Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 786-798 | ISSN:2251-6727

In Vitro Insecticidal Activity of Silica Nanoparticles and Simarouba Amara Against Arabica Coffee White Stem Borer, Xylotrechus Quadripes

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(Received: 16 March 2025 Revised: 20 April 2025 Accepted: 01 May2025)

KEYWORDS

Xylotrechus quadripes, Nanosilica, *Simarouba amara* bark extract, Artificial diet.

ABSTRACT:

Xylotrechus quadripes, Chevrolet (Coleoptera: Cerambycidae) is one of the most lethal pests infesting Arabica coffee plants. The complex feeding habits and masked behavior of coffee white stem borer (CWSB) make pest management difficult despite the application of insecticides. In addition, the pesticides are inaccessible to larvae because soon after hatching larvae burrow into the stem and feed inside until they emerge as adults. In this regard, we developed a novel environment friendly biopesticide to combat CWSB. The activities of Nanosilica (NSi) and *Simarouba amara* bark extract (SABE) were evaluated by incorporating these in the artificial diet, mimicking the host plant environment. We also studied the impact of biopesticides on fecundity, fertility, larval penetration in cut stems, toxicity and olfactory responses of *Xylotrechus quadripes*. Both NSi and SABE exhibited dose-dependent mortality rates, demonstrating their effectiveness as biopesticides. On an average, the number of eggs laid on treated and untreated (control) cellulose sheets was found to be 3-4 and 18 in the NSi- and SABE-treated groups, respectively, compared to the control (25-35 eggs). The eggs in fertility study revealed a significant hatching difference between the NSi and SABE-treated groups. NSi strongly inhibited the penetration, whereas SABE-treated stems allowed 2-3 penetrations of larvae, suggesting the operation of different larvae penetration mechanisms. The toxicity study of NSi and SABE showed LC50 values of 257.63 and 575.43 mg/L respectively. The highest Olfactory avoidance response was observed in the NSi powder form (83.34%), followed by NSi-coated cut stems (76.67%) and SABE in solution form (73.34%). Thus, both NSi and SABE provide an effective alternative for integrated pest management in *coffee arabica*.

1. Introduction

Two major varieties of coffee, Coffea arabica and Coffea canephora (Robusta), are cultivated in India, making the country the seventh largest producer of coffee worldwide. India exports coffee to international markets, generating an annual revenue of $1,116.95 million [1]. Approximately 98% of 2.5 lakh coffee growers are small-scale farmers across various Indian states [2]. Unlike many international coffee farms, Indian coffee is distinctively shade grown. The coffee white stem borer (CWSB) *Xylotrechus quadripes* (Coleoptera: Cerambycidae) is a serious pest of Arabica coffee. CWSB is a major pest that affects Coffea arabica, one of India's key commercial coffee varieties [3]. The severity of the damage caused by CWSB increased by approximately 25–80% in 2019 compared to its impact in 1950. Over the past 25 years, the area of Robusta coffee cultivation has expanded by 56%, leading to a significant shift from Arabica to Robusta [4].

The life cycle of the pest extends from 142 to 390 days in the field, depending on plant size [5,6]. Females deposit 50–100 eggs in coffee bark cracks, hatch into larvae, and start chewing on the bark before tunnelling through stems. Once the larvae enter the interior part of

the stem, they interfere with the transportation of nutrition and water inside the stem, causing death of the coffee plant [6]. The CWSB larvae persisted for approximately 120 days after being housed inside the stem. The larvae progressively feed on the stem, pack the fecal matter behind, protect themselves from the applied pesticides, and become resistant [5]. The beetles emerge twice a year, during April - May and October- December, when they mate and pick another host plant to lay eggs [6]. Although it is easy to obtain stem borer females laying eggs in the laboratory, it is difficult to raise them in the laboratory [7]. The borer pest has been successfully raised in the laboratory on cut stems of Arabica plants from the egg to adult stage with periodic monitoring [8]. However, it is very difficult to collect growing larvae at different stages to study their growth characteristics. This is because during stem bisection, the chances of larvae being damaged are quite high. It is also challenging to reinsert the same collected larvae into another stem to investigate this effect [9].

Traditionally, chemical insecticides have been used to combat stem-boring insects, including CWSB. In addition, indiscriminate use of chemical pesticides causes environmental and human health risks, pushing the demand for eco-friendly and sustainable alternatives. As a result, researchers are actively involved in nanotechnology-based formulations and botanicals as environmentally friendly insecticides with fewer detrimental effects [10]. In this direction, we attempted to integrate pesticides into an artificial diet and conducted a toxicity experiment using NSi and SABE as insecticides.

Simarouba amara, a tropical tree species, has been extensively known for its medicinal qualities and insecticidal capabilities [11]. Various components of Simarouba spp., including bark, leaves, and seeds, possess bioactive compounds with insecticidal activity against diverse insect pests [43] biopesticides can also act as repellents that hinder oviposition and development or exert larvicidal effects on agricultural crop insect pests. These compounds have been identified as secondary metabolites [12]. These natural insecticides successfully kill insects without the need to develop resistance. With limited exceptions, they are less hazardous to non-target insects such as vertebrates, pollinators, and natural predators. They are also easy to obtain and utilize, and are less expensive [13]. Therefore, natural insecticides from plants can be considered an alternative to chemical insecticides [12,4,5]. Among these choices, bio-insecticides extracted from the bark of *Simarouba amara* are a promising alternative to synthetic chemical insecticides owing to their high insecticidal efficacy and minimal toxicity to non-target species.

Silicon (Si) is the second most abundant element in the Earth's crust after oxygen [14]. In addition, Si has been listed as a "beneficial substance" by the International Plant Nutrition Institute [15] and has been widely reported to improve plant resistance to both abiotic and biotic stresses [16,17,18,19,20]. In a performance experiment, the application of Si to maize resulted in higher larval mortality of the true armyworm Pseudeletia unipuncta than in maize plants without Si [21]. Therefore, the application of Si is a possible management approach for suppressing a wide range of pests, including leaf chewing [22,23], sap-feeding [24,25,26] and stem boring insects [22,26]. One possible explanation for the increased pest resistance in the plants by the application of Si is that the insects could directly absorb soluble Si, which may cause physical and physiological damage to the insect. However, little information has been obtained on the direct impact of Si on insects and their related mechanisms. Thus, the scenario beyond Si directly mediating plant-insect interactions warrants further exploration [20].

2. Methods

Source of Biopesticide

Nanosized Silica powder obtained from Niranthara Scientific Solutions Private Limited (Bangalore, India) was used to understand its effect on CWSB larval growth parameters. SABE was prepared by boiling 10 g of bark powder in 100 ml of distilled water for 15 min, followed by cooling (10:100 w/v). After passing through No. 1 Whatman filter paper, the extract was condensed by lyophilization, and the resultant mixture was maintained in sealed vials at 4°C until further use [27,28]. Complete phytochemical analysis of Simarouba glauca plant extracts was performed using advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) and High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS). This analysis successfully identified and quantified key bioactive compounds, including alkaloids, flavonoids, phenols, saponins, and tannins (Aljawobaei et al. 2024).

Insect Culture

In this study, we used CWSB-infested coffee stems collected from the Central Coffee Research Institute (CCRI), Balehonnur, Chikkamagaluru Dist., Karnataka, India, located at approximately 13°22′8.89″N, 75°25′24.92″E. Coffee plots and agroforestry systems dominated the agricultural environment in this area. CWSB was the main pest in the tested region. The larvae fecal matter blocking the opening of larval galleries in the stems and ring on the bark is a sign of infestation. The infested stems and branches of the plants were carefully removed and brought to the laboratory. Before the natural flight periods of April–May and October–December, infested stems were stored in an insectary [8]. After emergence, the adults were coupled, housed in small plastic containers for mating, and encouraged to lay eggs.

Artificial Diet Preparation

An artificial diet was successfully prepared for rearing the borer from eggs to adults in the laboratory. The diet was prepared according to [9] using healthy arabica stems (var. Chandragiri). The diet surface was punctured with a sterile dissecting needle and the newly hatched larvae were placed in a well with a sterile brush. As soon as the larvae were placed in the diet, the containers were sealed, kept in the BOD chamber at a temperature of 28 ±2°C and 50-60% relative humidity [30,31,32,33].

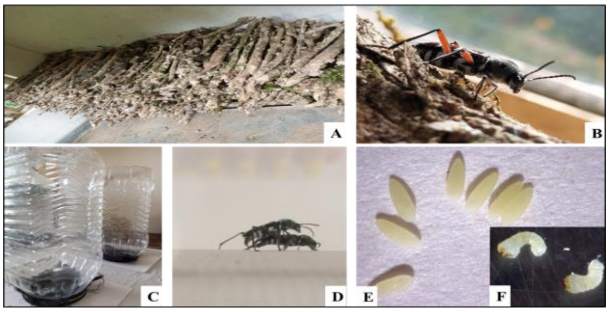

Fig. 1. Insect rearing. (A) Collection of CWSB-infested stems from plantations, (B) Emergence of CWSB adults from infested stems, (C) Pairing of male and female CWSB adults released into a mating chamber, (D) mating of males and females, (E) female insects released eggs on cellulose paper, and (F) Neonates hatched from the eggs. These neonates were collected and reared on an artificial diet.

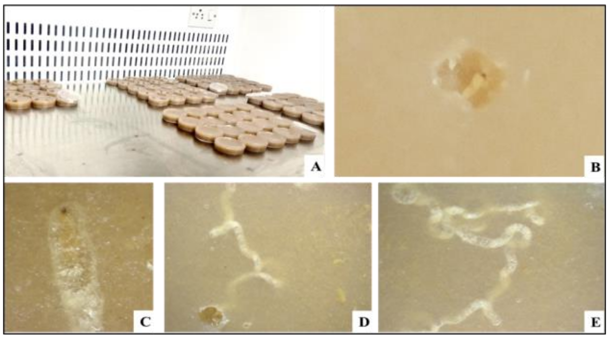

Fig. 2. Feeding behavior in the artificial diet. (A) Preparation of artificial diet for rearing CWSB, (B) introduction of freshly hatched neonates to the artificial diet, (C) Confirmed feeding behavior by neonate larvae in artificial diet, and (D & E) path made by larvae during feeding in artificial diet. NSi and SABE were added to the diet to determine their effectiveness.

Initial Insecticidal Potency Assessment

The study involved a series of assays to evaluate the efficacy of biopesticides against the coffee white-stem borer (CWSB) at different developmental stages. In the Artificial Diet Assay, biopesticides were incorporated into an artificial diet fed to the first-instar larvae, and the resulting mortality rates were recorded. This Fecundity study focused on assessing the reproductive fitness of adult beetles by monitoring their egg-laying behavior in response to biopesticide-treated paper surfaces. Subsequently, the fertility study evaluated the hatchability of eggs laid during the fecundity experiment under controlled conditions. In the Cut Stem Penetration Assay, the impact of biopesticides on the boring success of CWSB larvae was assessed using the cut stems of the Chandragiri coffee variety. Additionally, the Contact or Residual Assay examined the residual lethality of biopesticides by exposing eggs and neonate larvae to the treated surfaces. The Toxicity Assay involved directly exposing eggs to varying concentrations of biopesticides to determine dose-dependent effects on hatching success and larval mortality. Finally, the Y-Tube Olfactometer Assay was conducted to observe the behavioral response of beetles, specifically their preference or aversion to biopesticide-treated odor sources. These methods provide a comprehensive evaluation of biopesticide

effects across multiple developmental stages and the behavioral responses of the target pest.

Artificial Diet Assay

To assess the effect of biopesticides on the growth and survival of CWSB larvae, an artificial diet was prepared. Biopesticides were incorporated into the diet at concentrations ranging from 1 g/L to 5 g/L, and a control diet without biopesticides was maintained. First-instar larvae of CWSB sourced from laboratory-reared populations were used in this study. Mortality response was observed and recorded to determine the efficacy of each concentration [9].

Fecundity Study

The reproductive fitness of adult beetles was evaluated by monitoring the egg-laying behavior in response to the treated surfaces. One male and two female beetles were placed in a plastic container (30 cm diameter) sealed with a perforated cloth cover to allow proper aeration. The treated cellulose papers, coated with different concentrations of NSi and SABE, and air-dried for 30 min, were placed at the bottom of the containers. Control papers were treated with Milli-Q water. After 24 h of exposure, the number of eggs laid on the treated and untreated paper was recorded. This study assessed the effects of NSi and SABE on the fecundity of CWSB by quantifying the number of eggs laid in each treatment group [34].

Fertility (Hatchability) Study

Hatchability of eggs laid during the fecundity study was evaluated under controlled conditions. Eggs were carefully collected from treated and untreated cellulose papers and transferred to separate rearing containers, with papers treated with different concentrations of NSi and SABE. Untreated eggs were used as controls. The containers were maintained at a constant temperature and humidity to ensure uniform conditions for egg development. Eggs were monitored over time to assess hatching rates and any abnormalities in larval development were recorded. Hatchability percentage was calculated by dividing the number of hatched eggs by the total number of eggs laid in each treatment [34].

Cut Stem Penetration Assay

The effect of biopesticides on the boring success of CWSB larvae was assessed using cut stems of the Chandragiri coffee variety. Stems 7–10 cm in length were treated with NSi and SABE at concentrations ranging from 1% to 5%. The biopesticides were applied using a paint brush to coat the stem surface, while the control stems were rinsed with Milli-Q water and air-dried in the laboratory. After treatment, the stems were placed individually in Petri dishes and 10 newly hatched larvae were introduced into each plate using a camel hair brush. The penetration success of the larvae into the treated and control stems was visually observed and recorded over a period of 24 to 240 h. This setup allowed for a clear assessment of the deterrent effects of NSi and SABE on larval boring activity [35].

Toxicity Assay

Freshly deposited CWSB eggs were exposed to NSi and SABE at concentrations ranging from 1000 to 5000 mg/L for 10–15 s. Eggs were monitored for ten days to assess hatching success and subsequent larval mortality. LC_{50} values were calculated using probit analysis to determine the lethal concentration for 50% mortality in comparison with the control group of untreated eggs [36].

Contact or Residual Assay

Larval mortality was evaluated using sterile filter papers treated with NSi and SABE at concentrations of 1000, 2000, 3000, 4000, and 5000 mg/L. The treated paper was placed in Petri dishes and removed with distilled water. Individual first-instar larvae were introduced into the treated paper, and the dishes were incubated at 25 ± 1°C for 48h. Mortality rates were recorded, and untreated filter paper served as a control. Ten replicates were performed for each concentration [37].

Olfactometer Assay

A Y-tube olfactometer was used to evaluate the behavioral responses of adult beetles to biopesticides. Individual beetles were introduced into the Y-tube apparatus, where one arm contained biopesticide-treated odor sources and the other contained an untreated control. Responses were recorded and a Preference Index (PI) was calculated to quantify attraction or avoidance. This assay provides insights into the sensory effects of NSi and SABE on adult beetles.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10.1.0. The data were arcsine-transformed and pooled before the analysis. The effects of Si and SABE on biopesticide-impacted artificial diet, contact or residual assay, fecundity, fertility, and boring on cut stems were conducted under laboratory conditions and analysed by two-way ANOVA. The means of the sensory (olfactory) responses were determined using the chi-square test to determine the P value and statistical significance.

3. Results

Artificial diet assay

An artificial diet is crucial in entomological research to assess insect populations. By mimicking natural conditions, artificial diets enable the study of the effects of these pesticides on insect larval development, mortality rates, and behavioral patterns, thereby providing valuable insights into their effectiveness as control agents against pest infestations. The life cycle and feeding behavior of the insects in the artificial diet are shown in Figs. 1 and 2. We investigated the influence of various concentrations of NSi and SABE, when incorporated into an artificial diet, on the mortality rates of experimental larvae. The observed mortality rate was concentration-dependent after treatment with NSi and SABE. For NSi, mortality rates increased with increasing concentrations, ranging from 40% at 1 g/L NSi to a peak of 83.33% at 5 g/L, compared to a 20% mortality rate in the control group. Similarly, SABE concentrations showed escalating effects on mortality, with rates ranging from 26.66% at 1 g/L to the highest mortality rate of 73.33% at 5 g/L, in contrast to the control rate of 6.66% (Fig. 3). These outcomes underscore the potential pesticidal properties of NSi and SABE, suggesting the possibility of further exploration of their application in pest management.

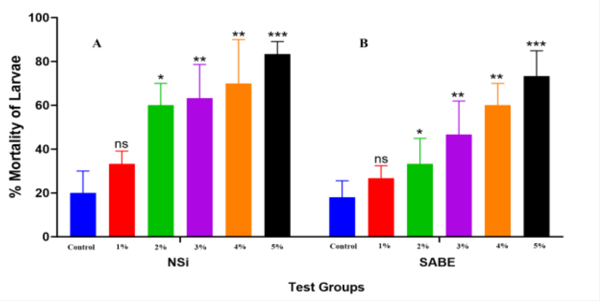

Fig. 3. Effect of the biopesticide-incorporated artificial diet on larval mortality. (A) NSi-treated artificial diet and (B) SABE-treated artificial diet. The data are expressed as mean ± SEM (n=30). Statistical significance is expressed as * (p<0.05), ** (p< 0.01), *** (p<0.001), and ns (P > 0.05).

Fecundity study

Fecundity studies test the number of eggs an insect lays, and how different factors affect fecundity. Fecundity tested on rough cellulose papers treated with bioactive compounds provides insights into the impact of these compounds on insect reproduction, revealing their ability to inhibit egg-laying behavior or the viability of laid eggs. In the present study, the different treatment groups exhibited significant trends in fecundity (Fig. 4). Among the treatment groups, NSi showed a mean fecundity of 3-4 eggs, whereas SABE had a much greater mean of 18 eggs per individual at a lower concentration. However, the untreated control group had a higher mean fecundity of 25–35 eggs (Fig. 4). Two-way ANOVA revealed a significant concentration-dependent effect, influenced by various factors. The interaction effect between the row and column factors accounted for 4.4% of the total variation with a p-value of 0.0002, indicating its significance. Both the row and column factors individually contributed significantly to the total variation of 29.70 and 63.62%, respectively, and both had significant p-values of <0.0001. However, the subject factor did not significantly contribute to the total variation, explaining only 0.22% with a relatively high p-value of 0.72, indicating non-significance. These results indicate a significant relationship between the changes in the proportion of biopesticides and reproductive output.

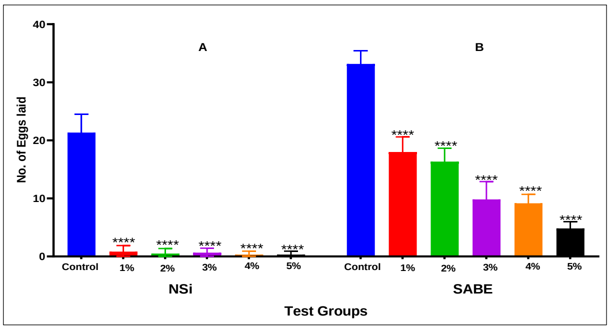

Fig. 4. Fecundity study test groups. (A) Rough drawing papers treated with NSi and (B) rough drawing papers treated with SABE, compared to the control data, are expressed as mean ± SEM (n=30). Two-way ANOVA

showed that the statistical significance of NSi and SABE treatment across the concentrations was expressed as **** (P < 0.0001), * (P > 0.01), and ns.

Fertility study

Fertility, particularly hatchability, is a critical aspect of reproductive success in insects. It directly affects population dynamics, genetic diversity, and species survival, playing a fundamental role in maintaining ecosystem balance and resilience. In this study, we investigated the impact of NSi and SABE repellent properties on CWSB egg hatchability. The hatching rates were closely monitored, and egg hatching from each group was quantified for hatchability assessment (Fig.5). Any anomalies were recorded, and statistical analyses were conducted using ANOVA to compare the effects of NSi and SABE to those of the control. We conducted a two-way ANOVA with the variables NSi and SABE as the row factors and different concentrations as the column factor, which indicated significant effects. SABE and NSi individually contributed significantly to the total variation, at 13.77 and 80.33%, respectively, with p-values of <0.001. However, the control does not significantly contribute to the total variation, showing only 0.7697% with a p-value of 0.2248. These results indicated that both NSi and SABE, as well as their interaction, have a significant impact on the outcome when compared to the control.

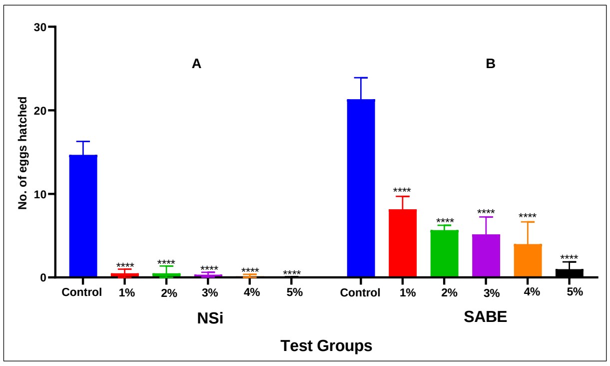

Fig. 5. Fertility study in test groups: The eggs were hatched on rough drawing paper treated with NSi (A) and with SABE (B) as compared to control. The data are expressed as mean ± SEM (n=30). Two-way ANOVA was used to express the statistical significance of NSi and SABE treatment across the concentration was expressed as **** (P < 0.0001), * (P > 0.01) and ns.

Larvae penetration study on cut stem

The larvae penetration on cut stems was treated with biopesticides to assess the larvae success on penetration in the treated stems or if they encounter difficulties due to the presence of biopesticide. If the biopesticide effectively deter larvae penetration, it suggests that the treatment is successful in protecting the plant from insect damage. In this study, larvae were tested for their penetration into the coffee stem. The significant variation in the penetration trends were observed in different treatment groups (Fig. 6). The NSi treatment showed no mean penetration of larvae in any of the individual percentage treatments, while the SABE treatment showed a mean penetration of 2-3 larvae in a lower percentage. Statistical analysis indicates that the biopesticide concentrations significantly contribute to the variance observed in the data, accounting for 83.91 % of the total variation and having a significant p-value of 0.0001. In contrast, the control group did not contribute significantly to the total variation, with 1.47% and a p-value of 0.6269, indicating a lack of significance. The statistical analysis confirmed that biopesticide concentration significantly influenced larval penetration, accounting for 83.91% of the total variation (p = 0.0001). In contrast, the control group contributed only 1.47% to the total variation, with a p-value of 0.6269, indicating no significant impact on larval penetration. These findings suggest that NSi provides complete protection against larval penetration, while SABE offers partial deterrence, particularly at higher concentrations.

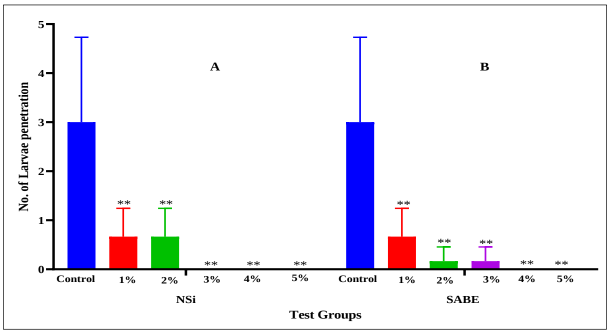

Fig. 6. Larvae penetration study on cut stem. (A) The larval penetration on coffee cut stems treated with NSi and (B) The larval penetration on coffee cut stems treated with SABE. The data are expressed as mean ± SEM (n=30). Two-way ANOVA was used to express the statistical significance of NSi and SABE treatment across

the concentration was expressed as **** ($P < 0.0001$), ** ($p< 0.01$), * ($P > 0.01$) and ns.

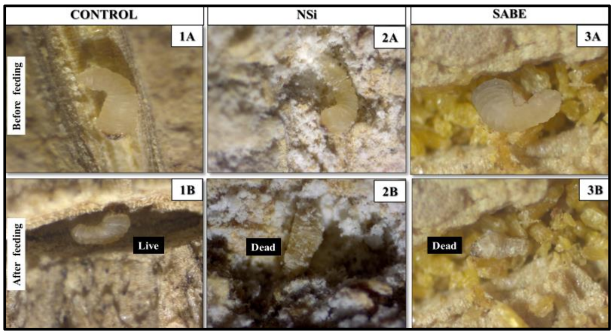

Fig. 7. Larvae penetration study on cut stem: The neonates were released into NSi- and SABE-treated cut stems, 1A-1B (Control), 2A-2B (NSi), and 3A-3B (SABE) test groups. The dead neonates after feeding in the cut stems is shown in Figures, 2B and 3B.

Toxicity assay

Determination of LC_{50} for NSi and SABE

Studies have indicated that biopesticides are eco-friendly, possess low toxicity properties, are biodegradable, and specific in action with little or no negative impact on non-target organisms. The dose-response test found no mortality in the control (0 mg/L) during the 96h and 240h exposure time period. Mortality percentage of larvae at different concentrations of NSi and SABE is presented in Tables 1 and 2. The resulting 96-h LC50value for NSi was found to be 225.63 mg/L and for SABE, it was found to be 575.43, which resulted in 50% mortality of the test larvae. Our findings provide valuable insights into the potential ecological consequences of NSi and SABE and mitigate its environmental impact.

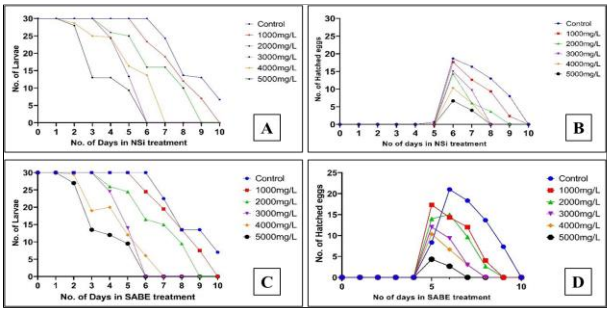

Fig. 8. Contact residual method: The neonate Larvae were treated and observed for mortality and egg hatchability of CWSB (up to 10 days) before (A and C) and after the treatment (B and D) with NSi and SABE by contact residual method, respectively.

Table 1. Median lethal concentration (LC50) of NSi after Test for (%) mortality response of CWSB larvae and eggs to the exposure of NSi.

Exposure Conc. (mg/L)	Total Test larvae	Total No. of Dead larvae	Mortality (%)	LC_{50} (mg/L)
0	30	0	0	257.63
100	30	8	26	
200	30	12	40	
300	30	16	53	
400	30	18	60	
500	30	22	73	
800	30	25	83	
1000	30	28	93	

The data were expressed as mean ± SEM (n = 30). The nonlinear regression analysis determined a LC50 of 257.63 mg/L (95% CI) with a Hill slope of 0.9111, indicating a moderately shallow dose-response curve. Despite a high coefficient of determination (R2=0.9952), the lack-of-fit test revealed a significant P-value of 0.0469.

Table 2. Median lethal concentration (LC50) of NSi after Test for (%) mortality response of CWSB larvae and eggs to the exposure of SABE.

Exposure Conc. (mg/L)	Total Test larvae	Total No. of Dead larvae	Mortality (%)	LC_{50} (mg/L)
0	30	0	0	575.43
100	30	5	16	
200	30	8	26	
300	30	12	40	
400	30	16	53	
500	30	19	63	
800	30	22	73	
1000	30	25	83	

The data were expressed as mean ± SEM (n = 30). The nonlinear regression analysis determined a LC50 of 575.43 mg/L (95% CI), The Hill slope was steeper at 1.387, the coefficient of determination (R2=0.9933) was high, but the lack-of-fit test revealed a significant P-value of 0.0138.

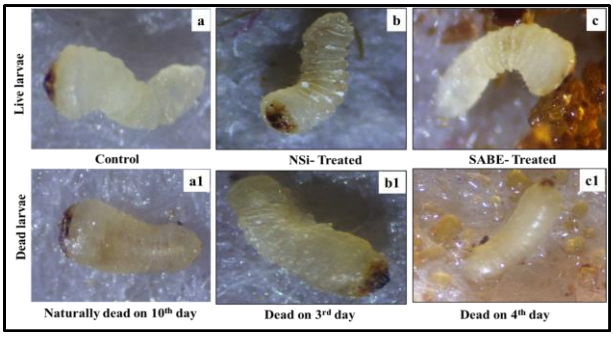

Fig. 9. Contact residual assay: Larvae mortality of fresh neonates observed over 10-day period following treatment with NSi and SABE. Live larvae of pretreatment were denoted as b and c, whereas the dead larvae after treatment were labelled b' and c'. Additionally, untreated larvae that naturally died on the 10th day were represented as a and a'.

Olfactometer assay

In olfactometer assay, the behavior of insects exposed to pesticides can be observed in controlled environments where their responses to odour cues are analysed. Pesticide-treated olfactometers may elicit altered behavioural responses in insects, such as avoidance or attraction, providing insights into the efficacy of the pesticides in influencing insect behaviour. After acclimation, 30 individual beetles were introduced into a Y-tube olfactometer. Preference index (PI) calculations demonstrated contrasting behavior. Cut stems coated with SABE, SABE powder, SABE solution, cut stems coated with NSi, NSi solution, NSi powder, and coffee arabica stem without treatment were used as control stimuli. The cut stem coated with SABE and NSi showed 56.67 % and 76.67 %, SABE and NSi as solution showed 73.34 % and 46.67 %, SABE and NSi powder form showed 50 % and 83.34 % avoidance response, respectively. The coffee arabica stem as a control showed 100% attraction response. Biopesticide treatments with NSi and SABE elicited a significant avoidance response ($P < 0.0001$), indicating that fewer beetles were attracted to the treated arm compared to the control. These results highlight the differing chemosensory sensitivities of the beetles to the two biopesticide treatments.

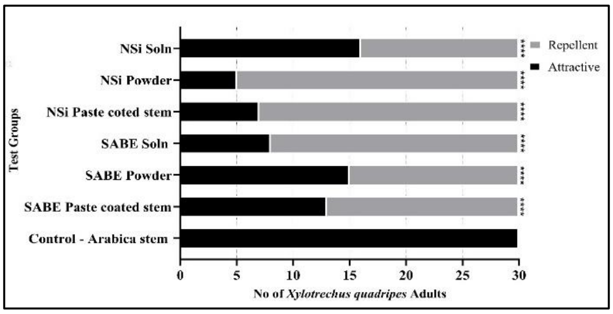

Fig. 10. Olfactory response of *Xylotrechus quadripes*: The test groups include both NSi and SABE in three different application strategies. The data expressed as mean ± SEM (n=30). Chi-square showed highly statistical significance was indicated by asterisks (**$p < 0.05$, **$p < 0.01$, ****$p < 0.001$).

Discussion

The advancements in insect-rearing techniques have significantly enabled detailed research on artificial diets and bioassay methods such as fecundity, fertility, cut stem assays, toxicity assays, and olfactometer assays. Our study utilized these methodologies to explore pest management strategies against coffee white stem borer (CWSB), emphasizing the toxicological and behavioural impacts of Nanosilica (NSi) and *Simarouba amara* bark extract (SABE) as potential biopesticides. Additionally, we looked into the toxicological effects of NSi and SABE as potential bio-pesticides. Silicon has been extensively reported to confer resistance against a wide range of pests and pathogens across various crops. Previous studies have demonstrated their role in enhancing the plant resistance by reducing larval boring efficacy, impairing feeding mechanisms and affecting pest enzymatic activities [38,39,40,20]. Earlier, the external application of silicon (Si) to rice plants has been found to enhance their resistance against various insect pests, including the yellow rice borer (S. incestuous) [40], rice striped stem borer (Chilosuppressalis) [20], sugarcane borer (Diatraea saccharalis) [41], stalk borer (Eldana saccharina) [42], green bug (Schizaphis graminum) [25], brown plant hopper (Nila Parvata lugens) and rice leaf folder (Cnaphalacrocis medinalis). The studies have established a negative correlation between the efficacy of larval boring and the Si concentration in rice plants [35]. Increased Si content in rice plants hinders the entry of Yellow Stem Borer (YSB)

larvae into the stem and also impairs their feeding by weakening their mandibles.

Botanical pesticides, derived from various plant parts, have demonstrated their capacity to alter insect behavior and physiology. For instance, A. indica extracts have been shown to reduce pest incidence and feeding activity [43]. This study showed that the utilization of A. indica extracts led to a reduction of 3.48-4.44% in the incidence of dead hearts disease caused by the rice yellow stem borer (Scirpophaga incertulas). As a result, the larvae eat fewer plant tissues and produce fewer faecal pellets [43,35]. Similarly, the pace at which D. saccharalis bores into two rice genotypes (Cocodrie and XL723) is significantly affected by the addition of Si to the soil [41]. Similarly, our study revealed that SABE significantly affected CWSB mortality and reproductive success, further supporting its potential as a plant-based bio-pesticide. Application of Si in rice crops, for example, has hindered stem borer penetration and feeding by weakening their mandibles, while simultaneously increasing the time required for larval penetration [44]. These findings align with our observations where NSi-treated plants exhibited reduced larval penetration success, corroborating silicon's deterrent effects. The treatment also suppressed the enzymatic activities of acetylcholinesterase, glutathione S-transferases, and cytochrome P450 levels in the midgut of C. suppressalis larvae [44]. Likewise, a reduced boring rate upon the application of Si was reported against Sphaerotheca fuliginea in melon, cucumber, and pumpkin [22]. Consistent with previous studies, the present study also demonstrated lesser boring success of yellow stem borer larvae in the stems of silica-treated plant.

The results of our present study shed light on the major influence of NSi and SABE when introduced into an artificial diet on the mortality rates of *Xylotrechus quadripes* larvae. Both NSi and SABE displayed concentration-dependent patterns, causing increased death rates at higher concentrations in the artificial diet. For NSi, the mortality rates fluctuated from 40% at 1000 mg/L to a peak of 83.33% at 5000 mg/L, compared to a 20% mortality rate in the control group. Similarly, SABE doses demonstrated positive effects on mortality, ranging from 26.66% at 1000 mg/L to a maximum of 73.33% at 5000 mg/L, when compared to the control group (6.66%). This highlights the possible pesticide qualities of both NSi and SABE, underlining their efficacy in pest management. The mortality rate of X. quadripes larvae increased with higher concentrations of these bioactives in artificial diet, reaching the maximum efficacy at 5000 mg/L. LC50 values of 257.63 mg/L for NSi and 575.43 mg/L for SABE indicate high toxicity levels even at relatively low concentrations. The effect of NSi and SABE on the reproductive capabilities of CWSB was found to be very significant. The average number of eggs laid on NSi- and SABE-treated papers was significantly lower (3–4 and 18, respectively) compared to 25–35 eggs in the control group. This reduction in egg-laying capability indicates that both NSi and SABE substances can disrupt the reproductive cycle of CWSB, and can contribute to long-term pest population control. Furthermore, the cut stem assay revealed that the larvae had lower penetration success in treated stems as compared to control. NSi was particularly highly effective, with fewer larvae successfully penetrating the treated stems, suggesting a potent deterrent effect. The olfactometer assays provided insights into the behavioural responses of adult beetles to NSi and SABE. The results indicated varied chemosensory sensitivities, with beetles showing avoidance behaviours when exposed to treated surfaces. This avoidance response suggested that these biopesticides can function as repellents and thereby protecting coffee plants from infestation.

The strength of our study lies in its comprehensive approach, evaluating not just the mortality but also the reproductive and behavioural aspects of CWSB. Yet, there is a need to look into the underlying mechanism of reported mortalities and determine the ideal doses for successful pest management with minimal environmental impact. The varied chemosensory sensitivities of beetles to NSi and SABE, as revealed by the olfactometer experiment, highlights the need for a detailed study of insect responses to these biopesticides. The comprehensive approach of our study in evaluating mortality, reproductive output, larval penetration, and behavioural responses, provided a complex understanding of the pesticide properties of NSi and SABE. However, further investigation is warranted to elucidate the underlying mechanisms of toxicity, optimize concentrations for field applications and assess potential ecological impacts. The suppression of enzymatic activities observed in other studies suggests

the need to explore biochemical pathways affected by these bioactives [44]. Additionally, studies on long-term ecological consequences and the development of intelligent regulatory measures are crucial for sustainable pest management.

Overall, our study attempted to evaluate the potential of NSi and SABE in pest management approaches against CWSB. The concentration-dependent impact on mortality response, reproductive output, larvae penetration, and modified chemosensory sensitivities provide a full grasp of the pesticide properties of NSi and SABE. The significance of our study lies in its contribution to the development of an effective and ecologically conscious pest control measures. However, as stated above, there is a need to understand the detailed mechanism of the biopesticide action and the likely ecological repercussions of its widespread use. Future research should delve into these factors, examining the ecological ramifications and refining concentrations for practical applications. The studies also should address the necessity of intelligent regulatory measures to balance the benefits of pest management with the potential environmental risks associated with NSi and SABE exposure.

In conclusion, the present study establishes NSi and SABE as promising candidates for eco-friendly pest control strategies against *Xylotrechus quadripes*. The effect of these on multiple life cycle stages of the pest, combined with their potential to reduce reliance on synthetic pesticides, underscore their importance in integrated pest management programs. Researchers and agricultural practitioners must collaborate to refine these findings for practical applications while ensuring minimal environmental impact. The outreach and education initiatives are indeed essential to promote the adoption of such biopesticides among farmers and stakeholders, paving the way for sustainable and effective pest control solutions. In light of these findings, it is crucial for policymakers, researchers, and agricultural practitioners to collaboratively work towards the development and implementation of sustainable pest control measures. Additionally, outreach and educational initiatives can help to disseminate the knowledge generated by the study, fostering awareness among farmers and stakeholders about the potential benefits and risks associated with the use of NSi and SABE in pest control.

Conflict of interest

Authors declare there is no conflicts of interest.

Funding Declaration

This research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

Author Contributions

Raghu A. M. conceptualized the study, designed, conducted the experiments and developed the manuscript. Seetharama H. G. facilitated access to the entomology laboratory and coordinated necessary permissions with the Director of CCRI. Krishna Reddy, M. S. Uma, and Roobak Kumar assisted in troubleshooting experimental challenges. Thippeswamy N. B. contributed by drafting official letters for obtaining research permissions and provided input on manuscript revision. Rajeshwara Achur assisted in refining and revising the manuscript. All authors reviewed and approved the final version of the manuscript.

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