



Toxic and repellent effect of *Zanthoxylum armatum* (Sapindales:Rutaceae) essential oil to *Sitophilus zeamais* (Coleoptera:Curculionidae)

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Abstract

The essential oil from *Zanthoxylum armatum* (Prickly ash) fruit was evaluated for its insecticidal properties against the *Sitophilus zeamais* (maize weevil) with different concentrations (10, 5, 2.5, 1.25, 0.62, 0.31, and 0.15%) in both a glass vial bioassay and in oil-treated maize grain. The repellency effect of the oil was tested through a filter paper test. The results revealed that the lethal concentration (LC₅₀) for the oil at 96 and 144 h was estimated as 11.6% ($y = 0.97x - 1.03$) and 4.5% ($y = 1.10x - 0.721.03$) percent concentration of oil. The lethal time (LT₅₀) at the highest oil concentration (10%) was calculated as 13.84 h, whereas, it took approximately 197 h to achieve 50% mortality at the lowest concentration in the glass vial bioassay. In essential oil treated maize bioassay experiment, a concentration of 0.01% of the oil ($y = 0.56x + 0.41$) was sufficient to kill 50% of the tested maize weevils within 10 days. Repellency tests exhibit strong deterrent effect of 97.5% repellency with 10% concentration after 24 h, while the lowest concentration (0.15%) repelled around 55% of the weevils. The results suggest that *Zanthoxylum armatum* essential oil could be an effective natural compound for managing maize weevil in grain storage, which can be an environmentally friendly alternative to conventional chemical pesticides.

Keywords Median lethal concentration · Median lethal time · Bioassay · Maize weevil · Mortality · Repellency

1 Introduction

Storage insect pests inflict substantial grain losses, as demonstrated by various studies. For instance, damage estimates were of 24% from Bhutan (Dorji et al. 2020), 10–20% from Nepal (Bhandari et al. 2015), and 80% from tropical countries (Pingali 2001). The maize weevil (*Sitophilus zeamais*) is one of the most common storage pests in Nepal, causing approximately 20% loss in maize and up to 42% in wheat under free-choice experimental conditions (Sharma et al. 2016).

The chemical control methods commonly used for storage pests (Kandel et al. 2021) may leave residues in food and feed, posing a potential health risk (Aryal et al. 2020; Aryal and Aryal 2023). Therefore, use of botanicals and

plant-derived products has been a traditional practice since time immemorial (Trivedi et al. 2018). Various plant parts, such as bark, leaves, roots, and fruits, have been used in their crude forms (chopped or powdered) (Basyal et al. 2022), or as essential oils (Aryal et al. 2023b). These preparations have been found effective against a range of insect pests (Aryal et al. 2023a; Khanal et al. 2021), with some showing less harmful effect to beneficial insects (Tembo et al. 2018).

One of promising plant, *Zanthoxylum armatum*, commonly known as Prickly-ash, Nepalese pepper (*Timur*) or Toothache tree. It is widely distributed across South and Southeast Asia. In Nepal, particularly, it is distributed from east to west at altitudes ranging from 1000–2500 m, growing both in forest ecosystems and in open landscapes (Phuyal et al. 2019). Of the approximately 250 species distributed worldwide (Mawthoh et al. 2023), *Z. armatum* is among the most valued (Agnihotri et al. 2022), which is highly prevalent in Nepal (DPR 2011; Hertog and Wiersum 2000). This plant holds economic potential due to its broad range of pharmacological (Wen et al. 2024), ethnobotanical (Mutinda et al. 2023), and toxicological (Okagu et al. 2021) properties.

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More importantly, *Zanthoxylum* essential oil, exhibits insecticidal activity (Li et al. 2023), functioning as a fumigant, contact poison, and repellent against various storage insect pests (Prieto et al. 2011; Thakur 2018; Wang et al. 2015; Zhang et al. 2017). It has also potential for ovicidal effect which have been documented against *Callosobruchus analis* (Brari and Thakur 2017). Previous studies in Nepal reported *Z. armatum* fruit powder causes intermediate mortality on *S. zeamais* (Basyal et al. 2022), while evidence suggests that the essential oil extract may exhibit stronger effect than crude powder (Gbate et al. 2021).. Considering all these existing evidences, the present study aims to evaluate the toxicity and repellency of *Z. armatum* essential oil against *S. zeamais* through glass vial bioassay, maize treatment bioassays, and repellency test.

2 Materials and methods

2.1 Host insect

Rearing of maize weevils (*Sitophilus zeamais*) was done on the Manakamana-4 variety of maize grain in 1 l capacity glass jar containing 500 g of maize grain with 14% grain moisture, which produced conducive environment for insect growth and development. We maintain the grain moisture by adding water according to (Adams and Schulten 1978).

$$\text{Weight of water to be added (g)} = \text{Weight of grain (g)} \times \frac{\text{Desired moisture\%} - \text{Present moisture\%}}{100 - \text{Desired moisture\%}}$$

We used one hundred adult weevils that were introduced into a glass jar. Weevils were obtained from the rearing culture maintained at the National Entomology Research Center (NERC), NARC. After seven days, the adult weevils were removed. Jars containing grain and weevils were prepared weekly to ensure a regular supply of weevils for experimental use. Temperature and relative humidity were monitored hourly using a data logger. The average temperature and relative humidity were 24.1 ± 0.05 °C and $70.7 \pm 0.3\%$, respectively. All experiments were conducted in the NERC laboratory during 2020/21.

2.2 Essential oil extraction

Fruits of *Zanthoxylum armatum* were fetched from the local market and ground into fine powder. One hundred grams of powder were subjected to an essential oil extraction system using a Clevenger-type apparatus as described by (Aryal et al. 2023), yielding 4.91 g of essential oil per 100 g of powder.

2.3 Glass vial bioassay

We prepared ten percent stock solution from extracted oil of *Z. armatum*, and a series of seven concentrations were obtained through two-fold serial dilution with methanol (10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625%). Each vial of 20 ml capacity was internally coated with 300 µl of respective concentration by gently rolling to ensure uniform distribution of oil along inner surface. Four replications were maintained for each concentration. Additionally, four control vials were also coated with methanol only. Coated vials were left to dry before introducing 15 unsexed *S. zeamais*, (aged 7–14 days) into each vial, comprising 60 insects per treatment. Initial mortality was recorded at 3 h post-inoculation, followed by assessments at 12-h intervals up to 48 h, and subsequently at 23-h intervals up to 288 h. Insect mortality was confirmed by probing with fine brush under stereomicroscope.

2.4 Maize treatment bioassay

Seven concentrations were prepared as described in the glass vial bioassay. For each treatment, 0.5 ml of each solution was added to jar containing 100 g of maize grain (Manakamana-4 variety) and mixed thoroughly to ensure even coating of the grains. Each treatment has four replications, and one control jar was treated with methanol only.

The experiment was arranged in CRD design. Into each jar, twenty adult weevils (1–7 days old, mixed sex) were introduced. The jars were covered tightly with lid. Mortality was observed daily up to 10 days, and then again on days 12, 16, and 24 after weevils were added. Dead weevils were confirmed in the same way as described in the glass vial bioassay section.

2.5 Repellency test

The repellent effect of *Z. armatum* essential oil was evaluated following the method described by Wang et al. (2019) under laboratory conditions maintained at 25 ± 1 °C and $65 \pm 5\%$ relative humidity. Whatman filter papers (8 cm diameter) were folded in half and placed in petri dishes, with the fold serving as a boundary between treated and control halves. Test solutions were prepared by dissolving extracted oil in methanol. Four concentrations (10, 5, 2.5, and 1.25%) were tested, each with four replications. For each replicate, 0.5 ml of essential oil was applied to one half of filter paper

using a micropipette and spread as uniformly as possible. The other half of the filter paper was treated with methanol as a control. After the solvent had evaporated, twenty unsexed adult *S. zeamais* (aged 7–14 days) were released in the center of filter paper. Observations were made 24 h after release to assess the distribution of insects between the treated and control areas. Percent repellency was calculated using the following formula (Liu and Ho 1999; Talukder and Howse 1993).

$$\text{Percent repellency (PR\%)} = 2(C - 50)$$

where C is the percentage of adult weevil present on the untreated half of petri dish. The number 50 represents a neutral or random distribution of insects. Positive value indicates repellency, while negative value express attractancy.

2.6 Statistical analysis

Data was subjected to probit analysis (Finney 1971) to calculate median lethal concentration (LC_{50}) and median lethal time (LT_{50}) for both the glass vial bioassay and maize treatment bioassay. For percent repellency data, the Kruskal–Wallis test was employed following the observation of significant differences in error variances ($F_{3,12} = 5.79$, $p = 0.011$) with Levene's test (Levene 1960) with SPSS 16.0 (SPSS 2007). Repellency data were square root transformed before they were subjected to analysis (JMP Pro 18.0.2). Means were compared with Tukey's test ($p = 0.05\%$).

3 Results

3.1 Lethal concentration on mortality of *Sitophilus zeamais* with essential oils of Prickly ash Pepper in glass vial bioassay

Probit regression analysis was performed to estimate the median lethal concentration of Prickly ash pepper essential oil required to achieve 50% and 90% mortality of maize weevil populations over time. At 96 h, LC_{50} was 11.6%, while a much higher concentration of 241.4% was estimated for LC_{90} ($y = 0.973x - 1.04$). By 144 h, the LC_{50} was decreased to 4.5%, and the LC_{90} was also reduced to 64.56%

($y = 1.109x - 0.73$). No mortality was observed in the control treatment throughout the experiment period (Table 1).

3.2 Median lethal time (Hours) on mortality of *Sitophilus zeamais* with essential oils of Prickly ash Pepper in glass vial bioassay

Probit regression analysis was employed to determine the median lethal time (LT_{50}) required to cause 50% mortality in maize *S. zeamais* across seven different concentrations of Prickly ash pepper essential oil. At a concentration of 10%, only 13.8 h were estimated to be required to achieve 50% mortality ($y = 1.04x - 1.18$). At 5%, LT_{50} increased to 38.8 h ($y = 1.22x - 1.94$). Similarly, LT_{50} values at 2.5%, 1.25%, and 0.625% concentrations were 117.87 ($y = 1.77x - 3.67$), 156.47 h ($y = 1.51x - 3.32$), and 178.2 h ($y = 2.15x - 4.83$), respectively. Interestingly, the LT_{50} concentration at 0.3125 was 149.22 h ($y = 1.36x - 2.95$), slightly lower than that at 0.625%. At the lowest concentration tested, i.e., 0.1562%, 196.89 h were required to reach 50% mortality ($y = 3.62x - 8.30$) (Table 2).

These results demonstrate a general trend where lower concentrations of essential oil require longer exposure times to achieve 50% mortality, and conversely, higher concentrations can act more quickly. No mortality was observed in the control treatment throughout the observation period.

3.3 Lethal concentration on mortality of *Sitophilus zeamais* with essential oils of Prickly ash Pepper in maize treatment bioassay

Probit regression analysis was conducted to estimate lethal concentrations (LC_{25} , LC_{50} and LC_{90}) of *Z. armatum* essential oil applied to maize grain against *S. zeamais*. At 6 days, LC_{50} of 8.07% of essential oil concentration was estimated, while the LC_{25} was 0.23% ($y = 0.43x - 0.39$). By 10 days, the concentrations required for 50, 25 and 90% mortality were 0.18, 0.01, and 34.96%, respectively ($y = 0.56 + 0.02$). At 16 days, LC_{50} decreased further up to 0.05%, LC_{25} remaining at 0.01%, and LC_{90} dropping to 0.067% ($y = 1.17x + 1.48$). Mortality was not observed in the control treatment during observation period (Table 3).

Table 1 LC_{50} and LC_{90} (%) of the essential oil extract of *Zanthoxylum armatum* in glass-vial bioassay tested against *Sitophilus zeamais* after 96 and 144 h

Hours	LC_{50} (CL ₉₅ %)	LC_{90} (CL ₉₅ %)	χ^2	slope ($\pm$ SE)	intercept ($\pm$ SE)	P
96	11.62 (7.13–23.27)	241.37 (82.32–17.03)	390.58	0.97 ($\pm$ 0.04)	−1.03 ($\pm$ 0.04)	<0.001
144	4.51 (2.74–7.38)	64.56 (28.85–31.11)	466.84	1.1 ($\pm$ 0.05)	−0.726 ($\pm$ 0.04)	<0.001

3.4 Median lethal time (days) on mortality of *Sitophilus zeamais* with essential oil of Prickly ash Pepper in maize treatment bioassay

Probit regression analysis was performed to estimate the median lethal time (LT_{50}) required to cause 50% mortality of *S. zeamais* at five concentrations of *Z. armatum* essential oil. At a concentration of 10%, only 7.8 days were required to achieve 50% mortality ($y = 5.3x - 4.76$). At 5%, LT_{50} increased to 9.45 days ($y = 4.91x - 4.79$), and at 2.5%, it further increased to 10.04 days ($y = 4.64x - 4.65$). At lower concentrations, LT_{50} decreases sharply, reaching 37.06 days at 1.25% ($y = 3.76x - 5.9$) and 42.8 days at 0.025% ($y = 4.171x - 6.81$). These results indicate a clear trend: as the concentration of essential oil decreases, the time required to achieve 50% mortality increases. No mortality was observed in the control treatment during the observation period (Table 4).

3.5 Mortality concentration curve

When the glass vial bioassay was conducted without providing food to the insects, the effect of essential oils on insect survival was observed up to 168 h and 216 h for the two

highest concentrations of oil (10% and 5%, respectively) (Fig. 1A). In the maize grain treatment, the two highest concentrations of (10% and 5%, respectively) cause 100% mortality at 16 and 24, respectively (Fig. 1B).

3.6 Repellent effect of essential oil Prickly ash Pepper to *Sitophilus Zeamais*

The repellent effect of *Z. armatum* essential oil on *S. zeamais* was significantly different across various concentrations ($\chi^2 = 8.70$, $df = 3$, $p = 0.034$). The highest concentration (10% and 5%) resulted in average repellency percentages of 97.5% and 95%, respectively, while the lowest concentration (1.15%) exhibited a repellency of only 55% (Fig. 2).

4 Discussion

This study assessed the toxicity and repellency of *Zanthoxylum armatum* essential oil against *Sitophilus zeamais*, a major pest of stored grains. Essential oils have been increasingly studied for managing storage insect pests (Zimmermann et al. 2021), maize grain bioassay, focusing on mortality and repellency responses.

Table 2 LT_{50} of the essential oil extract of Prickly ash pepper in glass-vial bioassay tested against *Sitophilus zeamais* with seven different concentrations

Conc (%)	LT_{50} (hours)	χ^2	Slope ($\pm$ SE)	Intercept ($\pm$ SE)	p
10	13.84 (5.65–23.72)	1328.59	1.04 ± 0.03	-1.18 ± 0.06	<0.001
5	38.78 (17.86–63.16)	2471.78	1.22 ± 0.04	-1.94 ± 0.07	<0.001
2.5	117.87 (89.39–154.98)	1523.96	1.77 ± 0.05	-3.67 ± 0.11	<0.001
1.25	156.47 (116.29–225.35)	1476.46	1.51 ± 0.05	-3.32 ± 0.10	<0.001
0.625	178.22 (138.66–246.93)	1909.21	2.15 ± 0.07	-4.83 ± 0.15	<0.001
0.3125	149.61 (111.54–213.18)	1201.62	1.36 ± 0.04	-2.95 ± 0.09	<0.001
0.15625	196.96 (169.95–236.29)	1564.35	3.62 ± 0.11	-8.30 ± 0.26	<0.001

Table 3 LC_{50} and LC_{90} (%) of the essential oil extract of Prickly ash Pepper treated with maize in bioassay tested against *Sitophilus zeamais* after 6, 10, 16 days

Days	LC_{50}	LC_{25}	LC_{90}	χ^2	Slope ($\pm$ SE)	Intercept	P
6	8.07 (5.61–13.15)	0.23 (0.14–0.33)	273.0 (214–344)	17.20	0.43 ± 0.41	-0.39 ± 0.02	<0.001
10	0.18 (0.09–0.27)	0.01 (0.003–0.03)	34.96 (17.36–102.3)	53.34	0.56 ± 0.04	0.41 ± 0.03	<0.001
16	0.05 (0.03–0.7)	0.02 (0.005–0.03)	0.67 (0.53–0.88)	66.00	1.17 ± 0.08	1.48 ± 0.05	<0.001

Table 4 LT_{50} of the essential oil extract of Prickly ash pepper in plastic jar containing maize bioassay tested against *Sitophilus zeamais* with seven different concentrations

Conc (%)	LT_{50} (Days)	LT_{90}	χ^2	slope	intercept	prob
10	7.84 (7.64–8.05)	13.65 (13.05–14.36)	79.71	5.3	-4.76	<0.001
5	9.45 (9.24–9.67)	17.24 (16.54–18.05)	49.88	4.91	-4.79	<0.001
2.5	10.04 (9.70–10.42)	18.96 (17.77–20.47)	109.56	4.64	-4.65	<0.001
1.25	37.06 (33.21–42.59)	81.25 (66.87–104.48)	50.36	3.76	-5.9	<0.001
0.625	42.84 (37.09–52.40)	86.81 (67.78–123.90)	23.56	4.17	-6.81	<0.001

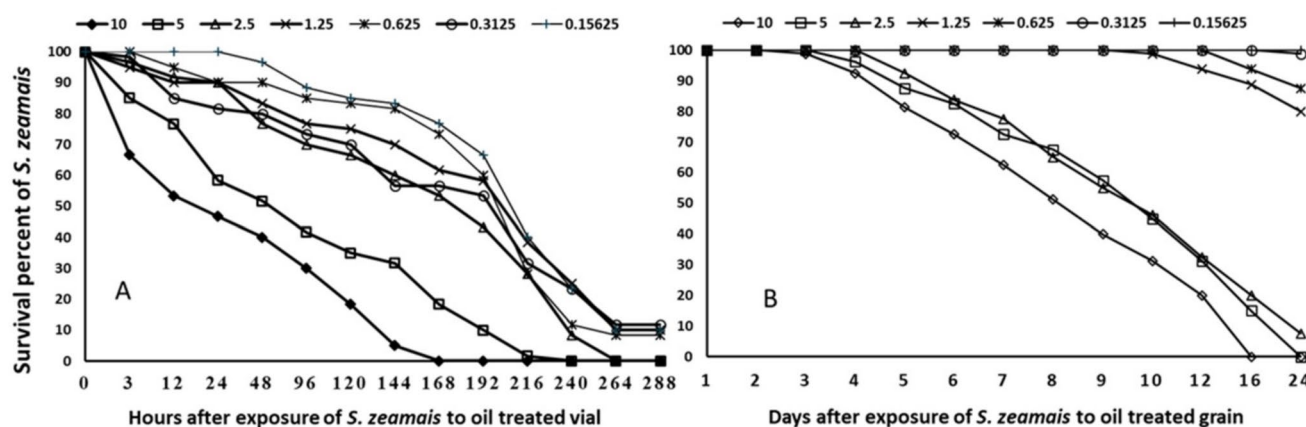


Fig. 1 Survival of *Sitophilus zeamais* to different concentrations of essential oil in (A) glass vial bioassay and (B) Maize treatment bioassay

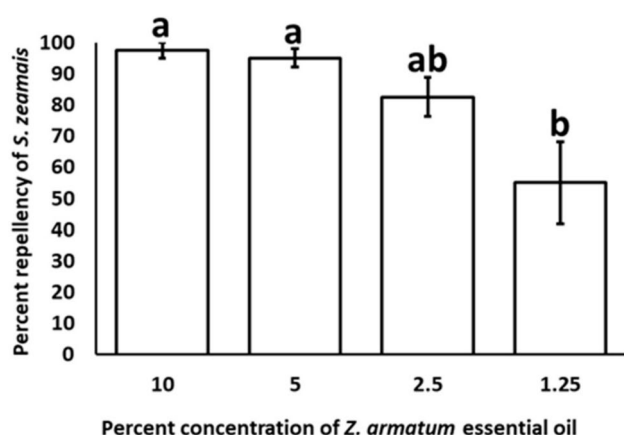


Fig. 2 Percent repellency of *Z. armatum* essential oil to *S. zeamais*. Error bars represent $\pm$ SE. Different alphabets above the error bar indicate significant difference among treatments (Tukey-Kramer HSD after Kruskal-Wallis non-parametric test). Similar alphabets above bar do not indicate significant different among the essential oil concentration percent

4.1 Comparative efficacy of *Z. armatum*

The essential oil of *Z. armatum* demonstrated moderate contact toxicity to *S. zeamais* in the glass vial bioassay. The median lethal concentration (LC_{50}) was found to be 11.62% at 96 h and 4.51% at 144 h of exposure. These values depict lower toxicity when compared to the LC_{50} of other essential oils reported in some of the literature, such as sweet flag essential oil (0.16% at 24 h) and clove essential oil (0.85% at 96 h) against the same pest species (Azab 2018; Aryal et al. 2023b). Additionally, Mady et al. (2021) reported that insect immersed 1 min in 10% essential oil of *Origanum majorana* required 12 h of exposure to achieve against *Rhynchophorus ferrugineus* 50% mortality. This variable toxicity highlights the importance of plant species, their chemical profiles,

and the method of extraction in determining bioactivity. Intraspecific differences in toxicity within the *Zanthoxylum* genus have also been observed. For example, *Z. monophyllum* showed strong fumigant effects, whereas *Z. rhoifolium* exhibited weak toxicity of *S. oryzae* (Prieto et al. 2011). Wang et al. (2015) reported that *Z. armatum* essential oil showed strong fumigant and contact toxicity to *Lasioderma serricorne* and *Tribolium castaneum*, with LC_{50} values of 13.83 and 4.28 mg/L air, respectively. It also showed notable contact toxicity, with LD_{50} values of 18.74 μ g/adult for *L. serricorne* and 32.16 μ g/adult for *T. castaneum*, highlighting that toxicity can vary depending on the insect species tested.

4.2 Bioactivity of *Z. armatum*

The variation in efficacy may be attributed to the diverse chemical composition of essential oils, which can differ based on geographical origin, climate, and even climatic season (Cicchetti et al. 2017; Liang et al. 2022). Moreover, the source and condition of plant material also influence toxicity. For example, essential oils extracted from fresh fruits of *Z. armatum* were found to be more exotic to *S. zeamais* than those obtained from dried, purchased fruits (Tanoh et al. 2020). In Nepalese *Z. armatum*, major bioactive constituents include limonene, α -pinene, myrcene, linalool, citronellal, 6-methyl heptanal, (E)-methyl cinnamate, (Z)-ethyl cinnamate, and others (Cicchetti et al. 2017), where limonene was reported for its strong toxicity to *T. castaneum* adults and larvae with LD_{50} of 17.06 μ g/adult and 16.42 μ g/larvae, respectively.

In our study, when applied to maize grains, *Z. armatum* essential oil required 7.84 days at 10% concentration and 37.06 days at 1.25% concentration to achieve 50% mortality of *S. zeamais*. These results particularly showed a slower toxicity response than observed in the glass vial bioassay. The differences are likely to be due to the mode of exposure.

For example, Eesiah et al. (2022) reported that 10% or higher concentration of cinnamon or clove or thyme caused mortality levels of *S. zeamais* comparable to or exceeding those achieved with chemical insecticides. In a glass vial, insects come into immediate contact with a high concentration of the oil, whereas in grain treatment, the toxicant is more gradually ingested as part of the food source. A similar pattern was also observed by Germinara et al. (2017), who reported reduced toxicity of essential oils to *Sitophilus* granaries when applied to wheat grains, compared to direct exposure. While both methods exhibit the insecticidal potential of *Z. armatum*, the glass vial bioassay gives a rapid toxicity measure, whereas the maize treatment reflects a more realistic scenario for storage pest control.

4.3 Applications and Repellent activity

Our results indicated that Prickly ash pepper essential oil showed significant pest repellent activity against *Sitophilus zeamais*. Repellency was highly dose-dependent, with concentrations eliciting strong avoidance behavior. Our result aligns with the findings from other studies, where *Z. armatum* oil significantly repelled *T. castaneum* across a range of doses of 62.5 µg to 1000 µg in an olfactometer setup (Khalil et al. 2024). Similar results with *T. castanum* were observed by (Wang et al. 2011) with significant repellency effects. Essential oil of *Z. armatum* has demonstrated both toxic and repellent effects, supporting its potential to be botanical pesticide. Given its efficacy and eco-friendly nature, it is timely and necessary to consider *Z. armatum* and other promising essential oils and their active constituents for the development of botanical-based bioinsecticides as safer alternatives to conventional biopesticides (Isman 2020).

4.4 Limitations and opportunities

Essential oil concentration and exposure time to cause weevil mortality were dose-dependent. Higher concentration led to faster mortality, as evidenced by the decreasing LT_{50} value with increasing oil concentration and vice versa. The trend is consistent and obvious with previous findings, where higher doses often overcome insect defense more quickly (Kharsahnoh and Kayang 2023). In our study, 5 and 10% of *Z. armatum* essential oil resulted in 100% mortality of *S. zeamais* at 168 and 240 h, respectively. Although the specific essential oils differ by plant species, plant-derived essential oils are effective bioinsecticides against *S. zeamais*. The results from this were obtained under the controlled laboratory conditions, which may not fully represent the farmers' storage environment, therefore field trial in actual storage are necessary to validate the efficacy of *Z. armatum* oil in further research. These findings support the broader

applicability of essential oils as biopesticides in stored pest management.

5 Conclusion

The use of plant-derived components, especially essential oils, in managing storage pests has gained momentum as a sustainable alternative to chemical pesticides these days. In this study, the essential oil of *Zanthoxylum armatum*, a readily available local botanicals in Nepal, was evaluated for its insecticide and repellent effects against *S. zeamais*, a major storage pest of maize. Glass vial bioassays indicated that the oil has both time- and dose-dependent toxicity, with an LC_{50} of 11.6% at 96 h and 4.5% at 144 h. The LT_{50} was as low as 13.84 h at the highest concentration tested. Furthermore, in maize treatment bioassays, even a concentration as low as 0.01% was sufficient to achieve 50% mortality over 10 days. The essential oil also exhibited significant repellency, reaching up to 97.5% repulsion observed at 10% concentration after 24 h. Our findings suggest that *Z. armatum* essential oil may offer both potent lethal and behavioral deterrent effects, making it promising eco-friendly alternative for controlling maize weevils in stored grain. which can offer both lethal and deterrent effects. Additionally, long term and repeated exposure to the oil and their effect on insect behavioral and physiological changes in current and successive generation could be a new valuable research question to explore. Further, research should focus on development of commercial formulations, evaluation of long-term efficacy, and assessment impact on grain quality in terms of residue impacts on human health.

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Author contributions SA and AP conceptualized the experiment, conducted experiment, Analyzed data. SA and AP Prepared the first draft. ASRB and KC conceptualized and edited the draft and further helped editing refined the final manuscript.

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Data availability Data are available from the corresponding author on reasonable request.

Declarations

Ethical Approval No ethical approval required since this study involves insect species (*Sitophilus zeamais*).

Consent for publication All authors provide consent for publication.

Competing interests The authors declare that they have no conflicts of interest.

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