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Biopesticide Potential of Clove Oil Against Maize and Sorghum Storage Weevils *Sitophilus* species in Kenya

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Abstract

In Kenya, maize is a staple food grown widely by 90% of farmers. The government has identified sorghum for flour blending to reduce dependence on maize and to improve food security and nutrition. Despite the importance of these cereals, the weevils, *Sitophilus* species cause 40% damage to maize and sorghum in Kenya. Thus, the study aimed to evaluate Clove oil biopesticide potential against *Sitophilus* spp. on maize and sorghum. The experiment comprised of control (T1), clove oil treatments T2 (1.0 µl), T3 (0.5 µl), T4(0.25 µl), and T5(0.125 µl). Maize and sorghum grains (20gm) were mixed with clove oil, and 20 unsexed weevils. Weevils' mortality data was recorded after 24 and 48 hours. Data were subjected to ANOVA, and means were separated using Fisher's protected least significant difference test ($p \leq 0.05$). There was a significant difference ($P<0.001$) between the treated maize and the control after 24 and 48 hours of treatment. In T2, mean weevil mortality >17.8±1.3 was recorded in sorghum at 24 and 48 hours. There was a significant difference ($P<0.001$) between the control and T2 and T3 sorghum grains. The *Sitophilus* spp weevils were susceptible to clove oil, a potential biopesticide against *Sitophilus* spp on stored maize and sorghum.

Keywords: Maize and Sorghum; Weevils, *Sitophilus* spp; Clove oils; Bio-pesticide

Introduction

Globally cereal crops; Sorghum, Millet, Wheat, Maize, and Rice are important foods for the population (Macauley, 2015). Maize is a major staple food crop grown in a wide range of environments and consumed by people with different food preferences in sub-Saharan Africa (SSA). Sorghum is ranked second after maize in order of importance and it is produced in 22% of the cereal total area (Taylor 2003, Macauley 2015, Popescu et al. 2018). In Kenya, Maize is the main staple food, grown in 90% of all farms. The Government of Kenya has identified Sorghum among other cereals for diversification and flour blending to reduce over-reliance on maize and improve food security and nutrition (Njeru et al. 2023, AFA 2024).

In SSA the insect pests are the key constraints to cereal crop production and utilization. The field and storage pests cause an estimated 10-88% loss of the total maize produced per season in the region (Shaaya et al. 1997, Ojola and Omoloye 2012, Midega et al. 2015) Grain damage results in food safety and health issues, loss in weight, nutritional and aesthetic value, and marketability (Husain et al. 2017). Among the insect pests that damage stored grains are grain weevils *Sitophilus* species (Sweelam et al. 2019).

The grain weevils *Sitophilus* spp. (rice weevil (*Sitophilus oryzae*), granary weevil (*Sitophilus granarius*), and maize weevil (*Sitophilus zeamais*,) are the destructive pests of stored grains worldwide (Mofokeng 2016, Mason 2018, Sweelam et al. 2019, Bhargude et al. 2021). The weevil damage can start with egg-laying in the field once on mature grains and >20%moisture. The pests make small holes into the grain and lay eggs singly per cavity, secretes a glue-like substance to seal the cavity after egg laying. Eggs hatch, and larvae feed in destroying the grain interior. The *Sitophilus* spp. reduce stored grains to a mass of hulls and excreta (Sahoo and Sahoo 2018, CABI 2017, NPPC 2022).

In Kenya, weevil damage impacts greatly on stored sorghum and maize which are food security cereals. Weevil damage causes grain losses up to 40% during storage. This insect pest causes contamination of grains, reduce grains viability leading to poor germination, aesthetic value of the grains hence poor marketability, and decreased nutritional value (De Groote et al. 2023, Kasina and Likhayo P 2025). Management of weevils in stored grains in Kenya is mainly by use of registered synthetic chemicals (PCPB 2019). The products used belong to various toxicological chemical classes. Conventional pesticides are relatively efficient, but are associated with chemical residues in foods, human health concerns, environmental contamination, and increased costs of production (EU 2021, Leskovac and Petrovi´c 2023). Thus, there is a need for an integrated pest management approach that is safer and available. Such strategies include using bio-pesticides that cost less, some are derived from readily available natural sources depending on the type and locality, are easy to use, no qualified personnel needed to apply them, and are safe for human health and the environment (Ayilara et al. 2023).

The plant species in the families; Asteraceae, Ranunculaceae, Brassicaceae, Apiaceae, Piperaceae, Lamiaceae, Lauraceae, Verbenaceae, and Myrtaceae, have shown insecticidal activity against insect pests. Products in the form of powders, extracts, and essential oils from such plants have been used to manage stored grain pests. Among such plants with insecticidal phytochemicals is the Clove plant (Mittal et al. 2014).

The clove plant *Syzygium aromaticum* (L.) Merril. & Perry, in the family Myrtaceae is an evergreen plant grown for its unopened small reddish-brown flower buds; used as a food flavor enhancer. In addition, the buds are a source of oil from which clove essential oil is extracted (Mittal et al. 2014, Radunz et al.2018, Haro-González et al. 2021).

Clove essential oil's main phytochemicals are eugenol (60-90%), eugenol acetate (2-27%), and 5-12% β-caryophyllene; while the minor compounds are methyl amyl ketone, methyl salicylate, and benzaldehyde. The clove bud's pungent taste is attributable to the eugenol compound (Mittal et al. 2014, Faisal et al. 2020, Seema et al. 2020, Haro-González et al. 2021). Clove oil compounds are known for their antioxidant, antibacterial, anti-carcinogenic, antifungal, and insecticidal biological capacities (Amelia et al. 2017, Kumar et al. 2024). In addition, clove oil, has been used as a food flavoring agent, in dentistry, germicides, and cosmetics among others (Mittal et al. 2014, Amelia et al. 2017, Radunz et al. 2018, El-Saber Batiha et al. 2020, Faisal et al. 2020).

The clove essential oils generally recognized as safe (GRAS, have been used as insecticides against arthropod pests (aphids, armyworms, beetles, cutworms, mites, flies, wasps, and weevils) (Tian 2015, Baker et al. 2018). Potential use of the Clove powder and extracts (oil) against weevils has been reported on stored grains (Sorghum, Maize, beans, cowpea) (Ileke et al. 2014, Carlos et al. 2016, Baker et al. 2018, Sousa et al. 2023). Essential oils exhibit a wide range of biological activities against insects; feeding deterrence, acute toxicity, repellency, growth inhibition, and development and reproduction limitations (Carlos et al. 2016, Sharifi-Rad et al. 2017, Halliru and Suleiman 2022). Though the insecticidal properties of clove oil on pests have been reported, its effectiveness in the management of weevils on stored grains in Kenya is not known.

Thus, the study aimed to evaluate the bio-pesticide potential of Clove oil against Maize and Sorghum storage weevils *Sitophilus* species under laboratory conditions in Kenya.

Materials and Methods

The experiment was conducted at the Kenya Agricultural and Livestock Research Organisation (KALRO), Horticulture Research Institute Thika, Entomology

Laboratory in 2024. The storage grain weevils *Sitophilus* species were used in the experiment. The specimens were locally sourced from grain stores. The weevils were reared on sorghum grains in KALRO-HRI laboratory. The weevils were maintained in 1 kg glass jars with the capacity fitted with gauze and fastened with a rubber band. The rearing of unsexed weevils lasted one (1) month, from which dead weevils were removed, and a new generation was initiated on fresh grains from eggs that remained. This resulted in weevils of the same age. The clove oil was sourced from a stockist within Thika Town.

The maize (KALRO-Ukamez variety) and Sorghum (Seredo variety) both that are drought tolerant and fast growing varieties were used in the experiment; where 20 g of grains were weighed and mixed with defined doses/volumes of crude clove oil in microlitres (µl) in a 9cm diameter Petri dish. The control treatment consisted of untreated 20 g of sorghum and maize grains. Twenty unsexed adults grain weevils aged 15-20 days were placed in each Petri dish. The experiment was set in a completely randomized design, with four treatment arms and a control. Each treatment was replicated four times. Clove oil treatments; T2 (1.0µl), T3 (0.5µl), T4 (0.25µl), and T5 (0.125µl). The experiment was run for 48hours

Data collection

The data on the insecticidal effect of clove oil on weevils was collected at 24 and 48 hours after treatment. The dead weevils were counted every 24 hours and the cumulative number was recorded. The weevils were considered dead if they remained immobile for two minutes (Antunes et al. 2013).

Data Analysis

Mortality data were subjected to statistical analysis of variance (ANOVA), and means were separated using Fisher's protected least significant difference test ($p \leq 0.05$) using Genstat 15th edition.

Results

Susceptibility of *Sitophilus* spp. weevils to clove oil-treated maize grains

Mortality of weevils was observed in all the essential oil-treated maize but there was no death in the control experiment which was untreated in the first 24 hours. All the oil treatments (T2, T3, T4 and T5) recorded a mean number of dead weevils of >19.00. There was a significant difference ($P < 0.001$) between the oil treatments and the control. There was no significant difference among the oil treatments (Table 1).

Table.1: Number of dead weevils in Maize at 24 hours in Clove oil post-treatment

Treatment		Mean ±S.E
T1(Control)		0.0 ± 0.0^{b}
T2		20.0 ± 0.0^{a}
T3		20.0 ± 0.0^{a}
T4		19.5 ± 0.5^{a}
T5		19.5 ± 0.5^{a}
P-Value	<0.001	

Treatments: T1 (control), T2 (1.0µl), T3 (0.5µl), T4 (0.25µl), and T5 (0.125µl) (ul; Clove oil doses) and S.E: Standard Error

In the 48 hours of treatment, the mean weevil mortality of 20.0±0.0 was observed in all the maize clove oil treatments. A mean weevil mortality of 0.0±0.0 was recorded in the control experiment (Figure 1). There was a significant difference ($P<0.001$) in weevil mortality between the control (untreated) and all the clove oil treatments. There was no significant difference in mean (20±0.0) weevil mortality among the oil treatments; T2 (1.0 µl), T3 (0.5 µl), T4 (0.25 µl), and T5 (0.125 µl).

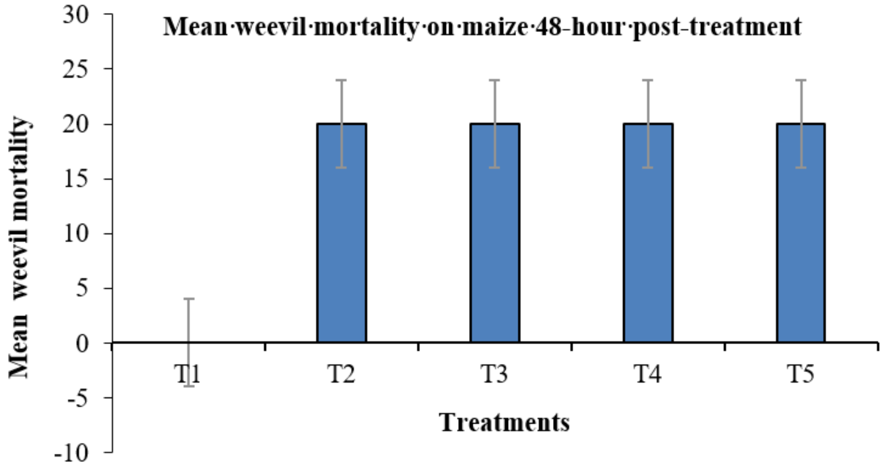

Figure 1: Mean Weevil Mortality 48-hour post-treatment**.** Treatments; T1 (control), T2 (1.0 µl), T3 (0.5 µl), T4 (0.25 µl), and T5 (0.125 µl) (µl; clove oil doses)

Susceptibility of *Sitophilus* spp weevils on clove oil-treated Sorghum grains

Weevil mortality was registered in all the clove-treated sorghum grains 24 hours post-biopesticide exposure. High mean number of dead weevils (17.8±1.3) was recorded in the highest clove oil treatment T2 (1.0µl) followed by T3 (0.5 µl), T4 (0.25 µl), T5 (0.125 µl) and control (untreated). There was a significant difference (P<0.001) between the control and oil-treated sorghum grains T2 and T3. A significant difference (P<0.001), between clove oil treatments T2; T3, and T4:T5 was observed. In addition, there was no significant difference (P>0.001) between in the mean weevil mortality in the control and clove oil treatment T4 and T5 which registered mean mortality of 3 .5±1.7; 0.75±0.479 respectively (Figure 2).

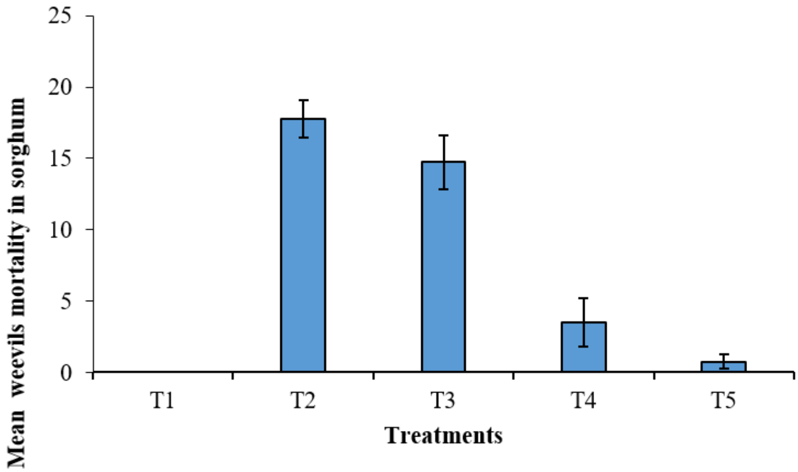

Figure 2: Mean weevil Mortality on clove oil-treated sorghum 48-hour post-treatment**.** Treatments: T1 (Control), T2 (1.0 µl), T3 (0.5 µl), T4 (0.25 µl), and T5 (0.125 µl) ul; Clove oil doses).

Weevil mortality was recorded in all clove oil-treated sorghum grains set up, 48 hours after treatment. The highest to lowest mean mortality was in the order of 19.5±0.29, 16.25±1.65, 3.75±1.75, 1±0.41, and 0±0 (T2, T3, T4, T5, and T1-control respectively). A significant difference (P<0.001) among all clove-treated grains was registered. In addition, a significant difference (P<0.001) between the control (T1) and treatments T2, T3, and T4 was noted. There was no significant difference in mean weevil mortality between the control and treatment T5 (0±0;1±0.4 respectively) (Table 2).

Table.2: Mean number of dead weevils on sorghum at 48 hours of Clove oil post-treatment

Treatment		Mean ±S.E
T1(Control)		0±0^{d}
T2		19.5±0.3^{a}
T3		16.3±1.7^{b}
T4		3.8±1.8^{c}
T5		1±0.4^{d}
P-Value	<0.001	

Treatments: T1 (control),T2 (1.0 µl), T3 (0.5 µl), T4 (0.25 µl), and T5 (0.125 µl) ul; Clove oil doses).

A comparison between susceptibility of weevils on clove oil-treated Maize and Sorghum grains.

The highest mean number of dead weevils (20.0±0.0;19.5±0.3) on maize and sorghum grains respectively was recorded in the highest concentration of clove oil T2 (1.0 µl); while in the control in both grains no mortality was recorded at the 48hr after treatment. In addition, there was significant difference (P<0.001) between clove oil treatment T2 and T1 the control in maize and Sorghum grains. There was no weevil death observed in the control (T1) in maize and sorghum (Figure 3). In the clove oil treatment T5 (0.125µl), mean weevil mortality on maize was 20.0±0.0 while on sorghum was 1±0.4). A significant difference (P<0.001) was observed between clove treated maize and sorghum grains in T4 (0.250µl) and T5 (0.125µl).

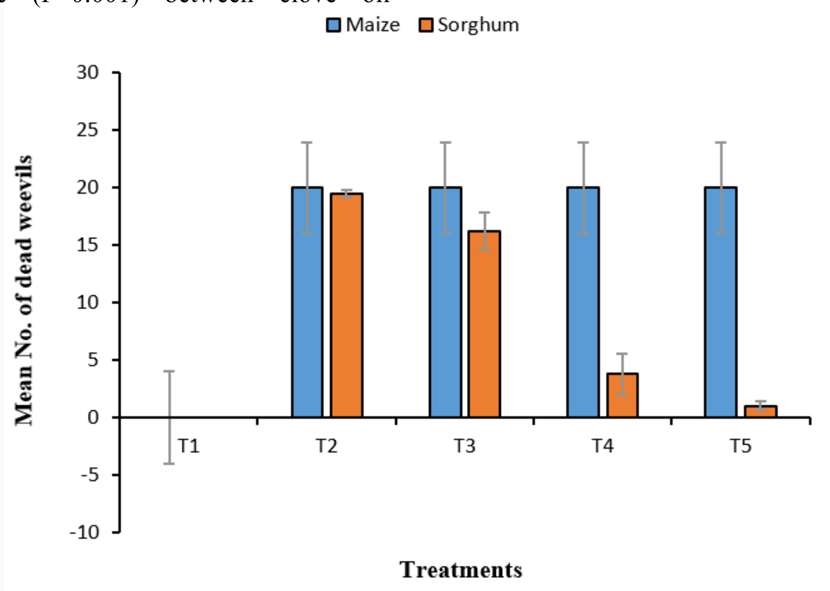

Figure 3: Comparison between mean weevil mortality on clove treated maize and sorghum 48-hour after treatment. Treatments: T1 (control), T2 (1.0 µl), T3 (0.5 µl), T4 (0.25 µl), and T5 (0.125 µl) ul: Clove oil doses)

Discussion

The study result indicated susceptibility of *Sitophilus* spp. weevils to clove oil-treated maize grains. This is because weevil mortality was recorded in all oil treatments 24- and 48-hours post-treatment. No weevil mortality was observed in the control experiment (Untreated maize grains). There was no significant difference in the effect of oil treatment in both exposure periods. A study by Ashamo and Odeyemi (2001), reported 100 % mortality of the *Sitophilus* spp. on maize within 24 hours of treatment with peanut oil *Arachis hypogaea*. According to Eesiah et al. (2022), a study on insecticidal activity of selected biopesticides (essential oils (EOs) indicated maize weevil mortality and grain storage period increased concentrations with cinnamon, clove, and thyme oils being more effective. In addition, no weevil mortality was observed at the 1% of plant oil extract concentration. In another study, 100% mortality of bean and maize weevil was reported 48 h post-treatment with Clove oil concentrations of 17.9 and 35 µL g-1(Garcia and Jairoce 2016). According to Sousa et al. (2023), a combination of Clove bud and Pennyroyal essential oils reduced grain losses by >45% and, the survival ability of *Sitophilus zeamais* (Motschulsky) by >90%.

Results from the study showed susceptibility of *Sitophilus* spp. weevils to clove oil-treated Sorghum grains. Weevil mortality was observed in all clove oil treatments 24- and 48-hours post-exposure. No mortality was recorded in the untreated control (T1). Nevertheless, higher weevil mean mortality was observed in clove oil treatment T2 (1.0µl) compared to the rest of the treatments. Thus, a significant difference between mean weevil mortality in the highest clove oil concentration T2 (1.0µl) and lowest concentration T5 (0.125µl) and the untreated control was observed in 24 and 48 hours of exposure. There was an indication of the effect of clove oil concentration on mean weevil mortality. There was no difference reported between the lowest concentration and untreated control. Similary, according to Bhanderi et al. (2015); Bhargude et al. (2021) rice weevil, *Sitophilus oryzae* on sorghum was susceptible to sweet flag, custard apple seed, and neem seed kernel powders' treatments. In addition, Sunil et al. (2023) found that, the sweet flag rhizome oil at 10% concentration resulted in 98.75% repellent activity on maize weevil at 24 hours after treatment. The results from other researchers emphasize on the effectiveness and potential of use of some plant extracts against insect pests of stored products including maize and sorghum.

A comparison between the susceptibility of weevils on clove oil-treated Maize and Sorghum (T5) grains indicated a mean (20.0±0.0) weevil mortality 48hr after treatment; while in sorghum low clove oil treatment resulted in low mean (1±0.4) weevil mortality. This could be attributed to the difference in chemical components of maize and sorghum kernel. In common maize 70-75% and 100% in waxy maize starch is amylopectin. The maize variety may have been the waxy type that may have translated to low clove oil absorption capacity, hence more oil remained on the grain surfaces. This may have increased weevils' contact with the clove oil and vice versa for sorghum that may have had high oil absorption capacity. The study results suggested that insecticidal oil may have positive or negative effect on pests. Similarly, the effectiveness of clove oil on stored grain weevil management is dependent on grain type and oil concentration used. The physical and chemical properties (hardness, moisture content, and surface texture) of different grains, impacts on clove oil penetration and interaction with the grain and the weevils (Carlos et al. 2016, Rai et al. 2021).

Conclusion

The study concludes that Maize and Sorghum stored grain weevils *Sitophilus* spp are susceptible to clove essential oil. Weevils on maize were more susceptible to clove oil treatment than Sorghum. Clove oil has great potential as a biopesticide for use as an alternative for synthetic insecticide against maize and Sorghum stored grains weevils *Sitophilus* spp. This could address food security, safety, and environmental health. There is a need to determine the composition of the clove oil used in the study; and efficacy assessment of large-scale maize and sorghum storage.

Declaration of interest

There is no potential competing or conflicting interest(s) in regard to publication of this work

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