

Green Extracts of *Nelumbo nucifera* Gaertn. (Lotus): Promising Alternatives for *Spodoptera litura* (Fab.) Management through Chemical Profiling and Insecticidal Evaluation

P. Srinivasan

srinibiotech92@gmail.com

Saveetha Institute of Medical and Technical Sciences (Deemed to be University) Saveetha Dental College: SIMATS Deemed University Saveetha Dental College <https://orcid.org/0000-0002-5694-0929>

Natesan Krishnamurthy

Selvamm Arts and Science College

Kannan Revathi

Muthayammal College of Arts and Science

Sami Al Obaid

King Saud University College of Science

Hossam M. Aljawdah

King Saud University College of Science

Prasath Ramasamy Chandrasekaran

National Kaohsiung University of Science and Technology - Nanzih Campus

Research Article

Keywords: Lotus extracts, Agriculture pests, Lepidopteran, n-Hexadecanoicacid, Larvicide, Non-targets

Posted Date: November 26th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4996261/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Waste and Biomass Valorization on January 11th, 2025. See the published version at <https://doi.org/10.1007/s12649-024-02857-x>.

Abstract

This study delves into the potential insecticidal properties of *Nelumbo nucifera* (Lotus) against the cutworm *Spodoptera litura* L., a significant agricultural pest. Analysis of the chemical profile of *N. nucifera* revealed that the peak area was highest for 6a- β -Aporphine, 1,2-dimethoxy- (33.79%), followed by n-Hexadecanoic acid (21.14%). Notably, larval mortality was most pronounced at the peak dosage of *N. nucifera* (2.5 mg/L) on the second (96%), third (87%), and fourth (85%) instars. Additionally, sub-lethal dosages of *N. nucifera* (1.5 milligrams per liter) significantly hindered the growth of *S. litura* by extending the larval and pupal durations while also reducing fecundity and Oviposition rates in a dose-dependent manner. Furthermore, exposure to sub-lethal dosages of *N. nucifera* led to both inhibition and upregulation of major enzymes (esterases, CYP450 and GST). Severe damage as well as dysregulation of midgut tissues were observed in fourth instars of *S. litura* exposed to *N. nucifera* (2.5 milligrams per liter). Moreover, our results indicate that *N. nucifera* extracts pose no harm to soil earthworms and beneficial honey bees, as confirmed through in vitro assays and *in silico* predictions, respectively. Overall, these findings offer promising prospects for the development of new bio-based agents for targeting and managing the lepidopteran pest *S. litura*.

1. Introduction

Plant-derived materials have gained significant attention in recent years as viable alternatives to synthetic pesticides in managing agricultural pests [1]. These materials, often derived from botanical sources, offer several advantages, including decreased environmental effect, lower toxicity to non-target organisms, as well as potential compatibility with organic farming practices [2–4]. The study of natural compounds for their potential insecticidal properties has garnered significant interest in recent years, driven by the need for sustainable and eco-friendly pest management solutions [5–6]. In general, botanical insecticides are less persistent in the environment than synthetic pesticides, which lowers the possibility of bioaccumulation and long-term ecological harm [7–8]. Plant-derived products have several advantages, however their effectiveness varies according on pest species, application techniques, and environmental circumstances [9–10]. Standardization of botanical extracts and formulations is essential to ensure consistent efficacy and minimize risks to non-target organisms [11]. Their diverse modes of action, lower environmental impact, and compatibility with sustainable farming practices make them valuable components of integrated pest management strategies aimed at reducing reliance on conventional chemical pesticides [12].

Spodoptera litura (Fab.), generally referred to as the Cotton leafworm or tobacco cutworm, is a highly destructive polyphagous insect pest notorious for its wide host range, which includes crops like cotton, tobacco, maize, soybean, and various vegetables [4, 13]. Its voracious feeding habits and rapid reproduction make it a significant threat to agricultural production, causing substantial economic losses globally [14]. Historically, conventional chemical insecticides have been the cornerstone of *S. litura* control strategies [15–16]. However, overreliance on these synthetic insecticides has raised worries about contamination in the environment, adverse impact on organisms not the target, and the

advancement of insecticide resistance among pest populations [17]. Consequently, there is a growing imperative to explore sustainable alternatives to synthetic pesticides for managing *S. litura* infestations while minimizing ecological harm and preserving long-term agricultural productivity.

Nelumbo nucifera Gaertn., commonly known as lotus, boasts a rich assortment of bioactive compounds, including alkaloids, flavonoids, and other secondary metabolites, renowned for their therapeutic properties in traditional medicine. Recent scholarly inquiry has shifted towards exploring the potential insecticidal attributes of *N. nucifera*, assessing its effectiveness in deterring or managing pests [18]. Lotus extracts exhibit strong antioxidant capabilities attributed to the presence of flavonoids, phenolic compounds, and vitamins. These antioxidants effectively scavenge free radicals, thereby mitigating oxidative stress and potentially affording protection against various chronic ailments [19]. Furthermore, the metabolites present in lotus extracts have demonstrated notable anti-inflammatory, antimicrobial, and neuroprotective effects, possibly ameliorating neurodegenerative conditions by enhancing neuronal survival and reducing oxidative damage [20–22]. Additionally, lotus extracts harbor valuable metabolites endowed with insect-repellent properties, growth regulation abilities, and toxicity against a plethora of agricultural pests, encompassing caterpillars, aphids, and beetles. The toxic nature of these extracts disrupts the physiological processes of pests, resulting in their mortality or diminished reproductive capacity [23–25]. Revered for its cultural and medicinal significance, lotus is now under scrutiny for its potential insecticidal effects against a spectrum of agricultural pests, including the notorious cutworm, *Spodoptera litura* (*S. litura*).

This investigation aims to uncover the insecticidal potential of *N. nucifera* against *S. litura* through serial experiments. By carefully evaluating key parameters such as larval mortality, growth inhibition, and behavioral responses, our goal is to gain a comprehensive understanding of the impact of *N. nucifera* and their major metabolites on *S. litura* and its potential as a natural, environmentally friendly alternative to conventional chemical insecticides. The outcomes of this study offer the prospect of not only enhancing our comprehension of *N. nucifera* insecticidal attributes but also presenting a sustainable and eco-conscious remedy to mitigate the influence of *Spodoptera litura* on agricultural crops. This research significantly contributes to the ongoing endeavors aimed at formulating effective, environmentally friendly pest management strategies that resonate with the principles of sustainable agriculture.

2. Experimental Section

2.1. Sample collection

Fresh leaves of *N. nucifera* were harvested from Namakkal, Tamil Nadu, India, by a neighborhood pond (11°13'15.1"N 78°10'00.1" E). The samples were meticulously collected and promptly placed in clean polythene bags to preserve their freshness. Subsequently, they were transported to Muthayammal College of Arts & Science (Autonomous) for additional examination.

2.2. Preparation of extract

The dried leaves of *N. nucifera* have been completely crushed with an electric grinder to a fine powder. Subsequently, 35grams of the powdered leaf were placed into 100milliliters of solvent made of methanol and subjected to extraction in Soxhlet extractor made of glass. After extraction, A rotary evaporator was used to condense the resultant solutions. This apparatus aids in removing the solvent, resulting in a more condensed extract with a total yield of 5.60 grams. The final residue was dissolved in methanol to create the stock solution. The herbal extract was then put into a sterile glass tube as well as retained at 4°C[29].

2.3. GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The examination of the *N. nucifera* extract was carried out with a GC-MS (Gas Chromatography-Mass Spectrometry) system. Mass spectral parameters were configured under the following conditions: a detector voltage of 70 V, source temperature and injector temperature adjusted accordingly, and injection mode set to split less with a 0.5µL sample injected. Furthermore, the oven temperature has been maintained at 85°C for 1 minute initially, with an initial time set at 3 minutes and a starting temperature of 40°C. Subsequently, the temperature was incrementally increased until reaching a final set temperature, held for 1 minute, resulting in a total run time of 30 minutes. Retention times of the active compounds in the samples were assessed and compared alongside their mass spectra in comparison to the library. Quantitative data was gathered from the results using a peak normalization technique incorporating FID response[29].

2.4. Insect culture

Bioassays were conducted with II-IV instar *S. litura* larvae by employing Castor leaves treated with proportions of 0.5, 1, 1.5, 2, & 2.5 ml of *N. nucifera* extract. Prepared samples were applied to leaves during the natural cooling process ($50 \pm 2^\circ\text{C}$). The control group received methanol only. Each treatment involved a group of 20 larvae per concentration with three replicates. Deaths were reported on a 24-hour basis, & final death was noted. Percentage mortality was calculated with corrections for natural mortality[10].

Oviposition assay

The procedure was followed in order to determine the ovicidal activity. 25 recently laid down eggs of *S. litura* were individually exposed to five different concentrations (0.5, 1, 1.5, 2, 2.5ml) prepared with methanol. Five replications of each concentration were made. A separate control group (methanol in water) was kept up, as well as the death of eggs was examined under a microscope. The evaluation of ovicidal activity was done 120 hours after therapy. Using the following formula: Percent ovicidal activity[10]: $\frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$

2.5. Enzyme analysis

2.5.1. CarE (Carboxylesterase) assay

Carboxylesterase activity was determined using the Senthil-Nathan et al. technique.[26] with a few minor adjustments. Each food treatment yielded twenty 7-day-old larvae, which were collected as well as homogenized in five millilitres of 0.1M phosphate buffer(pH 7.4), which comprises one mM of ethylene diamine tetra acetic acid (EDTA), phenyl methyl sulfonyl fluoride (PMSF), Phenyl thio urea (PTU), & 20percent glycerol. After centrifuging the homogenate for 20 minutes at 4 degree celcius at 10,000rpm, the supernatant has been utilized as the” enzymatic source.

The reaction mixture was created by combining 5.0 ml of anaphthyl acetate at 0.3 mM with 40 µl of the enzymatic source. It was then incubated for twenty minutes at room temp., in the dark, and with periodic shaking. Then, 1.0ml of a combination of 5% sodium dodecyl sulfate (SDS) solution, made with double-distilled water in a 2:5 ratio,& fast blue B (1.0% w/v salt in a pH 6.8 phosphate buffer (0.04 M)) was mixed. The mixture was then incubated for an additional 20 minutes at room temperature. The absorbance value (OD) at 590nm has been measured and compared to a blank solution prepared using the buffer that was used. Using a standard curve, the esterase activity in various treatments was computed. [26].

2.5.2. Glutathione S-transferase (GST) assay

GST activity has been measured using 1-chloro-2,4-dinitrobenzene (CDNB) based on the procedure of Habig et al. [27]. Simple 0.1 M phosphate buffer with one mM EDTA & 20 per cent glycerol. was used to make larval homogenates. 3 ml of phosphate buffer, 50mM CDNB, 150 mM reduced glutathione, as well as 30 mM enzyme solution, made up the reaction mixture. It was then gently mixed and incubated for two to three minutes. After five minutes of recording the change in absorbance rate (OD) at 340nm, the GST activity (µmol/mg/min) has been computed by employing the relevant coefficients. Using BSA as the standard, the Coomassie Brilliant Blue G (CBBG) method was used to determine the homogenate's protein concentration. A spectrophotometer was used to record the absorbance at 595 nm.

2.5.3. Cytochrome P450 (CYP450) assay

Spodoptera litura larvae in the II to IV instar stage, after exposure to sub-lethal doses of *N. nucifera* extract, were dissected following rinsing in double distilled water. Prior to dissection, the larvae have been chilled and “immersed in 40 mM sodium phosphate buffer (pH 7.2). Dissection involved removal of the heads, last abdominal segment, and digestive system, leaving carcasses for Cytochrome P450 (CYP450) activity determination. Black, round-bottomed 96-well microplates were used to measure the activity of ethoxycoumarin-O-de-ethylase within the body's barrier in order to determine the cytochrome P450 activity. Individual larval carcasses were placed in each well, which held 100µl of a 0.4mM 7-ethoxycoumarin solution in a fifty mM sodium phosphate buffer(pH7.2). After four hours of incubation at 30 degrees Celsius, the reaction was stopped using 50 µl of ethanol and one mM of glycine buffer (pH 10.4). Throughout the reading, larval carcasses remained at the well's bottom. 100 µl of phosphate buffer, 50 µl of glycine buffer, & Fifty µl of ethanol were placed in blank wells. A Synergy HT microplate reader (Bio-Tek Instruments, India) equipped with 400nm excitation as well as 480nm emission filters

was used to evaluate the fluorescence of the reaction media. The measure of enzyme activity was μmol of 7-hydroxycoumarin (7-OH) per milligram of larvae per minute [10].

2.6. Midgut histology

The study concentrated on the effects of a lethal dosage (2.5 milligrams per liter) of *N. nucifera* crude extract on the 4th instar larvae's intestine tissues, which were immobilized in Bouin's chemical reagent overnight, along with control samples. After that, they had been chilled to about 26°C for two hours, and then a cryo-microtome was used to cut them into 1.5 μm pieces (Leica, Cryocut 1800, Germany). Using a B-600 TiFI-Italy teaching microscope, the complete midgut slices were placed on microscopic slides, examined, and caught on camera using a mercury short-arc lamp light installed in an Optika Fluo Series HBO [28] (Murfadunnisa et al., 2019).

2.7. Earthworm toxicity

The *Eudrilis eugeniae* earthworm species was kept chemical-free at the Muthayammal College of Arts and Science (Autonomous), Rasipuram, Tamil Nadu, India. The whole population of earthworms was cultivated at $28.9 \pm 0.36^\circ\text{C}$ the ambient temperature. The Artificial Soil Test and Filter Paper Test (FPT) were then carried out using the procedures described in Vasantha-Srinivasan et al. [29]. The study compared the effects of 1.5 and 2.5 milligrams per liter of *N. nucifera* extract with 0.5 and 1.0 milligrams per liter of the commercial pesticide Cypermethrin at treatment levels. The OECD standard procedure was followed for conducting the Artificial Soil Test (AST) in this investigation, which comprised 20% kaolin clay, 10% sphagnum peat moss, along with 70% sand [30].

2.8. In-silico toxicity prediction

BeeTox, a free online server utilizing AI (artificial intelligence) and designed in order to estimate the short-term toxicity of substances & ligands to honey bees, was applied to evaluate the toxicity of specific chemicals obtained from the extract of *N. nucifera*. The creators of this tool are Moreira-Filho et al. [31].

2.9. Statistical analysis

The mortality data underwent an extensive statistical analysis, which included logarithmic and variance analysis of arcsine, along with square root converted percentages. Then, using 5 duplicates of the observed readings, mean values were computed. Tukey's multiple-range tests were used to evaluate differences among distinct larvicidal treatment groups, with a significance level of $p < 0.05$. The Minitab®17 software has been used for the statistical analysis. Statistical calculations had been carried out using Sigma Plot Twelve, a Microcal software tool, to estimate mid-gut enzyme levels. An accurate and thorough knowledge of the larvae's lethal dosage reactions to the experimental settings was made possible by this investigation.

3. Results and Analysis

3.1. Chemical Profile of *N. nucifera*

IPM (Integrated pest management) strategies are widely performed to manage pest populations along with mitigate crop damage. These tactics cover a range of methods, including as the application of chemical pesticides, applying biological control agents (natural enemies), cultural customs, as well as the development of resistant crop types. As insect growth regulators (IGRs), synthetic compounds such as pyrethroids hinder the action of lepidopteran hormones, causing disruptions to larval development and molting, which in turn causes malformations or mortality. Furthermore, organophosphates, such malathion and chlorpyrifos, interfere with the insects' nervous system by interfering with the transmission of nerve signals. This neurotoxic impact has the potential to cause death and paralysis. The insect lepidopteran *S.litura* affects crops around the world in different ways in accordance with on a number of variables, such as climate, location, farming practices, and host plant susceptibility. The chemical screening of *N. nuciferacrude* extract delivered total of fifteen major metabolites, the chromatogram and the GC-MS compounds was displayed (Fig. 1A&B) The peak area percentage was maximum for 6a-. beta. -Aporphine,1,2-dimethoxy- (33.79%), followed by n-Hexadecanoicacid (21.14%) respectively. Apart from that the chemical profile of *N. nucifera* also covers Hydrazinecarbothioamide (0.92%), "Phenol,4,4'-methylenebis [2,6-dimethyl- (1.4%), Hexadecane (1.17%), Neophytadiene (0.95%), Hexadecanoicacid, methylester (6.47%), Methyl 10-trans,12-cis-octadecadienoate (5.13%), E,E,Z-1,3,12-Nonadecatriene-5,14-diol (3.22%), 9,12-Octadecadienoicacid(Z,Z)- (8.2%), Z,Z-8,10-Hexadecadien-1-ol (6.81%), Octadecanoic acid (5.0%), Silane,diethyl(2,4,6-trichlorophenoxy)pentyl- (1.44%), 1,3,5-Trisilacyclohexane (1.39%), and (R)-1-Methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-diben" (2.98%) respectively. The total peak area (PA), retention time (RT),compound name tabulated (Table 1).

Commonly found in tropical and subtropical areas, this pest's presence often results in substantial economic losses in agriculture [32]. In several studies reported that invasive aquatic plant and through GC-MS analysis, the possible hepatoprotective bioactive chemical components from the n-butanol fractionated hydroethanolic extract of *P. stratiotes* leaves were identified[33]. The mechanisms of insecticidal resistance in the crop pest *S. litura* against major commercial compounds is also a greater challenge in managing this crucial pest [15]. This mechanism involves alterations in the target sites of insecticides, such as receptors or enzymes, rendering them less susceptible to the toxic effects of the compounds [34]. Also, the insect develops enhanced detoxification mechanisms to metabolize or sequester the insecticide before it can exert its toxic effects [35]. This can involve the upregulation of detoxification enzymes, such as cytochrome P450s, esterase's, or glutathione S-transferases, which facilitate the breakdown or sequestration of insecticides within the insect's body, reducing their toxicity [36].

3.2. Larval toxicity

The larval toxicity of *N. nucifera* extracts exhibited dose-dependent mortality rates "against II, III, and IV instar larvae of *S. litura*, with a notably higher mortality rate observed at the prominent dosage of *N. nucifera* (2.5 milligrams per liter) across all instar stages (II: 96.5%, $F_{4,20} = 23.22$, $P \leq 0.001$; III: 87.5%, $F_{4,20} = 23.66$, $P \leq 0.001$ "; IV: 85.4%, $F_{4,20} = 18.42$, $P \leq 0.001$). Each treatment dosage significantly differed from both the control group and other treatment dosages. Conversely, the minimal dosage of *N. nucifera*

extract (0.5 milligrams per liter) resulted in comparatively lower larval mortality rates across all instar “stages (II: 30.4%, $F_{4,20} = 23.22$, $P \leq 0.001$; III: 24.5%, $F_{4,20} = 23.66$, $P \leq 0.001$; IV: 23.1%, $F_{4,20} = 18.42$, $P \leq 0.001$) (Fig. 2). The lethal concentration (LC_{50}) value was observed at 1.3 milligrams per liter in II instar, 1.8 milligrams per liter in III instar, and 2.1 milligrams per liter for IV instar respectively”.

Incorporating herbal extract-based pesticides into pest management strategies presents numerous advantages for mitigating resistance to commercial chemical pesticides [37]. Their varied chemical compositions, distinct modes of action, and diminished environmental impact render them valuable assets for sustainable pest control, thereby contributing to the preservation of effective pest management practices for future generations [38–39]. *Often referred to as the sacred lotus, N. nucifera is a perennial aquatic plant in the Nelumbonaceae family.* Renowned for its medicinal properties, this plant has found extensive use in traditional medicine systems. Various parts of *N. nucifera*, such as the roots, fruits, stamen, tender leaves, stems, as well as flowers, have been reported to possess diverse biological and pharmacological activities [40–41]. Previous studies investigating the phytochemical screening of *N. nucifera* leaf extracts have confirmed the presence of Tannins, Saponins, and Flavonoids [42].

3.3. Development inhibition of *N. nucifera*

Exposure of *S. litura* larvae to *N. nucifera* extract resulted in a dose-dependent extension of larval duration, with a significant increase observed at the higher sub-lethal dosage of 1.5 milligrams per liter, reaching 31 days ($F_{4,20} = 23.66$, $P \leq 0.001$), compared to 18 days for control larvae (Fig. 3). This trend persisted in pupal development, where the duration was significantly prolonged with increasing dosage of *N. nucifera*, peaking at the highest dosage (1.5 milligrams per liter) with 22 days ($F_{4,20} = 13.65$, $P \leq 0.001$), in contrast to both other treatment dosages as well as the control group (8 days, $F_{4,20} = 13.65$, $P \leq 0.001$) (Fig. 3).

Furthermore, the Oviposition Deterrent Index (ODI) revealed a significant inhibition of *S. litura* Oviposition following therapy with *N. nucifera* sample, with the highest inhibition percentage observed at the higher sub-lethal dosage (1.5 milligrams per liter), reaching 87.5% ($F_{4,20} = 29.44$, $P \leq 0.001$), compared to other dosages: 1.0 milligrams per liter (67.5%), 0.5 milligrams per liter (43.3%), 0.25 milligrams per liter (15.4%), and control (5.3%) (Fig. 4A)[43]. Moreover, the reproductive rate, indicated by the mean fecundity of *S. litura*, has been significantly influenced in a dose-response manner to *N. nucifera* extract, with notable effects observed at various concentrations: 1.5 milligrams per liter (535 number of eggs, $F_{4,20} = 29.05$, $P \leq 0.001$), 1.0 milligrams per liter (632 number of eggs), 0.5 milligrams per liter (745 number of eggs), 0.25 milligrams per liter (978 number of eggs), and control (1250 number of eggs) (Fig. 4B).

3.4. Enzyme inhibition of *N. nucifera*

The alpha (α) carboxyl esterase activity was significantly inhibited by the prominent dosage of *N. nucifera* (1.5 milligrams per liter), resulting in significant inhibition across the II instar (0.41 mM/mg -

$F_{4,20}=18.65$, $P\leq 0.001$), III “instar (0.443 mM/mg - $F_{4,20}=15.23$, $P\leq 0.001$), and IV instar (0.512 mM/mg - $F_{4,20}=18.04$, $P\leq 0.001$) stages compared to the respective control levels of II instar (0.713 mM/mg), III instar (0.742 mM/mg), and IV instar (0.793 mM/mg) (Fig. 5A). Similarly, β carboxyl esterase activity decreased in a dose-dependent manner with *N. nucifera* treatment, exhibiting significance at higher dosages (1.5 milligrams per liter) in the II instar (1.133 mM/mg - $F_{4,20}=18.55$, $P\leq 0.001$), III instar (1.312 mM/mg - $F_{4,20}=17.77$, $P\leq 0.001$), and IV instar (1.451 mM/mg - $F_{4,20}=19.83$, $P\leq 0.001$) stages compared to the respective control levels” of II instar (1.754 mM/mg), III instar (1.856 mM/mg), and IV instar (1.932 mM/mg) (Fig. 5B).

In contrast, detoxifying enzyme GST’s level increased in a dose-dependent manner with exposure to *N. nucifera*, with significance observed at a sub-lethal dosage of 1.5 milligrams per liter across the II instar (0.526 μ mol/mg - $F_{4,20}=15.31$, $P\leq 0.001$), III instar (0.563 μ mol/mg - $F_{4,20}=18.21$, $P\leq 0.001$), & IV instar (0.631 μ mol/mg - $F_{4,20}=19.55$, $P\leq 0.001$) stages as compared to the respective control levels of II instar (0.421 μ mol/mg), III instar (0.465 μ mol/mg), & IV instar (0.487 μ mol/mg) (Fig. 5C). Similarly, the CYP450 level increased in response to *N. nucifera* in a dose-dependent manner, with significant elevation noted at the sub-lethal dosage (1.5 milligrams per liter) across the II instar (7.41 mmol 7-OH/mg - $F_{4,20}=23.14$, $P\leq 0.001$), III instar (7.91 mmol 7-OH/mg - $F_{4,20}=18.24$, $P\leq 0.001$), & IV instar (8.04 mmol 7-OH/mg - $F_{4,20}=22.54$, $P\leq 0.001$) stages as compared to the respective control levels of II (5.13 mmol 7-OH/mg), III (5.21 mmol 7-OH/mg), and IV (5.30 mmol 7-OH/mg) (Fig. 5D).

3.5. Midgut toxicity of *N. nucifera*

The midgut toxicity of IV instar larvae of *S. litura* delivered severe dysregulation in the midgut cell and tissues treated with lethal dosage of *N. nucifera* (2.5 milligrams per liter). There was severe dysregulation in the alignment of the peritrophic membrane (pM), epithelial layer (el), as well as brush border membrane (bbm) of midgut larvae of *S. litura* exposed to *N. nucifera*. Also, the ciliated border was also damaged severely and the midgut content was collapsed significantly (Fig. 6B). In contrast, the control larvae displayed normal appearance of the midgut cells and tissues (Fig. 6A).

Midgut enzymes such as esterases, GST, and CYP450 play pivotal roles in the detoxification system of insects, facilitating the metabolism and elimination of xenobiotics, including insecticides and plant secondary metabolites. Esterases are particularly critical for the hydrolysis of ester bonds, essential for detoxifying insecticides like organophosphates and carbamates [44]. This enzymatic action confers resistance to insecticides in lepidopteran pests by breaking down these compounds into less toxic metabolites [45]. However, plant extracts containing various phyto-compounds can inhibit esterase activity, diminishing the insect's ability to detoxify insecticides and heightening susceptibility to chemical control measures [32]. The exposure to *N. nucifera* extracts (1.5 milligrams per liter) significantly inhibited esterase (α and β) activity in different larval instars of *S. litura*. GSTs, enzymes catalyzing the conjugation of glutathione to xenobiotics, aid in their excretion or sequestration [46]. In response to toxic compounds, insect pests elevate the expression of GSTs and CYP450 enzymes to enhance their capacity for toxin metabolism and elimination [47].

3.6. Earthworm toxicity of *N. nucifera*

The Filter Paper Test (FPT) conducted on earthworm *E. foetida* exposed to lethal dosages of *N. nucifera* (1.5 & 2.5 milligrams per liter) and cypermethrin (0.5 & 1.0 milligrams per liter) demonstrated that the herbal extract treatment with *N. nucifera* was comparatively less toxic or even harmless, even at the maximum dosage exposure (2.5 milligrams per liter) after 24 hours (7.3% mortality - $F_{4,20}=27.14$, $P \leq 0.001$) & 48 hours (5.4% mortality - $F_{4,20}=24.51$, $P \leq 0.001$), respectively. In contrast, the commercial pesticide cypermethrin (1.0 milligrams per liter) exhibited a higher mortality rate after 24 hours (23.4% - $F_{4,20}=27.14$, $P \leq 0.001$) & 48 hours (20.2% - $F_{4,20}=24.51$, $P \leq 0.001$), respectively (Fig. 7A). Furthermore, there was no noble change between the mortality rates of the *N. nucifera* treatments and the control after 24 hours (3.4%) and 48 hours (3.2%).

The results of the Artificial Soil Test (AST) also indicated that *N. nucifera* treatment (2.5 milligrams per liter) was less toxic to *E. foetida* after 2 days (5.1% mortality - $F_{4,20}=21.55$, $P \leq 0.001$), 7 days (4.6% mortality - $F_{4,20}=28.33$, $P \leq 0.001$), and 14 days (4.1% mortality - $F_{4,20}=29.55$, $P \leq 0.001$) of exposure, respectively. In contrast, the commercial compound cypermethrin (1.0 milligrams per liter) resulted in a significant mortality rate after 2 days (27.5% - $F_{4,20}=21.55$, $P \leq 0.001$), 7 days (21.3% - $F_{4,20}=28.33$, $P \leq 0.001$), and 14 days (17.3% - $F_{4,20}=29.55$, $P \leq 0.001$), respectively (Fig. 7B). Similarly, as observed in the AST results, there was no noble change among the mortality rates of the *N. nucifera* treatments and the control after 2 days (2.3%), 7 days (2.1%), and 14 days (1.7%). Epithelial layer (EL), are equipped with various transporters and pumps that actively regulate the movement of nutrients and xenobiotics across the gut epithelium [48]. Additionally, the epithelial layer can undergo structural modifications in response to toxic compounds.

Earthworms are frequently regarded as barometers of soil health, with their existence and proliferation serving as markers of a robust and harmonious soil ecosystem [49]. Consistent with our research, Dinesh-Kumar et al. [50] similarly demonstrated that the Plant *Swietenia mahagoni*, family Meliaceae, in conjunction with its plant chemicals constituent Bis (2-ethylhexyl) phthalate, exhibited lower toxicity compared to the synthetic insecticide Cypermethrin when tested on the earthworm *Eudrilus eugeniae* at a 200 milligrams per kilogram is the maximum dosage. The *in-silico* toxicity prediction tools facilitate rapid and efficient screening of pesticide compounds across a broad spectrum of non-target organisms.

This capability empowers researchers and regulatory agencies to promptly discern potentially hazardous compounds and prioritize further testing and evaluation. Online *in-silico* prediction tools such as BeeTox provide streamlined mathematical techniques for predicting a chemical compound's toxicological characteristics, enabling scientists & authorities to quickly assess the possible dangers connected to a variety of substances [51].

In-silico bee toxicity of *N. nucifera* metabolites

The *in-silico* bee toxicity of *N. nucifera* metabolites, the BeeTox AI online server predicted that the major metabolite constituents of *N. nucifera* pose no harm to the honeybee *Apis mellifera* with regard to of contact & acute oral toxicity. Comprehensive mapping of toxicity contribution of the phyto-compounds in *N. nucifera* is presented in Table 2. Our findings, conducted through BeeTox, indicate that the bioactive compounds of *N. nucifera* pose no toxicity to honey bees and are environmentally safer for beneficial species.

4. Conclusions

The current toxicological screening of its active bio-rational extracts presents an opportunity to integrate this botanical treasure into integrated pest management strategies. This approach holds promise for developing novel bio-based agents to target the lepidopteran pest *S. litura*, thereby promoting eco-friendly solutions and reducing chemical burdens. Furthermore, the availability of *N. nucifera* as a locally-sourced aquatic plant encourages the application of local or native plants to control pests, in line with organic along with community-supported farming methods. Future research efforts should prioritize the effective formulation of plant compounds such as 6a-. beta. -Aporphine, 1,2-dimethoxy-, and n-Hexadecanoic acid, considering their potential biological properties and minimal impact on non-target organisms. Examining the synergistic relationships among various botanical substances or between synthetic pesticides and botanicals may improve effectiveness while reducing side effects.

Declarations

A conflict of interest

We certify that no known conflicts of interest are connected to this study, and no substantial financial support has been provided that could have affected the results. The authenticity of the work has been guaranteed with great care.

The accessibility of information and resources

This published publication contains all of the data gathered or analyzed during this investigation. Furthermore, the accompanying author can provide the data that back up the study's conclusions upon request.

Acknowledgments

We thank that the "research was supported by Researchers Supporting Project number(RSP2024R315) King Saud University, Riyadh, Saudi Arabia.We thank the "Regional Innovation Strategy (RIS)" through the NRF (National Research Foundation of Korea) funded by the MOE (Ministry of Education) (2021RIS-002)

and Additionally, this study was funded by the FIST program of the Department of Science and Technology, India" (DST-FIST) (SR/FIST/LS-1/2019/522).

References

1. Karthick, K., Muthusamy, J., Prabhakaran, V.S., Pandiyan, R., Narayanaswamy, R., Muthu, K., Sengodan, K., Raja, G., Sengottayan, S.N., Selvaraj, G.K., Rajakrishnan, R.: In-vitro and in-silico analysis of methanolic crude extracts of Mountain knotgrass *Aerva lanta* (L.) against two lepidopteran pests and non-target species. *Toxin Rev.* 1–16. (2023)
2. Kamaraj, C., Karthi, S., Reegan, A.D., Balasubramani, G., Ramkumar, G., Kalaivani, K., Zahir, A.A., Deepak, P., Senthil-Nathan, S., Rahman, M.M., Islam, A.R.M.T.: Green synthesis of gold nanoparticles using *Gracilaria crassa* leaf extract and their ecotoxicological potential: Issues to be considered. *Environ. Res.* **213**, 113711 (2022)
3. Vasantha-Srinivasan, P., Shanmuga-Priya, S., Han, Y.S., Radhakrishnan, N., Karthi, S., Elsadek, M.F., Mustafa, A.E.Z.M., Senthil-Nathan, S.: Phyto-chemical screening, insecticidal potential and detoxifying enzyme inhibition of *Ficus auriculata* (Lour.) extracts, against the mosquito vector and non-target aquatic predator. *Biocatal. Agri Biotechnol.* **53**, 102864 (2023)
4. Kalaivani, K., Senthil-Nathan, S., Stanley–Raja, V., Vasantha-Srinivasan, P.: Physiological and biochemical alterations in *Vigna radiata* L. triggered by sesame derived elicitors as defense mechanism against *Rhizoctonia* and *Macrophomina* infestation. *Sci. Rep.* **13**(1), 13884 (2023)
5. Lengai, G.M., Muthomi, J.W., Mbega, E.R.: Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Sci. Afr.* **7**, e00239. (2020)
6. Ngegba, P.M., Cui, G., Khalid, M.Z., Zhong, G.: Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture.* **12**(5), 600 (2022)
7. Chengala, L., Singh, N.: Botanical pesticides-A major alternative to chemical pesticides: A review. *Int. J. Life Sci.* **5**(4), 722–729 (2017)
8. Unni, P.S., Kirupaanntha-Rajan, P., Vasantha-Srinivasan, P., Srinivasan, S., Han, Y.S., Karthi, S., Radhakrishnan, N., Park, K.B., Rajagopal, R., Senthil-Nathan, S.: Chemical composition and toxicity of commercial *Mentha spicata* and *Eucalyptus citriodora* essential oils on *Culex quinquefasciatus* and non-target insects. *Environ. Sci. Pollut Res.* 1–22. (2024)
9. El-Wakeil, N.E.: Botanical pesticides and their mode of action. *Gesunde Pflanzen*, **65**(4). (2013)
10. Senthil-Nathan, S.: A review of biopesticides and their mode of action against insect pests, pp. 49–63. *Role of green technologies, Environmental sustainability* (2014)
11. Radhakrishnan, N., Karthi, S., Raghuraman, P., Ganesan, R., Srinivasan, K., Edwin, E.S., Ganesh-Kumar, S., Mohd Esa, N., Senthil-Nathan, S., Vasantha-Srinivasan, P., Krutmuang, P.: Chemical screening and mosquitocidal activity of essential oil derived from *Mikania scandens* (L.) Willd. against *Anopheles gambiae* Giles and their non-toxicity on mosquito predators. *All Life.* **16**(1), 2169959 (2023)

12. Amir, F., Wiyatiningsih, S.: The Utilisation of Botanical Pesticide as Pest Control in The Management of Organic Vegetable Farming. Nusantara Science and Technology Proceedings, 71–75. (2023)
13. Islam, S.M.N., Chowdhury, M.Z.H., Mim, M.F., Momtaz, M.B., Islam, T.: Biocontrol potential of native isolates of *Beauveria bassiana* against cotton leafworm *Spodoptera litura* (Fabricius). Sci. Rep. **13**(1), 8331 (2023)
14. Yang, H.H., Li, J.Q., Ma, S., Yao, W.C., Chen, Y.W., El Wakil, A., Dewar, Y., Zhu, X.Y., Sun, L., Zhang, Y.N.: RNAi-mediated silencing of SlitPer disrupts sex pheromone communication behavior in *Spodoptera litura*. Pest Manag Sci. **79**(10), 3993–3998 (2023)
15. Che, W., Li, Y., Zhang, D., Qu, C., Luo, C., Wang, R.: Monitoring and characterization of field-evolved resistance to diamide insecticides in *Spodoptera litura* collected from eastern China. J. Appl. Entomol. **148**, 253–260 (2023)
16. Li, W., Yang, W., Shi, Y., Yang, X., Liu, S., Liao, X., Shi, L.: Comprehensive analysis of the overexpressed cytochrome P450-based insecticide resistance mechanism in *Spodoptera litura*. J. Haz Mat. **461**, 132605 (2024)
17. Muthusamy, R., Ramkumar, G., Kumarasamy, S., Albeshr, M.F., Alrefaei, A.F., Ma, Y., Narayanan, M.: Resistance to synthetic pyrethroid and neonicotinoid is associated with reduced reproductive efficiency in the field population of *Spodoptera litura* (Insecta: Lepidoptera). Biocatal. Agri Biotechnol. **56**, 103031 (2024)
18. Wang, M., Hu, W.J., Wang, Q.H., Yang, B.Y., Kuang, H.X.: Extraction, purification, structural characteristics, biological activities, and application of the polysaccharides from *Nelumbo nucifera* Gaertn.(lotus): A review. Inter J. Biol. Macromol. **226**, 562–579 (2023)
19. Zhang, Y., Xu, Y., Wang, Q., Zhang, J., Dai, X., Miao, S., Lu, X.: The antioxidant capacity and nutrient composition characteristics of lotus (*Nelumbo nucifera* Gaertn.) seed juice and their relationship with color at different storage temperatures. Food Chem. X. **18**, 100669 (2023)
20. Nutho, B., Tungmunthum, D.: Exploring Major Flavonoid Phytochemicals from *Nelumbo nucifera* Gaertn. as Potential Skin Anti-Aging Agents: *In silico* and *In vitro* Evaluations. Inter. J. Mol. Sci. **24**(23), 16571. (2023)
21. Intui, K., Nuchniyom, P., Laoung-On, J., Jaikang, C., Quiggins, R., Sudwan, P.: Neuroprotective Effect of White *Nelumbo nucifera* Gaertn. Petal Tea in Rats Poisoned with Mancozeb. Foods. **12**(11), 2175 (2023)
22. Ibrahim, R.E., Fouda, M.M., Younis, E.M., Abdelwarith, A.A., Salem, G.A., Elkady, A.A., Ismail, S.H., Davies, S.J., Rahman, A.N.A.: The anti-bacterial efficacy of zinc oxide nanoparticles synthesized by *Nelumbo nucifera* leaves against *Clostridium perfringens* challenge in *Oreochromis niloticus*. Aquacul. **578**, 740030. (2024)
23. Santhoshkumar, T., Rahuman, A.A., Rajakumar, G., Marimuthu, S., Bagavan, A., Jayaseelan, C., Zahir, A.A., Elango, G., Kamaraj, C.: Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol. Res. **108**, 693–702 (2011)

24. Ray, A.S., Bhattacharya, K., Singh, A., Chandra, G.: Larvicidal activity of *Nelumbo nucifera* Gaertn. (Nymphaeaceae) against *Anopheles stephensi* (Liston 1901) and its effect on non-target organisms. J. Mosq. Res. **4**(1). (2014)
25. Seehavet, S., Phonkacha, P., Hongpakdee, P., Tangkawanit, U.: Species composition, population dynamics, and assessment of biopesticide application on Thrips (Thysanoptera) in sacred lotus *Nelumbo nucifera* gaertn. Songklanakarin J. Sci. Technol. **44**, 459–465 (2022)
26. Senthil-Nathan, S.: Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against lepidopteran insects. Fro Physiol. **4**, 72246 (2013)
27. Habig, W.H., Pabst, M.J., Jakoby, W.B.: Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Boil Chem. **249**(22), 7130–7139 (1974)
28. Murfadunnisa, S., Vasantha-Srinivasan, P., Ganesan, R., Senthil-Nathan, S., Kim, T.J., Ponsankar, A., Kumar, S.D., Chandramohan, D., Krutmuang, P.: Larvicidal and enzyme inhibition of essential oil from *Spheranthus amaranthroids* (Burm.) against lepidopteran pest *Spodoptera litura* (Fab.) and their impact on non-target earthworms. Biocatal. Agri Biotechnol. **21**, 101324 (2019)
29. Chandrasekaran, R., Revathi, K., Nisha, Senthil-Nathan, S.: Physiological effect of chitinase purified from *Bacillus subtilis* against the tobacco cutworm *Spodoptera litura* Fab Pesticide Biochemistry and Physiology:1(). (2012)
30. OECD, T.N.: 207: Earthworm, acute toxicity tests, vol. 2. OECD Guidelines for the Testing of Chemicals, Section (1984)
31. Moreira-Filho, J.T., Braga, R.C., Lemos, J.M., Alves, V.M., Borba, J.V., Costa, W.S., Kleinstreuer, N., Muratov, E.N., Andrade, C.H., Neves, B.J.: Bee ToxAI: An artificial intelligence-based web app to assess acute toxicity of chemicals to honey bees. Artif. Intel Life Sci. **1**, 100013 (2021)
32. Kalaivani, S., Jayanthi, S., Revathi, K., Chandrasekaran, R.: Phytochemical profile of *Euphorbia hirta* plant extract and its in vitro anticancer activity against the liver cancer HepG2 cells, pp. 1–8. Vegetos (2023)
33. Varsha, L., TyagiSaya, G.S., Rashmi, T.: Hexadecanoic acid methyl ester, a potent hepatoprotective compound in leaves of *Pistia stratiotes*. Appl. Biology Chem. J. **4**(4), 118–120 (2023)
34. Zhang, Z., Gao, B., Qu, C., Gong, J., Li, W., Luo, C., Wang, R.: Resistance monitoring for six insecticides in vegetable field-collected populations of *Spodoptera litura* from China. Horticulturae. **8**(3), 255 (2022)
35. Cheema, H.K., Kang, B.K., Jindal, V., Kaur, S., Gupta, V.K.: Biochemical mechanisms and molecular analysis of fenvalerate resistant population of *Spodoptera litura* (Fabricius). Crop Prot. **127**, 104951 (2020)
36. Amala, K., Karthi, S., Ganesan, R., Radhakrishnan, N., Srinivasan, K., Mostafa, A.E.Z.M., Al-Ghamdi, A.A., Alkahtani, J., Elshikh, M.S., Senthil-Nathan, S., Vasantha-Srinivasan, P.: Bioefficacy of *Epaltes divaricata* (L.) n-Hexane extracts and their major metabolites against the Lepidopteran pests *Spodoptera litura* (fab.) and dengue mosquito *Aedes aegypti* (Linn). Molecules. **26**(12), 3695 (2021)

37. Hoesain, M., Suharto, Prastowo, S., Pradana, A.P., Alfarisy, F.K., Adiwena, M.: Investigating the plant metabolite potential as botanical insecticides against *Spodoptera litura* with different application methods. *Cogent Food Agri.* **9**(1), 2229580 (2023)
38. Divekar, P.: Botanical pesticides: An eco-friendly approach for Management of Insect Pests. *Acta Scientific Agri.* (ISSN: 2581-365X), **7**(2). (2023)
39. Tesari, T., Leksono, A.S., Mustafa, I.: Effectiveness of botanical pesticide combined with *Beauveria bassiana* on mortality, nutritional index and fecundity of *Spodoptera litura* L. *Cogent Food Agri.* **10**(1), 2320816 (2024)
40. Sheikh, S.A.: Ethno-medicinal uses and pharmacological activities of lotus (*Nelumbo nucifera*). *J. Med. Plants Stud.* **2**, 42–46 (2014)
41. Laoung-On, J., Jaikang, C., Saenphet, K., Sudwan, P.: Phytochemical screening, antioxidant and sperm viability of *Nelumbo nucifera* petal extracts. *Plants.* **10**(7), 1375 (2021)
42. Baehaki, A., Lestari, S.D., Apriyanti, W.: Phytochemical screening and antioxidant activity of seeds extract of water plant (*Nymphaea stellata* and *Nelumbo nucifera*). *J. Chem. Pharm. Res.* **7**(11), 221–224 (2015)
43. Wani, Y., Ganie, N.A., Mir, M.R., Baqual, M.F., Murtaza, I.: Activities of acid phosphatase and alkaline phosphatase in various silkworm races and correlation studies. *J. Entomol. Res.* **47**(4), 749–756 (2023)
44. Cruse, C., Moural, T.W., Zhu, F.: Dynamic roles of insect carboxyl/cholinesterases in chemical adaptation. *Insects.* **14**(2), 194 (2023)
45. Bhatt, P., Bhatt, K., Huang, Y., Lin, Z., Chen, S.: Esterase is a powerful tool for the biodegradation of pyrethroid insecticides. *Chemosphere.* **244**, 125507 (2020)
46. Hilliou, F., Chertemps, T., Maïbèche, M., Le Goff, G.: Resistance in the genus *Spodoptera*: Key insect detoxification genes. *Insects.* **12**(6), 544 (2021)
47. Nauen, R., Bass, C., Feyereisen, R., Vontas, J.: The role of cytochrome P450s in insect toxicology and resistance. *Rev. Entomol.* **67**, 105–124 (2022)
48. Napoleão, T.H., Albuquerque, L.P., Santos, N.D., Nova, I.C., Lima, T.A., Paiva, P.M., Pontual, E.V.: Insect midgut structures and molecules as targets of plant-derived protease inhibitors and lectins. *Pest Manag Sci.* **75**(5), 1212–1222 (2019)
49. Ji, C., Miao, J., Xia, B., Dai, Y., Yang, J., Zhang, G., Zhang, Q., Wang, F., Tang, T., Zhao, M.: Evaluation of the toxic effects of fluindapyr, a novel SDHI fungicide, to the earthworms *Eisenia fetida*. *Sci. Total Environ.* **899**, 165697 (2023)
50. Dinesh-Kumar, A., Srimaan, E., Chellappandian, M., Vasantha-Srinivasan, P., Karthi, S., Thanigaivel, A., Ponsankar, A., Chanthini, K.M.P., Shyam-Sundar, N., Annamalai, M., Kalaivani, K.: Target and non-target response of *Swietenia Mahagoni* Jacq. chemical constituents against tobacco cutworm *Spodoptera litura* Fab. and earthworm, *Eudrilus eugeniae* Kinb. *Chemosphere*, 199, 35–43. (2018)
51. Narayanaswamy, R., Prabhakaran, V.S., Al-Ansari, M.M., Al-Humaid, L.A., Tiwari, P.: An In Silico analysis of synthetic and natural compounds as inhibitors of Nitrous Oxide Reductase (N2OR) and

Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

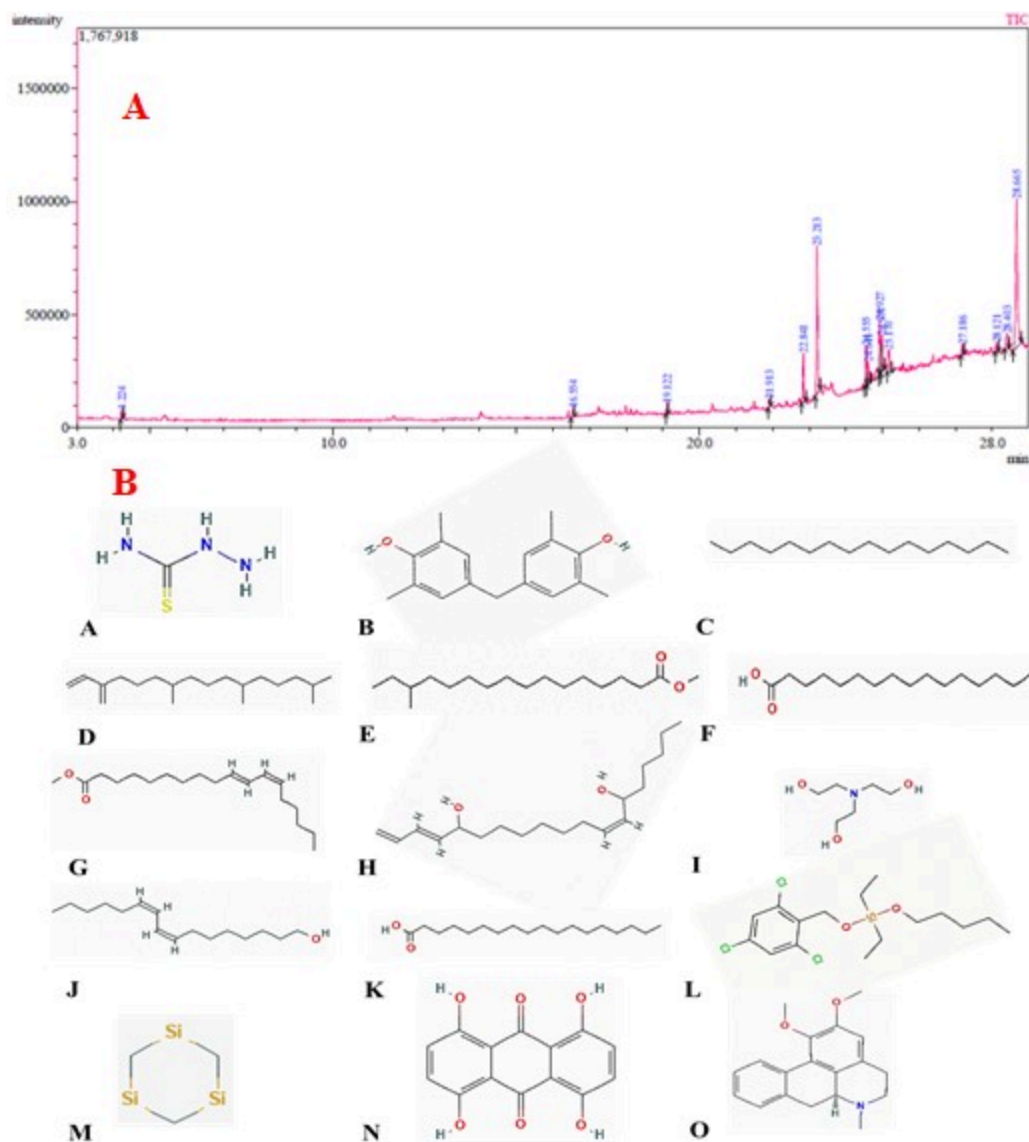


Figure 1

GC-MS chromatogram and the major metabolites derived from *N. nucifera* crude extract. Were the alphabets representing in (B) are the chemical names as follows (A) Hydrazinecarbothioamide, (B) "Phenol,4,4'-methylenebis [2,6-dimethyl-, (C) Hexadecane, (D) Neophytadiene, (E) Hexadecanoicacid, methyl ester, (F) n-Hexadecanoic acid, (G) Methyl 10-trans,12-cis-octadecadienoate, (H) E,E,Z-1,3,12-Nonadecatriene-5,14-diol, (I) 9,12-Octadecadienoicacid(Z,Z)-, (J) Z,Z-8,10-Hexadecadien-1-ol, (K)

Octadecanoic acid, (L) Silane,diethyl(2,4,6-trichlorophenoxy)pentyl-oxo-, (M) 1,3,5-Trisilacyclohexane, (N) (S)-1,2,9,10-tetrahydroxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline, and (O) 6a-.beta.-Aporphine,1,2-dimethoxy- respectively”.

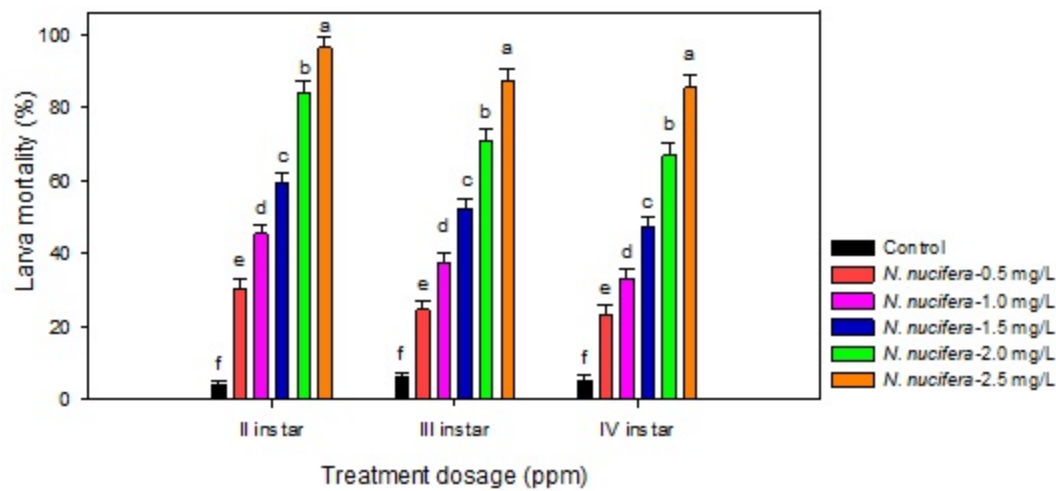


Figure 2

Larvicidal activity of *N. nucifera* crude extract dosages against 2nd, 3rd and 4th instar larvae of *S.litura*. Tukey's test results show that there is no notable distinction ($P \leq 0.05$) when the means ($\pm$ SEM) are followed by the identical letters above the bars.

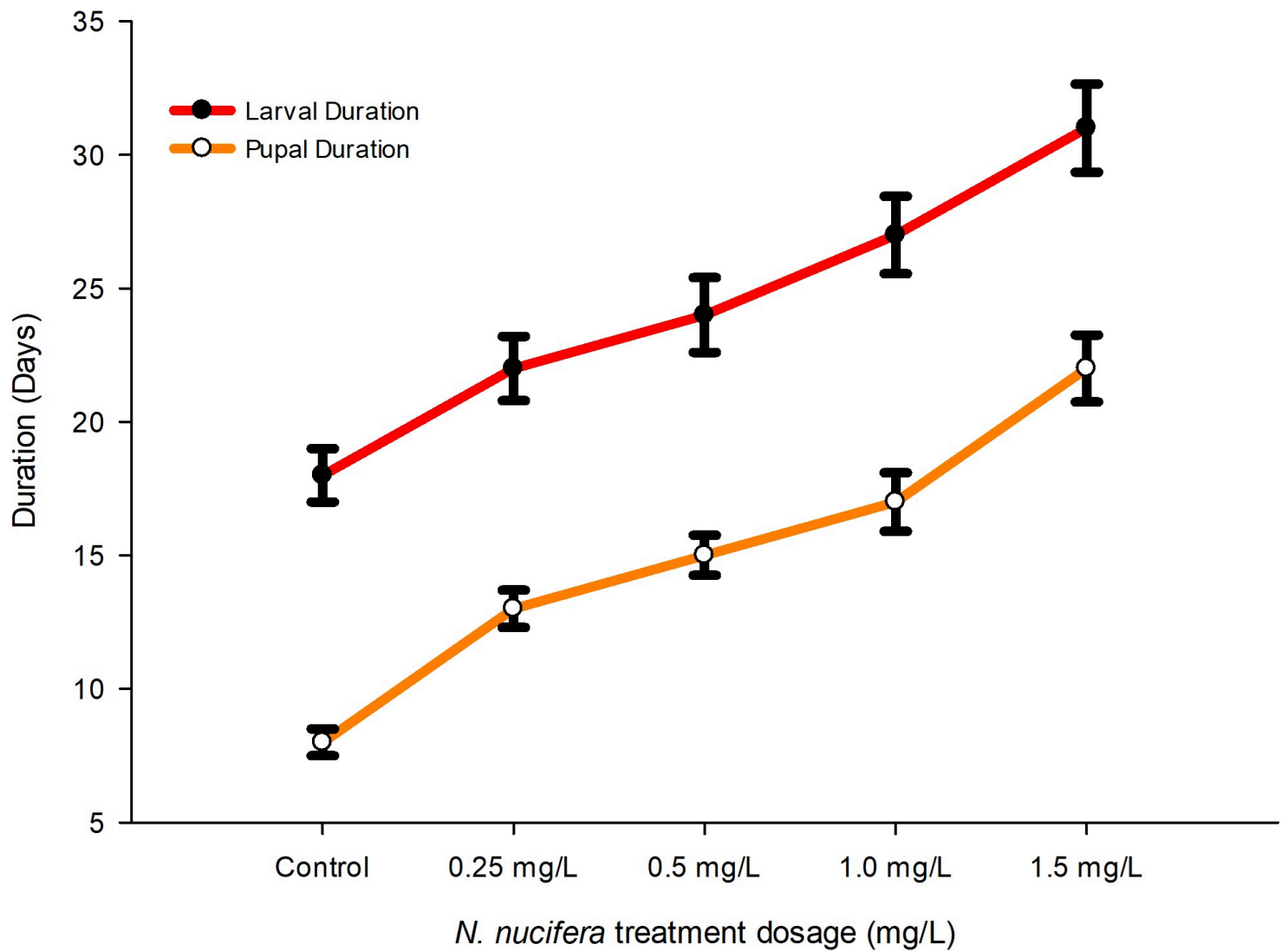


Figure 3

Days needed for *S. litura* larvae to develop & pupae after exposure with sub-lethal dosages of *N. nucifera* crude extracts. Means ($\pm$ SEM) show that there is no significant variation ($P \leq 0.05$) on the basis of the findings of Tukey's test.

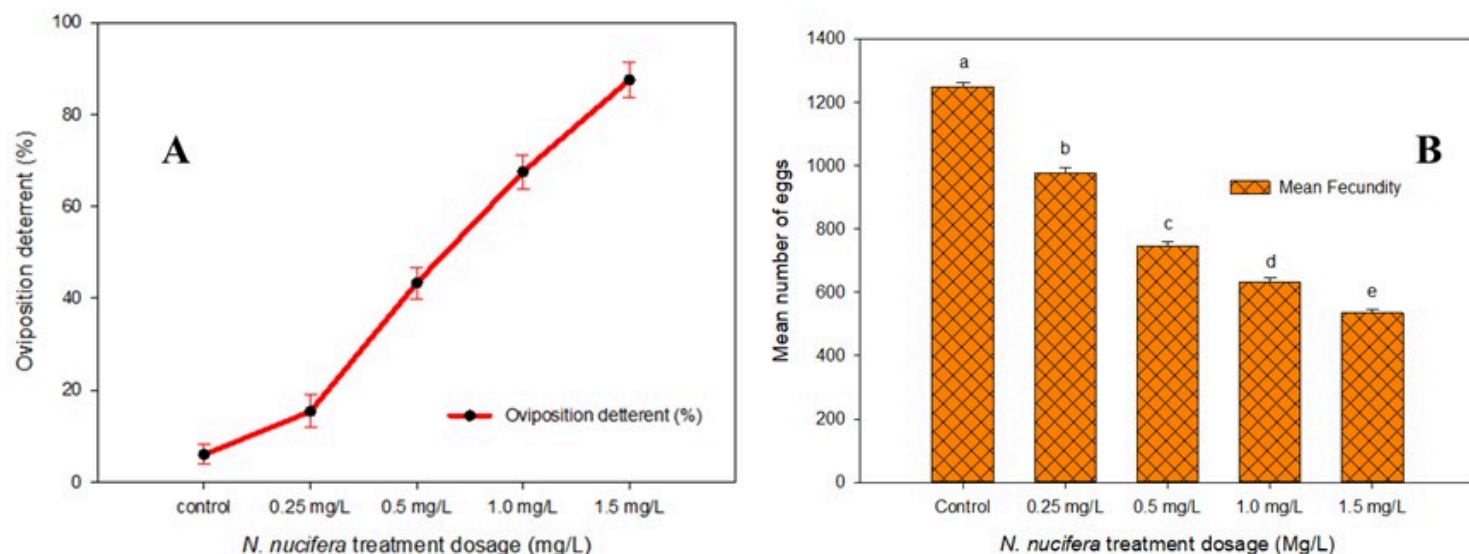


Figure 4

Oviposition deterrent index (ODI) (**A**) and Fecundity (mean no eggs laid) (**B**) of *Spodoptera litura* exposed to sub-lethal dosages of *N. nucifera* crude extracts. Tukey's test results show that there is Nothing noteworthy difference ($P \leq 0.05$) when the means ($\pm$ SEM) are followed by the identical letters above the bars.

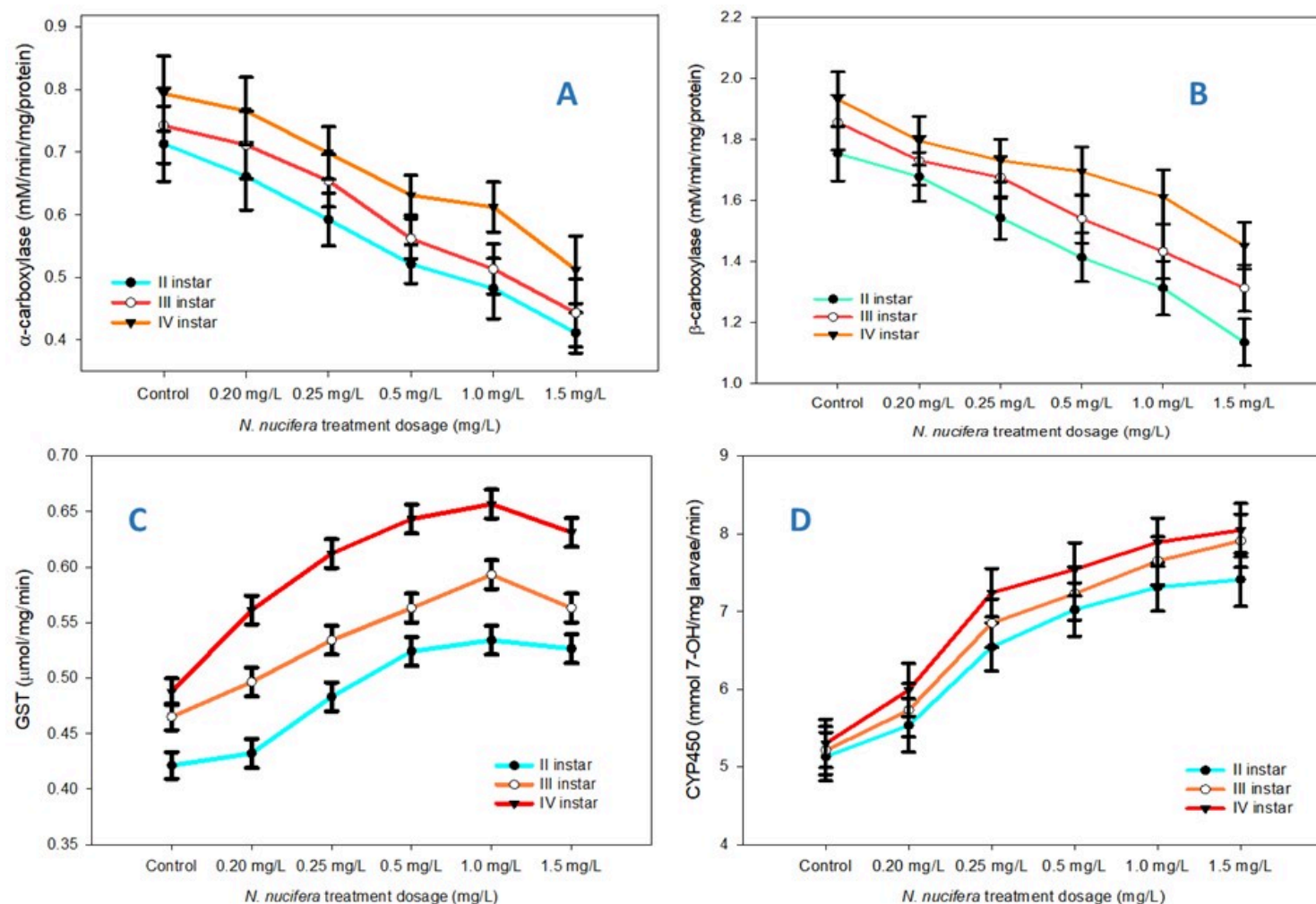


Figure 5

Expression of major detoxifying enzymes (A) α -carboxylesterase, (B) β -carboxylesterase, (C) glutathione S-transferase (GST), & (D) cytochrome-P450 (CYP450) in II, III & IV instars of *S. litura* exposed to sub-lethal dosages of *N. nucifera* crude extracts. Means ($\pm$ SEM) show that there is no notable distinction ($P \leq 0.05$) on the basis of the findings of Tukey's test.

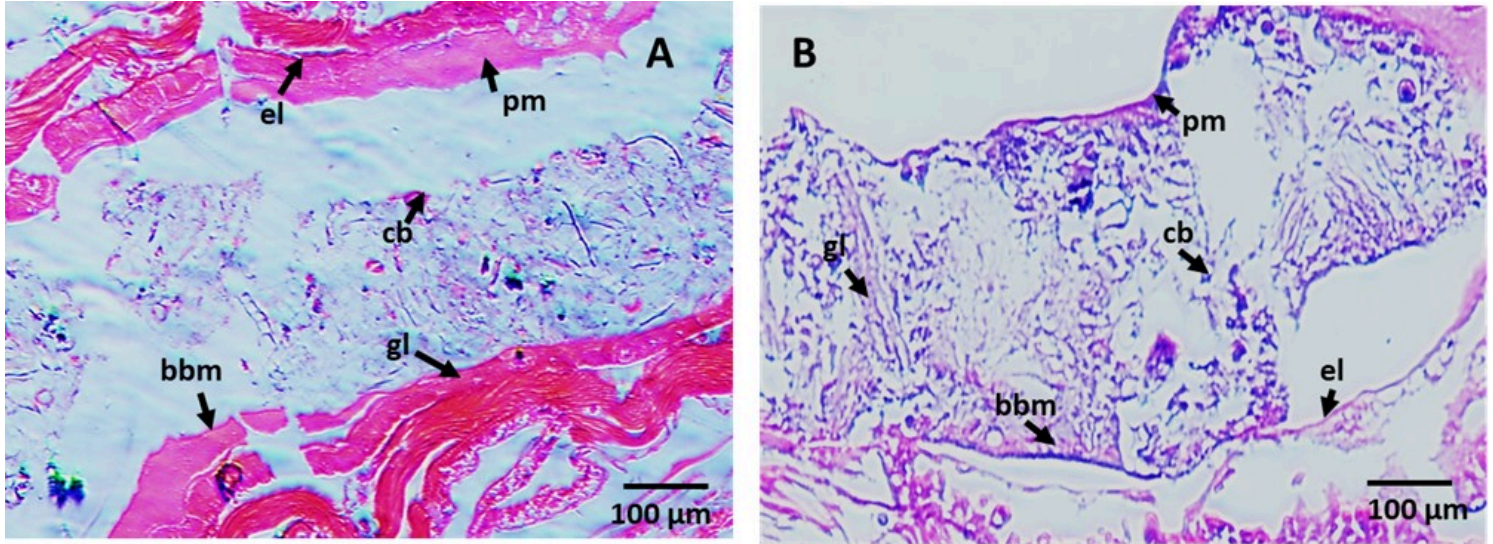


Figure 6

Midgut histological view of *S. litura* IV instar larvae (A) control, (B) *N. nucifera* crude extract treatment (2.5 milligrams per liter). el: epithelial layer; cb: ciliated border; gl: gut lumen; bbm: brush border membrane; nopm: peritrophic membrane.

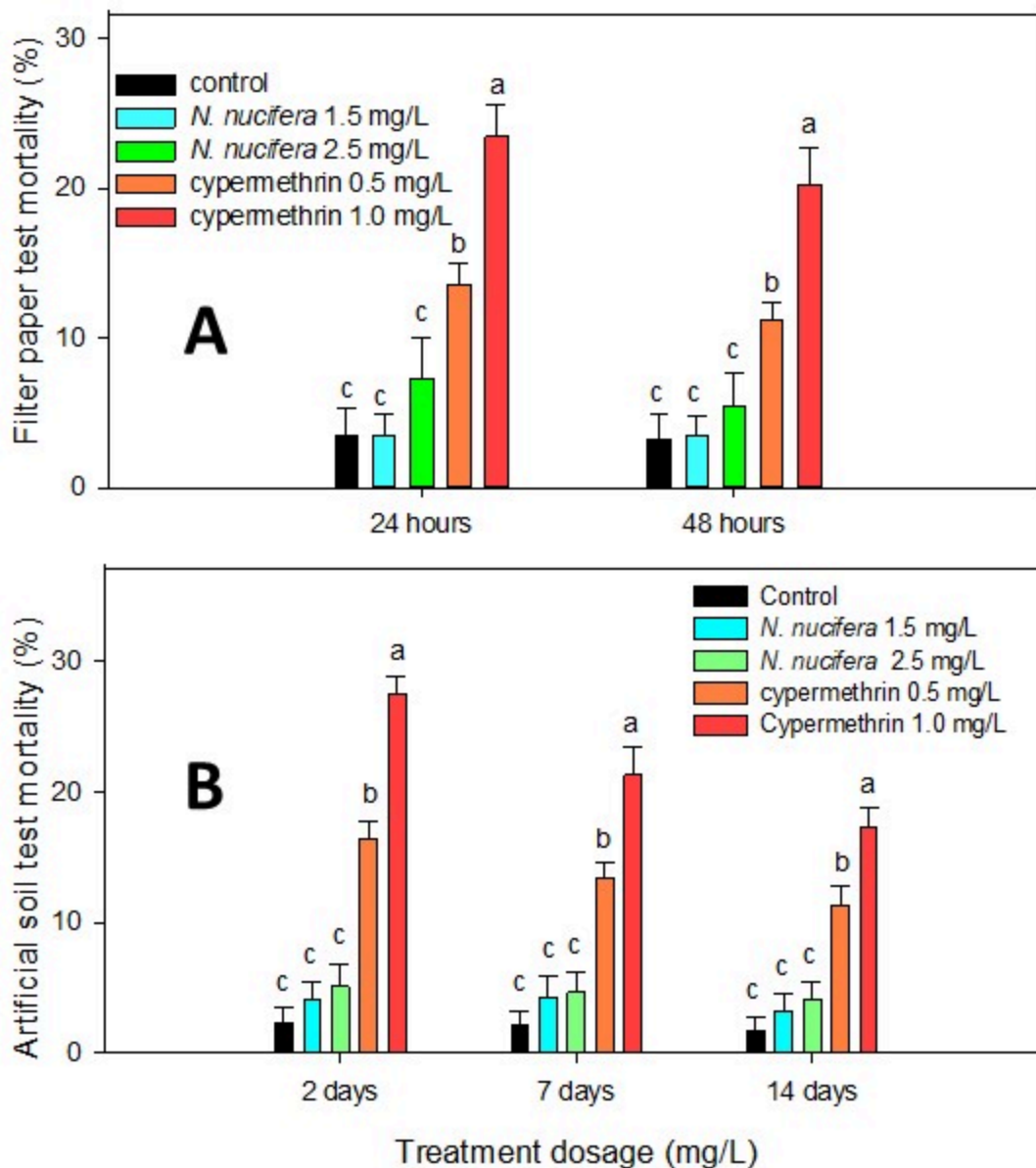


Figure 7

Non-target earthworm *Eisenia fetida* toxicity (A) filter paper test (FPT) for 24 & 48 hours and (B) Artificial soil test for 2, 7 & 14 days of exposure to crude extracts of *N. nucifera* and Cypermethrin is a chemical insecticide. Tukey's test results show that there is no significant variation ($P \leq 0.05$) when the means ($\pm$ SEM) are followed by the identical letters above the bars.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.docx](#)