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Bioefficacy of Selected Plant Extracts against *Sitophilus zeamais* on Post-Harvest Management of *Zea mays*

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ABSTRACT

Maize (*Zea mays* L.) is the most important cereal crop in Kenya with 90% of the total population relying on it as the main staple food. Post-harvest loss in maize is caused by insect pests such Sitophilus zeamais and Larger grain borer and the can sometimes be up to 90 percent. Synthetic chemicals are used to manage weevil infestation in maize. However, chemical residues have been detected on the stored maize prior to consumption. Additionally, continuous uses of synthetic chemicals have led to development of pest resistance reducing their effectiveness. This study therefore sought to evaluate a possible use of *Ocimum kilimandscharicum* essential oil to manage Sitophilus zeamais. The study also determined the effect of pretreating baglets with aqueous extracts of *A. indica* and *W. ugandensis* to enhance efficacy of *O. kilimandscharicum* oil. Laboratory experiments were carried out to establish insecticidal property of *O. kilimandscharicum* oil. Additionally, on farm experiments were carried out to establish the oil's effectiveness and longevity. Maize grains were treated with *O. kilimandscharicum* oil in the first experiment and in the second treated maize grains were put in miniature synthetic bags treated with aqueous extracts of *A. indica* and *W. ugandensis* and stored in a granary for six months. On-farm experiment revealed that *O. kilimandscharicum* oil doses were effective against *S. zeamais* as compared with untreated maize grains. However, 10 ml per 1kg of maize grains was the best dosage. Weight loss in maize grains was proportional to the number of holed grains. Replenishment of the oil after the third month (at 6 ml, 8 ml and 10 ml) reduced significantly ($p<0.0001$) the number of holed grains as well as weight loss. Pretreatment of baglets containing maize grains with aqueous extracts of *A. indica* and *W. ugandensis* significantly improved protection of maize grains treated with *O. kilimandscharicum* oil. From the results of this study it can be concluded that plant extracts can offer a possible substitute to synthetic chemicals in post-harvest management of *S. zeamais* in stored maize. Use of essential oil of *O. kilimandscharicum* for post-harvest protection of maize grains against *S. zeamais* is therefore recommended.

Keywords: *Ocimum kimandscharicum*, *A. indica*, *W. ugandensis*, Oil extracts, Aqueous extracts.

INTRODUCTION

Maize is a basic human food in Kenya, grown in almost all agro-ecological zones and on two out of every three farms. It accounts for nearly half of calories consumed [1] and has per capita consumption of 94 kilograms; this translates to between 30 and 34 million bags (2.7 to 3.1 million metric tons) of annual maize consumption in Kenya [2].

It is also used as an animal feed as well as raw materials for production of many industrial products. Maize gives more carbohydrates compared to wheat and sorghum, and it is an important source of phosphorous [3]. Maize contains small amounts minerals such as Calcium and iron, vitamins such as thiamine and niacin, as well as small amounts of fat [4]. Furthermore, maize crop is important in ensuring food security to consumers since it gives more yields per unit area of land [3].

Maize weevil (*Sitophilus zeamais*) is one of the most serious pests infesting maturing cobs as well as stored maize [5,6]. Other stored cereal grains are also infested acting as alternative pest hosts. Wheat is among its notable secondary hosts that have become one of the basic human foods in Africa that help to alleviate protein deficiency and malnutrition young children.

Maize is most prone to insects' damage during early stages of growth and soil insects can pose up to 30% losses from tasseling to harvesting [5,7,8]. Pests that attack maize during development to maturity include *Spodoptera exempta*, *helicoverpa zea*, *Cicadulina mbila*, *Monoptera australis*, *Rhopalosiphum maidis*, *Sitophilus zeamais* and *Frankliniela occidentalis* [5].

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is one of the most serious post-harvest insect pests in the tropics. Loss in grain weight Grain ranging 20–30% on average has been documented [9]; and according to [10,11] up to 80% loss may result for untreated maize grain in traditional facilities depending on the storage period.

The use of synthetic chemicals to manage weevils has in most instances led to development of pesticide resistance. High and acute toxicity, long degradation period, environmental pollution and carcinogenic concerns are some of the problems associated with synthetic chemicals used to control postharvest diseases.

Control failures and resistance to phosphine are very high in India and Australia [12]. Ozone depletion has been associated with methyl bromide [13] and has been banned in developed countries. Malathion, chlorpyrifos, or deltamethrin are contact insecticides which offer protection to stored products insect infestation for several months. However, resistance by insect pests has been a growing concern [14]. Furthermore, adverse effects on food, side effect on human, residual toxicity and environmental pollution has been reported [15,16]. The continuous and indiscriminate use of these insecticides has also led to development of resistant strains and accumulation of toxic residues on food grains for human consumption leading to health concerns [17].

Synthetic chemicals such as phosphine, carbon disulphide, malation, carbaryl or permethin are used as fumigants that adversely subdue the destructive nature of entomons and other storage insect pests. These fumigants have been documented to effectively combat the destructive activities of stored products pests [18,19]. The usual fumigation methods are being re-evaluated in the developed countries due to various reasons such as depletion of the O-zone layer and ability of phosphine and methyl bromide to cause cancer [19,20]. Lack of application knowledge, resistance by pests, great application cost, insects developing genetic resistance, harm beneficial insects as well as being harmful to the user directly, are some of the concerns linked to many synthetic insecticides [21-23].

Uses of safer, cheaper as well as eco-friendly methods of managing pests attack in stored products are gaining popularity within the tropics [24]. Use of edible plant materials as grain protectants are being given great attention currently [19,25,26] and some of these plant species of which are also used for medicinal purposes are in great abundance in tropics.

Natural plant extracts may provide an alternative way to protect stored maize from insect damage. This study therefore sought to evaluate the possibility of *O. kilimandscharicum* oil, aqueous extract of *A. indica* and *W. ugandensis* for post harvest management of *S. zeamais*.

MATERIALS AND METHODS

Collection and preparation of plant materials

Ocimum kilimandscharicum aerial plant parts were collected from wild population in Ruiru, Kiambu County, Kenya. Taxonomic identification of the plant was done in Plant Sciences Herbarium in which voucher specimen was deposited. One kilogram of both fresh *A. indica* and *W. ugandensis* leaves was collected from several trees at Kenyatta University medicinal garden.

Extraction of essential oil and aqueous extracts

The collected plant materials of *Ocimum kilimandscharicum* were air dried under shade in a well ventilated area for five days before extraction. The essential oils were extracted by hydro-distillation using Clevenger's type apparatus for 4 hours at ICIPE (Nairobi). Extraction of the distillate by hexane was followed by drying over anhydrous sodium sulphate and the removal of solvent using rotary evaporator. Oil collected was stored in air-tight bottles and kept in a refrigerator. The collected leaves of *A. indica* and *W. ugandensis* were washed under tap water, rinsed three times in sterile distilled water and blotted dry by use of sterile blotting paper. The leaves were mixed with one litre of distilled water in a sterilized glass beaker, boiled for 2 hours to obtain water extract. The water extract was put in sterilized beakers after cooling.

Insecticidal property of *O. kilimandscharicum* oil on *S. zeamais*

Twenty (20) grams of maize kernels were further sampled and treated with 5µl, 15µl, 30µl, 45µl, 60 µl, 90 µl and 105 µl of oil which had been shaken with hexane to ensure uniform distribution of the oil over the grain surface. The control treatment comprised of untreated grains. Each treatment was replicated four times and arranged in a completely randomized design. The maize grains were put in 50 ml glass jars and covered with cheese cloth fastened with a rubber band to allow aeration. Ten adult *Sitophilus zeamais* were introduced into each of the jars containing treated and untreated grains. The numbers of dead weevils were counted at an interval of 2 days for one week to determine *S. zeamais* mortality due to presence of *O. kilimandscharicum*. The weevil was considered dead when the limbs did not move when probed with a soft camel brush [27].

The insecticidal property of *O. kilimandscharicum* oil was further investigated on-farm in Kangema Subcounty to determine its effectiveness and longevity. Conventional insecticides such Pirimophos methyl (Actellic super™) remains effective for approximately six months on stored maize. The method of [28] was adopted with modification. The treatment involved different amounts of essential oils (2ml, 4ml, 6ml, 8ml and 10ml) dissolved in 1ml hexane and mixed with maize grains. To ensure proper admixture, grains were put in a plastic bucket and top lid replaced. The grains were swirled within the container until a thorough admixture was achieved. The maize grains were left for one hour to allow the solvent to evaporate.

The treated maize grains were then put in small gunny bags (30 cm by 10 cm) and replicated four times (plate 3.6). Control treatments were set using untreated maize and Actellic super™ at the recommended dose of 50g/90kg as a positive control. The experiments were arranged in a completely randomized design. The small gunny bags with treatments and controls were put under the same conditions that would simulate those of a typical granary. *Sitophilus zeamais* infestation and weight loss in the small bags was then checked monthly for six months. One hundred grams of the grains from each treatment replicate were sampled and analysed for infestation by weevils following the international count and weigh method described by [29].

In second experiment, one kilogram of maize grains was treated with *O. kilimandscharicum* oil at 6ml, 8ml and 10ml and after three months the same concentrations were replenished. The treatments were replicated four times with a control set using untreated maize grains. The experiments were then arranged in a completely randomized design. One hundred grams of the grains from each treatment replicate were sampled and analysed for infestation by weevils following the international count and weigh method described by [29] for six months.

In the third experiment the baglets were soaked in 500ml of aqueous extracts of *A. indica* and *W. ugandensis* overnight and extracts allowed to dry. Six millilitre of *O. kilimandscharicum* oil was dissolved in one millilitre hexane, mixed with one kilogram maize grains and left for one hour for the solvent to evaporate. Maize grains were then put in the pre-treated baglets and replicated four times. Control treatments were set using untreated maize grains in unpre-treated baglets. The experiments were arranged in completely randomized design. The baglets together with maize grains were kept in granary and infestation by *S. zeamais* was checked monthly for six months using international count and weigh method described by [29].

Data management and statistical analysis

Data on number of holed grains and weight loss obtained were entered in a spread sheet, Microsoft Excel and normality determined. Transformation by logarithm, $Log_{10}(X+1)$ was done followed by two way ANOVA using SAS 9.0 software. The means were separated using Tukey's HSD test at 5% level of significance. Results were finally presented in tables and graphs.

RESULTS

Insecticidal activity of *O. kilimandscharicum* on *S. zeamais* in maize grains under laboratory conditions

Maize grains artificially infested with adult *S. zeamais* and treated with *O. kilimandscharicum* oil indicated insecticidal properties of *O. kilimandscharicum* oil. At 5µl concentration level of *O. kilimandscharicum* oil, mortality of *S. zeamais* was significantly ($p<0.0001$) lower than all other concentration levels (Table 1). There was however no mortality of *S. zeamais* in untreated maize grains. Mortality of *S. zeamais* at 105µl, 90µl, 60µl, 45µl, 30 µl and 5 µl concentration levels of *O. kilimandscharicum* oil was significantly higher ($p<0.0001$) than in untreated maize grains. In addition, mortality of *S. zeamais* was significantly different ($p<0.0001$) in 105µl, 45µl, 30 µl and 5 µl concentration levels. However, mortality of *S. zeamais* was not significantly different at 60 µl and 90 µl concentration levels of *O. kilimandscharicum.*

Mortality was high on first day of exposure in maize treated with *O. kilimandscharicum* oil and reduced as days increased. Mortality of *S. zeamais* significantly differed ($p<0.0001$) in day 1, 3 and 5. Significant interactive effects ($p<0.0001$) were observed between concentrations and days on mortality of *S. zeamais* (Figure 1).

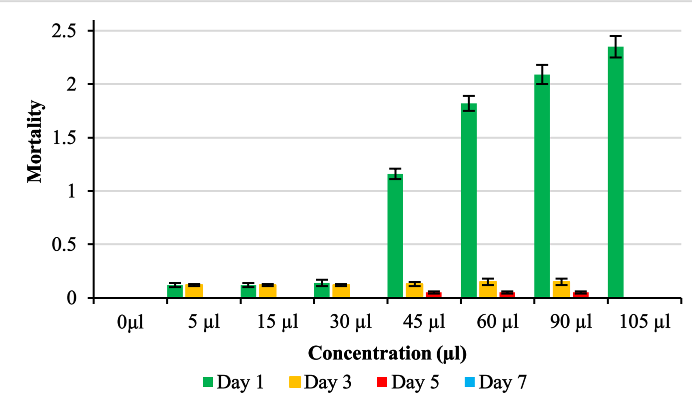

Figure 1: Interactive effects of concentration of *O. kilimandscharicum* oil with day on mortality of *S. zeamais*

Table 1: Mortality (mean ± S.E) of *S. zeamais* on day 1, 3, 5 and 7 after artificially infested maize samples were treated with different amounts of *O. kilimandscharicum* oil

Treatment	Mortality of *S. zeamais*
105µl	0.45±0.40[a]
90µl	0.35±0.12[b]
60µl	0.34±0.16[b]
45µl	0.28±0.90[bc]
30µl	0.14±0.50[c]
15µl	0.09±0.40[cd]
5µl	0.07±0.40[d]
untreated maize	0
Days	
Day 1	1.13±0.18[a]
Day 3	0.17±0.06[b]
Day 5	0.07±0.04[c]
Day 7	0
P- values	
Concentration	0.0001
Day	0.0001
Concentration*Day	0.0001

Values are expressed means ± SEM for four replicates. Means within respect columns followed by similar small case letter(s) are not significantly different at P <0.0001 by Tukey's HSD test.

Insecticidal activity of *O. kilimandscharicum* oil against *S. zeamais* in maize grains stored in granary.

The on-farm experiments revealed that *O. kilimandscharicum* oil exhibited grain protection ability against *S. zeamais* infestations. At 10ml concentration level of *O. kilimandscharicum* oil, the numbers of holed grains were significantly lower ($p<0.0001$) than all other *O. kilimandscharicum* concentration levels (Table 2). In maize grains treated with Actellic Super™ the number of holed grains were significantly lower ($p<0.0001$) than maize grains treated with *O. kilimandscharicum* oil (Table 2). The number of holed grains in Actellic Super™ and all *O. kilimandscharicum* oil concentration levels were however significantly lower ($p<0.0001$) than in untreated maize grains. Significant interactive effects ($p<0.0001$) were observed between concentrations and number of months on the number of holed kernels (Figure 2).

Weight loss (in grams) revealed that as infestation of maize grains by *S. zeamais* increased, so did the weight loss. In 10ml concentration level of *O. kilimandscharicum* oil, weight loss (in grams) was significantly ($p<0.0001$) lower than all other *O. kilimandscharicum* oil doses (Table 2). Weight loss in maize grains was significantly higher ($p<0.0001$) in untreated maize grains than oil doses (Table 2). However, *O. kilimandscharicum* oil concentration levels differed significantly ($p<0.0001$). Weight loss in maize treated with Actellic super was significantly lower ($p<0.0001$) than the oil doses. Significant interactive effects ($p<0.0001$) were observed between concentrations and number of months on weight loss (Figure 3).

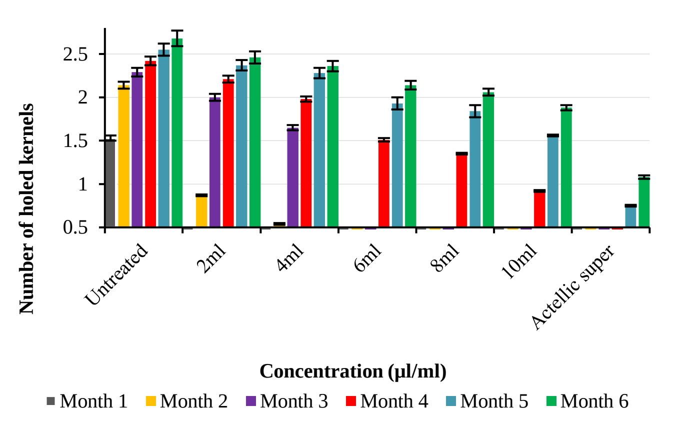

Figure 2: Interactive effects of concentration of *O. kilimandscharicum* oil with month on number of holed kernels by *S. zeamais*

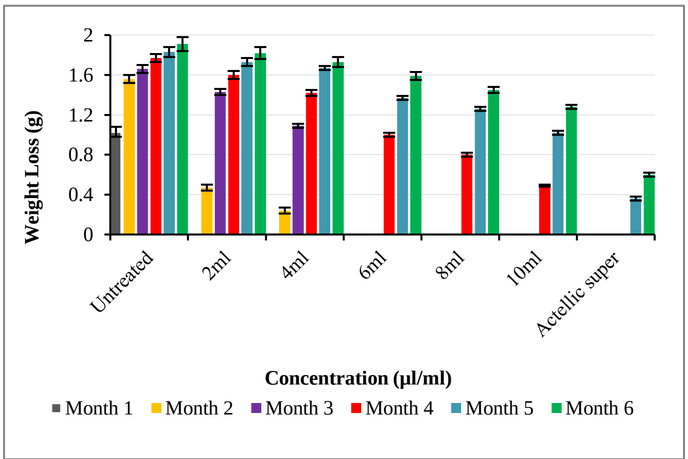

Figure 3: Interactive effects of concentration of *O. kilimandscharicum* oil with month on weight loss of stored maize grains by infestation of *S. zeamais*

Table 2: Mean number of holed maize grains and weight loss in maize treated with different amounts of *O. kilimandscharicum* oil for six months storage.

Treatment	Number of Holed kernels	Weight Loss
Untreated maize	2.27±0.08[a]	1.35±0.14[a]
2ml	1.65±0.18[b]	1.17±0.14[b]
4ml	1.47±0.18[c]	1.02±0.14[c]
6ml	0.92±0.19[d]	0.66±0.14[d]
8ml	0.73±0.18[e]	0.58±0.12[e]
10ml	0.73±0.16[f]	0.46±0.11[f]
Actellic super	0.30±0.09[g]	0.34±0.09[e]
Months		
Month 1	0.21±0.10[f]	0.15±0.07[f]
Month 2	0.51±0.14[e]	0.33±0.10[e]
Month 3	0.85±0.19[d]	0.52±0.12[d]
Month 4	1.48±0.15[c]	1.02±0.11[c]
Month 5	1.89±0.11[b]	1.32±0.09[b]
Month 6	2.09±0.09[a]	1.48±0.08[a]
P- values		
Concentration	0.0001	0.0001
Month	0.0001	0.0001
Concentration*Month	0.0001	0.0001

Values are expressed means ± SEM for four replicates. Means within respect columns followed by similar small case letters are not significantly different at P <0.0001 by Tukey's HSD test.

Replenishment of *O. kilimandscharicum* oil in maize grains at the end of third month

When *O. kilimandscharicum* oil was replenished in the fourth month at 10 ml, 8 ml and 6 ml the number of holed maize grains and weight loss reduced remarkably (Table 3). The number of holed maize grains were significantly higher (p<0.0001) in untreated maize grains than in all *O. kilimandscharicum* oil doses. However, the doses of *O. kilimandscharicum* oil differed significantly (p<0.0001) from one another (Table 3). Significant interactive effects (p<0.0001) were observed between concentration and month on the number of holed kernels (Figure 4). Similarly, weight loss of stored maize grains after replenishing *O. kilimandscharicum* oil after the third month was significantly higher (p<0.0001) in untreated maize grains than all oil doses. Significant interactive effects (p<0.0001) were observed between concentration and month on weight loss of stored maize grains (Figure 5).

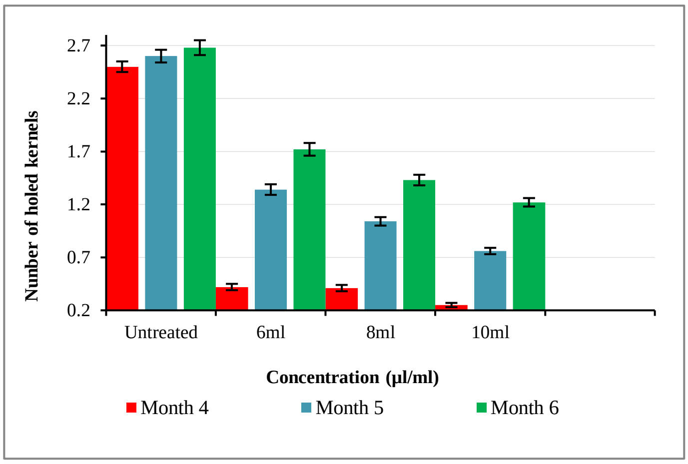

Figure 4: Interactive effects of concentration of *O. kilimandscharicum* oil with month on number of holed kernels by *S. zeamais* on replenishing oil

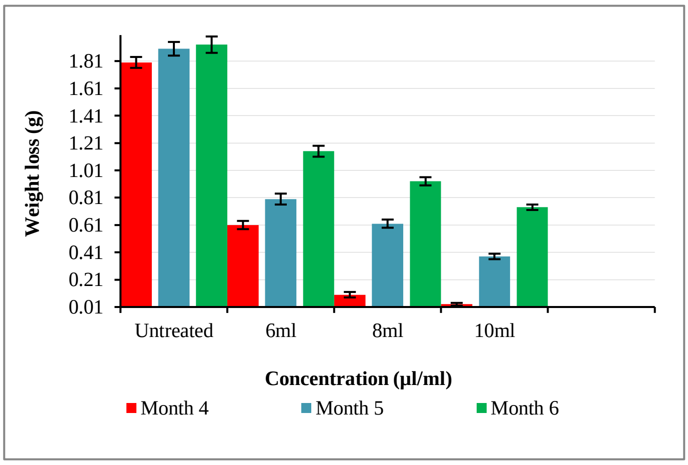

Figure 5: Interactive effects of concentration of *O. kilimandscharicum* oil with month on weight loss of stored maize grains by infestation *S. zeamais* after replenishing oil

Table 3: Mean number of holed maize grains and weight loss in months on replenishing *O. kilimandscharicum* oil in maize after the third month.

Treatment	Number of Holed kernels	Weight loss
Untreated maize	2.36±0.07[a]	4.99±1.70[a]
6ml	0.58±0.14[b]	1.03±0.35[b]
8ml	0.47±0.11[c]	0.59±0.28[c]
10ml	0.34±0.10[d]	0.35±0.19[d]
Months		
Month 4	0.81±0.25[c]	0.52±0.19[c]
Month 5	1.43±0.18[b]	0.92±0.15[b]
Month 6	1.76±0.14[a]	1.19±0.12[a]
P- values		
Concentration	0.0001	0.0001
Month	0.0001	0.0001
Concentration*Month	0.0001	0.0001

Values are expressed as means ± SEM for four replicates. Means within respect columns followed by similar small case letters are not significantly different at P <0.0001 by Tukey's HSD test.

Pre-treatment of baglets of maize grains with *A. indica* and *W. ugandensis* extracts and grains with *O. kilimandscharicum* oil.

Results of pre-treating the baglets containing maize grains treated with *O. kilimandscharicum* oil with aqueous extract of *A. indica* and *W. ugandensis* showed improved protection of maize grains against *S. zeamais.* The number of holed maize grains was significantly higher (p<0.0001) in untreated maize grains stored in unpretreated baglets than in treated maize grains stored in pre –treated baglets (Table 4). There were no significant differences in the number of holed kernels in baglets pre-treated with aqueous extracts (Table 4). Significant interactive effect (p<0.0001) was observed between treatment and month on the number of holed kernels (Figure 6).

Table 4: Mean number of holed maize grains and weight loss in maize treated with *O. kilimandscharicum* oil and stored in baglets treated the aqueous extracts of *A. indica* and *W. ugandensis* for six months.

Treatment	Number of Holed kernels	Weight loss
Untreated maize	2.19±0.01[a]	1.57±0.07[a]
W. ugandensis + oil	0.91±0.19[b]	0.60±0.13[b]
A. indica + oil	0.89±0.19[b]	0.59±0.12[b]
Months		
Month 1	0.51±0.22[f]	0.31±0.13[f]
Month 2	0.66±0.28[e]	0.48±0.21[e]
Month 3	0.73±0.31[d]	0.52±0.22[d]
Month 4	1.76±0.13[c]	1.15±0.12[c]
Month 5	2.07±0.09[b]	1.43±0.09[b]
Month 6	2.27±0.07[a]	1.61±0.06[a]
P- values		
Concentration	0.0001	0.0001
Month	0.0001	0.0001
Concentration*Month	0.0001	0.0001

Values are expressed as means ± SEM for four replicates. Means within respect columns followed by similar small case letters are not significantly different at P<0.0001 by Tukey's HSD test.

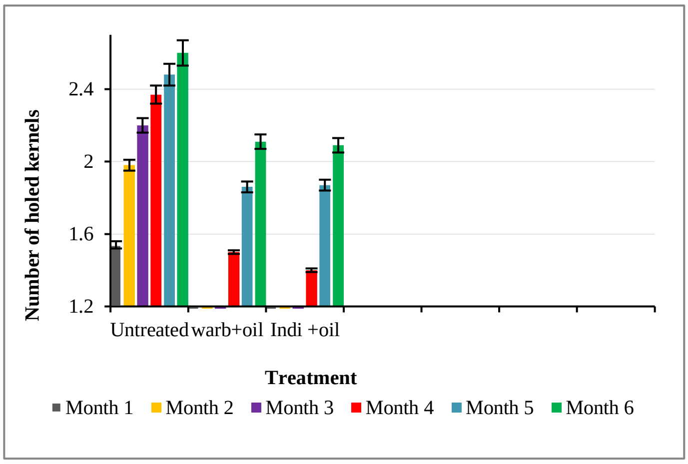

Figure 6: Interactive effects of maize treated with *O. kilimandscharicum* oil, stored in baglets treated the aqueous extracts of *A. indica* and *W. ugandensis* with month on number of holed kernels by *S. zeamais*

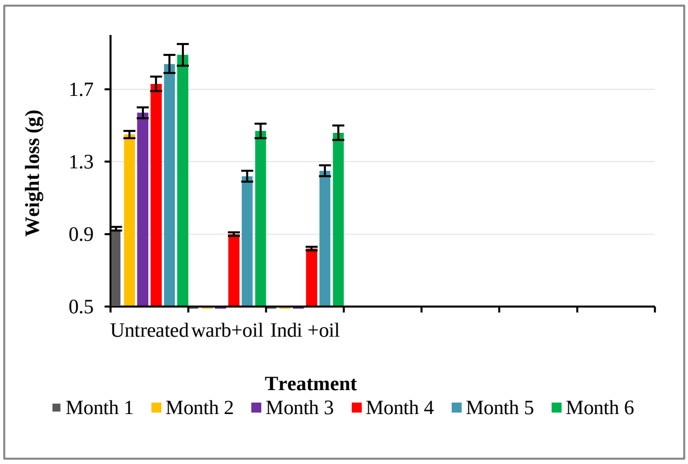

Figure 7: Interactive effects of maize treated with *O. kilimandscharicum* oil, stored in baglets treated the aqueous extracts of *A. indica* and *W. ugandensis* with month on weight loss

Weight loss (in grams) in maize treated with *O. kilimandscharicum* oil and stored in baglets pre-treated with aqueous extracts of *A. indica* and *W. ugandensis* was significantly (p<0.0001) lower than in untreated maize grains stored in unpre-treated baglets (Table 4). There was no significant difference in weight loss in baglets treated with aqueous extracts of *W. ugandensis* and *A. indica*. Significant interactive effect (p<0.0001) were observed between treatments and month on weight loss of stored maize grains (Figure 7).

DISCUSSION

Insecticidal activity of *O. kilimandscharicum* on artificially infested maize grains

Results from this study revealed that the oil has contact toxicity property since mortality was high after 24 hours exposure. There was 100% mortality of *S. zeamais* at 105µl/ml concentration level of *O. kilimandscharicum* and 0% mortality at 5 µl/ml concentration level after 24 hours exposure. Mortality of *S. zeamais* was also 0% on untreated maize grains. Mortality of *S. zeamais* was high on the first day of exposure in maize treated with *O. kilimandscharicum* at 105 µl, 90µl, 60µl, 45µl and 30µl doses and reduced as days increased. High mortality in the first day of exposure might be due to contact toxicity of its major constituent camphor.

This study agrees with [30] who documented that *O. kilimandscharicum* at 100mg/filter paper and 100µg/insect caused over 93% and 100% mortalities in *Sitophilus granarius*, *S. zeamais* and *Prostephanus truncatus*. However, only 70% and 100% mortality in *Tribolium castaneum* was observed after 24 hours exposure. According to [31] dried ground leaves and essential oil of *O. kilimandscharicum* in doses of 25.0g leaves and 0.3g essential oil per 250g grain (sorghum or maize) killed 100% of *Sitotroga cerealella*, *Rhyzopertha dominica* and *Sitophilus zeamais* in 2 days. The best repellent activity was seen by 0.3 g essential oil/250 g grains against *Sitophilus zeamais*.

Toxic effects of plant extracts in the Institute for stored Product Protection were initially done by [30] on extract of the medicinal plants of *O. kilimandscharicum, O. suave and O. kenyense*. Depending on the Ocimum species, eugenol, 1, 8 cineol and camphor has been identified as main compounds of the essential oils. The compounds were found to greatly affect beetles of the species *Tribolium castaneum*, *Lasioderma serricorne*, *Prostephanus truncatus*, *Sitophilus granarius* and *S. zeamais* when mixed to grain. Complete control was achieved after 24 hours at a dosage of 0.5 µl/kg or 0.5 mg/kg of grain. Furthermore, it was established that admixture of these compounds with low quantities of vegetable oils like sunflower seed oil or sesame oil increased toxicity to insects and persistency [30].

Long term insecticidal activity of *O. kilimandscharicum* on *S. zeamais*

On farm studies revealed that *O. kilimandscharicum* oil was effective against *S. zeamais*. However, insecticidal activity of the oil varied with dose used. At 10ml, 8ml and 6ml doses of *O. kilimandscharicum* efficacy against *S. zeamais* lasted for three months. At 4ml and 2ml doses of *O. kilimandscharicum* efficacy against *S. zeamais* lasted for one month after treatment. However, in 10ml, 8ml, 6ml, 4ml, and 2ml doses of *O. kilimandscharicum* oil the number of holed grains differed significantly ($p<0.0001$) from control treatment. The percentage weight loss in maize was proportional to the number of holed grains. The weight loss was below 50% in 10ml, 8ml and 6ml doses of *O. kilimandscharicum* after six months of storage. In 4ml and 2ml doses of *O. kilimandscharicum*, percentage weight loss in maize grains was above 50% after six months of storage. Percentage weight loss in untreated maize grain (control treatment) differed significantly ($p<0.0001$) with every *O. kilimandscharicum* dosage.

When *O. kilimandscharicum* oil was replenished after the third month of storage, the number of holed maize grains significantly ($p<0.0001$) reduced in 10ml, 8ml and 6ml doses of *O. kilimandscharicum*. In addition, efficacy of the oil against *S. zeamais* improved with an extra month. Percentage weight loss was also proportional to the number of holed grains. The weight loss was below 20% in 10ml, 8ml, and 6ml doses of *O. kilimandscharicum*. Every *O. kilimandscharicum* dose replenished after the third month differed significantly ($p<0.0001$) with untreated maize grains.

According to [32,33] extracts from Lamiaceae Family are effective in causing mortality of weevils may be as a result of active compounds such as flavanoids, alkaloids, steroids, glycosides and terpenoids against weevils. Furthermore, compounds such as saponins, glycosides, flavanoids and phenols have toxic effects on Coleopterans [34]. It's also possible that some of the extracts came into contact with insects' spiracle to further mortality.

Death of some weevils might have been due to starvation [35] because coating the grains with the extracts minimized contact between the grains and the weevils. Compounds found in Lamiaceae such as monomeric flavanoids, glycosides, terpenoids and tannins acted as feeding deterrent [34,36] and thus enhaced mortality by starvation.

Pretreatment of baglets containing treated maize grains with aqueous extracts of *A. indica* and *W. ugandensis*

Pretreatment of baglets with aqueous extract of *A. indica* and *W. ugandensis* improved the efficacy of the *O. kilimandscharicum* oil against *S. zeamais*. The numbers of holed grains were lower in treated maize stored in pretreated baglets than in untreated maize stored in unpretreated baglets after six months of storage. In addition, significant difference ($p<0.0001$) was observed in the number of holed grains in treated maize grains stored in pretreated baglets than in treated maize in unpretreated baglets. Percentage weight loss was also proportional to the number of holed grains. Weight loss was below 30% in pretreated baglets and below 40% in treated maize grains stored in unpretreated baglets after six months of storage. Percentage weight loss in untreated maize grains in unpretreated baglets was above 50%. Reduced weight loss in pretreated baglets could as a result of active ingredients present in the three plant extracts.

This study agrees with [37] who found that neem leaf treatment of corn stored in sacks or unpeeled corn cobs held in bins was quite simple and effective against *S. zeamais*, *Tribolium* spp., *Rhyzopertha dominica*, and *Cathartus* spp. [38], documented mixing neem leaves with paddy grain in a proportion of 2 to 100 parts (wt/wt), bag treatment with neem leaf extract, or placing barriers of neem leaves between bags and storage floor, significantly reduced the infestation by *S. oryzae* and *R. dominica* to paddy grain stored in 40 kg jute bags for 3 months.

Azadirachtin is a compound derived from *A. indica* tree [39]. Azadirachtin is documented to be active against stored product pests acting as repellent, sterilant, insect growth regulator, feeding deterrent and as anti-ovipositional. Neem extracts eliminates insect pests with soft cuticle or their juveniles, although it is basically anti-ovipositional, insect growth regulator, feeding deterrent and repellent [40].

According to [41], methanolic extract of *W. ugandensis* was found to be potential insect antifeedant activity against *Spodoptera Exempta*. Based on fractionation guided by the assay, three antifeedants were isolated from the fruits, leaves and bark of *W. ugandensis*. Feeding of larvae of two species of Africam armyworm, the monophagous *Spodoptera exempta* and the polyphagous *Spodoptera littoralis* was inhibited by Muzigadial and warburganal at 0.1ppm in a regular leaf disk method [42].

According to [43] ugandensial and polygadial are potent antifeedant for Spodoptera but less active. Drimane sesquiterpenoids are also shown to have insect antifeedant property [44]. According to [45], stem bark of *W. ugandensis* exhibit antiplasmodial acivity against *Plamodium knowlesi* and *Plamodium berghei*. Warburgia species possess high pharmaceutical value, both for humans and livestock. This is due the abundance of drimane and colorotane sesquiterpenoids [46].

Opiyo *et al.* documented that essential oil of *W. ugandensis* was effective against *S. zeamais* and exhibited 100% mortality after 21 days. The extracts were effective as actellic dust and completely inhibited emergence of adults' insects. Polygadial, ugandensolide and warburganal were best in growth inhibition [47].

CONCLUSION

Essential oil of *O. kilimandscharicum* has insecticidal property and more specifically being active against *S. zeamais*. The activity was also concentration dependent totally. In artificially infested maize grains, mortality of *S. zeamais* was high on the first day of exposure to the essential and this probably demonstrates the contact toxicity of essential oil.

Long-term insecticidal activity of essential oil of *O. kilimandscharicum* shows that the oil can protect maize against infestation of *S. zeamais* in granary for three months. The activity was concentration dependent where 10ml per 1Kg of maize was selected as the best dosage. Replenishing essential oil on maize grains after 3 months of storage increased its efficacy.

Pre-treatment of baglets with aqueous extracts of *A. indica* and *W. ugandensis* is useful as it enhances protection of stored threshed maize grains against *S. zeamais.* Pre- treatment has therefore an added advantage to treating maize with *O. kilimandscharicum* oil.

Conflicts of Interest

No conflict of interest among authors.

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