replicates. Ten adult *S. zeamais* were artificially introduced to each jar with treatments and controls. The number of dead weevils was counted at an interval of one day for seven days to determine *S. zeamais* mortality of extract formulation and for controls. Abbott's formula [25] was used to calculate the percentage of mortality.

2.5.3. Long-Term Evaluation of Extract Formulation Efficacy on *Sitophilus zeamais* in Stored Maize

Efficacy was determined based on the protocol of Don-Pedro et al. [23] with modifications, which involved the use of miniature bags instead of 90 kg gunny bags for simulation. In the first experiment, the first four replicate miniature bags were each treated with 1.5 g of prepared plant extract formulations per 100 g of untreated maize. The next four replicates were treated, each with 0.0556 g/100 g Actellic gold dust as a positive control. The last four replicates were set using untreated miniature bags as negative controls. One hundred grams of newly harvested untreated maize was then added to each of the bags in the three categories described (plant extract formulation-treated baglets, baglets treated with Actellic gold dust, and untreated miniature bags) before storage.

In the second experiment, 100 g of maize treated with plant extract formulation was transferred to miniature bags (30 cm by 10 cm) treated with plant extract formulation and replicated four times. The method was repeated but using treated maize in unpretreated miniature bags. The small miniature bags (30 cm by 10 cm) with controls and treatments

were put in the same conditions that would simulate those of a typical granary, all in quadruplets. *Sitophilus zeamais* infestation and the number of holed grains in the small bags were checked, and the numbers were recorded monthly for six months following the international count and weigh method used by Food and Agriculture Organization in 2021 [26]. Fifty maize grains were randomly taken from treated and untreated gunny bags and separated into insect-damaged and undamaged grains on a sterilized tray. The number of damaged and undamaged grains obtained was used to calculate percentage damage (PD) and Weevil perforation index (WPI) according to the formula used by Ileke et al. [27].

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

$$\text{WPI} = \frac{\% \text{ Treated grains perforated}}{\% \text{ control grains perforated} + \text{Treated grains perforated}} \times 100$$

where:

WPI > 50 = negative protectant of plant material tested

WPI < 50 = positive protectant

2.6. Prediction of Active Compounds' Molecular Targets via Molecular Docking

2.6.1. Sources of Data on Ligands and Targets

Phytochemicals of *Z. usambarense* and *W. ugandensis* were identified using the GC-MS instrument (Thermo Finnigan, Lutz, FL, USA) equipped with an ionization detector and a capillary column of HP-5MS (30 m × 0.25 mm × 0.25 µm) and optimized using ChEMBl, STITCH, HIT and PubChem databases based on their pesticidal activities on *S. zeamais* (Table S1). 3D structures of candidate ligands were retrieved from PubChem drug bank in structure data file (SDF) format using their specific phytochemical compounds identifiers. Five compounds of interest were retrieved; 1,8-cineole (CID_2758), Terpinen-4-ol (CID_2724161), Linalool (CID_6549), Eugenol (CID_3314) and D-Limonene (CID_440917). Open babel software version 3.1.1-x64 was used to convert the 3D structure in (SDF) format of ligand to protein data bank (PDB) format to enable docking and scoring analysis using ArgusLab software version 4.0.1. Proteins selected as putative targets were downloaded from the PDB database in their 3D structures using their four-letter unique identifier codes.

2.6.2. Prediction of Active Sites and Molecular Docking Simulation

CASTp software version 3.0 was used to affirm the amino acids, which are the active sites on target proteins where specific docking with ligand occurs on specific locations along their target proteins' 3D structures′ length provided in the PDB database. ArgusLab version 4.0.1 software was used for blind ligand-receptor docking and scoring analysis to determine; the fitness of each ligand to each binding site of target proteins that could allow ranking from the lowest to the highest, evaluation of intermolecular interaction involved in each candidate binding mode, which could either be hydrogen or hydrophobic bonds and the best ligand binding pose in kcal/mol. The type of bonds involved in ligand-receptor docking was determined along with their structural conformation. The conformation of ligands and their target protein 3D structures were modified for better visualization and interpretation before exporting as portable network graphics (PNG) files from Arguslab software version 4.0.1. Two dimensional Ligplots diagrams of the putative ligand-protein interactions were downloaded using their respective four letters PDB protein codes from PDBsum-EMBL-EBI. PyMOL software (version 1.1) was used to visualize the candidate ligands in the binding pockets of their respective target receptors.

2.7. Data Analysis

R-software version 4.2.1 was used for all statistical analyses [28]. Chi-squared (χ^2) test was used to evaluate repellence, and the LD_{50} values for each of the four experimental

setups of organic extracts from the two plants were determined. Lethal Time (LT_{50}) was also determined for each treatment concentration level used for the mortality study. Statistical significance was estimated at $p \leq 0.05$.

3. Results

3.1. Molecular Identification of Sitophilus zeamais

The molecular identification of the *S. zeamais* insects used in this study showed that the representative samples selected had 86 to 99.77% sequence homology with publicly available 28S rRNA sequences of *S. zeamais*. Furthermore, alignments of the sequences from this study showed 100% homology with each other. The *S. zeamais* sequences obtained were deposited in NCBI GenBank.

3.2. Qualitative Phytochemical Characterization Analysis Using GC-MS

This study identified 22 and 42 phytochemicals in the hexane extracts of *W. ugandensis* and *Z. usambarense*, respectively (Figures S1 and S2), while the methanol extracts of *W. ugandensis* and *Z. usambarense* contained 14 bioactive compounds in each (Figures S3 and S4, Tables S2–S5).

3.3. Repellent Activity of Organic Extracts of Zanthroxylum usambarense and Warburgia ugandensis on Sitophilus zeamais

Warburgia ugandensis hexane extracts (Figure 1A) indicated the highest repellence of 76.7% ($p \leq 0.05$), followed by hexane extracts of *Z. usambarense* (Figure 1B) with 73.3% ($p \leq 0.05$). Methanol extracts for *W. ugandensis* (Figure 1C) and *Z. usambarense* (Figure 1D) had similar repellency effectiveness of 63.3% ($p > 0.05$) on *S. zeamais*. All extracts caused repellency on *S. zeamais*.

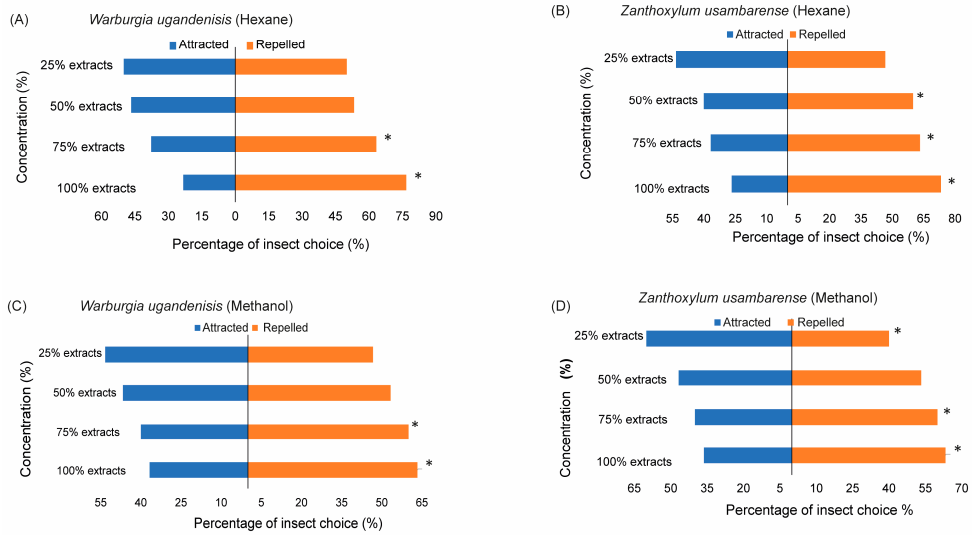

Figure 1. Repellence of *Sitophilus zeamais* by (**A**) *Warburgia ugandensis* hexane extract, (**B**) *Zanthroxylum usambarense* hexane extract, (**C**) *Warburgia ugandensis* methanol extract and (**D**) *Zanthroxylum usambarense* methanol extract. *—statistically significant repellence at $p \leq 0.05$.

The repellent activity of the combined plant extract formulation consisting of appropriate ratios of *W. ugandensis* and *Z. usambarense* together with talc carrier on *S. zeamais* showed that the plant extract formulation had a mean average repellence of 85%. However, the standard (DEET) had a higher repellence of 97% (Figure 2; Table S6).

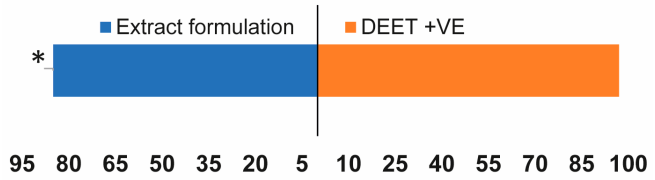

Figure 2. Repellence of *Sitophilus zeamais* using the formulation of the combined extracts of *Zanthroxylum usambarense* and *Warburgia ugandensis*. *—statistically significant repellence at $p \leq 0.05$.

3.4. Mortality Activity of Hexane and Methanol Extracts of Zanthroxylum usambarense and Warburgia ugandensis on Sitophilus zeamais

Z. usambarense and *W. ugandensis* hexane extracts both showed 100% mortality at 100% extracts concentration (after day one for *W. ugandensis*) and at 75% extract concentration for *W. ugandensis* after four days of exposure (Figures 3 and 4). However, all other extracts' concentrations apart from the three mentioned above showed varied mortality from each other ($p \leq 0.05$). The hexane extracts of *Z. usambarense* had higher mortality rates than the methanol extracts, with the 75% hexane extract concentration causing mortality ranging from 21% to 73% from day one (1) to day four (4) (Figure 3a) and there was an increase in the mortality trend for all extract formulations with an increase in time (Figure 3b). The methanol extracts indicated lower mortalities that ranged from 10% to 53% at 25% and 100% extract concentrations respectively (Figure 3c) with an increase in the mortality trend for all extract formulations with an increase in time (Figure 3d). For Actellic Super^{TM} diluted with extraction solvent hexane, the 75% and 100% aliquots had 100% and 98.35% mean mortality respectively after 96 h (Table S7).

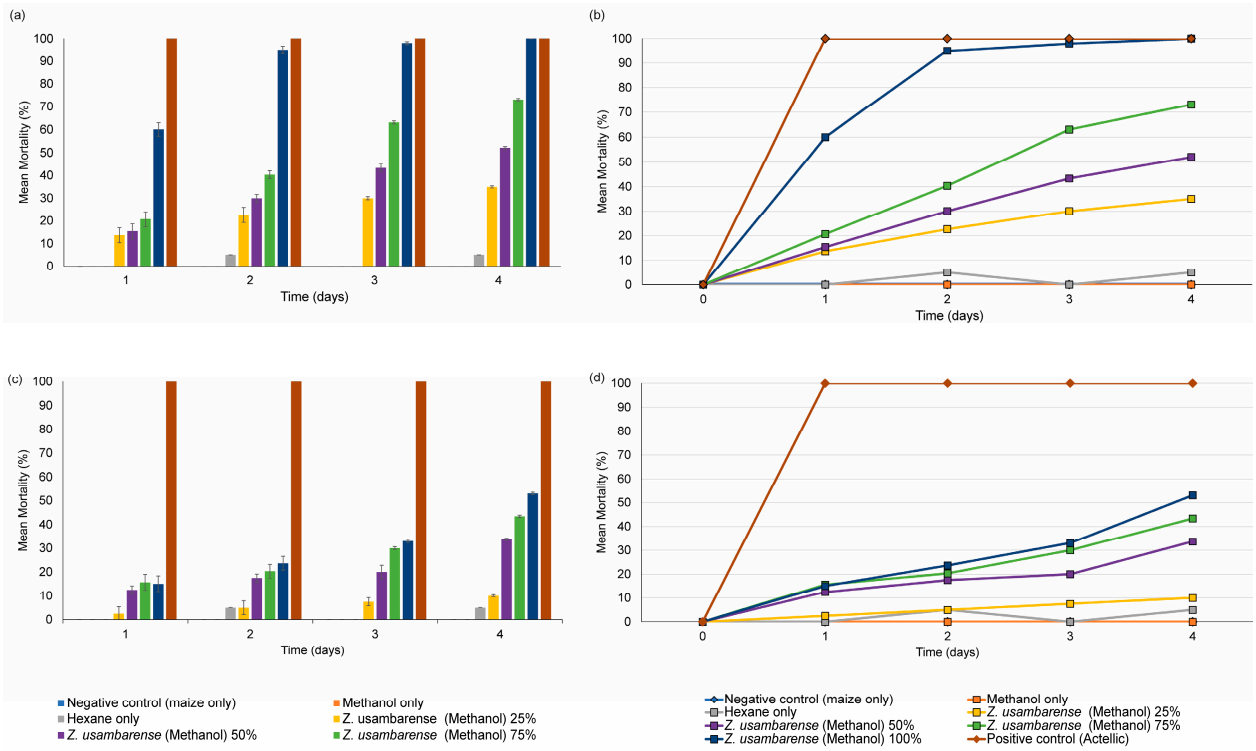

Figure 3. Mortality of *Sitophilus zeamais* using (**a**,**b**) *Zanthroxylum usambarense* Hexane extracts and (**c**,**d**) *Zanthroxylum usambarense* Methanol extracts over four days of exposure. Actellic Super^{TM} was used as the standard.

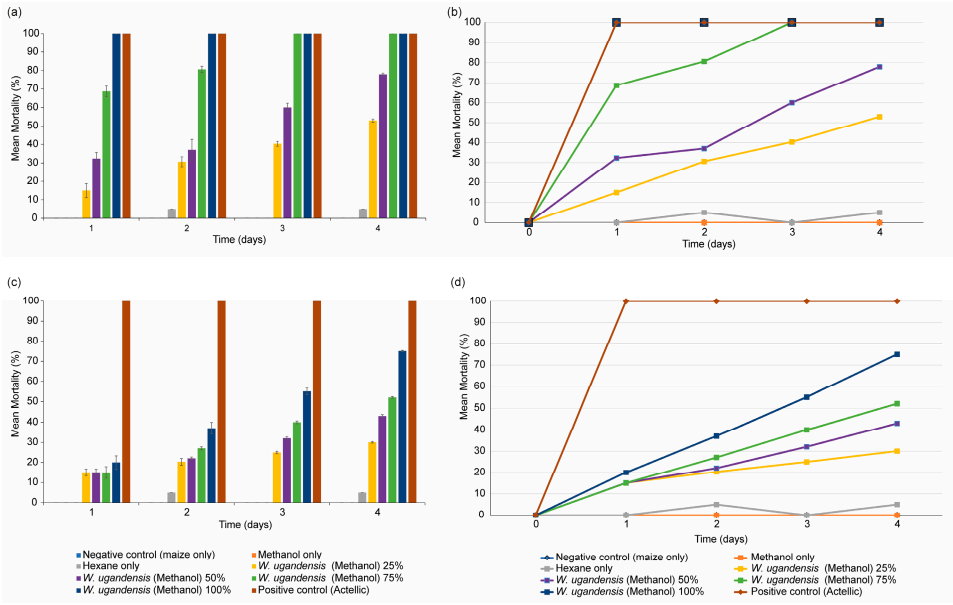

Figure 4. Mortality of *Sitophilus zeamais* using (**a**,**b**) *Warburgia ugandensis* Hexane extracts and (**c**,**d**) *Warburgia ugandensis* Methanol extracts over four days of exposure. Actellic Super^{TM} was used as the standard.

The hexane extracts of *Warburgia ugandensis* also had higher mortality rates than the methanol extracts, with the 75% hexane extract concentration causing mortality ranging from 69% to 100% between day one (1) to day four (4) (Figure 4a). There was an increase in the mortality trend for all extract formulations with an increase in time (Figure 4b,d). The methanol extracts of *W. ugandensis* caused mortality that ranged from 30% to 75% after 96 h of exposure ($p \leq 0.05$). For Actellic Super^{TM} diluted with methanol solvent, at 75% and 100% aliquots levels, 95% and 84.35% mean mortality, respectively, was observed after 96 h (Table S8). The mortality of *S. zeamais* by the *Z. usambarense* and *W. ugandensis* methanol extracts at all dose levels were significantly different from each other and from that caused by Actellic Super^{TM} ($p < 0.05$) (Table S8).

3.5. Probit Analysis of Zanthroxylum usambarense and Warburgia ugandensis Extracts' Mortality on Sitophilus zeamais

Probit analysis for each experiment to determine the LD_{50} of both plant extracts after 96 h of exposure showed that the hexane extract of *W. ugandensis* was more effective at lower LD_{50} (69.25 µg/mL) compared to *Z. usambarense* (114.89 µg/mL). In Methanol extracts, *W. ugandensis* showed higher toxicity at lower LD_{50} (163.52 µg/mL) compared to *Z. usambarense* LD_{50} (197.19 µg/mL) (Table 1). Another probit analysis was carried out for each treatment concentration used in mortality evaluation to determine their respective LT_{50}(h) using R-software version 4.2.1.

Table 1. Lethal Dose (µg/mL) at 50% concentration of all four *Zanthroxylum usambarense* and *Warburgia ugadensis* extracts together with Actellic Super^{TM} on *Sitophilus zeamais* (n = 40).

Sample	Hexane	Methanol
W. ugandensis	69.25 µg/mL ± 7.16	163.52 µg/mL ± 12.96
Z. usambarense	114.89 g/mL ± 9.06	197.19 g/mL ± 19.09
Actellic super^{TM}	27.03 g/mL ± 4.83	42.13 g/mL ± 10.83

3.6. Insecticidal Activity of Extracts Formulation on Sitophilus zeamais in Maize Grains under Laboratory Conditions

The mortality rate in the plant extract formulations and Actellic super^{TM} treated maize increased with exposure time. The combined extract formulation was more efficient than

stem bark extracts of both plants used individually. Actellic super^{TM} treated maize showed 100% mortality on the third day of exposure and extract formulation on the fifth day, respectively. Maize treated with extraction solvents methanol and hexane showed 3% and 5% mortality, respectively. In maize only group, no mortality was observed after seven days of storage (Figure 5).

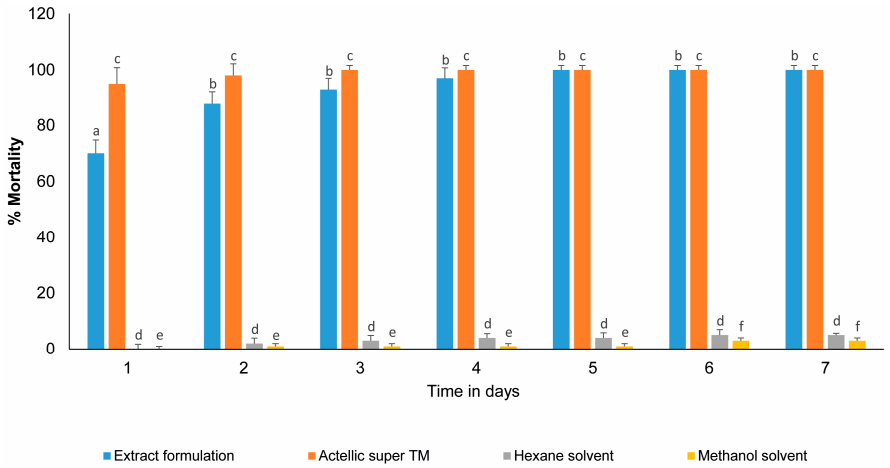

Figure 5. Mortality of *Sitophilus zeamais using* (0.25:1) ratios of *Zanthroxylum usabarense* and *Warburgia ugandensis* combined extract formulation over seven days of exposure. Actellic super^{TM} was used as the positive control while the extraction solvents (Hexane and Methanol) were used as the negative controls. The letters above error bars (a–f) represent statistical significance between treatments at $p \leq 0.05$.

3.7. Extract Formulation Insecticidal Activity on S. zeamais in Maize Grains Stored in a Granary for Six Months

For the untreated grains in untreated baglets, *S. zeamais* were present in their tenths of hundreds in all four replicates with complete damage to maize grains which appeared flour-like. A weevil perforation index (WPI) (Table S9) of 100.1 ± 0.15 and maize damage of 88 ± 5.09% was recorded. Within the same period, the treated grains on the treated baglets and untreated maize on the treated baglets appeared whole with a WPI value below <50%. Seed damage and WPI values for treated maize in treated baglets were 10 ± 6.09 and 21.36 ± 0.14, for treated bags with untreated maize were 16 ± 5.56 and 34.18 ± 0.17 and for treated maize in untreated bags were 14 ± 4.19 and 29.91 ± 0.11 respectively. In Actellic Super^{TM} treated maize, the number of damaged grains was lower than those treated with plant extracts formulation within the six months with seed damage and WPI values of 2 ± 3.53 and 2.02 ± 0.10 respectively (Figure 6: Table S9).

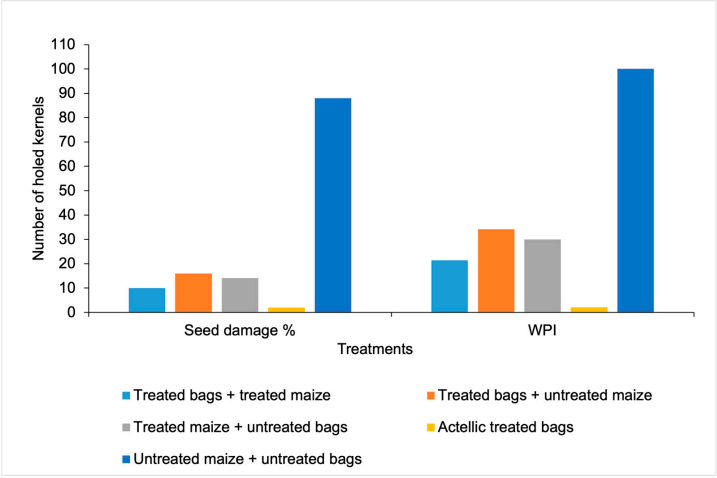

Figure 6. Insecticidal activity of *Zanthroxylum usambarense* and *Warburgia ugandensis* extracts formulation on Long-term storage (six months) of maize grains. WPI: weevil Perforation Index.

3.8. Analysis of Molecular Targets for Repellent and Toxic Bioactive Compounds in Sitophilus zeamais

Docking and scoring analysis results ranked active compounds by affinity (ΔG); D-Limonene (binding site-Tyrosine [TYR6]) > Eugenol (binding site-Glutamic acid [Glu88]) > Terpinen-4-ol (binding site-Valine [Val91]) > 1,8-cineole (binding site-Leucine [Leu237]) > Linalool (binding site-Serine [Ser21]) in kcal/mol. D-Limonene formed many intermolecular interactions with Glutathione-s-transferase epsilon 2 including hydrogen and hydrophobic bonds. Eugenol, Terpinen-4-ol,1,8-cineole and linalool also formed many chemical bonds with their target proteins which are; Cytochrome c oxidase subunit 2, Phenoloxidase-activating factor 2, Mitochondrial, Calcium uniporter protein and Cathepsin L-like proteinases respectively like D-Limonene. Visualization displayed the interaction of target protein amino acids with ligands through hydrophobic interaction and hydrogen bonds using PyMOL software version 1.1 (Tables 2 and S10). Ligplots displayed the region where actual bonding occurred between the putative proteins and ligands for the pro check. The docking results showed D-Limonene having the highest binding score of −13.49 kcal/mol, with linalool having the least binding score of −7.00 kcal/mol (Table 2).

Table 2. Molecular docking analysis for optimized compounds against their putative target proteins and their corresponding binding energies.

Compound/Molecular Formula	Target Proteins	Binding Energy (ΔG) (kcal/mol)
D-Limonene ($C_{10}H_{16}$)	Glutathione-s-transferase epsilon 2 (GSTe2)	−13.49
Eugenol ($C_{10}H_{12}O_2$)	Cytochrome c oxidase subunit 2	−11.13
Terpinen-4-ol ($C_{10}H_{18}O$)	Phenoloxidase-activating factor 2	−9.97
1,8-cineole ($C_{12}H_{20}O_3$)	Mitochondrial, Calcium uniporter protein	−7.15
Linalool ($C_{10}H_{18}O_2$)	Cathepsin L-like proteinases	−7.00

4. Discussion

This study evaluated the presence and identity of biologically active components of 70% methanol and n-hexane stembark extracts of *Zanthroxylum usambarense* and *Warburgia ugandensis* using GC-MS, which have previously been associated with pesticidal effects on insect pests [29]. The study used extract concentration within the dose ranges used by Acero et al. [30]. The workers used 25%, 50%, 75% and 100% *w*/*v* extract concentrations to assess the biorational properties of *Chromolaena odorata* and *Artocarpus heterophyllus* on a closely related weevil, *Sitophilus oryzae*. In this study, all the organic stembark extracts of both plants at different concentrations demonstrated potent repellence potential on *S. zeamais* as each repellency index value was less than one (<1), therefore classified as non-attractant to insects. Generally, repellence activities were slightly higher in *W. ugandensis* compared to Z. *usambarense* extracts, this may be due to variations in phytochemical composition within these plant species, as proved by GC-MS [31]. After all, variations in plant extract composition mainly depend on, geographical location, genotype, plant target part, season, and method of extraction [31]. However, both hexane extracts of *W. ugandensis* and *Z. usambarense* showed higher repulsive activity of 76.7 and 73.3%, respectively, compared to their methanol extracts, which showed 63.3% each. The high repellency of hexane extracts of both plants in the study is an attestation of the abundance of phytochemicals with repellency effects as compared to their methanol extracts which included monoterpenes D-limonene (6.57%), Terpinen-4-ol (18.01%) and 1,8-cineole (5.12%) known to cause repellence.

D-limonene is commonly used for controlling structural pests such as termites [32], while Terpinen-4-ol is known to repel selected important mosquito vectors, including *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* [33]. Repellence activities could also be linked to the presence of 1,8-cineole in the organic stem bark of extracts of *W. ugandensis*. According to Rajashekar et al. [31], 1,8-cineole have been used as a repellent agent against various urban coleoptera species. The phytochemicals in *W. ugandensis* than *Z. usambarense* stembark extracts may have used a similar mechanism of action to induce repulsion activity to *S. zeamais*. This finding corroborates the study by Kosini et al. [34], who proved that hexane extract of *Gnidia kraussiana* showed a higher repellency against cowpea weevil than their methanol extracts.

Hexane extracts of both plants which exhibited the highest repellence activities of 76.7% for *W. ugandensis* and 73.3% for *Z. usambarense*, were mixed at appropriate ratios of (0.25:1) for *W. ugandensis* and *Z. usambarense* respectively, together with talc carrier by sorption process to form formulations. Extract formulation indicated higher repellence of a mean average of 85% compared to each extract alone. This may have been attributed to the effectiveness and efficiency attained due to the combination and interaction between some minor and major constituents' phytochemicals from both plants' extracts [35]. Receptors responsible for digression in insects could have been activated by these phytochemicals with repulsive properties rather than food-attractancy behavior. This resulted in maize grains being unpalatable and unattractive to the insect pest, making them continue foraging [36].

Toxic effects of organic stembarks extract of *Z. usambarense* and *W. ugandensis* against *S. zeamais* was also assessed after desiccation of the 70% methanol extracts by freeze drying before inclusion of the talc carrier to concentrate the extract in order to achieve the highest toxicity level of the extract and to ensure an effective sorption process of the carrier. In general, the toxicity of methanol and hexane extract of *Z. usambarense* and *W. ugandensis* was proportional to their concentrations. *S. zeamais* mortality rate escalated exponentially with an increase in concentration and time. This is because more interaction time between the insect's target sites and plants' active compounds allows for the effective mechanism of action for toxicity to take place [37]. These findings agree with the study by Zhang et al. [38], who reported that mortality percentages are enhanced by increasing periods of exposure.

The toxicities of extracts of *Z. usambarense* and *W. ugandensis* on *S. zeamais* also varied based on the extraction solvent used. Hexane stembark extracts of *Z. usambarense* and *W. ugandensis* generally evoked the highest mortality against S. *zeamais* with lower LD_{50} values; this manifested that hexane extracts had more phytochemicals with toxic activities than methanol extracts [31]. This agrees with the study by Ouko et al. [39], who reported higher mortality of hexane stembark extracts of *Vernonia lasiopus* and *Tithonia diversifolia* as compared to their methanol extracts.

High mortality of hexane stembarks extracts of both plants at lower concentrations can be attributed to eugenol (19.28%) and linalool (21.42%) that were present in both plants' extracts as observed from GC-MS chromatograms, which have been shown to be very toxic to insect pests. Eugenol-based extracts have been found to be insecticidal and patented for pesticidal activities against cockroaches and aphids [40]. The lowest toxicity and repellence activities in methanol extracts were mostly observed in the *Z. usambarense* extracts. This may be attributed to the lower concentration of polar phytochemicals (β-Ocimene Andrographolide and Citronellic acid) mostly lost during extraction and drying [30], which could have shown desirable activities in weevils. Similarly, Otusanya et al. [29] showed that stembark extracts of *T. diversifolia* did not offer protection against infestation by *S. oryzae* as post-harvest protectants of maize grains. Methanol and hexane, which were organic solvents for test extracts and worked as a negative control, had the best mean mortality of 3% and 5%, respectively, on *S. zeamais*, which was attributed to natural factors since the mean value of <6% was considered as non-substantial [41]. Maize alone was used to account for optimum mortality time, thus enabling easy calculation of optimal time LT_{50} for each test concentration. The plant extracts of *Z. usambarense* and *W. ugandensis*, which exhibited the highest mortality activities, were mixed at appropriate ratios with talc Carrie

powder by sorption to form formulations. The mortality activity of extract formulation under laboratory conditions showed that prepared dust formulation is more efficient compared to Z. *usambarense* and *W. ugandensis* stembark extracts while used individually and had activity comparable to that of Actellic super^{TM} (50 g/90 kg maize), which was used as standard pesticide after seven days of exposure.

Infestation and damage by *S. zeamais* were observed once a month for six months, and a huge difference in the number of weevils present and the number of damaged grains was revealed between maize treated with plant extracts formulation and the untreated. Long-term insecticidal activity results of extract formulation in stored maize grains clearly indicated that the stembark extracts of *Z. usambarense* and *W. ugandensis* had potential as positive protectants with weevil perforation index (WPI) of <50% against *S. zeamais*. Therefore, the extract formulation protects the grains by discouraging weevils from feeding on the treated grains leading to starvation. However, there was minimal damage by *S. zeamais* on the treated maize grains. This may have been caused by an uneven distribution of extract formulation on the surface of grains, or the extract formulation may have sunk to the base of the baglets and therefore left some grains unprotected hence reducing their concentration and effective coverage of the grains [38]. This study agrees with Opiyo et al. [42], who documented that plant hexane extract formulation of *W. ugandensis* and *Allium sativum* was effective against *Prostephanus truncates* during storage.

Furthermore, this study employed computer-aided methods to evaluate the binding efficiencies and binding pose of active compounds from the two plants and their target proteins. All the compounds that were identified by GC-MS were filtered through databases such as PubChem, HIT, ChEMBl and STITCH, based on information of interaction with their target proteins. Molecular docking was carried out to identify the pesticidal phytochemicals with binding activity to their target protein receptors from which we can justify the observed pharmacodynamics properties (Toxicity and repellency) of D-Limonene, Eugenol, Terpinen-4-ol, 1,8-cineole and Linalool active compounds.

The main targets of botanical pesticides on *S. zeamais* are the insect chemosensory systems, including gustation and olfaction systems contained within cuticle structures [43]. From this study, the presence of 1,8-cineole in the extracts may have been involved in the subversion of the mitochondrial electron transport chain (ETC) by inhibiting ATP synthase that catalyzes the phosphorylation process where ADP is converted to ATP in *S. zeamais* [44]. Due to this reason, the insects may lack the energy to carry on with life-sustaining physiological processes and hence die. For repellence, GABA neurotransmitters that normally stimulate feeding and attraction responses among herbivorous beetles may have been targeted by 1,8-cineole, antagonizing binding GABA phagostimulants by causing the closure of chloride ion channels, thus inducing repellence of *S. zeamais* [8]. Additionally, exposure to linalool may have inhibited acetylcholinesterase enzyme causing death due to failure of acetylcholine hydrolysis and, thus ataxia [45]. The eugenol in these extracts may have targeted voltage-dependent ion channels, that results in over-excitation of the insect's cerebrospinal nervous system and finally, death [46]. Voltage-gated sodium channels are also known to be targets for Terpinen-4-ol phytochemical that causes repellence. The presence of Terpinen-4-ol may prevent the closure of the voltage-gated sodium channels, eventually blocking the olfactory system of the *S. zeamais* from recognizing attractive cues from the plant's extracts [46]. Lastly, the presence of D-Limonene may cause repellence by inhibiting sugar-sensing cells on the mouth region of insects that stimulate feeding and thus distaste of treated maize grains The signal to the brain of the *S. zeamais* provoked avoidance from further approach or feeding [32]. Although the above-hypothesized mode of action is based on inferential data, it is not supported by direct corroborative validation from our study. Therefore, we recommend further studies to validate this mechanism in *S. zeamais*.

Zanthroxylum usambarense and *W. ugandensis* are used heavily for medicinal purposes to manage many illnesses in places where they grow naturally [47]. Safety tests carried on test organisms during preclinical trials for analysis of medicinal activities of *W. ugandensis* and *Z. usambarense* against *Plasmodium berghei* and *Plasmodium knowlesi*, affirm that these

two plants to be safe, therefore recommended to be used as traditional medicine [48]. However, the use of conventional insecticide has a lot of implications for health because most of these chemicals are taken and retained in food crops thus, consumption of such crops imposes health complications on humans and herbivorous animals [49].

5. Conclusions

In conclusion, these results indicate that both extracts from stem barks of *W. ugandensis* and *Z. usambarense* have the potential for the protection of stored maize grain. Additionally, *Z. usambarense* and *W. ugandensis* stembark extracts when in formulation exhibit notable potential as a protectant for long term storage of maize for up to six months. Formulating organic extracts of *Z. usambarense* and *W. ugandensis* lead to higher protective ability, possibly due to synergistic activities of the combined extract components from both plants. Higher efficiency was probably achieved by combining different modes of action of the phytochemicals, which ultimately reduced the chances of resistance and resurgence on insects during storage. Thus, the use of stem bark extracts formulation of *W. ugandensis* and *Z. usambarense* should be further investigated for integration in weevil management programs and to be embraced as a preference biorational to chemical pesticides which are not environmentally friendly yet pose no risk of resistance development. This analysis showed that: D-Limonene, Eugenol,1,8-cineole, Linalool and Terpinen-4-ol were the most suitable compounds found in medicinal plants as potential repellence and toxic biological compounds, which should be explored in future research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151410833/s1, Table S1: Optimized phytochemicals, structure of target protein, ligand, and binding site; Table S2: Class of phytochemicals and their Retention time from *W. ugandensis* hexane extracts; Table S3: Class of phytochemicals and their Retention time from Z. usambarense hexane; Table S4: Retention time and the class of compound extracted compounds from *W. ugandensis* methanol; Table S5: Class of phytochemicals and their Retention time from *Z.usambarense* methanol extracts; Table S6: Cumulative mean repellents of adult *S. zeamais* as influenced by the plants extracts and their formation; Table S7: Cumulative hexanes mean mortality of *S. zeamais*; Table S8: Cumulative methanol means mortality of *S. zeamais*; Table S9: Long term insecticidal activity of extract formulation in stored maize grains; Table S10: Molecular docking for optimized compounds against their target proteins; Figure S1: GC-MS profile of *W. ugandensis* hexane extracts; Figure S2: GC-MS profile of *Z. usamberanse* n-hexane extracts; Figure S3: GC-MS profile of *W. ugandensis* methanol extracts; Figure S4: GC-MS profile of *Z. usambarense* methanol extracts.

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