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Insecticidal activity of *Delonix regia* (Fabaceae) against the cotton leafworm, *Spodoptera littoralis* (Bois) with reference to its phytochemical composition

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Overuse of synthetic pesticides causes problems for humans and the environment or leads to insect resistance to insecticides, so plant extracts and essential oils have gained popularity as an environmentally acceptable alternative to chemical pesticides. This study investigated the toxicity of *Delonix regia* leaf and seed extracts, protein patterns, and genetic distance analysis against the 5th instar larvae of cotton leafworm, *Spodoptera littoralis* Boisaduval. Methanol, petroleum ether, and acetone extracts of leaves and seeds of *D. regia* were used to coat castor leaves for ingestion by the 5th instar of *S. littoralis* larvae. The seeds' methanol and petroleum ether extracts were the most effective (100 and 98 Mortality%), with LC_{50} values of 0.887 and 1.795 g/L, respectively, 24 h post-treatments. Data showed that *D. regia* extracts affected consequences of protein changes compared to untreated *S. littoralis* larvae resulted in genetic changes, as well as inhibition of the insect's important α-amylases, forming protein complexes, and influencing normal growth and development. GC-MS analysis of the chemical composition of the seed extract revealed 18 compounds with high levels of stigmasterol, 2-methyl-4-vinylphenol, benzoic acid, 3-hydroxy, and squalene with percentages of 43.07%, 21.33%, 14.51%, and 14.12%, respectively. As a result, we concluded that *D. regia* seed extracts had the potential to control *Spodoptera littoralis* larvae.

Keywords *Spodoptera Littoralis*, *Delonix regia*, Biopesticide, Native-PAGE, Control, Cotton

With 30 valid and recognized polyphagous species, the widespread genus *Spodoptera* Guenée 1852 attacks diverse grain crops, pasture lands, and vegetable crops worldwide[1,2]. This genus includes several species that cause extensive damage to a broad range of essential crops, including cotton, maize, soybean, rice, cabbage, tomato, lettuce, pepper, strawberry, eggplant, tobacco, and sugar beet[3–5]. In addition to the primary insect complex consisting of *S. exigua*, *S. frugiperda*, and *S. litura*, the Egyptian cotton leafworm, *S. littoralis*, poses a threat to the production of the aforementioned crops[6]. With a widespread distribution in Egypt and other African nations, *S. littoralis* has emerged as one of the most dangerous pests in this region, necessitating the search for nontraditional and conventional pesticides within the context of environmental concern[7]. In Egypt, the Egyptian cotton leafworm, *S. littoralis* (Boisd.), is considered a major destructive pest, causing massive

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economic damage to ornamentals and orchard trees[8], In addition to cotton, vegetables, and fruits, the Egyptian cotton leafworm, *S. littoralis*, is considered a major destructive pest in Egypt[9,10]. *Spodoptera littoralis*, which gives priority to the larval stage, has a host range of more than 100 plants and causes a 50% loss in yield[11,12]. Over the past years, *S. littoralis* has developed resistance against several synthetic insecticides and some biocontrol agents, such as *Bacillus thuringiensis*, which challenged their various control plans[13].

Globally, there is a direction to find alternatives to synthetic pesticides for managing insect pests in order to avoid their disadvantages, such as human and environmental problems[14–16] and the impact on the non–target organisms[17,18]. In addition to the biological control and intercropping[19,20], the use of botanical insecticides in the ancient civilizations, including Egypt, India, Greece, and China[21,22], as well as more than 150 years ago in Europe and North America appears to be a solid foundation[23].

Two millennia ago, ancient Egyptian, Chinese, Greek, and Indian civilizations documented botanical extracts as effective substances in the control of pests[22]. Generally, four types of botanicals are used in insect control, viz. pyrethrum, rotenone, neem, and essential oils[23]. Various plant extracts of the royal poinciana, *Delonix regia*, have been found to be characterized by insecticidal and biocidal activities against many insects and acarines.

The royal poinciana, *D. regia* (Bojer ex Hook) Raffin (Family: *Fabaceae*), is native to India, Africa, Madagascar, and Northern Australia. The plant was traditionally used to treat a variety of diseases, including malaria, jaundice, ulcers, wound arthritis, and diarrhea[24]. In addition, its abundance of flavonoids, saponins, tannins, steroids, alkaloids, triterpenoids, and carotene hydrocarbons contributed to its application in the control of numerous insect and acarine pests, such as the Indian white termite, *Odontotermes obesus*, the German cockroach, *Blattella germanica*, the deer tick, *Ixodes scapularis*[25], the diamondback moth, *Plutella xylostella*[26], the maize weevil, *Sitophilus zeamais*[27], the southern house mosquito, *Culex quinquefasciatus*[28], the pepper weevil, *Anthonomus eugenii*[29], the cowpea aphid, *Aphis craccivora*[30].

In a comparative study against the destructive fifth larval instar of *S. littoralis* (Boisd.), we evaluated the leaf and seed extracts of *D. regia* (Bojer ex Hook) using various solvents, including petroleum ether, methanol, and acetone. Moreover, the effects of genetic variations caused by these applications relative to untreated larvae were reported. To our knowledge, this is the first study to assess the extracts of *D. regia* as a control botanical alternative against the Egyptian cotton leaf worms, *S. littoralis*.

Materials and methods

Plant materials and extraction of chemical constituents

The collected plant was identified and authenticated by Dr. Reem Hamdy, a plant taxonomy consultant at the Cairo herbarium, and a voucher specimen was deposited at the herbarium of the Plant Department, Faculty of Science, Cairo University, under the code: (PH 11-09-2024). Leaves and seeds of the royal poinciana, *D. regia* (Caesalpinioideae, Fabaceae) were collected from the North Coast, Egypt (31° 12’ 37.8” N; 29° 54’ 45.108” E) (Fig. 1). For the extraction of chemical components from the leaves, three different solvents (petroleum ether,

Fig. 1. Leaves and seeds of *Delonix regia* (taken by Dr. Mohamed Baz).

acetone, and methanol) were used, whereas only petroleum ether and methanol were used for the extraction of the components from the seeds since the acetone has the same ability to petroleum ether in extracting *D. regia* seed components. A Soxhlet extractor (Sklo Union, Teplice, Czech Republic) was utilized for eight continuous hours to extract the chemical components from 150 g of the crushed seeds and 100 g of the leaves. Excess solvents were extracted with the aid of an HS–3000 electric aspirator (Bibby Scientific Ltd., Staffordshire, UK). Stock extractions were then centrifuged for 20 min at 1,000 rpm to separate the extracted substances.

Experimental insects

Larvae of the Egyptian cotton leafworm, *S. littoralis* (Boisduval, 1833), were obtained from the laboratory colonies of the Department of Applied Entomology & Zoology, Faculty of Agriculture, Alexandria University, Egypt (31° 12' 0.3312" N; 29° 55' 7.4604" E). Under controlled laboratory conditions (25 ± 2 °C and 65 ± 5 RH%; 12 h light: 12 h darkness), the larvae were reared by feeding on castor plant leaves, *Ricinus communis*. The larvae of the fifth instar were subjected to starvation six h prior to the bioassay[31].

Bioassay

A total of 750 larval *S. littoralis* was processed to evaluate *D. regia* extracts. The petroleum ether and methanolic extracts of both the seeds and the leaves were prepared in a series of five concentrations (1, 5, 10, 20, and 50 g/L). Every concentration was applied to three replicates of ten larvae each. Methanol, acetone, and petroleum ether were used solely as controls. Fresh castor leaves were submerged in each concentration for three min to ensure a uniform coating of the extract on the castor leaves, then allowed to dry before the introduction to be ingested by the starved larvae in Petri–dishes. The mortality rates were evaluated after 24, 48, and 72 h post-feeding. Specimens of the control and treated larvae were preserved in 70% ethanol for further analysis. Mortality values were corrected according to the corrected Abbott formula[32]

$$\%\,Corrected\,mortality\;=\;\left(\frac{\%\,mt-\%\,mta\;X\;100}{100-\%\,mta}\right)$$

Where:

mt = mortality in treatment.

mta = mortality in witness treatment.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses of organic crude extracts of the seeds of *D. regia* (the most effective extracts) were performed by mixing two ml of the oil thoroughly with 7 ml of alcoholic sodium hydroxide ($C_2H_7NaO_2$), followed by addition of 7 ml alcoholic sulfuric acid with well-vortexing and kept overnight. One ml of sodium chloride was then added to the mixture and mixed well. Two ml of methanol were added to the mixture and vortexed, followed by dilution in 5 ml of diethyl ether and dried by anhydrous sodium sulphite (Na_2SO_3). One µl of the diluted mixture was placed in the GC-MS vial. The pH of the extraction buffer in the Native electrophoretic protein pattern is 7.8. Various analyses was performed with the help of Shimadzo GC-MS-QP2010 Ultra machine (Kawasaki-shi, Japan) with a RTX-5MS column (30 m, length ; 0.25 mm diameter; 0.25 µm, thickness) in 150 °C oven temperature, 250 °C injection temperature with injection mode of split; total flow: 55 ml/min; linear velocity pressure: 142 KPa; linear velocity: 49.6 cm/sec.; column flow: 1.74 ml/ sec. and purge flow: 3.5 ml/min. Helium have been used in the GCMS as a carrier gas with 1 ml/min flow rate.

Native electrophoretic protein pattern

Weights of 0.2 gm of every preserved larva/each treatment were rapidly frozen in liquid nitrogen, followed by homogenization with one ml of the extraction buffer. The homogenates were centrifuged for 5 min at 10,000 rpm, and the supernatants were transferred into another tube. Determination of the total protein in all pooled samples was performed based on the method previously described by Bradford[33].

Mixtures of homogenates samples and loading buffers were run in the vertical slab polyacrylamide gel electrophoresis (PAGE) according to Laemmli[34] using mini–gel electrophoresis unit (BioRad, USA) with 180 V/ 30 min followed by 150 V/ 45 min. A modification of lacking SDS in gel 8% (Acrylamide/Bis 30% T, 2.67% C; Tris–HCL 1.5 M, ph 8.8; Tris–HCL 0.5 M, ph 6.8; Ammonium persulfate 10%; and N, N,M, M–Tetramethylethylnediamine (TEMED) and running buffer (Tris- (24 mM) and glycine (194 mM)) were to determine the relative molecular weight of isolated proteins. After documentation, protein bands were visualized by staining with Coomassie Brilliant Blue G–250 and destained overnight with 7% (v/v) glacial acetic acid[35]. The relative mobilities (Rf), band intensity, percent of band intensity (B%), and band quantity (Qty) of the electrophoretically separated bands were determined.

Photographing, scanning, and band analysis were performed using Quantity One software (Version 4.6.2) to determine the relative motilities and amounts of the peptide chains as well as scanned graphical presentation of the fractionated bands of each lane. Additionally, it is used to determine percentages of the similarity index (SI%) and genetic distance (GD%).

Statistical analysis

We applied the one-way analysis of variance (ANOVA) for data analysis. Multiple comparisons were carried out applying Tukey's test and probit analysis for calculating the lethal values using the computer program MedCalc statistical software v. 19.2.6 (MedCalc Software Ltd., Ostend, Belgium)[36].

Results

Larvicidal activity of *D. Regia* extracts on *S. littoralis* larvae

The study evaluated the larvicidal effects of *D. regia* extracts against the 5th larval instar of *S. littoralis*, suggesting an insecticidal activity against *S. littoralis* larvae. The data of this study demonstrated that the methanol extract of *D. regia* seeds had stronger harmful effects than other plant extracts against *S. littoralis*. The mortality percent (MO%) of *S. littoralis* treated with 20 g/L methanol seed extracts of *D. regia* at 24 h post-treatment (PT) was complete (Fig. 2) with corresponding LC_{50} (50%, median lethal concentration) = 0.887 g/L (Table 1), while the corresponding values for *D. regia* seeds (Pe), *D. regia* leaves (Pe), *D. regia* leaves (M), and *D. regia* leaves (A) extracts were of LC_{50} values = 1.795, 3.37, 5.876, and 18.787 g/L, respectively, 24 h PT.

The larval mortality was maximum after 72 h of PT, while the mortality reached to the maximum at 20 g/L in plant extracts. In terms of fatal concentrations, *D. regia* methanol and petroleum ether seed extracts were revealed to be the most effective against *S. littoralis* larvae (LC_{50} = 0.587 and 1.251 g/L), followed by the petroleum ether extract of the leaves (2.661 g/L), the methanol extract of the leaves (3.726 g/L), and the acetone extract of the leaves (9.798 g/L) (Table 1). The application of solvents as control treatments resulted in no recorded mortalities. Several morphological irregularities were observed either in the treated larvae or in the pupation of the survivors (Figs. 3 and 4).

GC-MS analysis

GC-MS analysis of the chemical composition of the seed extract revealed a cocktail of 18 compounds with eight substances of insecticidal properties. *D. regia* seed methanolic contains high levels of Stigmasterol, 2-Methoxy-4-vinylphenol, Benzoic acid, 3-hydroxy, and Squalene with percentages of 43.07%, 21.33%, 14.51%, and 14.12%, respectively (Table 2).

Native polyacrylamide gel electrophoresis

The absence of some protein bands was observed in all the treatments compared to the control. The methanolic extract of *D. regia* seeds (T_2) was the most effective, resulting in the absence of four different protein bands. The petroleum ether of the seeds (T_1) was in second place, and it caused the disappearance of three protein bands. Both the methanolic (T_4) and petroleum ether (T_3), and acetone (T_5) leaf extracts caused the absence of one protein (Fig. 5). Related data of relative mobility, band intensity, and band quantity are depicted in Table 3.

For the first two protein bands, larvae treated with the methanolic extract of the leaves (T4) were the least mobile (0.09 and 0.148, respectively). The petroleum ether treatment of the leaves caused a decrease in the mobility of the third (0.34), fourth (0.58), and fifth bands (0.64). No significant differences were detected between the relative mobilities of other protein bands of the treated larvae (Table 3).

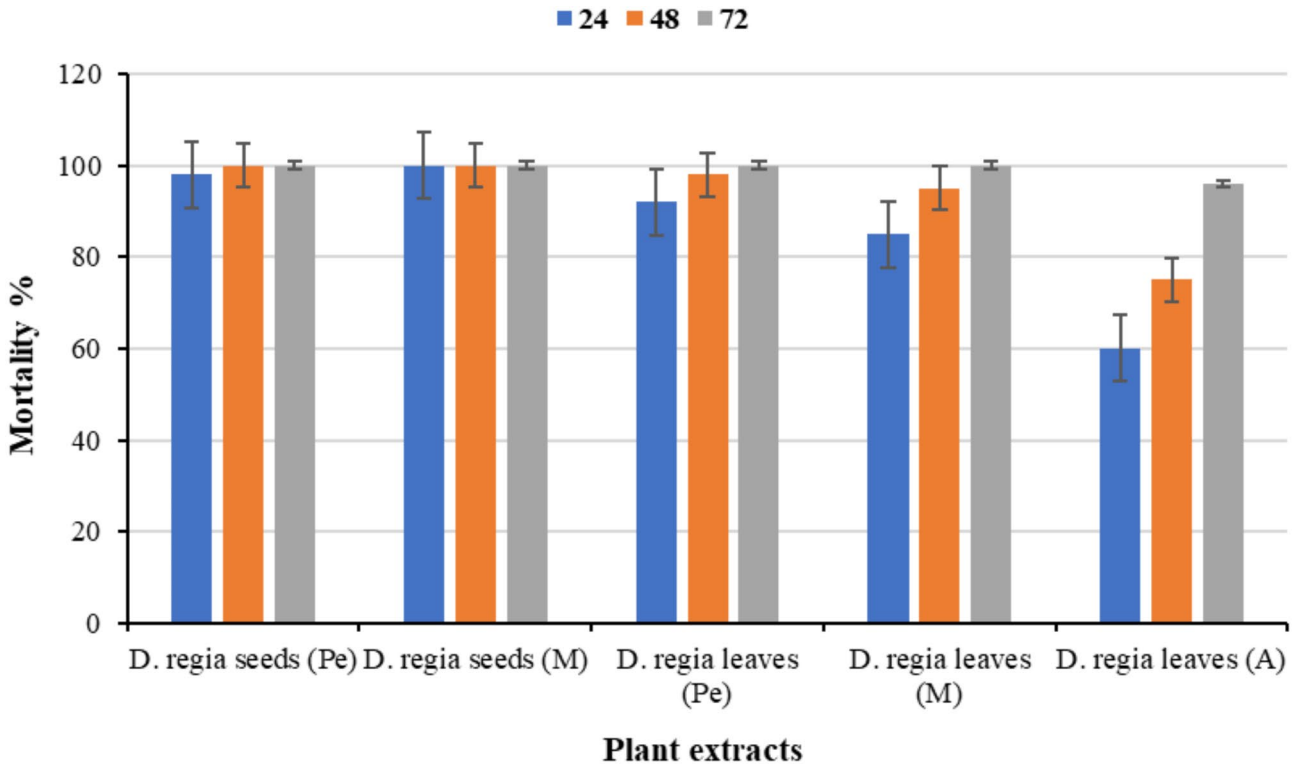

Fig. 2. Efficacy of *Delonix regia* extracts on *Spodoptera littoralis* larval mortality, 24, 48, and 72 h post-treatment.

Treatment	Intervals (hr)	LC_{50}[a] (g/L) 95% CL[b]	LC_{95}[c] (g/L) 95% CL	(Slope ± SE)[d]	(χ^2)[e]
D. regia seeds (Pe)	24	1.795 (0.173–4.174)	677.753 (116.346–400.000)	0.7465 ± 0.19	2.077
	48	1.6029 (0.179–3.664)	419.0165 (88.522–71.000)	0.7527 ± 0.19	2.180
	72	1.2519 (0.072–3.165)	478.4484 (89.857–220.000)	0.7517 ± 0.19	1.973
D. regia seeds (M)	24	0.887 (0.260–1.657)	17.930 (9.792–57.718)	0.9632 ± 0.26	1.260
	48	0.803 (0.222–1.520)	15.319 (8.461–48.135)	0.9884 ± 0.27	1.284
	72	0.587 (0.1087–1.2411)	13.563 (7.275–47.731)	1.01 ± 0.28	1.206
D. regia leaves (Pe)	24	3.375 (0.948–12.384)	84.000 ($872.338–17.3\times10^{15}$)	0.7189 ± 0.18	1.320
	48	2.661 (1.890–9.639)	83.000 ($819.269–637\times10^{19}$)	0.8275 ± 0.16	1.253
	72	2.661 (1.89–9.6394)	83,000 ($819.2699–637\times10^{19}$)	0.8275 ± 0.16	1.253
D. regia leaves (M)	24	5.876 (0.938–16.655)	10.000 ($500–538\times10^{7}$)	0.7289 ± 0.18	1.901
	48	4.385 (1.118–17.200)	28×10^{4} ($49.000–16\times10^{5}$)	0.7172 ± 0.15	1.243
	72	3.726 (1.873–14.567)	10×10^{4} ($950–33\times10^{6}$)	0.7188 ± 0.16	1.320
D. regia leaves (A)	24	18.787 (5.613–5.900)	18×10^{4} ($1.600–13\times10^{6}$)	0.7308 ± 0.18	1.764
	48	14.426 (3.235–64.330)	27×10^{5} ($33\times10^{4}–22\times10^{6}$)	0.7202 ± 0.13	1.294
	72	9.798 (2.501–38.382)	66×10^{4} ($10.000–40\times10^{6}$)	0.7204 ± 0.18	1.359

Table 1. Toxicity of various *D. regia* extracts on the larvae of *S. Littoralis*. **Pe**: Petroleum ether; **M**: Methanolic; **A**: Acetone. [a] The concentration causing 50% mortality. [b] Confidence limits. [c] The concentration causing 95% mortality. [d] Slope of the concentration-mortality regression line ± standard error. [e] Chi square value.

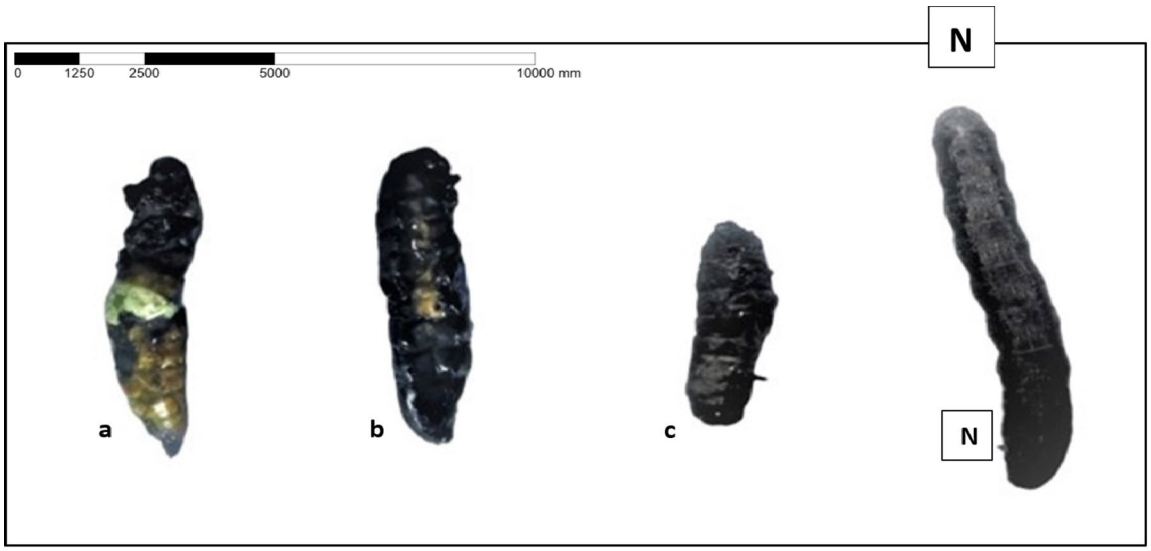

Fig. 3. *S. littoralis* larval anomalies in response to the application of *D. regia* extracts. Degeneration, atrophy, and deformities of the anterior part and the rest of the body of exposed larvae (a, b), total atrophy in larval muscle (c) compared to the untreated control (N).

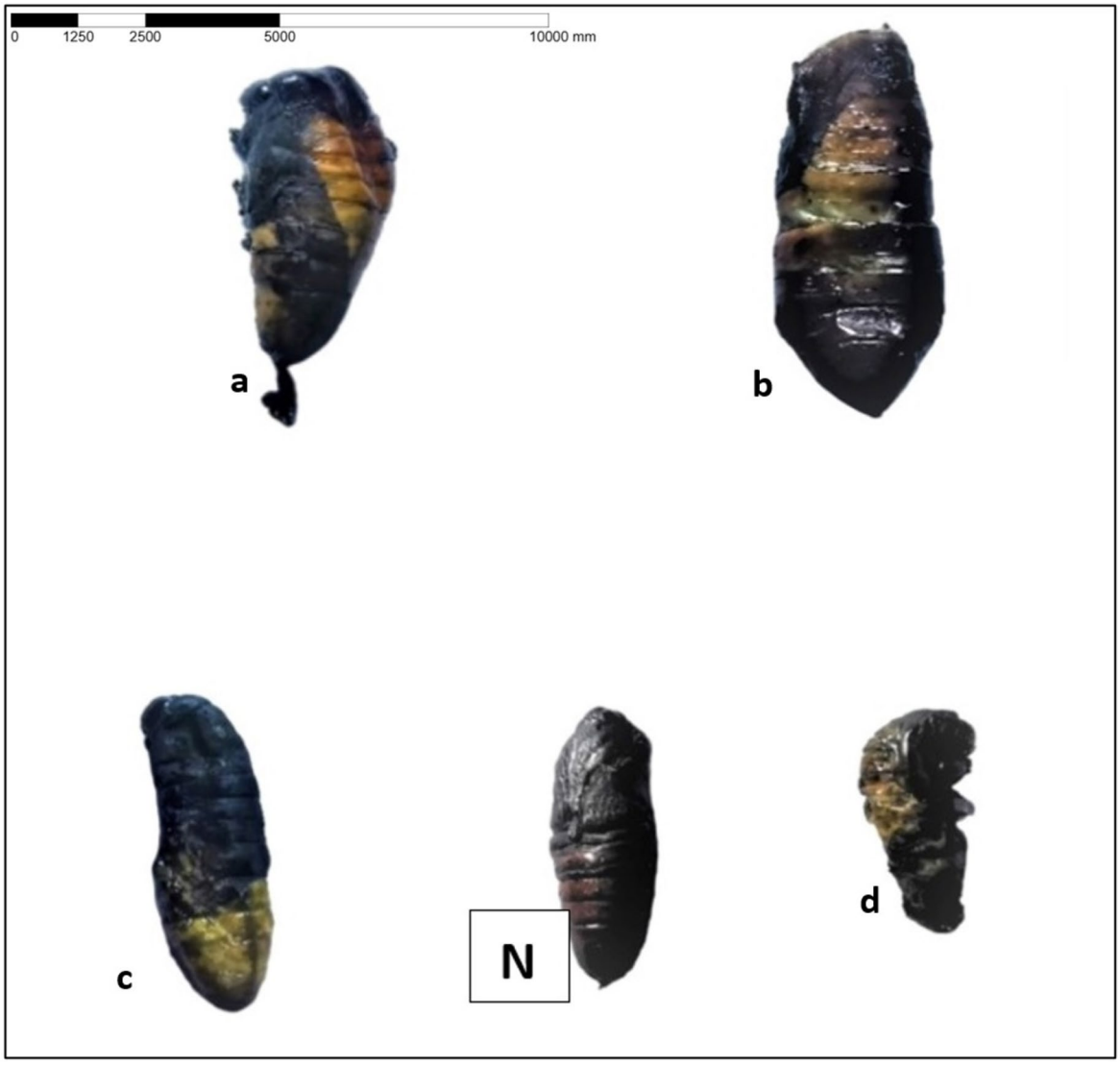

Fig. 4. Failure of pupation of the treated *S. littoralis* larvae. Occurrence of damage and deformities in multiple parts of the pupa body (a-d); production of intermediate larvae-pupa or larvae-pupa (c, d). (N = normal)

Similarity index and genetic distance

Similarity–wise calculations revealed that T_4 (methanolic extract of the leaves) was the least similar to control by only 58.10%, whereas T_3 (petroleum ether of the leaves) showed the highest similarity to control by 74.60%. In–between the treatments, the highest similarity pattern was between T_5 and T_3 by 80.70%. Genetic distances between band patterns ranged between 19.30% and 80.50% (Table 4).

Discussion

The present study revealed promising insecticidal activities of *D. regia* different extracts against *S. littoralis* larvae. The study showed that the methanol extract of *D. regia* seeds was more harmful to *S. littoralis* than other plant extracts. Methanol seed extracts of *D. regia* killed all of the *S. littoralis* stage in 24 h, with an LC_{50} value of 0.887 g/L. The maximum mortality occurred at a concentration of 20 g/L in the plant extracts after 72 h post-treatment. Methanol (LC_{50} = 0.587 g/L) and petroleum ether (1.251 g/L) were the two seed extracts from *D. regia* that killed the most *S. littoralis* larvae. The petroleum ether leaf extract (2.661 g/L), the methanol leaf extract (3.726 g/L), and the acetone leaf extract (9.798 g/L) came next.

The extraction yield and phytochemical composition were influenced by the polarity of the extracting solvents. The use of different solvents resulted in varying extraction yields. Changes in the polarity of the solvent can help explain why the concentration of bioactive compounds in the extract changed. This is due to the presence of substantial amounts of polar molecules in plant components that are soluble in highly polar liquids. A different study found that the acetone extract had more alkanes, flavonoids, terpenes, ketones, and phenols than the water-based extract[37].

No.	Compound	Retention Time (RT)	Peak area (%)	Reported action	Tested insects	Reference
1	4 H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	14.18	0.13	Anti-diabetic and antioxidant activity		73
2	n-Hexadecanoic acid	15.31	0.04	Pesticide	*Spodoptera litura*	74
3	E, Z-1,3,12 Nonadecatriene	16.08	0.03	anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.		75
4	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	16.14	0.03	Insecticidal activity	*Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti*	37
5	Campesterol	16.19	0.38	Antibacterial activity		76
6	2-Methoxy-4-vinylphenol	16.39	21.33	Insecticidal activity	*Myzus persicae*	29
7	7,10-Octadecadienoic acid, methyl ester	17.20	0.08	Anti-inflammatory, anxiolytic		
8	9,12-Octadecadienoic acid (Z, Z)	17.42	0.16	Insecticidal Activity	*Tribolium castaneum*, *Callosbruchus analis*	77
9	Stigmasterol	18.17	43.07	Acetylcholinesterase inhibitory activity	*Culex quinquefasciatus*, *Aedes aegypti*, *Chironomus riparius*	78
10	Benzoic acid, 3-hydroxy	18.20	14.51	Major Insecticidal activity	*Aedes atropalpus*	79
11	Benzoic acid, 4-hydroxy	18.24	2.04	Not reported		
12	Squalene	18.41	14.12	Insecticidal activity	*Musca domestica*	80
13	Heptadecanoic acid	19.91	1.32	Lipid synthesis inhibitors	*Spodoptera littoralis*	81
14	β-Sitosterol	20.04	0.41	Antiviral, Antiplasmodial		82
15	Heneicosanoic acid, methyl ester	20.25	1.18	Not reported		
16	Vitamin E	21.12	0.04			
17	Stigmasterol, 22,23-dihydro-γ -Sitosterol	21.96	0.63	Not reported		
18	Ergost-5-en-3-ol, (3. β)	23.54	0.50	Not reported		

Table 2. Chemical constituents of the methanolic extracted fraction of *Delonix regia* seeds with notes on reported toxicological studies.

Phytochemical analyses showed several components that serve as bioactive secondary metabolites in *D. regia* extract, including tannins, sterols, flavonoids, saponins, alkaloids, steroids, anthocyanins, triterpenoides, and carotene hydrocarbons[24]. Earlier reports approved the insecticidal activity of *D. regia* extract against beetles and caterpillars (LC_{50} = 5.7 g/L)[38], *Pericallia ricini* (LC_{50} = 9.47 g/L)[39], *Anopheles gambiae* (LC_{50} = 7.16 g/L)[40], the teak defoliator, *Hyblaea puera* (LC_{50} = 14.58 g/L)[41]. In addition to the toxicity, an antifeedant effect was reported when applied against the pulse beetle, *Callosobruchus maculatus*[42]. Apparently, *D. regia* extracts have topical insecticidal properties[43]; however, the cytotoxic properties, including the oxidative stress and decreasing the cell viability could be the main reason for the mortalities caused by carbon tetrachloride and dichloromethane fractions of *D. regia* extracts have been reported as cytotoxic components[44]. Previous studies have reported that the essential oils have a blocking effect on the insect spiracles, leading to the strangulation and death of the insect through their topical toxicity[45,46]. Other effects of some plant extracts included apparently ovicidal actions, resulting in decreased hatchability or oviposition rates as a result of impeding the adult insect's locomotion and mating success[46].

Based on earlier studies regarding the phytoconstituents of *D. regia*, seeds of this plant contain high concentrations of fatty acids viz. linoleic, heptanoic, octanoic, myristic, palmitic, stearic, and oleic acids that are absent in the leaves[47]. These acids have been previously investigated in terms of their toxicological effects on some insect pests, including spiny bollworm and *Earias insulana*, as well as whether they decrease or increase the total protein content of the tested insects out of the normal levels. It affects protein synthesis through the formation of the protein complex, influencing the insect's growth, development, performing vital activities, and eventual death[48]. Moreover, various fatty acids and esters of plant origin were previously showed toxic effects against the codling moth, *Cydia pomonella*[49], the southern house mosquito, *Culex quinquefasciatus*[50], the malaria vector, *Anopheles funestus*, and the Egyptian cotton leaf worms, *S. littoralis* itself[51,52]. The results of GC-Mass analysis of the methanolic seed extracts of *D. regia* reported high contain of Stigmasterol, 2-Methoxy-4-vinylphenol, Benzoic acid, 3-hydroxy and Squalene. Stigmasterol has been reported to have an inhibitory influence on the activity of one of the most familiar detoxifying enzymes in insects, the acetylcholinesterase (AChE)[53], suppressing the insect defense action in response to toxic substances and explaining the potential insecticidal efficacy on *S. littoralis* larvae of this study. Likewise, the 2-Methoxy-4-vinylphenol was reported as a compound with insecticidal activity[54].

In general, sterols such as β-sitosterol, stigmasterol, and sitosterol present in both seeds and leaves of *D. regia* are responsible for the insecticidal properties of fixed essential oils[55]. The tannins concentration of *D. regia* seeds and leaves as well[56] may be a determining factor in insecticidal and biocidal activity. Since they have been reported to be of hardening influence, leading to the limitation of cell transfer and eventually death, apart from their larvicidal properties, affecting the growth, development, and fecundity of many phytophagous insects[57,58].

Phytosterols, like stigmasterol and alkanols, control the killing of larvae. They can be found in the bioactive part of plants like *Delonix regia*, *Chromolaena odorata*, *Ricinus communis* and many plants. Stigmasterol and 1-hexacosanol were the primary chemicals responsible for killing the insect larvae, as they caused damage to

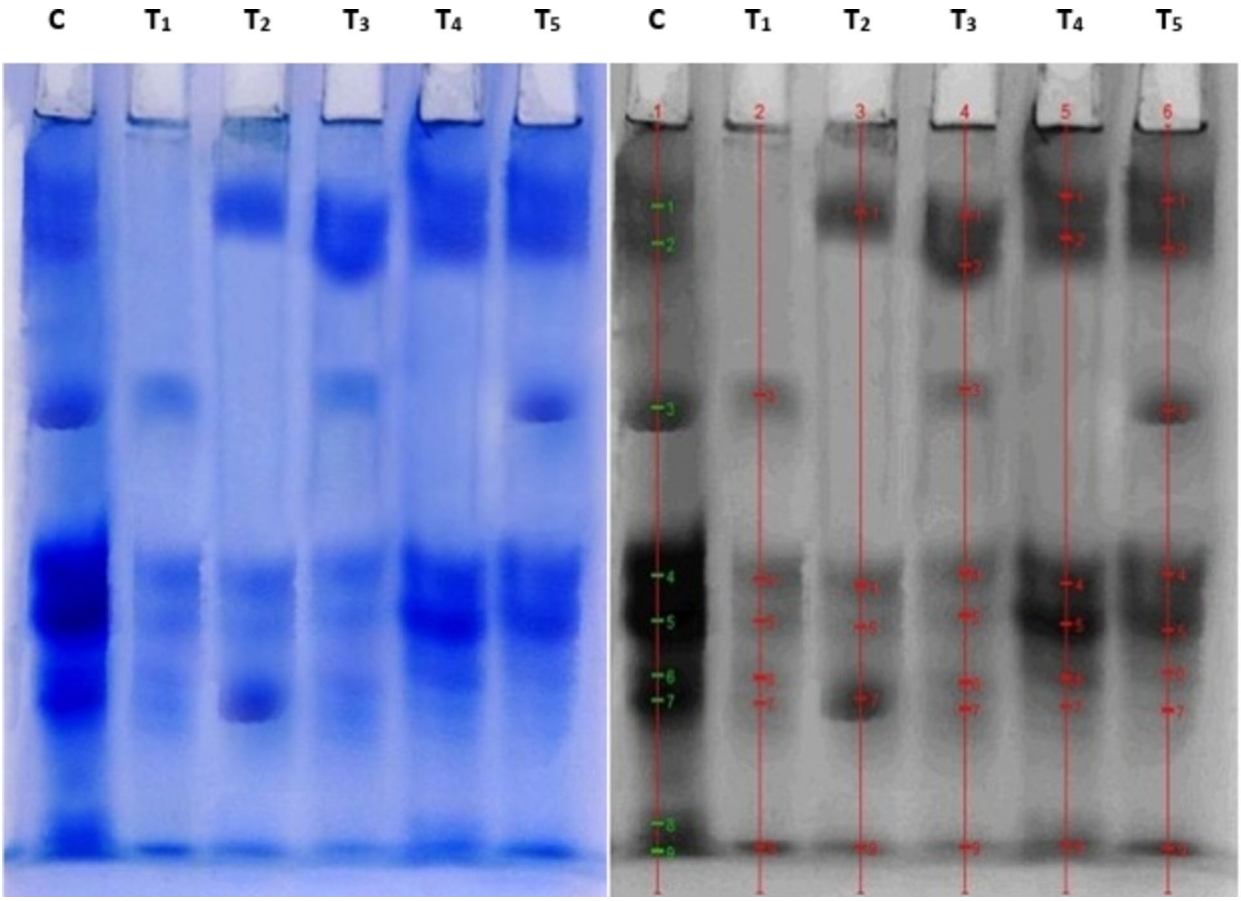

Fig. 5. Native electrophoretic protein pattern of the control and treated larvae of *S. littoralis* (M: molecular marker; C: control; T_1: Petroleum ether extract of *D. regia* seeds; T_2: Methanolic extract of *D. regia* seeds; T_3: Petroleum ether extract of *D. regia* leaves; T_4: Methanolic extract of *D. regia* leaves; T_5: Acetone extract of *D. regia* leaves).

the nerve cells. Researchers found that stigmasterol and 1-hexacosanol both stop acetylcholinesterase activity in *Culex quinquefasciatus* and *Aedes aegypti*. It was shown to block acetylcholinesterase both in the laboratory with recombinant acetylcholinesterase and in the wild with *Culex* and *Aedes* larval homogenates. Electrophysiological studies using electroantennography have shown that these substances make the brain respond more strongly (Gade et al., 2017).

Likewise, flavonoids and isoflavonoids[59], significantly influence the insect's behavior, development, and growth[60]. Falvonoides along and saponins previously showed increased mortality of the gypsy moth, *Lymantria dispar*[61], the tobacco armyworm, *S. litura*, the cowpea seed beetle, *C. maculatus*[62], the pea aphid, *Acyrthosiphon pisum* (Goławska et al. 2014). Flavonoids may kill nematodes by blocking acetylcholinesterase (AChE). This is because nematodes, insects, and mammals share many neurotransmitters, such as acetylcholine, serotonin, and glutamate[63]. Botanical extracts are also known by their side effects other than toxicants, including antifeedants, deterrents, and anti-growth/development[64,65], resulting in death eventually due to starvation and/or growth failure. This finding might also explain the behavioral changes, anomalies, and molting failure of the treated larvae in this study.

Variations in lethality between the different treatments could be either due to the plant part and the concentrations of the components inside each or due to the solvent used. Although the plant's metabolites vary in their chemical nature and composition according to the species, these properties are fixed in all the parts of the same plant species, and basically, secondary metabolites are the main components[66]. The concentrations of these secondary metabolites also vary according to the plant part[67], which might explain the more potent efficacy of the *D. regia* seed extracts than the leaf extracts in the obtained results. Furthermore, the nature of the solvent used is apparently playing a role in determining the final efficacy of the plant extract as it either varies in the efficiency of extracting the bioactive molecules from the plant or acts as a synergistic factor in some cases[68]. Methanol appears to be the optimal solvent for maximizing plant extraction yield in addition to the synergistic effect it plays[69,70]. This finding may explain why methanolic extracts demonstrated the best results in the current study, followed by other solvents.

Molecularly, the protein fraction (RF) of *D. regia* seeds that contain cationic proteins evidently inhibits the α–amylase enzymes being produced by insects, such as *C. maculatus* (by 97.7%), *Anthonomus grandis* (by 84.7%) and *Acanthoscelides obtectus* (by 48.5%) with more specificity to insect α–amylase and no inhibitory effects on the

Control				T_1				T_2				T_3				T_4				T_5			
Rf	Int.	B%	Qty	Rf	Int.	B%	Qty	Rf	Int.	B%	Qty	Rf	Int.	B%	Qty	Rf	Int.	B%	Qty	Rf	Int.	B%	Qty
0.11	181.24	10.41	7.98	–	–	–	–	0.12	152.39	20.73	16.35	0.12	146.82	12.58	7.46	0.09	179.14	14.77	6.03	0.10	180.80	14.52	7.83
0.16	193.11	11.09	2.37	–	–	–	–	–	–	–	–	0.18	165.70	14.20	10.70	0.148	172.65	14.23	9.91	0.16	161.96	13.00	6.601
0.37	176.07	10.11	6.67	0.35	130.72	15.73	11.64	–	–	–	–	0.34	141.17	12.10	4.66	–	–	–	–	0.37	153.53	12.33	10.56
0.59	210.96	12.12	15.13	0.59	140.85	16.95	10.56	0.60	144.95	19.72	10.15	0.58	151.03	12.94	9.34	0.596	185.00	15.25	10.16	0.59	158.68	12.74	9.096
0.65	243.61	13.99	3.99	0.65	145.43	17.50	7.27	0.65	148.25	20.17	5.08	0.64	150.07	12.86	7.01	0.65	219.43	18.09	10.13	0.66	183.48	14.73	6.544
0.72	228.33	13.12	2.34	0.72	144.38	17.37	3.61	–	–	–	–	0.726	147.92	12.68	2.64	0.72	174.57	14.39	4.14	0.71	151.24	12.14	4.816
0.75	205.01	11.78	11.13	0.75	141.38	17.01	3.09	0.75	175.19	23.83	9.40	0.76	138.68	11.89	7.43	0.757	151.21	12.46	3.21	0.76	130.67	10.49	1.664
0.91	167.24	9.61	3.60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.94	125.19	10.05	7.176
0.95	135.39	7.78	2.50	0.94	128.25	15.43	7.21	0.94	114.38	15.56	7.84	0.94	125.38	10.75	5.17	0.939	131.26	10.82	9.33	–	–	–	–

Table 3. Different parameters of the native electrophoretic protein pattern of the *S. littoralis* treated larvae. **Rf.**: Relative Mobility, **Int.**: Band Intensity, **B%**: Percent of Band Intensity, **Qty**: Band Quantity.

		Control	T_1	T_2	T_3	T_4	T_5
		SI%					
Control	GD%	100.00	59.30	64.60	74.60	58.10	61.80
T_1		40.70	100.00	50.00	66.50	61.00	80.50
T_2		35.40	50.00	100.00	66.50	56.60	56.90
T_3		25.40	33.50	33.50	100.00	80.50	80.70
T_4		41.90	39.00	43.40	19.50	100.00	76.20
T_5		38.20	19.50	43.10	19.30	23.80	100.00

Table 4. The similarity index (SI%) and genetic distance (GD%) of the native electrophoretic protein pattern. SI%: Percent of Similarity Index, GD%: Percent of Genetic Distance.

serine proteinases[71]. *Delonix regia* has been proven to produce diversified inhibitors which perhaps involved in the impact on the insect's defense mechanisms[71]. The alpha-amylases are principally digestive enzymes involved in the preliminary pathway of maltopolysaccaride digestion and are produced with the help of multiple gene copies. Multiple copying of insect amylases enables insects to organize their tissue- and stage-specific regulation, improve their enzymological abilities, and circumvent the plants' inhibitory defenses. The inhibition of these enzymes by inhibitors produced by *D. regia* plants may be the primary cause of the failure of these processes and eventual insect death[72]. The appearance and disappearance of proteins produced by *S. littoralis* larvae undoubtedly explain the hidden effect of the *D. regia* extracts' mode of action. Consequently, in the following experiment, we will design a study to investigate the nature of these products.

In the present study, we demonstrated a botanical alternative to conventional synthetic insecticides for controlling one of the main destructive phytophagous insects, *S. littoralis*. The toxicological effect of *D. regia* extracts on *S. littoralis* larvae was apparently not due to the topical application mentioned above. However, the effects of genetic and protein production reactions, such as the inhibition of the insect's α–amylases by these extracts, could be a determining factor in the development and survival of the insect in terms of anti–feeding (the inhibition of their feeding ability hours before the death), aggregation behavior (formation of mass number of larvae), immobility (lack of regular active movement), failure of stage–regulation or death actions (elongation of the larval stage period greater than the normal) as observed and discussed in the current study; however, further investigations regarding the α–amylases activity are needed to confirm this fact. Therefore, we recommend additional research into *D. regia* extracts to be used as botanicals with insecticidal properties against various insects.

This study attempted to manage *S. littoralis*, which is an economically significant pest in Egypt. *Delonix regia*'s various extracts of leaves and seeds were found promising in suppressing such insects with maximum impact of the methanolic seed extract (LC_{50} of 0.887 g/L). Additionally, several genetic and protein repercussions were noticed by the Native-PAGE profile, demanding future follow-ups of the current work. Therefore, we would encourage future research into the use and formulations of *D. regia* extracts against insect pests.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Ethical statement

The Ethics Committee of the Faculty of Agriculture, Alexandria University approved the work protocol (Code: Alex.Agri.112310305). We conducted the study in accordance with local legislation and institutional requirements.

Additional information

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