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Research Article

Toxicity analysis of three different essential (clove, taramira & neem oil) against sugarcane black bug (*Cavelerius excavatus*)

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ABSTRACT

Sugarcane is a significant industrial cash crop that accounts for almost 80% of the world's sugar production, sugarcane is also very important economically in Pakistan. Sugarcane black bug is one of the sucking pest of sugarcane crop and creating significant yield and quality losses. Although conventional chemical pesticides are effective, their misuse contamination endanger human health, soil biology, and the environment. Naturally derived biopesticides offer a safer and more sustainable alternative. This study evaluated the efficacy of neem, clove, and taramira oils against sugarcane black bug nymphs using a leaf-dip bioassay. Numbers of dead individuals were recorded daily till seven days. The findings demonstrated that clove oil had the best and fastest insecticidal effect, resulting in 93.3% death after 24 hours at 4 ppm and attaining total mortality within 48 hours, despite continuously low LC50 values. Neem oil showed mild but long-lasting effects, but taramira oil acted more slowly and took longer to reach full efficacy. While neem and taramira oils would be better suited for long-term or preventative management strategies, clove oil ultimately showed the most potential for rapid insect control.

Keywords: Biopesticides, Essential oils, Sugarcane black bug, Insecticidal activity, pest management, Sustainable.



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INTRODUCTION

An important crop for food, fodder, and energy, sugarcane (*Saccharum* spp.) is a unique agricultural plant that evolved from a wild grass species through anthropogenic evolution. Sugarcane has high stem sucrose buildup, as opposed to other grass species (Dinesh Babu et al., 2022). It is grown all over the world, and sugarcane accounts for around 80% of all sugar production (Pereira et al., 2024).

The primary sugar crop, sugar cane, has grown to over 1.6 billion tonnes worldwide since 2010, and it reached over 2 billion tonnes in 2023. With 39 percent of the world's sugar cane production in 2023, Brazil led the pack, followed by India (24%), China, Pakistan, and Thailand (approximately 5% each) (FAO, 2024). In Pakistan, sugarcane accounts for roughly 18.9% of the country's GDP and 60% of its foreign exchange profits (Chandio et al., 2016). In Pakistan, sugarcane is a highly valued and significant industrial cash crop (Afghan et al., 2022). In 2022–2023, 1,319 thousand hectares of sugarcane were planted in Pakistan, an increase of 4.7% over 1,260 thousand hectares the year before (Gop, 2024). From 2011–12 to 2021–22, all provinces saw positive yearly growth rates in sugarcane crop area, yield, and

production.

The second estimates from the Provincial Agriculture Departments (Crop Reporting Service) indicate that the nation's sugarcane production for the 2021–22 crop is 88.759 million tons, which is a 9.6% increase over the 81.009 million tons produced the previous year. While the yield played a negligible impact, contributing only 0.3% over the previous year, the 9.2% expansion in the area under the crop is responsible for the increase in production (MNFSR, 2022). But we are not getting yield up to the mark due to pest and disease attack. The most damaging pests in sugarcane are lepidopteran borers, which lower stalk weight, juice quality, and sugar recovery (Li, A. M. et al., 2024). But here we are discussing about a sucking pest which cause significant loss. Extreme summer drought is conducive to the growth of termites, black bugs, mites, and shoot borer. The widely dispersed black bug (*Cavelerius excavatus*; Hemiptera: Blissidae) lives in the whorl of sugarcane crops and suck cell sap (Atwal & Dhaliwal 2009).

Adults have black bodies with white splotches, nymphs resemble adults. Monitor the plant's base, where these are primarily found. It is present all year round, but it is most active during the hot, dry season when there is no rain. Early crop stage attacks take place from April to June (Naseer, 2022). Both adults and nymphs harm plants by consuming their cell sap. Affected plants develop yellow leaves with brown splotches. The plant's growth and the juice's quality suffer as a result (Zada et al., 2013; Omkar, & Tripathi, 2020). The *C. excavatus* adults and nymphs collectively consume the cell sap from the immature plants (Atwal & Dhaliwal 2009).

Black bug impact more on ratoon varieties as compared to fresh crop (Pandey, & Singh, 2014) when it came to abiotic parameters, temperature had a substantial, positive, and moderate impact on the population of sugarcane black bug adults and nymphs (Koonthar et al., 2024). To get rid of this pest attack we go for easy solution of chemical pesticide which has toxic and detrimental impact on human health (Pathak et al., 2022). Most of the farmers in Pakistan used synthetic chemical for its management (Raza et al, 2020). Pesticides of different groups like chlorpyrifos, fipronil, lamda cyhalothrin and bifenthrin are using to control black bug population.

The overuse of pesticides in agriculture harms the environment, contaminating 90% of water sources and reducing soil respiration by 35%. Biodiversity is declining with 70% fewer insects and 50% fewer farmland birds in Europe, while 40% of pollinators, like bees. Pesticides also disrupt food chains through bioaccumulation, threatening ecosystems (Ali et al., 2021). The majority of synthetic insecticides are members of the organochlorine, organophosphate, carbamate, and pyrethroid families (Mdeni et al., 2022; Ahamad and Kumar, 2023), which have distinct modes of action against pests and can persist in various environmental mediums for one day to several years (Bilal et al., 2019).

Agrochemical residues that are dispersed throughout the environment and agricultural land contaminate ecosystems (dust, soils, air, sediments, and water) and contaminate human diets worldwide (Ali et al., 2021). Children, expectant mothers, and aged or ill people are particularly vulnerable to the pesticide's aftereffects. These chemicals have bioaccumulated at the upper tropic levels and entered natural food systems (Chaturvedi et al., 2023; Annamalai et al, 2024)

It causes human malignance; for example, glyphosate is linked to breast cancer (Thongprakaisang et al., 2013). Pesticide exposure is linked to a number of illnesses, including non-Hodgkin lymphoma (NHL) and Hodgkin's disease (HD) (Descatha et al., 2005; Lope et al., 2008). On the other hand, Biopesticides are long-lasting, residue-free, selective in their mode of action, affordable, environmentally safe, and unrelated to greenhouse gas emissions. (Borges et al, 2021; Archana et al., 2022; Chowdhury et al., 2024). Biopesticides are derived from natural sources such as plants, bacteria, and fungi, provide a more environmentally friendly alternative to synthetic pesticides because they effectively target pests and reduce environmental hazards (Ayilara et al., 2023).

By reducing pollutants and chemical residues, its expanding application in integrated pest management promotes sustainable agriculture (Fenibo et al., 2022). Many applications now use biopesticides instead of synthetic ones due to increased commercialization brought about by advancements in biopesticide research. They can come from plants, microbes, or nanoparticles (Kidd et al., 2017; Samada and Tambunan, 2020). Certain metabolites released by microbes serve as microbial insecticides and shield plants from pests (Samada and Tambunan, 2020). Terpenes, alkaloids, and phenols are active plant chemicals that are employed as Phyto pesticides (Abubakar et al., 2020).

So, we do the screening of three biopesticides or plant extracts of neem (*Azadirachta indica*, Sapindales: Meliaceae), clove (*Syzygium aromaticum*, Myrtales: Myrtaceae) and taramira (*Eruca sativa*, Brassicales: Brassicaceae) on black bug to check the mortality. As discussed above discussion these biopesticides have minimal impact on human health. Sugarcane is used as food. If we use synthetic pesticides instead of bio pesticides, residues remain in the food, it can lethally impact on our lives.

MATERIALS AND METHODS

Sugarcane black bug population was collected from sugarcane field located in MNS University of Agriculture Multan Pakistan was carried out to Lab to assess the toxicity of three botanical pesticides (extracts of neem, clove, and taramira) on sugarcane black bug (*C. excavatus*) nymphs in their fifth instar. In the area of Muzaffargarh district, sugarcane plantations provided the specimens that were gathered in the field. Special attention was paid to choosing healthy, active fifth instar nymphs of consistent size for bioassay testing. Standardized exposure of test subjects to the botanical treatments was ensured through the meticulous implementation of the leaf-dip bioassay methodology. Using acetone of 20% dilution as the solvent carrier, five concentrations (4.0, 2.0, 1.0, 0.5, and 0.25 ml/L) were created for neem. A control treatment comprising water and acetone solution was also included.

Each concentration has given number from 1 to 5 and 6 representing the control was clearly marked to ensure precise identification throughout the experiment. Distilled water was used to gently clean fresh sugarcane leaves. After that, they spent 30 minutes completely immersed in the appropriate treatment solutions. To prevent contamination, the leaves were carefully removed from the treatment and allowed to air-dry on sterile surfaces in a controlled laboratory environment (maintained at $26\pm 2^{\circ}\text{C}$ with 70-75% relative humidity). The treated leaves were methodically put on sterile Petri dishes with a 90 mm diameter when they had sufficiently dried. Five replicate Petri plates were constructed for each concentration level, and three carefully selected fifth instar nymphs were inserted in each plate. This replication strategy maintained a reasonable experimental scale while providing sufficient sample size for accurate statistical analysis. Using the same rigorous methodology, the extracts of clove and taramira were subsequently evaluated using the same concentration gradients (4.0, 2.0, 1.0, 0.5, and 0.25 ml/L) and control treatment in order to ensure direct comparability of results across all three botanical drugs. Mortality data was recorded after 24, 48, 72, 96, 120, 144, and 168 h in order to account for the characteristic delayed response and prolonged efficacy of botanical pesticides. Nymphs were considered dead if they did not respond to tactile stimuli or move when softly pushed with a soft brush. After the first 24-hour post-treatment period, first data were documented. Mortality data was calculated for seven days.

Statistical Analysis

Probit analysis was performed for toxicity of three different essential oils in SPSS 22.

RESULTS

The leaf dip bioassay was used to test three botanical insecticides against sugarcane black bug nymphs: neem oil, taramira oil, and clove oil. Their concentration- and time-dependent efficacy patterns varied significantly, according to the study.

Clove Oil

Clove oil demonstrated remarkable insecticidal effectiveness at a concentration of 4 parts per million, reaching 100% death in 48 hours and 93.3% mortality in just 24 hours. Even at lower dosages, excellent time-dependent efficacy was observed: 2 ppm initially caused 46.7% mortality (24 hours), increasing to 100% by 144 hours. The LC50 values, which showed steep slope values of (2.795 ± 0.565) at 24 hours) and steadily decreased from 1.636 ppm (24 hours) to 0.177 ppm (168 hours), demonstrated strong dose-response correlations. Because of its consistent performance, clove oil got the highest toxicity score.

Neem Oil

The mortality rate of neem oil was 53.3% at 4 ppm (24 hours) and 100% after 96 hours, indicating moderate efficacy. While its LC50 values decreased from 3.337 ppm (24h) to 0.244 ppm (168h), its slope values varied from 2.447 ± 0.666 (24h) to 1.919 ± 0.558 (168h). Particularly at lower concentrations (0.5 ppm showed 0% death at 24h but reached 80% by 168h), the mortality patterns showed a more gradual but more persistent effect than clove oil.

Taramira Oil

Among the biopesticides tested, Taramira Oil had the slowest rate of action. It took 144 hours to reach 100% efficacy, and only 33.3% of individuals died at 4 ppm in the first 24 hours. With LC50 values improving from 8.747 ppm (24h) to 0.482 ppm (168h) and typically shallower slope values (1.487 ± 0.578 at 24h), it displayed less consistent dose-response patterns than the other oils. The time-course analysis revealed a number of noteworthy trends: Over prolonged exposure times, the efficacy of all biopesticides increased. At all-time points, clove oil maintained the fastest knockdown effect. Exposure times for lower concentrations (0.25–1 ppm) must be significantly longer in order to achieve mortality comparable to higher dosages. According to this study, clove oil's rapid action and potent efficacy at low dosages make it potentially helpful in emergency pest management scenarios. While neem oil is better suited for preventive

applications because of its more gradual but continuous effects, tamira oil may be more suitable in situations where slower-acting control is acceptable.

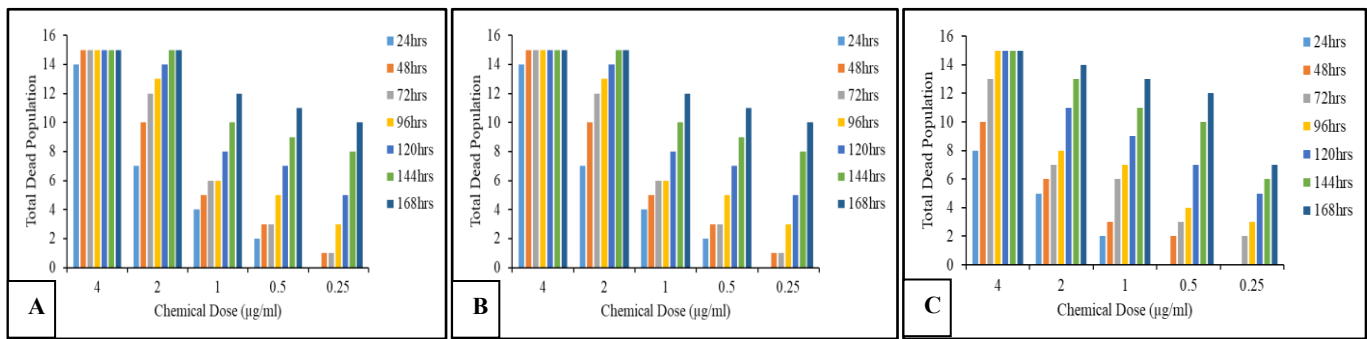


Figure 1. Mortality percentage in the 5th nymph of sugarcane black bug when treated with (A) clove oil (B) Taramira oil (C) Neem oil by leaf dip method.

Table 1. Sugarcane black bug

Botanicals	Hours	Total numbers	LC ₅₀ and 95% confidence limit (µg/ml)	Slope ±SE	Chi-square	Order of toxicity	df	rr
Clove oil	24	15	1.636(1.209-2.354)	2.795±0.565	2.612	15	3	9.242
	48	15	1.156(0.845-1.626)	2.664±0.510	2.674	12	3	6.531
	72	15	1.004(0.745-1.367)	2.944±0.550	1.554	10	3	5.672
	96	15	0.778(0.535-1.106)	2.317±0.470	4.159	9	3	4.395
	120	15	0.531(0.314-0.772)	2.055±0.470	3.889	6	3	3.00
	144	15	0.310(0.109-0.490)	1.829±0.508	4.768	3	3	1.751
	168	15	0.177(0.014-0.336)	1.644±0.563	2.574	1	3	
Tara Mira oil	24	15	8.747(3.742-1758.362)	1.487±0.578	0.967	21	3	49.418
	48	15	4.099(2.504-15.264)	1.919±0.558	0.985	20	3	23.158
	72	15	2.531(1.528-7.533)	1.495±0.416	3.027	18	3	14.299
	96	15	1.698(1.050-3.698)	1.487±0.395	2.004	16	3	9.593
	120	15	1.219(0.775-2.080)	1.668±0.401	1.149	13	3	6.887
	144	15	0.755(0.429-1.198)	1.624±0.404	4.310	8	3	4.265
	168	15	0.482(0.161-0.820)	1.336±0.398	3.846	5	3	2.723
Neem oil	24	15	3.337(2.298-7.341)	2.447±0.666	0.793	19	3	18.853
	48	15	2.500(1.675-5.086)	1.992±0.488	0.940	17	3	14.124
	72	15	1.440(0.935-2.569)	1.701±0.406	2.007	14	3	8.135
	96	15	1.007(0.655-1.580)	1.837±0.416	5.240	11	3	5.689
	120	15	0.562(0.284-0.874)	1.647±0.418	2.473	7	3	3.175
	144	15	0.346(0.126-0.552)	1.718±0.465	1.240	4	3	1.954
	168	15	0.244(0.066-0.399)	1.919±0.558	0.985	2	3	1.378

DISCUSSION

The relative potency ranking of clove oil, taramira oil, and neem oil remained unchanged. Exposure durations for lower concentrations (0.25–1 ppm) must be significantly longer in order to achieve mortality comparable to larger dosages. This study suggests that because clove oil acts quickly and is highly effective at low dosages, it could be particularly helpful in emergency pest management situations. Neem oil is more suited for preventive applications because of its more gradual but continuous effects, while taramira oil may be more suitable in situations where slower-acting control is acceptable. Clove oil proved to be more effective than other oils against the sugarcane black insect in the current investigation. The LC₅₀ values decreased significantly with time, from 1.636 µg/ml (24 hours) to 0.177 µg/ml (168 hours). These results are consistent with other studies showing the strong bioactivity of clove oil, particularly against coleopteran insects, grain pests, and mosquito larvae. As an illustration of the extraordinary vulnerability of black bugs

in comparison to mosquito larvae, the LC₅₀ of clove essential oil against *Anopheles stephensi* larvae was reported at roughly 57.5 ppm (µg/ml), which is much higher than our data (Osanloo et al., 2018; Aryal et al., 2023). This gradual decrease in LC50 suggests a cumulative toxic impact and shows that even very low concentrations of clove oil can effectively cause mortality in sugarcane black bug larvae over an extended period of time.

This finding is consistent with the known mode of action of many botanical insecticides, which often require longer exposure periods to exert their full effects (Mossa, 2016; Isman, M. B. (2020).) The trial looked at how well different essential oils controlled adult mortality at 24, 48, and 72 hours after exposure, as well as *Sitophilus oryzae*, the rice weevil. The treatments included eucalyptus oil (0.5 ml/lit), lemon grass oil (0.5 ml/lit), neem oil (0.5 ml/lit), clove oil (0.5 ml/lit), lavender oil (0.4 ml/lit), karanja oil (0.4 ml/lit), and tea tree oil (0.5 ml/lit).

The treatments with the highest mortality rates were neem oil (83.33%), karanja oil (77.77%), and lavender oil (31.11%). The remaining treatments were deemed to be in the middle, with clove oil (67.77%), eucalyptus oil (50.77%), lemon grass oil (54.44%), and tea tree oil (51.10%) coming in second and third, respectively (Ghosh et al., 2023).

In a study on *Sitophilus zeamais* revealed that 50% mortality occurred at 0.27–4.28% concentration over 10 days (equivalent to ~2.7–42.8 mg/ml), which is orders of magnitude higher than our maximal LC₅₀ of 1.636 µg/ml. Hence, sugarcane black bugs appear significantly more sensitive to clove oil than both mosquitoes and common grain weevils. Weevils have more ability to withstand against clove as compared to black bug.

The purpose of the study was to examine the toxicity, oviposition prevention, and compatibility of three biopesticides with the cowpea beetle of *Callosobruchus chinensis* (Coleoptera: Bruchidae): *Syzygium aromaticum*, *Citrus aurantifolia*, and *Allium sativum* essential oils.

The levels of toxicity (LC50 and LC95) were examined. The three tested essential oils were poisonous and inhibited *C. chinensis* oviposition. When the adults were exposed to the essential oil for 72 hours, *A. sativum* exhibited the maximum level of toxicity (LC50 = 0.07%), followed by *S. aromaticum* (LC50 = 0.08%) and *C. aurantifolia* (LC50 = 0.07%) (Nuryanti et al., 2022).

Our results in case of Neem oil exhibits significant and time-dependent mortality, similar to clove oil, with its toxicity increasing substantially with prolonged exposure. At 24 hours, the LC50 value of neem oil was 3.337 mug/ml; at 168 hours, it was a very strong 0.244 mug/ml (Table 1). This gradual drop in the necessary fatal dosage over time emphasizes neem oil's slow-acting and cumulative properties, which are key components of its pesticidal mechanism. Instead of causing quick knockdown toxicity, neem oil is known for its complex array of bioactive chemicals, especially azadirachtin, which confers diverse insecticidal activities, including antifeedant, repellent, insect growth regulatory (IGR), and ovicidal actions (Dodia et al, 2010).

At the experimental facility of Tando Jam Agriculture University, the study was carried out to combat the sucking complex (jassid, thrip, and whitefly), five biopesticides—neem oil (500 ml/acre), cooking oil (750 ml/acre), linseed oil (750 ml/acre), hinge (290 gm/acre), and cotton oil (750 ml/acre)—were applied twice. The results were compared to an untreated control. The population of pests was decreased by all products during both treatments. The best oils for minimizing sucking pests were neem (63.27%) and cotton (62.01%), on average, followed by hing (58.25%), cooking oil (57.18%), and linseed oil (55.24%), in that order (Lubna Bashir Rajput et al., 2017).

In our study as compared to above, At the highest tested concentration of 4 ppm, Neem oil induced 53.33% mortality within 24 hours, rapidly progressing to 100% mortality by 96 hours and sustaining this complete control through 168 hours. Importantly, Neem oil shown exceptional long-term efficacy even at lower concentrations; for example, a 2-ppm concentration caused 33.33% death at 24 hours but 93.33% mortality after 168 hours. This study found that specific botanical oil mixtures were effective at reducing pests that consume chiles. The next most successful combination was a 50:50 pongamia and neem oil blend, which was followed by a 2.0% blend of pongamia, neem, cotton seed, and citronella oils (50:25:15:10). These treatments, which mostly preserved pollinators and natural enemies (except from citronella oil), led to noticeably higher chilli yields. (Gadge and others, 2021). It demonstrates that neem is effective when combined with other oils.

In particular, the observed mortality percentages for treatments involving Taramira oil differed according to the exposure duration (24 to 168 hours) and the applied concentration (0.25, 0.5, 1, 2, and 4 ppm). Mortality rates were consistently higher at higher concentrations and for longer exposure periods, suggesting a definite dose- and time-dependent insecticidal effect.

In a study tested *Eruca sativa*, *Piper nigrum*, and *Withania somnifera* extracts against *Trogoderma granarium* at 2–8% concentrations. *Piper nigrum* caused the highest adult mortality (15.26%) and lowest F1 population growth (78.6%), while *Eruca sativa* showed the least efficacy (7.15% mortality, 115.47% population buildup). *Piper nigrum* also

significantly inhibited larval growth compared to other extracts (Javed et al, 2016). In my study of taramira oil it has 33 percent mortality at a higher concentration of 4ppm in laboratory conditions but at lower concentrations is as low of 6% after 24 h. It same as above study on the khapra beetle.

Essential oils from *Moringa oleifera*, *Eruca sativa* (arugula), *Raphanus sativus* (radish), and *Allium sativum* (garlic) were tested in a lab setting for their ability to repel rose aphids (*Macrosiphum rosae*) and black bean aphids (*Aphis fabae*) at different concentrations and exposure durations.

Mortality generally increased with higher concentrations and longer exposure. Arugula oil consistently showed the highest efficacy against both aphid species, reaching 97.5% mortality for *M. rosae*, followed by garlic, radish, and moringa oils, indicating their potential as natural aphicides(Akram, 2018).

CONCLUSIONS

Sugarcane is a significant industrial cash crop that accounts for almost 80% of the world's sugar production, sugarcane is also very important economically in Pakistan. Sugarcane black bug is one of the sucking pest of sugarcane crop and creating significant yield and quality losses. Although conventional chemical pesticides are effective, their misuse contamination endanger human health, soil biology, and the environment. Naturally derived biopesticides offer a safer and more sustainable alternative. This study evaluated the efficacy of neem, clove, and taramira oils against sugarcane black bug nymphs using a leaf-dip bioassay. Numbers of dead individuals were recorded daily till seven days. The findings demonstrated that clove oil had the best and fastest insecticidal effect, resulting in 93.3% death after 24 hours at 4 ppm and attaining total mortality within 48 hours, despite continuously low LC50 values. Neem oil showed mild but long-lasting effects, but taramira oil acted more slowly and took longer to reach full efficacy. While neem and taramira oils would be better suited for long-term or preventative management strategies, clove oil ultimately showed the most potential for rapid insect control.

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AUTHOR CONTRIBUTIONS

Muhammad Tayyab Abbas: Methodology, Software, Validation, Muhammad Zeeshan Khalid: Writing – review & editing. Data curation, Huzaifa Nauman: Visualization, Writing – original draft, Rashid Azad: Investigation, Formal analysis. Muhammad Naeem: Conceptualization, Methodology, Project administration, Nabeel Shafiq: Conceptualization, Investigation, Methodology, Muhammad Faizan: Project administration, Resources, Shah Zaib Noor: Writing - Review & editing, Huzaifa Imtiaz: Resources, Writing review & editing, Waqar Jaleel: Supervision, Validation, Writing – original draft,

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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