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Protein Expression Dynamics in *Xylotrechus quadripes* Larvae Reared on Artificial Diets Treated with *Simarouba amara* Bark Extract: A Model for Controlling Infestation on *Coffea arabica*

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KEYWORDS

Proteomics, *Simarouba amara* bark extract *Xylotrechus quadripes, Coffea arabica,* differentially expressed proteins (DEP).

ABSTRACT:

The Coffee White Stem Borer (*Xylotrechus quadripes*), a destructive pest of *Coffea arabica*, causes extensive damage to coffee plantations and threatens sustainable production. Conventional chemical pesticides offer limited long-term control and pose environmental risks, prompting the search for eco-friendly alternatives. This study investigates the insecticidal efficacy of *Simarouba amara* bark extract (SABE) when incorporated into artificial diets fed to *X. quadripes* larvae. The effects of SABE on larval growth, survival, and proteomic response were evaluated. Larvae reared on SABE-treated and control diets were subjected to protein extraction and analysis using Orbitrap High-Resolution Liquid Chromatography–Tandem Mass Spectrometry (OHRLC-MS/MS). Subsequent bioinformatics analysis was performed to identify differentially expressed proteins (DEPs) and their associated biological functions and metabolic pathways. SABE treatment significantly reduced larval survival compared to the untreated control. Proteomic analysis revealed the expression of 15 unique proteins in SABE-fed larvae versus 22 in the control group. Functional enrichment analysis of DEPs indicated the activation of detoxification-related proteins, including cytochrome P450s and glutathione-S-transferases, along with proteins involved in stress response and metabolic regulation. These changes suggest that SABE induces physiological stress and disrupts key metabolic processes, ultimately leading to larval mortality. The findings highlight the potential of *S. amara* bark extract as a botanical insecticide targeting *X. quadripes*. By interfering with essential molecular pathways, SABE offers a promising, biodegradable alternative to conventional chemical pesticides. This study provides a molecular foundation for the development of sustainable pest management strategies in coffee cultivation, contributing to the reduction of pesticide use and environmental impact.

1. Introduction

Coffea arabica (Arabica) and *Coffea canephora* (Robusta) are two major commercial coffee species cultivated globally. India ranks as the seventh-largest coffee producer worldwide [1], with Arabica and Robusta grown primarily in the southern states. Arabica, a member of the Rubiaceae family, is known for its superior quality but is more susceptible to pests and environmental stress. Since 1950, the area under Robusta cultivation in India has increased by over 80% [2], largely replacing Arabica farms that have been severely affected by pest infestations.

Indian coffee plantations follow complex agroforestry systems that integrate native and exotic shade tree species [3], promoting biodiversity but also providing a habitat for insect pests. The International Union for Conservation of Nature (IUCN) Red List classifies wild C. arabica as an endangered species, primarily due to forest conversion into coffee plantations. Among the most damaging pests of Arabica is the coffee white stem borer (*Xylotrechus quadripes*), a longhorn beetle (Cerambycidae) that causes annual crop losses estimated at USD 17–40 million [4]. Climate change has further contributed to the pest's distribution and population growth [5].

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Chemical pesticides have been widely used to manage X. quadripes, but with inconsistent results and harmful environmental consequences. Excessive pesticide use negatively impacts beneficial insects and ecological balance, prompting a shift toward sustainable alternatives. Botanical insecticides are particularly promising due to their larvicidal, pupicidal, and oviposition-inhibiting properties, as well as their biodegradability and low toxicity to non-target organisms [6,7,8].

The Simaroubaceae family comprises about 32 genera and 250 species of shrubs and trees, many of which possess bioactive secondary metabolites. Notably, Simarouba amara Aubl. has demonstrated insecticidal and repellent effects against species such as Aedes aegypti larvae and leaf-cutting ants (Atta sexdens rubropilosa) [9]. Its bioactive compounds including quassinoids, triterpenoids, alkaloids, and coumarins are associated with significant insecticidal activity [10,11,12]. These compounds interfere with insect hormonal signalling, digestion, and reproduction.

Although the pesticidal potential of S. amara is documented, little is known about its molecular impact on pest physiology. Proteomic analysis enables the identification of differentially expressed proteins (DEPs) that mediate the toxic effects of botanical extracts at the molecular level. Artificial diet systems provide a controlled environment to study the physiological responses of X. quadripes larvae.

In this study, we investigated the effects of Simarouba amara aqueous bark extract incorporated into artificial diets on the growth, survival, and protein expression of X. quadripes larvae. Using Orbitrap High-Resolution Liquid Chromatography–Tandem Mass Spectrometry (OHRLC-MS/MS) and Gene Ontology (GO)-based bioinformatics, we identified DEPs associated with toxicity, offering insights into the molecular mechanisms underlying the larvicidal activity of SABE.

2. Materials and Methods

Collection and Rearing of *X. quadripes* Larvae

For the rearing experiments, the larvae were collected from the Central Coffee Research Institute (CCRI), Balehonnur, Chikmagalur, Karnataka, India (13°22′8.89″ N, 75°25′24.92″ E). Infested stems were stored in the insectary before April-May and October-December natural fly periods. As a result, these adults were coupled, housed in small plastic containers for mating, and encouraged to lay eggs as per the protocol described by [13]. The insects have been successfully reared in the laboratory from the egg to adulthood on artificial food, according to [14].

Preparation of Artificial Diets with SABE

Arabica stems (var. Chandragiri) were flaked and used to prepare the artificial diet. *Simarouba amara* aqueous bark extract (SABE) was incorporated into the diet as the treatment, while an untreated diet served as the control. After preparation, the surface of the diet was punctured using a sterile dissecting needle to facilitate larval entry. Newly hatched *Xylotrechus quadripes* larvae were carefully transferred to the diet using a sterile brush. The containers were sealed and placed in a BOD incubator maintained at a constant temperature of 28°C and relative humidity of 50–60% [15]. Larvae were monitored for feeding behavior and survival.

Protein Extraction

Xylotrechus quadripes larvae fed on SABE-treated and control artificial diets were sampled, flash-frozen in liquid nitrogen, and stored at -80 °C until protein extraction. Each larval sample was individually ground in liquid nitrogen and sonicated three times on ice using a lysis buffer containing 6 M urea, 2 M thiourea, 0.025 M Tris-HCl, 1% SDS and 1% protease inhibitor cocktail [16]. The homogenate was then centrifuged at 20,000 × g for 10 minutes at 4 °C. The supernatant was collected, and total protein concentration was determined using the BCA assay.

For digestion, proteins were reduced with 10 mM DTT at 37 °C for 1 hour and alkylated with 20 mM IAA at 25 °C for 45 minutes. The sample was then diluted with 100 mM triethylammonium bicarbonate (TEAB) until the urea concentration was reduced below 2 M. Trypsin was added at a 50:1 (protein: enzyme) ratio and digestion was carried out overnight. A second round of digestion was performed using a 100:1 ratio for 4 hours to ensure complete cleavage. [16]. Mass spectrometric analysis of the peptide mixtures was performed using a Q-Exactive Plus Biopharma Orbitrap High-Resolution Liquid Chromatography Mass Spectrometer (O-HRLCMS), Thermo Scientific. The analysis was carried out at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay (IIT Bombay), India.

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Proteomic Analysis using Orbitrap HRLC-MS/MS

Tryptic peptides derived from *Xylotrechus quadripes* larvae fed on SABE-treated and control diets were fractionated using an Acclaim PepMap 100 nanoviper column (100 µm × 2 cm). Peptide separation was performed over a 120-minute gradient using acetonitrile at pH 9. The resulting peptide fractions were dried by vacuum centrifugation and subjected to tandem mass spectrometry (LC-MS/MS).

Analysis was conducted on a Q-Exactive Plus Biopharma Orbitrap mass spectrometer (Thermo Scientific). The mobile phase consisted of solvent A (0.1% formic acid in Milli-Q water) and solvent B (85:15 acetonitrile: Milli-Q water with 0.1% formic acid). An 8 µL peptide sample was injected at a flow rate of 5 µL/min, followed by sample loading at 12 µL/min, ensuring pressure did not exceed 750 bar. A gradient elution profile was applied: beginning at 2% B, ramping to 45% B by 100 minutes, increasing to 95% B at 105 minutes, and returning to 2% B by 120 minutes. Pre-column equilibration was carried out for 5 minutes with 2% B at a flow rate of 300 nL/min. Analytical separation was performed using a PepMap RSLC C18 column (2 µm, 100 Å, 50 cm), with a maximum system pressure of 700 bar.

For full MS acquisition, the parameters included: resolution of 70,000, AGC target of 2×10^5, maximum injection time of 120 min, 15 loop counts, 1 MSX count, TopN of 15, and an isolation window of 1.2 m/z. Higher-energy collisional dissociation (HCD) was applied at 27 eV. Data acquisition was in centroid mode with dynamic exclusion enabled (minimum AGC target 1.2×10^2, intensity threshold 1.0×10^3, peptide match enabled, isotopes excluded). The instrument was operated in positive polarity mode with lock masses set at 445.12003 m/z. Instrument performance was validated using the Nitrosamine_12062023.mstune tuning file [18].

Database search

Mass spectrometry data processing was carried out using Thermo Scientific Xcalibur software (version 4.2.28.14). Tandem mass spectra (MS/MS) were searched against two protein databases: *Xylotrechus quadripes* (*Uniprotkb_Xylotrechus_quadripes_2023_10_13. fasta*) and the closely related species *Agrilus planipennis* (*Uniprotkb_Agrilus_planipennis_2024_01_04. fasta*), which exhibits similar boring behavior. To ensure stringent identification, both databases were concatenated with a reverse decoy database for false discovery rate (FDR) control. Protein identification was performed using trypsin/P as the cleavage enzyme, allowing up to two missed cleavages and a maximum of five modifications per peptide. The mass tolerance was set to 5 ppm for precursor ions in both the first and main searches, and 0.02 Da for-fragment ions. Carbamidomethylation of cysteine residues was specified as a fixed modification, while oxidation of methionine and N-terminal acetylation were set as variable modifications. Peptide and protein identifications were filtered to maintain a 1% FDR threshold, ensuring high-confidence protein detection.

3. Results

A quantitative proteomic analysis was conducted using OHRLC-MS/MS to investigate the differential protein expression in *Xylotrechus quadripes* larvae reared on artificial diets supplemented with *Simarouba amara* bark extract (SABE) compared to a control diet. Protein identification was initially performed using a species-specific database (*Uniprotkb_Xylotrechus_quadripes_2023_10_13. fasta*), which yielded a total of 11 proteins in both SABE-treated and control groups (Fig. 1). The limited number of identifications suggested a relatively incomplete annotation of the *X. quadripes* proteome. To enhance protein discovery and gain deeper insights into larval proteomic responses, the same MS/MS spectra were re-analyzed using a comprehensive proteome of a closely related xylophagous insect, *Agrilus planipennis* (*Uniprotkb_Agrilus_planipennis_2024_01_04. fasta*) This analysis revealed an increased number of identified proteins: 22 in the control group and 15 in the SABE-treated group. The enhanced detection with the *A. planipennis* database reflects the evolutionary proximity and shared ecological niche between the two species, thereby providing improved coverage and annotation of homologous proteins. Proteins marked with the symbol "#" in Fig. 1 represent those that were not detected using the *X. quadripes* database but were successfully identified using the *A. planipennis* database. This discrepancy underscores the limitations of under annotated species-specific databases and highlights the importance of leveraging high-quality reference databases from phylogenetically related species in proteomic studies involving non-model organisms. The proteomic shifts

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observed in the SABE-treated group suggest potential alterations in larval physiological processes, possibly associated with stress response, detoxification, or metabolic disruption. These findings align with the hypothesis that SABE treatment modulates specific protein expression patterns, contributing to the insecticidal activity of the extract. The differential protein expression profile observed here provides valuable molecular insights into the mode of action of SABE and supports its role as a botanical agent for the management of *X. quadripes*. The protein coverage and molecular weight distribution of identified proteins in SABE-treated and control groups of *X. quadripes*. The SABE-treated group exhibited a higher proportion of proteins with greater peptide coverage compared to the control group, indicating enhanced protein identification. The distribution of proteins across molecular weight classes revealed that most proteins ranged between 10 and 70 kDa. The SABE treatment resulted in a broader and more even distribution across molecular weights, suggesting improved protein extraction and solubilization. Overall, SABE treatment enhanced the quality and depth of proteomic analysis in *X. quadripes* (Fig.2).

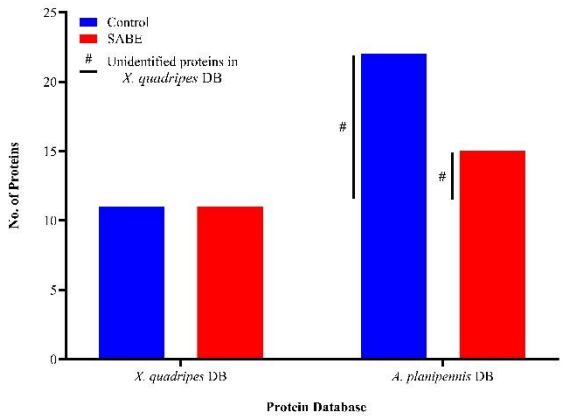

Fig. 1. Number of proteins identified by OHRLC-MS/MS analysis in *Xylotrechus quadripes* larvae reared on artificial diets, searched against two protein databases: *X. quadripes* and *A. planipennis*. The "#" symbol indicates proteins that were not identified using the *X. quadripes* database but were detected using the *A. planipennis* database.

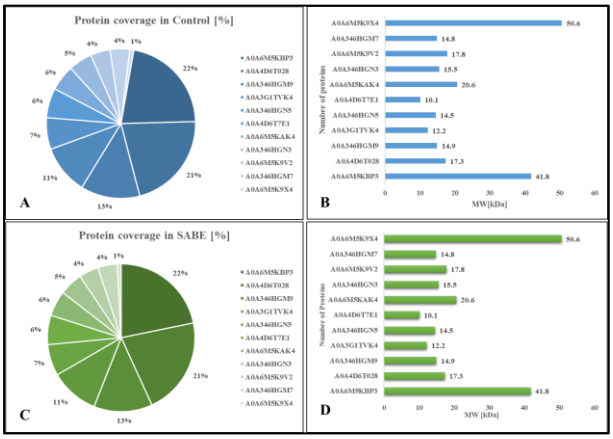

Fig. 2. Protein coverage and molecular weight distribution of identified proteins compared with X. quadripes database: (A, C) Coverage of proteins by identified peptides in SABE-treated and control groups; (B, D) Distribution of identified proteins across different molecular weight (kDa) classes.

Table 1: Proteins expressed in *X. quadripes* larvae after feeding an untreated artificial diet (Control).

Accession No	Protein Name
A0A6M5KBP3	Actin OS=*X. quadripes* OX=554073 PE=2 SV=1
A0A4D6T028	Uncharacterized protein (Fragment) OS=*X. quadripes* OX=554073 PE=2 SV=1
A0A346HGM9	Odorant binding protein 7 OS=*X. quadripes* OX=554073 GN=OBP7 PE=2 SV=1
A0A3G1TVK4	3-phosphoinositide-dependent protein kinase 2-like (Fragment) OS=Coffea canephora OX=49390 PE=2 SV=1
A0A346HGN5	Odorant binding protein 13 OS=*X. quadripes* OX=554073 GN=OBP13 PE=2 SV=1
A0A4D6T7E1	Histone H3 (Fragment) OS=Coffea canephora OX=49390 PE=2 SV=1

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A0A6M5KAK4	Tubulin alpha chain (Fragment) OS=*X. quadripes* OX=554073 PE=2 SV=1
A0A346HGN3	Odorant binding protein 11 OS=*X. quadripes* OX=554073 GN=OBP11 PE=2 SV=1
A0A6M5K9V2	Ubiquitin-40S ribosomal protein S27a OS=*X. quadripes* OX=554073 GN=RPS27a PE=2 SV=1
A0A346HGM7	Odorant binding protein 5 OS=*X. quadripes* OX=554073 GN=OBP5 PE=2 SV=1
A0A6M5K9X4	Elongation factor 1-alpha (Fragment) OS=*X. quadripes* OX=554073 GN=EF1a PE=2 SV=1

Table 2: Proteins expressed in *X. quadripes* larvae that succumbed after feeding on an artificial diet treated with *S. amara* bark extract (SABE).

Accession No	Protein Name
A0A6M5KBP3	Actin OS=*X. Quadripes* OX=554073 PE=2 SV=1
A0A4D6T028	Uncharacterized protein (Fragment) OS=*X. quadripes* OX=554073 PE=2 SV=1
A0A346HGM9	Odorant binding protein 7 OS=*X. Quadripes* OX=554073 GN=OBP7 PE=2 SV=1
A0A3G1TVK4	3-phosphoinositide-dependent protein kinase 2-like (Fragment) OS=Coffea canephora OX=49390 PE=2 SV=1
A0A346HGN5	Odorant binding protein 13 OS=*X. Quadripes* OX=554073 GN=OBP13 PE=2 SV=1
A0A4D6T7E1	Histone H3 (Fragment) OS=Coffea canephora OX=49390 PE=2 SV=1
A0A6M5KAK4	Tubulin alpha chain (Fragment) OS=*X. quadripes* OX=554073 PE=2 SV=1
A0A346HGN3	Odorant binding protein 11 OS=*X. Quadripes* OX=554073 GN=OBP11 PE=2 SV=1
A0A6M5K9V2	Ubiquitin-40S ribosomal protein S27a OS=*X. Quadripes* OX=554073 GN=RPS27a PE=2 SV=1
A0A346HGM7	Odorant binding protein 5 OS=*X. Quadripes* OX=554073 GN=OBP5 PE=2 SV=1
A0A6M5K9X4	Elongation factor 1-alpha (Fragment) OS=*X. Quadripes* OX=554073 GN=EF1a PE=2 SV=1

Differentially Expressed Proteins in *X. quadripes* Larvae Treated with SABE

Proteomic analysis of *Xylotrechus quadripes* larvae treated with *Simarouba amara* bark extract (SABE) revealed distinct alterations in protein expression profiles compared to untreated controls. Mass spectrometric data showed several unique peptide peaks and significant shifts in peak intensities in the SABE-treated group (Fig. 6), indicating differential protein expression in response to the treatment. A total of 15 proteins were identified as differentially expressed, with 11 upregulated (Table.1 and Table 2). Gene Ontology (GO) enrichment analysis revealed that the DEPs were predominantly associated with biological processes including oxidative stress response, detoxification, and hormone-mediated signaling. Notably, cytochrome P450 monooxygenases and glutathione S-transferases were significantly upregulated in the SABE group. Additionally, proteins related to cellular stress response and hormone biosynthesis pathways were differentially expressed. Several structural and metabolic proteins involved in energy production, including [insert example proteins], were either absent or significantly downregulated in

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SABE-treated larvae. The absence or downregulation of structural and energy metabolism proteins in the SABE group further indicates compromised physiological integrity and energy balance. Overall, the findings demonstrate that SABE induces a targeted molecular stress response in *X. quadripes*, disrupting key physiological pathways. This provides a mechanistic understanding of how SABE exerts its insecticidal effect and supports its potential as a sustainable, botanical-based control agent for managing coffee white stem borer infestations (Fig.3, Fig. 4 and Fig. 5).

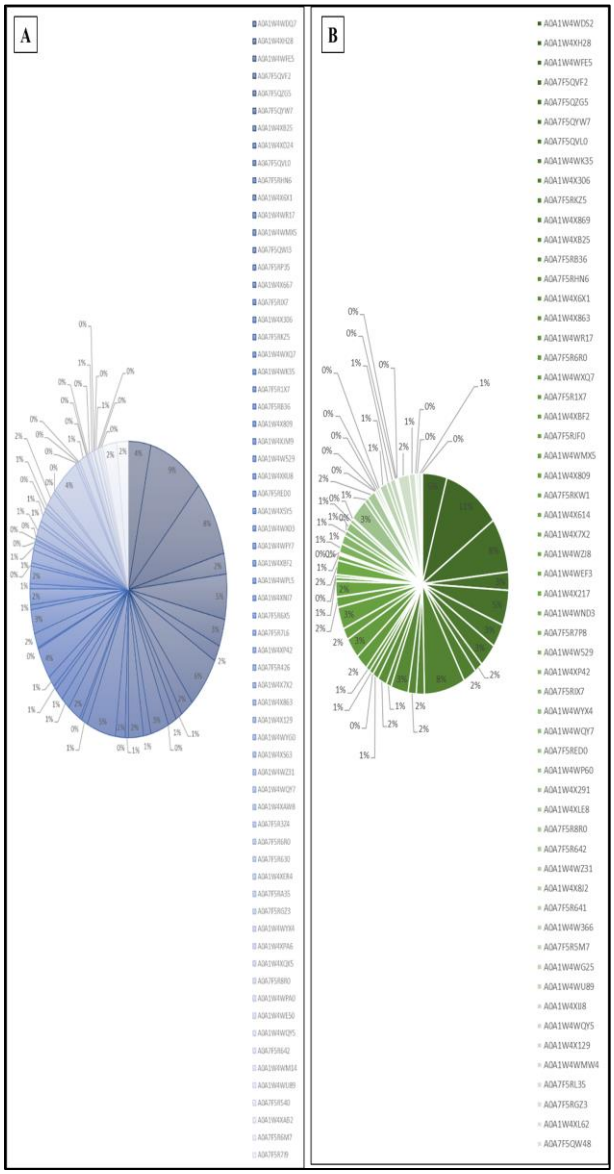

Fig. 3. Protein coverage and molecular weight distribution of identified proteins compared with *Agrilus planipennis* database: Coverage across (A) Control and (B) SABE treatments.

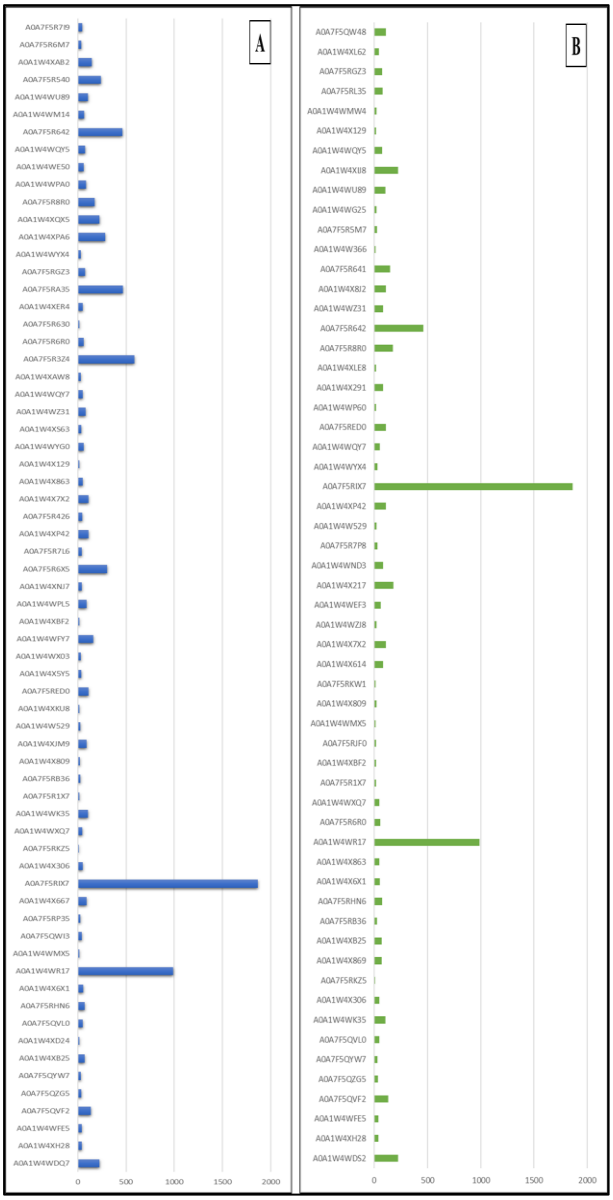

Fig. 4. Molecular weight of identified protein: Distribution of identified proteins among different molecular weight classes (kDa) compared with *A. planipennis* database ((A) Control and (B) SABE).

Bioinformatics Based Functional Analysis of DEPs

Bioinformatics analysis of the differentially expressed proteins (DEPs) revealed that *Xylotrechus quadripes* larvae treated with *Simarouba amara* bark extract (SABE) exhibited distinct molecular responses compared to the untreated control group. Functional

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enrichment through Gene Ontology (GO) and STRING network analysis demonstrated that the 15 proteins uniquely expressed in the SABE group were predominantly associated with signal transduction, oxidative stress response, enzyme inhibition, and immune regulation (Fig. 7,8).

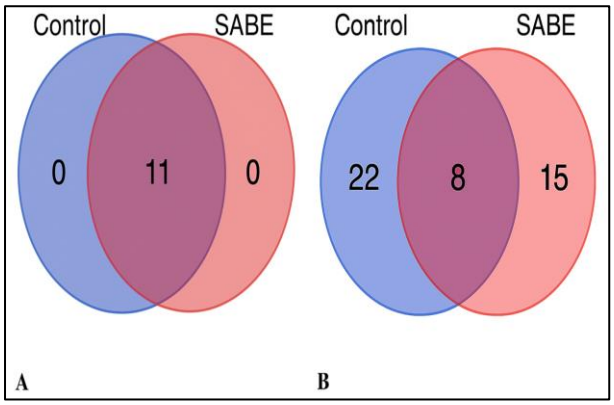

Fig.5. The differential protein expression in *Xylotrechus quadripes* larvae treatment with SABE: (A) Shared expression of 11 proteins with no uniquely induced proteins in SABE group; (B) Expression of 15 unique proteins in SABE group and 8 shared with control, indicating SABE-induced proteomic changes.

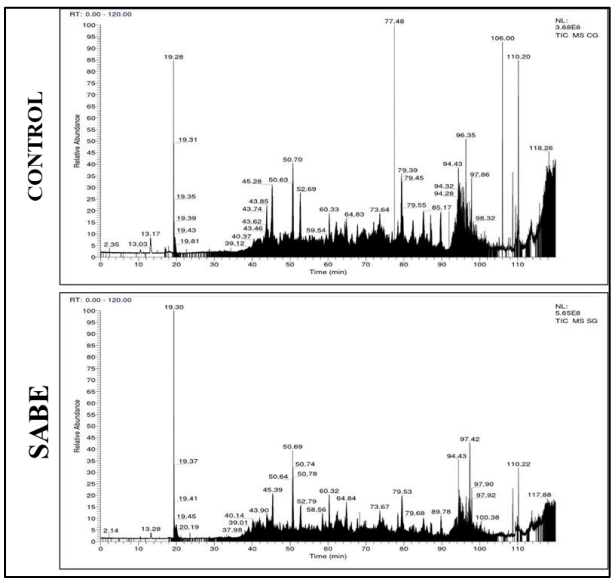

Fig. 6. OHRLCMS/MS chromatograms of protein extracts from *X. quadripes* larvae: Comparison between control and SABE-treated groups showing differences in peptide peak intensity and pattern.

Among these, several proteins were linked to kinase-mediated signaling pathways, suggesting that SABE bioactive compounds may interfere with phosphorylation-dependent regulatory processes within larval cells. Enrichment of detoxification-related proteins, including members of the cytochrome P450 family and glutathione-S-transferases, indicates that the larvae were activating defense mechanisms to metabolize or eliminate the xenobiotic compounds present in the extract.

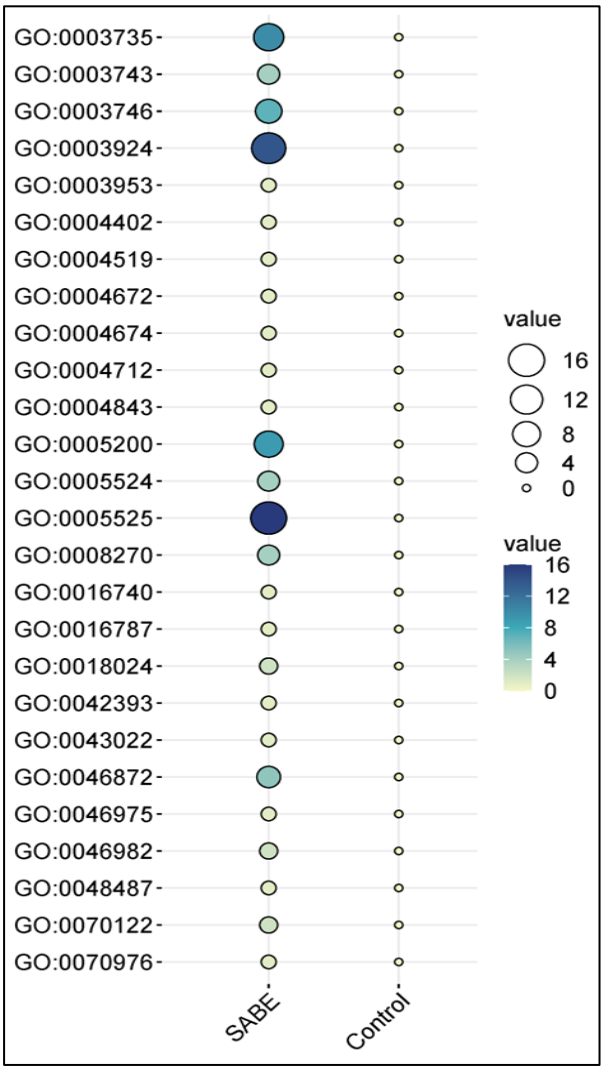

Fig. 7. GO-based enrichment analysis of differentially expressed proteins (DEPs): Functional classification of DEPs identified in SABE-treated and control groups, highlighting unique and shared molecular functions.

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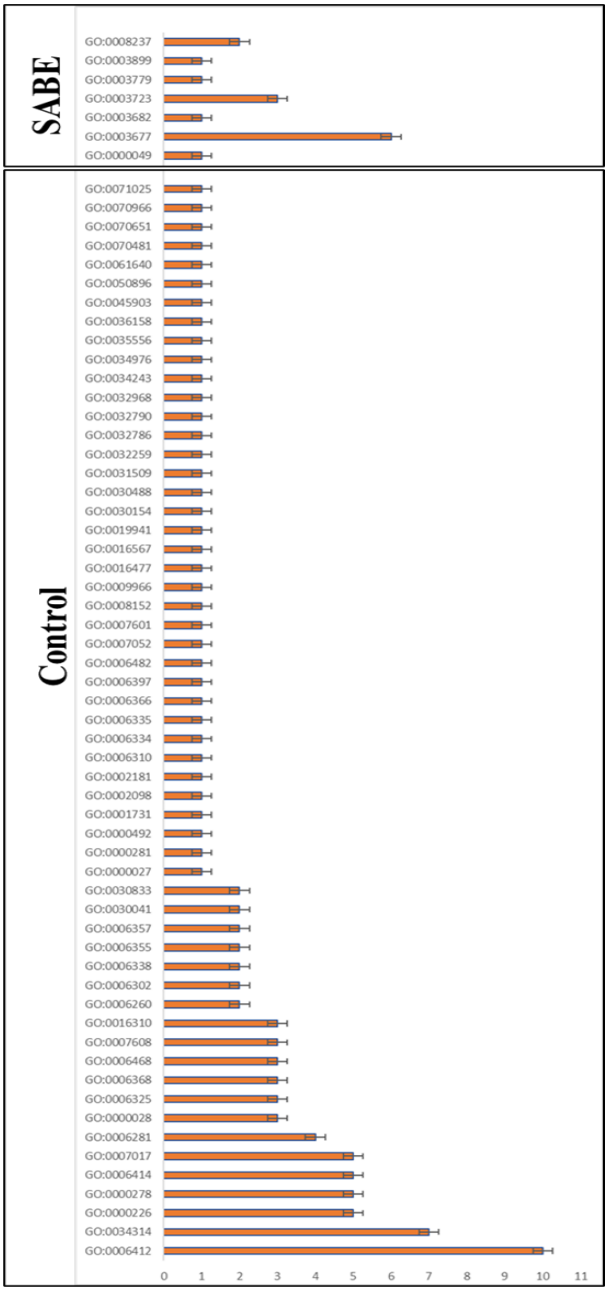

Fig. 8. Enrichment analysis of the DEPs in SABE treated and Control group: A GO-based enrichment analysis of DAVID provided unique functions of DEPs distinguished are Control (57) and SABE (7) treatments.

Additionally, proteins involved in immune regulation and protein degradation pathways were identified, implying that SABE disrupts larval immune balance and cellular protein homeostasis. These molecular alterations are consistent with the known bioactivity of SABE's phytochemicals, such as quassinoids and alkaloids, which are known to impair insect metabolism and development. In contrast, the control group exhibited only baseline expression of proteins with limited pathway enrichment, reflecting normal physiological status without external chemical stress. Collectively, these results show that SABE exposure induces targeted molecular stress responses rather than broad systemic disruption. This focused proteomic shift underscores SABE's potential as a selective botanical insecticide, capable of modulating key physiological pathways in *X. quadripes* without the broader collateral effects often observed with synthetic chemicals.

Discussion

This study investigated the insecticidal potential of Simarouba amara bark extract (SABE) against *Xylotrechus quadripes* larvae, combining bioassays with proteomic analysis to explore the underlying molecular responses. SABE treatment led to reduced larval survival and significant changes in protein expression profiles, providing insight into its biochemical mode of action and potential role as a sustainable botanical pesticide for managing Coffee White Stem Borer (CWSB) in Coffea arabica plantations.

Larval Mortality and Molecular Stress Responses

SABE-treated larvae exhibited increased mortality compared to the control group, supporting earlier findings on the larvicidal activity of Simarouba species. Previous studies have attributed this effect to quassinoids and alkaloids, which interfere with insect hormonal signaling, metabolism, and immune responses [10, 20]. The mortality observed in this study reinforces SABE's potential to act as an effective biopesticide, likely through multi-target disruption of physiological processes.

Proteomic Insights and Functional Pathway Disruption

Proteomic profiling revealed 15 unique proteins in the SABE-treated larvae, in contrast to 22 in the control group. Functional enrichment analysis showed that SABE-induced proteins were primarily associated with oxidative stress response, signal transduction, immune regulation, and enzyme inhibition. This suggests that SABE exposure prompts a targeted biochemical stress response, compelling the larvae to activate

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detoxification pathways to neutralize or metabolize bioactive plant compounds. Upregulation of detoxification-related proteins such as cytochrome P450s and glutathione-S-transferases points to metabolic efforts by the larvae to mitigate toxicity. The activation of oxidative stress-related pathways indicates ROS (reactive oxygen species) generation, a known effect of phytochemical exposure. Additionally, the presence of proteins involved in kinase-regulated signaling and protein degradation suggests interference with normal cell signaling and protein turnover [21].

Disruption of Sensory and Structural Functions

Odorant binding proteins (OBPs), including OBP7, OBP11, and OBP13, were differentially expressed in the SABE group, indicating that larval olfactory function may be compromised. OBPs are essential for host detection, mating behavior, and environmental sensing; their altered expression can significantly disrupt the insect's ability to locate host plants, contributing to behavioral suppression and reduced fitness. Similarly, the downregulation of structural proteins such as actin and tubulin in SABE-fed larvae suggests damage to cytoskeletal components or inhibition of structural protein synthesis. This may impair cell integrity and mobility, further compromising larval development and survival [22, 23,24].

Impact on Energy Metabolism

SABE also influenced proteins associated with energy metabolism, including ATP synthase and elongation factor 1-alpha. Disruption in these proteins indicates a disturbance in cellular energy production and protein translation, which could result in energy depletion and hinder critical physiological processes needed for survival and metamorphosis. [25, 8].

Implications for Sustainable Pest Management

The findings from this study support the use of SABE as a promising botanical insecticide with multiple modes of action. Unlike conventional chemical pesticides, SABE appears to selectively target key metabolic and physiological pathways in X. quadripes, minimizing environmental risks and potential harm to non-target organisms. Its bioactive compounds offer the potential for development into formulations aligned with Integrated Pest Management (IPM) principles, promoting sustainability and reducing dependence on synthetic pesticides. By disrupting larval survival through molecular and physiological mechanisms, SABE offers an eco-friendly alternative capable of contributing to long-term pest suppression in coffee agroecosystems. Its specificity, biodegradability, and origin from a plant-based source enhance its suitability for organic and sustainable agriculture [7].

Future Directions

To build upon these findings, further research should explore:

- Field evaluation of SABE efficacy under natural infestation conditions.
- Characterization of key regulatory proteins or biomarkers as targets for monitoring or improving SABE formulations.
- Assessment of ecological safety, including its effects on pollinators, beneficial insects, and soil microbiota.
- Optimization of extraction, formulation, and delivery methods for practical, scalable field application.

Conclusions

Simarouba amara bark extract demonstrates strong potential as a natural, eco-friendly insecticidal agent against *Xylotrechus quadripes*. The proteomic and bioinformatic data reveal that SABE disrupts multiple physiological pathways ranging from detoxification and sensory perception to structural integrity and energy metabolism ultimately leading to larval mortality. This multifaceted action profile makes SABE a valuable candidate for inclusion in IPM programs aimed at managing coffee white stem borer infestations. Its deployment can support sustainable coffee cultivation while reducing the environmental burden of synthetic pesticides. Continued research on SABE will help refine its application and confirm its long-term effectiveness and safety in real-world agricultural settings.

Conflicts of interest

The authors claim to have no conflicting interests.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims

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relating to the content of this article will be borne by them.

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Ethical Declaration

Not applicable.

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