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Toxicity evaluation of essential oil of clove (*Syzygium aromaticum*) bud against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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ABSTRACT

This study aimed to evaluate the efficacy, toxicity, and effectiveness of clove bud oils against *Sitophilus zeamais* in the stored maize variety Manakamana-4. The toxicity evaluation of the oil on maize weevils were conducted in completely randomized design (CRD) with eight treatments including seven different oil concentrations (10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125% and 0.15625%) and control replicated four times at laboratory conditions in National Entomology Research Center, Khumaltar, Lalitpur, Nepal during 2020/2021. The results exhibited higher toxicity of clove oil to weevils after 72 h of oil exposure. Higher concentrations and longer exposure periods resulted in an increased mortality rate. Lower concentrations required a longer time for 50% of the weevil population to kill, whereas higher concentrations required a shorter time. Moreover, the essential oil was effective in causing the mortality of the weevil population at all three time intervals of 6, 10, and 16 days with varying mortality rate as lower concentrations required extended exposure time. In the same way, repellency assays of clove oil by applying four different concentrations (10%, 5%, 2.5% and 1.25%) on Whatman filter paper showed significant pest repellant activity. Repellent action was also highly dependent upon oil concentration and exposure time showing highest repellent activity (98.75%) at 10% concentration at 24 h interval. Additionally, it indicated higher the concentrations of clove oil higher the seed germination and fewer the weevil progeny emergence, and vice-versa. Since clove oil is considered to have low mammalian toxicity and a minimum risk pesticide, it can be incorporated into integrated pest management system in storage against maize weevils.

Keywords: extraction, repellent, mortality, progeny, germination

सारांश

यस अध्ययनले भण्डारण गरिएको मकैको जात मनकामना-४ मा सिटोफिलस जियामेज विरुद्ध ल्वाङको तेलको प्रभावकारिता, विषाक्तता र प्रभावकारिता मूल्याङ्कन गर्ने उद्देश्य राखेको थियो । तेलको विषाक्तता मूल्याङ्कन गर्न सन् २०२०/२०२१ मा राष्ट्रिय कीट विज्ञान अनुसन्धान केन्द्र, खुमलटार, ललितपुर, नेपालको प्रयोगशालामा तेल बिनाको उपचार सहित सात विभिन्न तेल सघनताका उपचारहरु (१०%, ५%, २.५%, १.२५%, ०.६२५%, ०.३१२५% र ०.१५६२५%) को परीक्षण सञ्चालन गरिएको थियो। यी उपचार बिधि चार पटक दोहोर्याइएका थिए। ल्वांगको तेलले उपचार गरिएको ७२ घन्टा पछि घुनहरुमा उच्च विषाक्तता देखाएको थियो। तेलको उच्च सघनता र लामो समय तेलको सम्पर्कको असरले घुनको मृत्यु दर बढाएको पाइयो।कम सघनता भएको तेलले ५०% घुनको सङ्ख्या मार्नको लागि लामो समय

आवश्यक भएको पाइयो, जबकि उच्च सघनताको तेललाई कम समय चाहियो । यस बाहेक तेलले उपचार गरेको मकैमा ६, १०, र १६ दिनको अन्तरालमा घुनको मृत्युदर फरक फरक पाइयो । त्यसैगरी, व्हाटम्यान फिल्टर पेपरमा चारवटा फरक सघनताको (१०%, ५%, २.५% र १.२५%) ल्वांगको तेलको प्रयोग गरेर विकर्षणको प्रभाकारिता हेरिएको थियो ।जसमा, २४ घण्टाको अन्तराल पछि १०% सघन तेलमा उच्चतम विकर्षक (९८.७५ %) देखियो । विकर्षणको प्रभावकारिता पनि तेलको सघनता र उपचार समयमा अत्यधिक निर्भर रहेको पाइयो। ल्वाङको तेलको सघनता उच्च हुँदा बीउको अंकुरण प्रतिशत बढेको र कम हुदा घटेको पाइयो । थाहाभए मुताबिक ल्वाङको तेल स्तनधारी प्राणीहरुको लागि कम विषाक्त हुने र एकदमै न्यून जोखिमयुक्त कीटनाशकको रुपमा प्रयोग गर्न सकिने हुनाले यसलाई भण्डारणमा मकैमा लाग्ने घुनहरुको बिरुद्ध एकीकृत शत्रुजीब व्यवस्थापन प्रणालीमा समावेश गर्न सकिन्छ।

INTRODUCTION

There are about 2500 plant species from 235 families that have pesticide properties (Ngegba et al 2022), and among them 311 species are commonly available in Nepal (Neupane 1999). Plants contain different chemicals with various properties, which have significant importance in insect pest management. Plant chemicals either directly kill insect pests (Ayvaz et al 2010) or have repellent (Isman 2006, Lee 2018), antifeedant (Koul 2008), and fumigation effects (Trivedi et al 2017, Mwamburi 2022). Essential oil derived from different parts of plant species have been utilized to tests against various arthropods (Park and Tak 2016, Awad et al 2022) including insect pests (Innocent and Hassanali 2014, Lengai et al 2019, Ahmed et al 2022). Among different insect pests, Coleopterans were most studied against essential oils of plant followed by Lepidoptera (Campolo et al 2018). Essential oils have been especially effective against various storage insect pests (Zeng et al 2010, Correa et al 2015, Chaudhari et al 2021, Ibrahim, 2022, Aryal et al 2023). Essential oils are volatile natural complexes obtained from plant materials and are a rich source of biologically active compounds that have been shown to possess antibacterial, antifungal, antiviral, antioxidant, and insecticidal properties (Hammer et al 1999, Nerio et al 2010). These plant products have been suggested as suitable alternatives for controlling stored product pests including insecticide resistance ones worldwide because of their potential safety for the environment and human health (Regnault-Roger et al 2012). Plant essential oil also reduces effects to non-target arthropods (Tembo et al 2018) and has low mammalian toxicity (Mwamburi 2022).

Plant originated essential oils are effective against coleopteran insect pests in stored grain (Perez et al (2010)). Essential oil of clove has been studied against various insects and non-insect pests (Carletti et al 2011, Barua et al 2015, Correa et al 2015, Girisgin 2017, Lee 2018, Abd-Alla and Hamouda 2021) including storage pests (Jumbo et al 2014, Jairoce et al 2016, Hamza and Hamza 2018, Ikawati et al ??2020??, Aryal et al 2023). Similarly, repellent activity of the essential oil/pure compounds are also tested (Zeng et al 2010, Aryal et al 2023).

Clove (*Syzygium aromaticum*) essential oil and powder have been studied to manage agricultural pests as they possess pesticide properties (Kafle and Shih 2013). Major component of essential oil of clove is eugenol (Tian et al 2015, Charfi et al 2021), which is present about 76-84%, 77-88%, and 73-96% in leaf, stem and bud, respectively (Tisserand and Young 2014). Eugenol acetate, eugenol, and beta-caryophyllene are the major bioactive compounds of clove, and they are lipophilic in nature (Kafle and Shih 2013). Essential oil of clove bud as well as commercial eugenol is known to cause contact toxicity (Tian et al 2015) which is solely responsible to cause significant mortality (Zeng et al 2010). Clove essential oil also affects development of the eggs, larvae, and pupae of insect pests (Hong et al 2018, Martinez et al 2018). Clove oil is also effective against aphid and is safe to its predator ladybeetles (Toledo et al 2020). Environmental Protection Agency (EPA) has considered clove oil as a low risk pesticide and Food and Drug Administration (FDA) has allowed clove to add in human food, though it has some side effects like skin irritation, headache and decrease blood clotting (Lee 2018).

The reduction in the progeny growth of pulse beetle (*Callosobruchus maculatus* Fab.) may be due to early mortality and partial or complete retardation of the embryonic development in adult female weevils (Dike and Mbah 1992). Moreover, the physiological changes induced by the secondary metabolites of clove may cause poor egg laying capacity in weevils.

Insects at the larval or pupal stage died after the application of clove because they were unable to fully cast off their exoskeleton. The main mechanism of the plant extracts action is their ability to penetrate the chitin of insects (Ntonifor et al 2010). Studies show that clove extract can be absorbed through cuticle, which effects endocuticle with projected papillae and vacuolization (El Gohary et al 2021). Another mode of toxicity of those bioactive compounds of essential oil is through olfactory receptors (Tripathi and Mishra 2016). Octopaminergic mode of action of eugenol is investigated where nervous system of insects is affected (Enan 2001). This mechanism of killing insects through the bioactive compounds of clove is also applicable to maize weevils. Therefore, this study aims to establish median lethal concentration of clove bud essential oil, and its repellent effect to control weevil's damage.

MATERIALS AND METHODS

Insect Rearing

Rearing of maize weevils, *Sitophilus zeamais*, on Manakamana-4 variety of maize was conducted in 1l capacity cleaned and dried glass jars. Moisture of the maize grain was adjusted to 14% which produced conducive environment for insect growth and development (Adams and Schulten 1978).

$$Weight\ of\ water\ to\ be\ added\ (g) = weight\ of\ grain \times \frac{Required\ moisture\,\% - initial\ moisture\,\%}{100 - required\ moisture\,\%}$$

Pure culture of weevil was obtained from National Entomology Research Center (NERC), NARC. Hundred weevils were added in a clean jar containing 500 g of maize grain. The opening of jar was covered with black muslin cloth and made tight using rubber band. Such 8 glass jars were prepared on weekly basis to get regular supply of weevil for the experiments. Glass jar ready with maize grain weevils were kept for incubation at clean laboratory condition. Daily temperature and humidity of laboratory was recorded. Average temperature and relative humidity at rearing room during the study period was 23.7 ± 0.1 °C and 68.7 ± 0.3%, respectively. After 7 days the adult weevil were removed from the glass jar manually. All the experiments were conducted at NERC laboratory during 2020/21.

Oil extraction

Dried clove buds were purchased from market. Hundred g of the powdered clove buds were hydro-distilled for 4 hr at 60°C using Clevenger-type apparatus (Clevenger 1928, Eljazi et al 2017). Glass beads (7 - 8 in numbers) were added in the flask to prevent the bumping of the liquids. Extracted essential oil was dried using anhydrous sodium sulphate and kept in refrigerated condition at 4°C in amber color glass vials. Amount of oil obtained from 100 g of powder after distillation was 3.86 g (2.85 ml).

Toxicity evaluation using Scintillating Vial Test

Ten percent stock solution was prepared from extracted oil with methanol. Further, six different concentrations (5, 2.5, 1.25, 0.625, 0.3125 and 0.15625%) were prepared from stock solution with dilution factor of 2. Altogether 7 concentrations, one control (methanol only), 4 replications were designated to CRD design in laboratory. The inner surface of the vials and lids were coated with 300 μl with 7 concentrations each by rolling properly to make sure that the entire surface inside the vial and lid was contaminated (Snodgrass et al 2005). Four controls were also maintained coated only with methanol. Vials were kept some time for drying. After drying, 15 adult maize weevils (7 - 14 day aged) were placed inside each vials. No foods were provided. First and second observation was done after 3 and 12 h of treatment application and then on every 24 h interval. Weevils were prodded with camel hair brush and regarded as dead if no movement were observed. To confirm death, weevils were often observed under stereomicroscope.

Toxicity evaluation of essential oil on treated maize

Fifty gram sterilized maize at 100 ˚C for 30 min was kept in 1 L jar and moisture was adjusted to 14% as previously described. Seven dilutions were made from 10% of stock solution as in case of scintillating vial experiment. Half ml of each concentration was added in each jar containing 50 g of maize. Each concentration was repeated four times and control was maintained with addition of methanol only. Lid of jar was closed and shaken properly for uniform coating of the oils to the grains. Twenty adult (1 - 7 days old) weevils of mixed sex were released at the center of plastic jar. Then the jar was covered with perforated lid tightly and kept at laboratory condition. Mortality was assessed after 24 h of treatments to 24 days. The number of seeds damaged, weight loss and progeny emergence were also observed after 60 days of weevils' inoculation.

Repellency effect of oil to *S. zeamais*

Repellency assays of clove essential oil were carried out according to the experimental method described by Eljazi et al (2017) at 25 °C ± 1 °C and 65 ± 5% RH. Whatman filter papers (diameter 8 cm) were folded and marked in half and placed properly in clean petri dishes. Required concentration of test solutions were prepared by extracted oil with methanol, which comprised of solutions of concentration 10, 5, 2.5, 1.25%, etc. Solution of each concentration (0.5 ml) was applied to one half a filter paper discs uniformly with micropipette. The other half of the filter paper was treated with methanol only as a control. Each concentrations and control was repeated four times. Twenty unsexed adults of 7 - 14 days old were released at the center of the filter paper. The insect present at treated and untreated area of filter paper were counted after 24 h.

Germination test

The effect of treatments on seed germination due to weevil damage was examined after 60 days of the treatments. Ten random seed samples were picked from jar containing each treatments and test were employed according to the methods described by Haines (1991). Small tea glass sized plastic jar with holes to prevent water logging were used. Soil mixed with sand was filled in the container and one seed per container was placed. Containers for germination test were repeated four times. The number of germinated seedlings was counted and recorded after 7 days. The percent germination was computed according to Zibokere (1994) as follows:

$$Viability\ index\ (\%) = \frac{NG}{TG} * 100$$

Where NG = number of germinated seeds, TG = total number of test seeds.

Damage assessment and progeny development

Progeny emergence

The number of F1 progeny weevils emerging was recorded after sixty days by which all the progeny had emerged. Emerging adults were removed from the jar on each observation day.

Grain damage and weight loss

Number and weight of damaged and undamaged seeds were recorded after 60 days of treatment application. Seed were considered damage even small exit hole was made in grain by weevil. Seed weight loss was calculated by using the count-and-weight method (Adams and Schulten 1978):

$$Weight\ loss\ \% = \frac{Initial\ weight - final\ weight}{\text{Initial weight}} * 100$$

After re-weighing, the numbers of damaged grains were evaluated by counting wholesome and bored or seed with weevil emergent holes. Percentage seed damaged was also calculated as follows:

$$\text{Seed damage \%} = \frac{\text{Number of perforated grains}}{\text{Total number of grains counted}} * 100$$

Statistical analysis

The data from all the experiments were recorded and managed in a MS- excel sheet. The SPSS (16th version) was used for data analysis. The concentrations of each essential oil were transformed using log transformation at the base of 10. Data were subjected to probit analysis (Finney 1971). Chi-square test was used to test for heterogeneity within the data. In case of significant heterogeneity ($p < 0.05$), a heterogeneity factor was included to calculate variances and confidence limits. T-test was performed to see the repellency effect of clove essential. Data were square root transformed $\sqrt{x+0.5}$ to stabilize the variance for the variables like progeny emergence (number of insects), damage percent, weight loss (percent) and germination percent and subjected to one-way ANOVA and mean were compared with Tukey's post hoc (Yamamura et al 1999).

RESULTS

Mortality of S. zeamais with essential oils of cloves in vial test bioassay

Probit regression analysis at 72 h showed that 1.56% and 21.54% concentration of clove essential oil required for the 50% and 90% mortality of *S. zeamais* weevil population, respectively (y = 1.123x - 0.216) whereas at 96 hr, the concentration required to kill 50% and 90% of the test insects decreased up to 0.859% and 8.343%, respectively (y = 1.298x + 0.086). Mortality was not observed in control treatment during the time of experiment. (**Table**: 1). It took longer to cause weevil mortality as the concentration of oil decreases (**Figure** 1A).

Table 1. LC_{50} and LC_{90} (%) of the essential oil extract of clove (*S. aromaticum*) in glass-vial bioassay tested against *S. zeamais* after 72 and 96 h

Hr	LC50 (CL95%)	LC90 (CL95%)	χ2	slope (± SE)	intercept (± SE)	P
72	1.55 (1.10 - 2.05)	21.56 (14.46 - 38.03)	138.48	1.123(± 0.04)	-0.21 (± 0.03)	<0.001
96	0.85 (0.59 - 1.13)	8.34 (6.20 - 12.42)	119.53	1.29 (± 0.05)	0.08 (± 0.03)	<0.001

Median lethal time (LT50) for mortality of S. zeamais with essential oils of cloves in vial test bioassay

At 10% concentration only 2.86 h was enough to kill 50% of the *S. zeamais* weevil population (y = 1.43x - 0.65), whereas 8.49 h was required for 5% concentration to kill 50% of tested weevil population (y = 1.54x - 0.65). Similarly, at 2.5 (y = 1.91x - 2.55), 1.25 (y = 2.38x - 3.69), 0.625 (y = 2.17x - 3.53), 0.3125 (y = 2.75x - 4.94), and 0.156 % concentrations, 21.48, 35.53, 41.91, 62.60 and 75.35 h were required for the mortality of 50% population (**Table** 2). Mortality was not observed in control treatment during the time of experiment.

Table 2. LT_{50} of the essential oil extract of clove in glass-vial bioassay tested against *S. zeamais* with different seven concentrations.

Concentration (%)	LT50 (h)	χ2	Slope (± SE)	Intercept (± SE)	p
10	2.86 (1.85-3.99)	181.91	1.43 ± 0.05	-0.65 ± 0.08	<0.001
5	8.49 (6.05-10.92)	309.58	1.54 ± 0.04	-1.43 ± 0.07	<0.001
2.5	21.48 (16.715-26.56)	431.99	1.91 ± 0.05	-2.55 ± 0.08	<0.001
1.25	35.53 (30.09-41.07)	316.39	2.38 ± 0.06	-3.69 ± 0.11	<0.001
0.625	41.91 (36.14-47.77)	249.58	2.17 ± 0.06	-3.53 ± 0.10	<0.001
0.3125	62.60 (53.62-71.76)	455.38	2.75 ± 0.07	-4.94 ± 0.14	<0.001
0.15625	75.35 (67.3-83.76)	241.23	2.40 ± 0.07	-4.51 ± 0.14	<0.001

Mortality of S. zeamais with essential oils of clove treated grain

Probit mortalities were calculated after 6, 10 and 16 days. At 6 days, 0.58, 4.28, and 189 percent concentrations of essential oil were estimated to to cause 25, 50 and 90% mortality of *S. zeamais* weevil population, respectively (y = 0.77x - 0.49). At 10 days, 0.07, 0.27, and 3.14% concentrations were required for the mortality of 25, 50 and 90 % (y = 1.16x + 0.65), whereas at 16 days only 0.04, 0.13 and 1.04 % concentrations were required to kill 25, 50 and 90% (y = 1.42x + 1.25) of weevil mortality respectively (**Table** 3).

Table 3. LC_{25}, LC_{50} and LC_{90} (%) of the essential oil extract of clove in maize treated grain tested against *S. zeamais* at 6, 10, 16 days.

Days	LC25 (% concentration)	LC50 (% concentration)	LC90 (% concentration)	χ2	Slope (± SE)	Intercept	P
6	0.58(0.48-0.69)	4.28(3.5-5.23)	189.31(115-35)	20.59	0.77 ± 0.04	-0.49 ± 0.03	<0.001
10	0.07(0.04-0.1)	0.27(0.2-0.34)	3.41(2.56-4.96)	82.01	1.16 ± 0.05	0.65 ± 0.29	<0.001
16	0.045(0.03-0.07)	0.13(0.09-0.17)	1.04(0.08-1.34)	67.20	1.42 ± 0.07	1.25 ± 0.04	<0.001

Median lethal time (LT50) for mortality of S. zeamais with cloves essential treated grain

Probit regression analysis was done to calculate median lethal time (LT_{50}) of seven different concentrations of essential oil at which mortality of 50% maize weevil population were observed during certain time period. At 10% concentration only 4.08 days was enough to kill 50% of the weevil population (y = 3.55x - 2.71), whereas 4.8 (y = 3.85x - 2.63), 6.19 (y = 4.71x - 3.73), 7.99 (y = 4.26x - 3.84), 8.89 (y = 4.55x - 4.32), 10.29 (y = 3.86x - 3.91) and 11.8 (y = 3.68x - 3.95) days required for 5, 2.5, 1.25. 0.625, 0.31 and 0.156 % concentrations, respectively (**Table** 4). Mortality was not observed in control treatment during the time of experiment. It took longer to cause weevil mortality as the concentration of oil decreases however mortality in treated grain took 16 days to cause 100 % mortality in highest concentration (**Figure** 1B).

Table 4. LT_{50} of the essential oil extracted from clove in plastic jar containing maize bioassay tested against *S. zeamais* with different seven concentrations.

Concentration (%)	LT50 (days)	χ2	Slope (±SE)	Intercept	P
10	4.08 (3.77 - 4.39)	286.67	3.55 ± 0.89	-2.17 ± 0.67	<0.001
5	4.8 (4.98 - 5.10)	244.86	3.85 ± 0.98	-2.63 ± 0.77	<0.001
2.5	6.19 (5.97 - 6.42)	123.72	4.71 ± 0.12	-3.73 ± 0.1	<0.001
1.25	7.99 (7.81 - 8.19)	53.83	4.26 ± 0.11	-3.84 ± 0.1	<0.001
0.625	8.89 (8.62 - 9.17)	87.65	4.55 ± 0.12	-4.32 ± 0.11	<0.001
0.3125	10.29 (9.90 - 10.70)	94.69	3.86 ± 0.1	-3.91 ± 0.10	<0.001
0.15625	11.8 (11.4 - 12.4)	95.29	3.68 ± 0.1	-3.95 ± 0.10	<0.001

In our research, 100% mortality of weevil was observed at 72 hr exposure with 10% concentration of clove oil for the mortality of 100% population (**Figure** 1A) while it took 16 days in oil treated grains (**Figure** 1B).

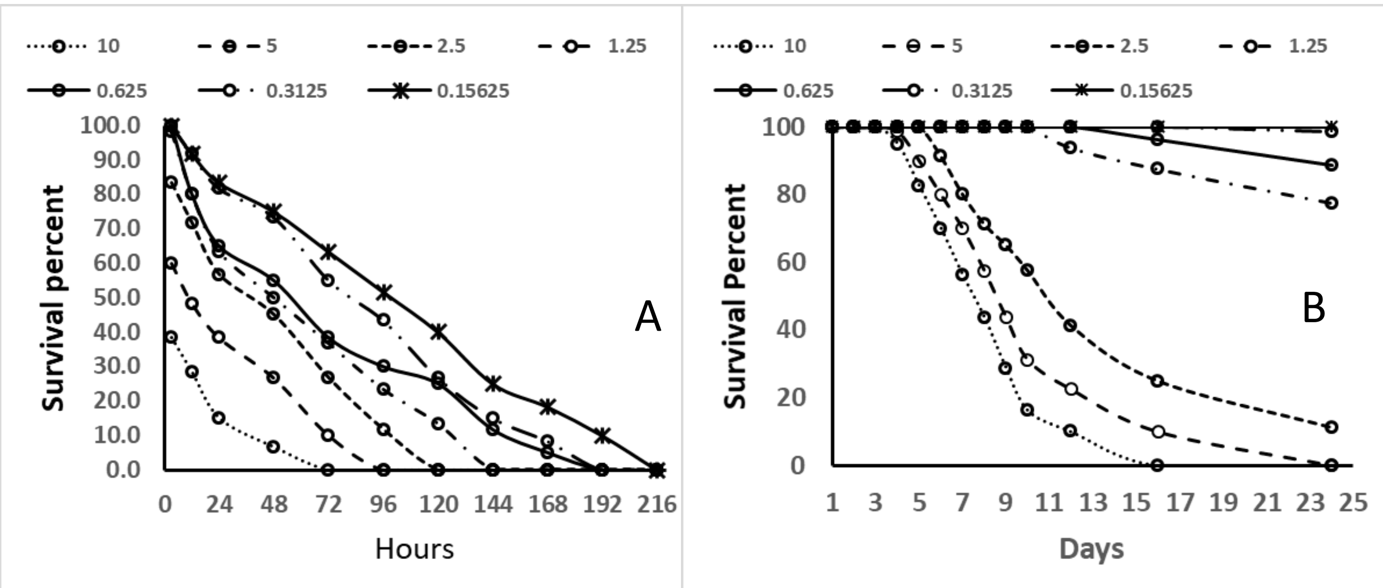

Figure 1. Survival of *S. zeamais* to different concentration of clove oil in (A) scintillating vial bioassay and (B) treated maize.

Repellent effect of essential oil of cloves to S. zeamais

Four different concentrations of cloves essential oil were used. Repellency tests results are shown in **Table** 5. Results indicated that cloves essential oil possessed significant pest repellent activity to *S. zeamais*. Repellent action was highly dependent upon oil concentration and exposure time. Essential oil of cloves at 10% concentration showed highest repellent activity (98.75%) against *S. zeamais* at 24 h interval (t = 39, df = 3, p = <0.001). Similarly, 5, 2.5 and 1.25% concentration oil inflicts percentage repellency of 93.75 (t = 35, df = 3, p = <0.001), 85 (t = 17, df = 3, p = <0.001), and 73.75 (t = 15.58, df = 3, p = <0.001) %, respectively after exposure time of 24 h.

Table 5. Percentage of weevil present at clove oil treated and untreated areas after 24 h (Repellency Effect)

Treatment	Mean ± SE (control)	Mean ± SE (treated)	df	t	P
10%	98.75 (± 2.5)	1.25 (±3.5)	3	39.00	<0.001
5%	93.75 (± 2.5)	6.25 (±2.5)	3	35.00	<0.001
2.5%	85.00 (± 4.08)	15.00 (±1.25)	3	17.00	<0.001
1.25%	73.75 (± 2.88)	28.75 (±3.28)	3	15.58	<0.001

Damage assessment, progeny development of S zeamais and germination percentages of treated grains

Percent weight loss ($F_{7, 32}$ = 448.23; p = <0.001), damage percent ($F_{7,32}$ = 374.27, p = <0.001), number of weevil progeny emerged ($F_{7, 32}$ = 407.72, p = <0.001) and germination percent of maize grain ($F_{7,32}$ = 42.96, p = <0.001) were significantly different after 60 days of treatment application (**Table** 5). Maximum weight loss (24.78 ± 0.58%) was observed in control treatment. Minimum weight loss (2.47 ± 0.14) was observed in treatment with 10% concentration. At 10% concentration, damage was minimum, which was statistically at par with 5% concentration. Maximum damage (59.38 ± 2.09%) was observed in control treatment (Table 5).

No progeny emergence was observed in 10%, 5%, 2.5% and 1.25% concentration. Concentration with 0.625%, 0.31% and 0.15% showed increase in number of progeny with increase in concentration percent. Only 3 ± 0.4 numbers of progeny emergence occurred at 0.625% concentration, 8.75 ± 0.62 number of progeny emergence occurred at 0.625% concentration and 16.7 ± 0.62 number of progeny emergence occurred at 0.625% concentration. These three concentrations are significantly different. 72 ± 1.83 numbers of live progenies were observed in untreated maize (**Table** 5).

Maximum germination was observed in 10% concentration (97.5 ± 2.5%) followed by 5% concentration (85 ± 2.89) and 2.5% concentration (82.5 ± 2.5) ($F_{7, 32}$ = 45.16, P = <0.001) (**Table** 6). The germination trend showed that with the increase in concentration of essential oil, rate of germination also increases or vice versa. Lowest germination (30% ± 4.08) was observed in untreated maize (**Table** 6).

Table 6. Damage assessment, progeny development of *S zeamais* and germination percentages in cloves treated grain

Concentrations (%)	Wt. loss%	Damage%	Progeny (n)	Germination %
10	2.47 (± 0.14)f	1.69 (± 0.24)h	3.50 (± 0.64)f	97.50 (± 2.5)d
5	3.12 (± 0.21)f	5.26 (± 0.59)g	14.50 (± 0.86)e	85.00 (± 2.89)c
2.5	4.41 (± 0.12)e	11.37 (± 0.85)f	24.00 (± 1.08)d	82.50 (± 2.5)bcd
1.25	5.26 (± 0.19)de	19.22 (± 1.38)e	35.25 (± 1.25)c	77.50 (± 2.5)bcd
0.625	6.30 (± 0.09)cd	24.93 (± 0.72)d	47.50 (± 1.04)b	70.00 (± 4.08)bc
0.31	7.32 (± 0.17)c	33.36 (± 0.87)c	53.50 (± 0.65)b	97.50 (± 2.5)ab
0.15	8.70 (± 0.52)b	42.68 (± 0.70)b	66.00 (± 0.68)a	55.00 (± 2.87)ab
Control	24.79 (± 0.58)a	59.38 (±2.09)a	72.00 (± 1.83)a	30.00 (± 4.08)a

n=number of weevil, data were analysed and mean separation were presented after data transformation but the data presented in the tables are actual mean with ±SE. Similar alphabets within the column indicates no significant differences among the concentrations.

DISCUSSIONS

We evaluated the toxicity of clove bud oil and its ability to reduce damage by *S. zeamais* on maize grain. In our research, 100% mortality of weevil was observed at 72 hr exposure with 10% concentration of clove oil, whereas 216 hrs was required for the mortality of 100% population with 0.15% concentration in vial bioassay. This shows the trend that with the decrease in concentration of essential oil, lethal time requirement for the mortality of 50% of weevil population is increased and vice versa. Oni and Ileke (2008) observed 100% mortality with 0.1 and 0.2% (v/w) concentrations within 24 hrs when tested clove oil as a fumigant against *Dinoderus porcellus*. Essential oil from the stem of clove could cause more than 96% of mortality after 5 days when tested on 100µl impregnated filter paper (Bandara and Senevirathne 2022) however Kerdchoechuen et al (2010) demonstrated 100% mortality of the maize weevil within 2 h when tested on 30 µl clove oil impregnated filter paper discs with LC_{50} of 0.22 µl cm^{-2}. Similarly, Clove powder applied at more than 3 mg/cm^2 of surface area of beaker provided 100% *Solenopsis invicta* mortality within 6 h after treatment (Kafle and Shih 2013). Filter paper based caused 100% mortality at 0.8% and soil based treatments caused 93% mortality at 1.2% concentration of *Macrotermes gilvus*, respectively (Andina et al 2022).

LC_{50} in scintillating vial bioassay in our study at 72 and 96 h are quite low. When exposure period is extended, mortality was increased which also lower the LC_{50} value. It is reported that clove oil showed its higher toxicity to *S. oryzae* only after 72 h (Saad et al 2017). Mishra et al (2012) evaluated the fumigation effect of clove oil against *S. oryzae* where LC_{50} of 17.32 and 15.34 µl were achieved at 24 and 48 h, respectively. *S. granarius* was treated on its body with different concentrations (1 to 32%) of clove essential oil and LC_{50} were calculated by Plata-Rueda et al (2018). They found that LC_{50} and LC_{90} of 11.9 and 23.4 (w/v) which were more toxic than cinnamon oil. Concentrations of clove oil required to cause 50% mortality for third instar larvae of *Aedes aegypti* was 0.005% in glass vial bioassay (Budiman et al 2022). LC_{50} of 0.192% and 0.267% clove oil concentration were reported for filter paper based and soil based treatment for termite (Andina et al 2022). Likewise, Ramlal et al (2020) reported LC_{50} of 224.45 µL/mL clove oil when treated to pea against *S. zeamais*. Higher toxicity might be due to the closed condition of the scintillating vial where both contact as well as fumigation effect may have occurred which triggered high mortality with low volume. Furthermore, differential results also depended upon the plant species, their parts used for extraction, plant parts used at different growth stages, extraction methods, chemical constituents present in oils, concentrations and time of exposure (Lee et al 2001, Eesiah et al 2022).

When maize was treated with clove oil, it took quite a longer duration to cause highest mortality to *S. zeamais* and LC_{50} value was also more than that of vial bioassay method. Our study revealed 100% mortality reached with 10% concentration at 16 days of treatments. About 87% mortality of *S. zeamais* with 10% clove oil reached at 7 weeks of the treatments to maize grains and mortality further increased as the concentration increases to 15% (Eesiah et al 2022). However, when maize grain was treated with clove oil, 100% mortality of *S. zeamais* was achieved with 35µl g^{-1} within 48 h (Jairoce et al 2016). In our research, median lethal concentration (LC_{50}) of cloves at 6, 10 and 16 days was 4.28, 0.27 and 0.13 %, respectively. Somewhat similar results Azab et al (2018) obtained where LC_{50} of 0.31 and 0.13 % of clove oil at 10 and 14 days were reported for *S. oryzae* when treated on wheat. LC_{50} of 0.11, 0.10, and 1.02 % concentration of clove oil (w/w%) were obtained for *S. oryzea*, *Rhyzopertha dominica* and *Tribolium castaneum*, respectively at 10 days when treated to wheat grain (El-Gizawy et al 2018). In another study, LD_{50} and LD_{99} of the *Acanthoscelides obtectus* were obtained, respectively, at 141 and 359.2 µl/kg of beans (Jumbo et al 2014). Similarly, Jairoce et al (2016) reported LC_{50} of 9.45 and 10.15 µl per g of maize grains against *A. obtectus* and *S. zeamais*, respectively at 48 h of treatments with clove essential oil.

Our results revealed considerable amount of weevils, 73 to 98% at 1.25 to 10% concentration of clove oil at 24 h attracted towards the untreated side of the filter paper. Significant repellency was observed when half of the petri dishes were treated with clove powder (Kafle and Shih, 2013) where they found 98.6 to 99.4% of ant repelled when clove powder doses were increased from 0.34 to 1 mg/cm^2 even as early as 3 h after treatments. Sabbour and Aziz (2010) observed repellency of 89% at 7 days with clove oil against *Bruchidius incarnates*. They further confirmed that bioactive compounds such as eugenol, eugenol acetate, and beta-caryophyllene all repelled ants with a proportion of 92.4 to 98.1% at 3 h after the treatment. *Lasioderma serricorne* was strongly repelled by α-caryophyllene (Wu et al 2017). Beta-caryophyllene also showed strong contact as well as repellent activity against store grain pests (Wang et al 2019). Plata-Rueda et al (2018) studied the behavioral repellency where they found that resting period was more on control area than treated area with essential oil of clove having both LC_{50} and LC_{90} values. Ramlal et al (2020) reported that clove oil showed repellent action against *S. zeamais* with dose of 4 and 8µL/mL after 2 and 4 h of treatment where they found the repellent index of 0.6 when treated to half of the filter paper. *S. zeamais* avoided the clove essential oil treated areas and changed their direction with reduced respiratory rates (Haddi et al 2015, Plata-Rueda et al 2018). However, attraction to clove oil measured by olfactometer by Eesiah et al (2022) showed very low percent repellency of *S. zeamais*. Jumbo et al (2014) also reported less attractiveness with the dosage of clove oil tested.

Major compounds of clove oil from clove bud are eugenol (73.5–96.9%), b-Caryophyllene (0.6–12.4%), Eugenyl acetate (0.5–10.7%), a-Caryophyllene (0.4–1.4%), Isoeugenol (0.1–0.2%), and Methyleugenol (0–0.2%) (Tisserand and Young, 2014). Eugenol (27.1%) and caryophyllene (24.5%) present in clove oil were the most toxic compounds against insect pests (Plata-Rueda et al 2018). In recent years, many scientists reported the amount of major compound of essential oils which were responsible for mortality, repellency and feeding deterrents in varying proportions (Charfi et al 2021, Awad et al 2022, Czarnobai et al, 2022). Varying degree of mortality and repellency reported by various scientists might be primarily due to the varying proportion of active compounds present in the essential oil of clove. Clove oil acts as a neuro-insecticides in German cockroaches (Enan 2001) and eugenol found targeted octopamine receptor in insects (Tripathi and Mishra, 2016) inducing hyperactivity in blood sucking bug (Reynoso et al 2019).

Proportion of progeny emerged from maize grain treated with different concentration varied significantly in our study where less progeny was developed from higher concentrations. Less progeny might be due to the hatching inhibition. Less progeny in higher concentration might be due to the early mortality of adult weevil or reduced eggs hatchability. Hong et al (2018) reported forty percent of egg hatching inhibition. Similarly, the highest reduction in hatchability of 1 and 4- day- old eggs of *Tuta absoluta* was recorded (Moadwad et al 2013). Jumbo et al (2014) and Silva et al (2016) observed delayed emergence of adult beetles due to clove essential oil. Other reason might be reduced egg laying by insect pests where essential oil passed through the chorion of the eggs and thereby disrupt normal developmental stages from eggs to adults (Ileke and Olotuah 2012). Our results showed the highest concentration of clove oil reducing weight loss by 4 times and damage percent by about 20 times in comparison to lowest concentration tested. It is much different if compared with control. Clove oils had a potential to reduce weight loss and damage percent of maize grains (Poudel et al 2023).

Chemical insecticides have not only detrimental effect on human and environment (Maharjan et al 2004), it also causes residue problem (Aryal et al 2021), insect may overcome those chemical pesticides with resistance development (Denholm and Devine 2013). Because of these adversaries essential oils are gaining popularity as it can overcome problem associated with chemical pesticides especially for storage insect pests. Essential oils can be one of the alternatives to chemical pesticide as they inherit low mammalian toxicity which are safe to non-target organisms (Koul et al 2008), hence can be used to control storage insect pest.

CONCLUSION

Maize weevil (*S. zeamais*) is one of the most nuisance pest that causes economic losses in stored grain. Since chemical pesticide used to control insect pest has negative impact on human and environment, essential oil of plants are gaining popularity these days. Our study also revealed that clove bud oil has toxic properties towards *S. zeamis* as well as it has repelling capacity. It also has low mammalian toxicity. Clove oil is listed as Generally Recognized as Safe (GRAS) compound to use as flavoring agents (Gooderham et al 2020). Due to various useful characteristics of clove oil, it could be regarded as a biorational insecticides which could be used in Integrated Pest Management for combating various problems related to chemical pesticides.

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