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Research Article

Bioefficacy and GC-MS analysis of *Chromolaena odorata* and *Leonotis nepetifolia* leaf extracts against *Spodoptera litura*

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Abstract: This study aimed to assess the insecticidal properties from leaf extracts of *Chromolaena odorata* L. and *Leonotis nepetifolia* (L) R.Br. on the third instar larvae of tobacco leaf-eating *Spodoptera litura* (F.). Leaves of both plant species were extracted with three solvents, acetone, methanol, and water, then tested for ovicidal, antifeedant, and larvicidal activity at 0.5, 1, 2.5, 5, and 7%. The methanol extract (5%) of *C. odorata* was found more active for ovicidal (73.33 ± 0.57%), antifeedant (82.45 ± 0.16%), and larvicidal (68.33 ± 0.05%) activities against *S. litura*. Similar results were noted from methanol extract (5%) of *L. nepetifolia* for ovicidal (71.33 ± 0.41%), antifeedant (71.77 ± 0.73%), and larvicidal (73.33 ± 0.08) activities. Phytochemical screening revealed a significant amount of alkaloids and phenolics in methanolic leaf extracts of both plants. Besides, thirty-one bioactive compounds from the methanolic extract of *C. odorata* and sixteen compounds from *L. nepetifolia* were identified by GC-MS analysis. The recorded compounds are phenols, fatty acids, esters, and essential oils with insecticidal properties. The insecticidal compounds detected from GC-MS and quantitative phytochemical analysis might be attributed to the high insecticidal potential (Ovicidal, antifeedant and larvicidal) of *C. odorata* and *L. nepetifolia*. Therefore extensive research on *C. odorata* and *L. nepetifolia* is needed in phytopesticide development against *Spodoptera litura*.

Keywords: *Chromolaena odorata, Leonotis nepetifolia, Spodoptera litura,* Insecticidal, Phytochemical

Introduction

India is an agricultural country, and more than 80% of the population depends on agriculture (Baskar *et al.*, 2014). Crop protection has immensely contributed to the success of the Green Revolution and sustained the production of food, fiber, fodder, and feed (Kumar, 2015). Biopesticide is a formulation made from naturally occurring substances that control pests by nontoxic mechanisms and in an eco-friendly manner, consequently gaining importance worldwide (Kumar, 2012).

The tobacco caterpillar, *Spodoptera litura* (Fab.), is one of the severe and prevailing polyphagous pests (Vetal and Pardeshi, 2019).

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This pest attacks more than 112 cultivated crops and causes severe losses (Baskar *et al*., 2011). Synthetic pesticides have been used for many decades in controlling pests due to their effective results in less time. However, their indiscriminate use resulted in several problems, such as resistance to pesticides, the resurgence of pests, elimination of natural enemies, and toxic residues in air, water, food, and soil which affected human health and disrupted the ecosystem. Finally, it led to a threat to the environment (Chinnamani and Jeyasankar, 2018). Therefore, searching for sustainable substituted methods for managing this pest is necessary.

Botanical insecticides have been recommended as a suitable alternative for plant protection with minimum adverse risk (Awasthi and Avasthi, 2017). Plant derivatives are highly toxic to many insect species, and more than 2000 plant species are known to possess some insecticidal properties. Some of the Meliaceae, Rutaceae, Asteraceae, Lamiaceae, Convolvulaceae, and Pedaliaceae are promising sources of insecticide-based properties (Isman, 1995; Sujatha *et al*., 2010). Lamiaceae species are recognized to include pharmacologically active phytochemicals with a broad spectrum of bioactivity. They are predicted to play more significant roles in drug discovery and food, cosmetic, and pesticide industries (Khodja *et al*., 2014).

Chromolaena odorata L. belongs to Asteraceae (Gautier, 1992), and *Leonotis nepetifolia* (L.) R. Br. belongs to the family Lamiaceae (Pushpan *et al*., 2012). *Chromolaena odorata* is reported for insecticidal properties (Yankanchi and Patil, 2009). Various plant parts of *L. nepetifolia* showed antiviral, antibacterial, fungicidal, pesticidal, anti-inflammatory, and anticancer activities (Almeida *et al*., 2018). Hence the present study aimed to evaluate the ovicidal, antifeedant, larvicidal activity, and phytochemical profiles from crude extracts of *C. odorata* and *L. nepetifolia* against the notorious polyphagous pest *Spodoptera litura* (F.). Further GC-MS analysis of the same plants was conducted to find the bioactive compounds with insecticidal properties.

Materials and Methods

Plant collection and extraction

Healthy leaves of *C. odorata* and *L. nepetifolia* were collected from Nipani, Karnataka, India. (16.404753 N latitude and 74.372758 E longitudes). The plant materials were identified, and specimens were deposited at the Herbarium of Department of Botany, Shivaji University, Kolhapur, Maharashtra, India (Voucher specimen No.VBG 01 and VBG 02, respectively). The plant materials were shade-dried at room temperature and powdered coarsely. The 3 g dried powder was sequentially extracted with acetone, methanol, and water using an orbital shaker for six h, 100 rpm (Neolab, India) at room temperature. The crude extracts were collected in clean borosil vials and stored in the refrigerator at 4 °C before being subjected to bioassays against *Spodoptera litura* (F.) (Baskar *et al*., 2010).

Insect culture

Spodoptera litura eggs were collected from the tobacco field nearby the Nipani area and were surface sterilized with 0.02% sodium hypochlorite solution, dried, and allowed to hatch. After hatching, the larvae were reared on a regular diet with castor leaf (*Ricinus communis* L.). Third, instar larvae were used for further study to minimize the handling effect. Sterilized soil was provided for pupation at room temperature (26 ± 2 °C) with LD 14: 10 h and 75 ± 5% relative humidity in insectary and allowable to multiply.

Ovicidal activity

The ovicidal activity of the crude extracts was studied according to Baskar *et al.* (2010) with little modifications by spraying them on freshly laid eggs of *S. litura*. The sprayed concentrations 0.5%, 1%, 2.5%, 5.0%, and 7.0% were prepared from crude extracts by diluting them with respective solvents. A spray solution of 0.5 ml was used per replication. Azadirachtin was used as a positive control. Acetone, methanol, and water were negative control (Baskar *et al*., 2009). Five replicates were maintained for each

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treatment with 20 eggs (total n = 100). The experiment was conducted under laboratory conditions (27 ± 2 °C) with LD 14:10 h and 75 ± 5% relative humidity. The number of eggs hatched in control and treatments was recorded up to 96 h. The percentage of egg mortality was calculated according to Abbott's formula.

Antifeedant activity

The antifeedant activity of plant extracts was studied using the leaf disc no-choice method (Isman *et al*., 1990). Fresh castor leaf discs of 4 cm diameter were punched using a cork borer and dipped in 0.5%, 1%, 2.5%, 5.0%, and 7.0% crude extracts individually. Leaf discs were treated with acetone, methanol, and water solvents. After air drying, each leaf disc was placed in a Petri dish containing wet filter paper to avoid early drying of the leaf disc, and a single 2 h pre-starved, third instar larva of *S. litura* was introduced. Five replicates were maintained for each concentration. After 24 h feeding, the leaf area not consumed by the larva was recorded from control and treated discs using Image J software. Azadirachtin was used as a positive control. The negative controls were acetone, methanol, and water (Baskar *et al*., 2009). The antifeedant activity was calculated using the formula:

Antifeedant activity % = [(C-T) ÷ (C+T)] x 100. Where "C" is the leaf area consumed in control and "T" is the leaf area consumed in treatment.

Larvicidal activity

Larvicidal activity of crude extracts of both plants at 0.5, 1, 2.5, 5, and 7.0% was determined by the topical application method described by Akhtar *et al.* (2012). The doses and concentrations of each plant extract were determined against third instars by preliminary experiments. Each larva of the third instar was treated with 3 µl on the thorax and abdominal regions dorsum using a micro-pipette. Control larvae received the same quantity of acetone, methanol, and water separately. Subsequently, larvae were transferred to rearing tubs (8 cm height × 18cm diameter) lined with wet paper towels and tubs closed with a muslin cloth. The treated and control larvae were maintained on normal castor leaves. Each concentration treatment contained 20 larvae with three replicates. Azadirachtin was used as a positive control. Acetone, methanol, and water were negative controls (Baskar *et al*., 2009). Larval mortalities were observed along with deformities at any stage in 3rd instars, and results were recorded. Mortality data were corrected using Abbott's (1925) formula and then used for statistical analysis.

Phytochemical screening

All the assays for phytochemical analysis of the extracts were performed in triplicates unless otherwise specified.

Qualitative analysis

Preliminary Phytochemical analysis for alkaloids, flavonoids, tannins, terpenoids, phenols, and saponins was done using both plants' acetone, methanol, and water extract. The plant extracts were evaluated for the presence of various phytoconstituents by performing different qualitative chemical tests as per the methods of Sofowara (1993), Trease and Evans (1989), and Harborne (1973).

Screening for alkaloids (Mayer's Test) 1 ml of the extract was measured into a watch glass, and little amount of dilute hydrochloric acid and Mayer's reagents were added to the solution; a white precipitate indicated the presence of alkaloids.

Screening for flavonoid (Shindo's Test) 1.3 ml of the extract was mixed with 0.5 g of magnesium turnings; the mixture was boiled for 5 min; the appearance of orange to red color indicated the presence of flavonoid.

Screening for phenol A few drops of ferric chloride solution was added to 2 ml of the extract in a watch glass; the appearance of bluish-green color indicated the presence of phenol.

Screening for saponin (Frothing Test) 2.5 ml of the extract was mixed with a few drops of distilled water, and the mixture was shaken vigorously. A copious lather formation was

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noticed, indicating saponin's presence, and the absence of the copious lather meant the absence of saponin.

Screening for tannin (Wohler's Test) A few drops of basic lead acetate solution were added to 1.6 ml of the extract; the appearance of a white precipitate indicated the presence of tannin.

Screening for terpenoids Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added; a reddish-brown coloration at the interface indicated the presence of terpenoids.

Statistical analysis

Data of ovicidal, antifeedant, and larvicidal activities were subjected to analysis of variance (ANOVA). The Significant differences between treatments were determined by Tukey's multiple range tests ($P \leq 0.05$) using SPSS software (Version16).

Quantitative analysis

Total alkaloids content (TAC)

TAC was determined as per the method reported by Ghane *et al*. (2018). The plant extract was treated with 1ml of 2N HCl and filtered. The filtrate was transferred to a new tube, and 5 ml of bromocresol green and 5 ml of phosphate buffer, and 4 ml chloroform were added. The mixture was shaken vigorously, collected in a 10 ml volumetric flask, and diluted to the volume with chloroform. A set of reference standard solutions for galanthamine was prepared in the same manner as described earlier. For measuring the absorbance of tests and standards against reagent black, the UV-Vis spectrophotometer instrument was set to 470 nm and noted the values. Galanthamine was standard, and content was expressed as mg galanthamine equivalent (GE)/g extract.

Total phenolics content (TPC)

Leaf extract solution (100 µl) was mixed with 500 µl of the Folin–Ciocalteu reagent. After 5 min, 0.8 ml (7.5% w/v) of sodium carbonate was added to the reaction mixture. The mixture was shaken thoroughly, distilled water was added to bring the volume up to 10 ml, incubated at room temperature for 60 min, and absorbance was read at 765 nm (Jasco V-730, Japan). Tannic acid was used to plot the calibration curve, and results were expressed as mg tannic acid equivalent (TAE)/g extract. TPC from all the extracts was determined by Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965).

Total flavonoids content (TFC)

TFC was estimated by the colorimetric method adopted by Attar and Ghane (2019). Aliquots of 200 µl (mg/ml) were taken, diluted with 75 µl distilled water, and mixed with 75 µl of 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3$ was added. The total mix was set aside for 5 min at room temperature, and then 500 µl 1 M NaOH was added. The reaction mixture was mixed well, and the absorbance was recorded immediately at 510 nm. Catechin was used to obtain a calibration curve, and results were expressed as mg catechin equivalents (CE)/g extract.

Total tannins content (TTC)

TTC was estimated using the vanillin-HCl method adopted by Attar and Ghane (2019) with minor modifications. Briefly, plant extract or standard catechin (100 µl) and 1 ml reagent consisting of 4% vanillin and 8% concentrated HCl (1:1) in methanol were mixed and incubated at room temperature. After 20 min incubation, absorbance was measured at 500 nm. Catechin was standard, and results were reported as mg catechin equivalents (CE)/g extract.

Total terpenoid content (TTEC)

To determine TTEC, the method was adopted from an earlier report (Chang and Lin, 2011) with few modifications. An appropriate aliquot of extract (100 µl from mg/ml working stock) was added to the 150 µl freshly prepared 5% (w/v) vanillin in glacial acetic acid, and Perchloric acid (500 ml) was added to the reaction mixture and heated in a water bath for 45 min at 60 °C. Further, all the reaction mixtures were placed in an ice bath. 2.25 ml glacial acetic acid was again added to the reaction mixture. Absorbance was measured at

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548 nm. Ursolic acid was standard, and results were expressed as mg ursolic acid equivalent (UAE)/g extract.

GC-MS analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis of methanol extracts of *Chromolaena odorata* L. and *Leonotis nepetifolia* (L.) R. Br. was performed using a GC-MS (Shimadzu TQ 8040 coupled with EI source) equipped with a column RXI-5SIL-MS (30m X 0.25id X 0.25df). The column oven temperature was programmed from 90 °C to 290 °C. The GC oven program was used as follows: oven temp was kept at 90 °C for 1 min and ramped at the rate 35 °C/min up to 130 °C and ramped to 240 °C at the rate 10 °C/min with 1 min hold further 290 °C at the rate 12 °C/ min with a final hold time of 3 min. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The temperature of the injector was fixed to 250 °C. The inlet pressure was 85.6 kPa. Helium (99.9995% purity) was the carrier gas fixed at a 1.2 ml/min flow rate. The mass range from 50-500 m/z was scanned at a rate of 3.0 scans/s. 1.0 µl of the methanol extract of *C. odorata* and *L. nepetifolia* were injected with a Hamilton syringe into the GC-MS manually for total ion chromatographic analysis in the splitless injection technique. The total running time of GC-MS is 21 min. The relative percentage of each extract constituent was expressed as a percentage.

Identification of constituents

The identity of the bioactive compounds in the methanol extracts of *C. odorata* and *L. nepetifolia was* carried out by mass spectroscopy based on comparing spectra fragmentation patterns with those stored in the computer library and published literature.

Results

Ovicidal activity

The present investigation revealed that the maximum ovicidal activities were 73.33 ± 0.57% and 71.33 ± 0.41% from methanol leaf extracts of *C. odorata* and *L. nepetifolia,* respectively, at $p < 0.05$, which was greater than the positive control (Table 1).

Table 1 The ovicidal activity of *Chromolaena odorata and Leonotis nepetifolia* on *Spodoptera litura* after 96 h.

Sr. No	Solvent	Treatment (%)	Ovicidal activity (%)	
			C. odorata	*L. nepetifolia*
1	Acetone	0.5	21.66 ± 0.21^{i}	33.33 ± 0.11^{j}
		1.0	26.66 ± 1.08^{g}	36.66 ± 0.05^{i}
		2.5	28.33 ± 0.30^{f}	38.33 ± 0.52^{h}
		5.0	46.66 ± 0.48^{cd}	53.33 ± 1.54^{e}
		7.0	45.20 ± 0.25^{cd}	53.10 ± 0.50^{e}
2	Methanol	0.5	33.33 ± 0.57^{g}	31.66 ± 0.35^{k}
		1.0	46.66 ± 0.52^{c}	38.33 ± 0.21^{g}
		2.5	48.33 ± 0.18^{b}	46.66 ± 0.25^{f}
		5.0	73.33 ± 0.57^{a}	71.33 ± 0.41^{a}
		7.0	73.10 ± 0.10^{a}	70.20 ± 0.20^{b}
3	Water	0.5	16.66 ± 0.52^{j}	26.66 ± 0.15^{l}
		1.0	21.66 ± 0.45^{i}	31.61 ± 0.18^{k}
		2.5	23.33 ± 0.30^{i}	46.66 ± 0.77^{f}
		5.0	28.33 ± 0.52^{h}	56.66 ± 0.32^{cd}
		7.0	27.90 ± 0.25^{g}	55.40 ± 0.20^{cd}
4	Azadirachtin	0.1	41.66 ± 0.31^{e}	42.16 ± 2.11^{g}
5	Negative control	Acetone	7.66 ± 0.12^{k}	5.86 ± 1.12^{n}
		Methanol	8.20 ± 0.24^{l}	6.10 ± 0.04^{m}
		Water	0.00 ± 0.00^{m}	0.00 ± 0.00^{o}

Values were the means of three replicates ± standard error. Mean values with different alphabets in same column showed statistically significant differences ($P \leq 0.05$) according to Tukey's test.

Antifeedan activity

The highest antifeedant activities 82.45 ± 0.16% and 71.77 ± 0.73%, were found in methanol leaf extracts of *C. odorata* and *L. nepetifolia*, respectively, at 5.0% and which was good as compared to a positive control (Table 2).

Larvicidal activity

The maximum mortality of 68.33 ± 0.05% and 73.33 ± 0.08% was reported at 5% of methanol leaf extracts of *C. odorata* and *L. nepetifolia,* respectively. In positive control, the mortality rate was less than 5% (Table 3). Also, methanol extracts caused malformations in the larvae, pupae, and adults of *S. litura* (Fig. 1).

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Table 2 The percentage of antifeedant activity of *Chromolaena odorata* and *Leonotis nepetifolia* on *Spodoptera litura* after 24h.

Sr. No	Solvent	Treatment (%)	Antifeedant activity (%)	
			C. odorata	*L. nepetifolia*
1	Acetone	0.5	11.97 ± 0.42kl	11.31 ± 0.26j
		1.0	41.25 ± 1.73h	13.48 ± 0.24i
		2.5	53.30 ± 0.32f	37.88 ± 0.12g
		5.0	62.11 ± 0.77cd	46.13 ± 0.4ef
		7.0	61.55 ± 0.05cd	45.90 ± 0.20ef
2	Methanol	0.5	4.30 ± 0.49n	5.50 ± 0.14k
		1.0	11.54 ± 1.11kl	12.01 ± 0.08ij
		2.5	45.71 ± 0.34g	47.87 ± 0.41ef
		5.0	82.45 ± 0.16a	71.77 ± 0.73a
		7.0	81.90 ± 0.15b	70.85 ± 0.65b
3	Water	0.5	11.58 ± 0.16kl	11.41 ± 0.88j
		1.0	26.49 ± 0.25j	12.46 ± 0.09i
		2.5	31.26 ± 0.18i	32.50 ± 0.46h
		5.0	58.25 ± 0.17e	61.77 ± 0.62cd
		7.0	57.64 ± 0.10e	60.25 ± 0.50cd
4	Azadirachtin	0.1	51.25 ± 0.34f	45.66 ± 0.53ef
5	Negative control	Acetone	4.26 ± 1.08n	2.75 ± 0.31m
		Methanol	5.10 ± 0.02m	3.02 ± 0.20l
		Water	0.00 ± 0.00m	0.00 ± 0.00m

Values were the means of three replicates ± standard error. Mean values with different alphabets in same column showed statistically significant differences ($P \leq 0.05$) according to Tukey's test.

Table 3 The percentage of larvicidal activity of *Chromolaena odorata* and *Leonotis nepetifolia* on *Spodoptera litura* after 96 h.

Solvent	Treatment (%)	Larvicidal activity (%)	
		C. odorata	*L. nepetifolia*
Acetone	0.5	33.33 ± 0.5^{ij}	26.66 ± 0.10^{k}
	1.0	36.66 ± 0.13^{h}	33.33 ± 0.18^{h}
	2.5	38.33 ± 0.22^{g}	36.66 ± 0.22^{g}
	5.0	53.33 ± 0.15^{cd}	46.66 ± 0.24^{d}
	7.0	52.65 ± 0.25^{cd}	45.25 ± 0.25^{f}
Methanol	0.5	31.66 ± 0.14^{ij}	33.63 ± 0.27^{h}
	1.0	38.33 ± 0.31^{g}	46.66 ± 0.12^{d}
	2.5	46.66 ± 0.15^{e}	48.33 ± 0.18^{c}
	5.0	68.33 ± 0.05^{a}	73.33 ± 0.08^{ab}
	7.0	67.40 ± 0.60^{b}	72.85 ± 0.02^{ab}
Water	0.5	21.66 ± 0.15^{m}	16.66 ± 0.13^{n}
	1.0	27.66 ± 0.08^{k}	21.66 ± 0.78^{m}
	2.5	30.66 ± 0.10^{l}	23.33 ± 0.30^{l}
	5.0	33.66 ± 0.21^{ij}	28.33 ± 1.52^{ij}
	7.0	32.55 ± 0.20^{ij}	27.50 ± 0.50^{ij}
Azadirachtin	0.1	41.66 ± 0.15^{f}	40.16 ± 0.74^{e}
Negative control	Acetone	8.33 ± 2.08^{o}	5.21 ± 0.12^{p}
	Methanol	9.10 ± 1.04^{n}	6.10 ± 0.05^{o}
	Water	0.00 ± 0.00^{p}	0.00 ± 0.00^{q}

Values were the means of three replicates ± standard error. Mean values with different alphabets in the same column showed statistically significant differences ($P \leq 0.05$) according to Tukey's test.

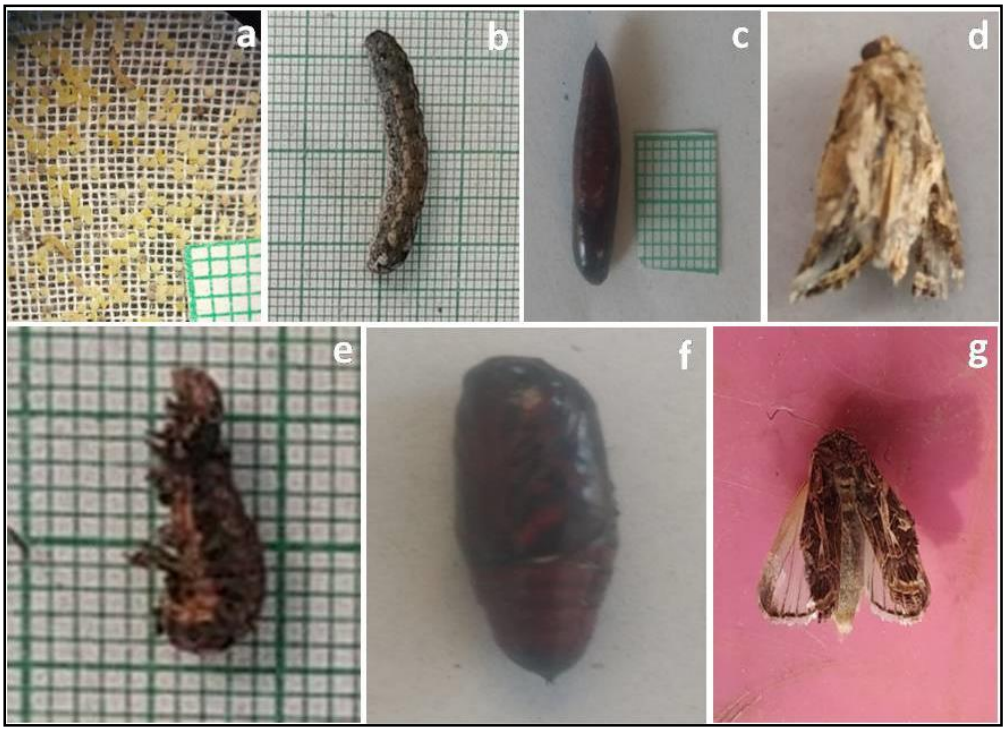

Figure 1 Healthy (a) eggs and neonate larvae, (b) larva, (c) pupa, (d) adult; Methanol extract treated (e) larva, (f) pupa (g) adult of *Spodoptera litura*.

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Preliminary phytochemical analysis

Preliminary phytochemical analysis for alkaloids, flavonoids, tannins, terpenoids, phenols, and saponins was done from acetone, methanol, and water solvents. Both plant samples were evaluated for the presence of various phytoconstituents by performing different qualitative chemical tests per the abovementioned methods. The preliminary Phytochemical analysis of the plant species revealed (Table 4) that *C. odorata* shows positive for alkaloids, flavonoids, tannins, and phenols in all extracts. Terpenoids exhibit negative only in aqueous extract. Test for saponins shows positive only in aqueous extract. In the case of *L. nepetifolia*, alkaloids, flavonoids, tannins, and phenols were present in all extracts. Terpenoids showed positive in acetone and methanol extracts and negative in aqueous extract. Saponins showed negative in all extracts.

Quantitative phytochemical screening

The extraction yields of TAC, TPC, TFC, TTC, and TTEC were studied from leaf extracts of *C. odorata* and *L. nepetifolia* (Table 5). The TAC of *C. odorata* solvent extract was in the range of 0.88-4.06 mg GEE/g DW. Acetone extract exhibited the highest TAC (4.06 ± 0.86 mg GEE/g DW); however, the lowest content was noted in water extract (0.88 ± 0.03 mg GEE/g DW). The level of TPC ranged from 8.36 to 11.95 mg TAE/g extract. The maximum (11.95 ± 0.19 mg TAE/g DW) and minimum (8.36 ± 0.01 mg TAE/g DW) TPC were exhibited from methanol and acetone extracts. The level of TFC ranged from 0.20-2.69 mg CE/g DW and methanol extract showed the highest (2.69 ± 0.01 mg CE/g DW) content, and water extract showed the lowest (0.20 ± 0.03 mg CE/g DW) content. The level of TTC ranged from 1.96-8.29 mg TAE/g DW. The maximum (8.29 ± 0.24 mg TAE/g DW) and minimum (1.96 ± 0.05 mg TAE/g DW). TTC was found in both water solvent and methanol solvent. The level of TTEC ranged from 1.64-4.09 mg UAE/g DW. The highest content was found in (4.09 ± 0.24 mg UAE/g DW) water extract, and the lowest content (1.64 ± 0.17 mg UAE/g DW) was found in methanol extract. The TAC of *L. nepetifolia* solvents studied was 0.88-5.51 mg GEE/g DW extract. Among all the solvents, acetone extract exhibited the highest TAC (5.51 ± 0.16 mg GEE/g DW); however, the lowest was noted in water extract (0.88 ± 0.12 mg GEE/g DW). The level of TPC ranged from 7.98 to 9.39 mg TAE/g DW extract. The maximum (9.40 ± 0.01 mg TAE/g DW) and minimum (7.98 ± 0.02 mg TAE/g DW) were noted in methanol and acetone extract. The level of TFC ranged from 0.26-4.24 mg CE/g DW, water extract showed the highest (4.24 ± 0.03 mg CE/g DW) content, and acetone extract showed the lowest (0.26 ± 0.00 mg CE/g DW) content. The level of TTC ranged from 2.59-3.83 mg TAE/g DW. The maximum (3.83 ± 0.13 mg TAE/g DW) in acetone extract and the minimum (2.59 ± 0.15 mg TAE/g DW) in methanol extract. The level of TTEC ranged from 1.17-3.34 mg UAE/g DW. The highest content was found in (3.34 ± 0.19 mg UAE/g DW) acetone extract, and the lowest content (1.17 ± 0.12 mg UAE/g DW) was found in the water extract.

Comparing both plants highest TAC (5.51 ± 0.16 mg GEE/g DW) was exhibited in acetone extract of *L. nepetifolia*, and the lowest TAC (0.88 ± 0.03 mg GEE/g DW) was shown in water extract of *C. odorata*. The maximum TPC (11.95 ± 0.19 mg TAE/g DW) was found in the methanol extract of *C. odorata*, and the minimum TPC (8.36 ± 0.01 mg TAE/g DW) was found in its acetone extract. The highest TFC (4.24 ± 0.03 mg CE/g DW) was noted in the water extract of *L. nepetifolia*, and the lowest TFC (0.20 ± 0.03 mg CE/g DW) in an extract of *C. odorata*. It is shown as maximum (8.29 ± 0.24 mg TAE/g DW) and minimum TTC (1.96 ± 0.05 mg TAE/g DW) in water and methanol extract of *C. odorata*. The highest TTEC (4.09 ± 0.24 mg UAE/g DW) was exhibited from the water extract of *C. odorata*, and the lowest TTEC (1.17 ± 0.12 mg UAE/g DW) from *L. nepetifolia*.

GC-MS analysis

Thirty-one compounds were detected from methanol extract of *C. odorata* (Table 6). The results revealed that Undec-10-ynoic acid, dodecyl

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ester (7.9%) was found to be the major component followed by octadecanoic acid (5.02%), nopyl acetate (3.63%), cyclononasiloxane, octadecamethyl-(1.44%), n-hexadecanoic acid (1.38%), 3H-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3, 10-dimethylene-3a,4,7,8,9, 10,11,11a-octahedron- (1.21%), tetra cosamethyl-cyclo dodecasil oxane (0.49%), which shows various biological activities. Further, 3-ethyl-2-pentanol (0.15%), octacosane (0.13%), diethyl phthalate (0.12%), 2,6-di isopropyl naphthalene (0.11%), methyl 8-oxo octanoate (0.07%), nonanedioic acid, dimethyl ester (0.04%), caprini cacid (0.03%).

Sixteen compounds were detected from the methanol extract of *L. nepetifolia* (Table 7). The results showed that n-Hexadecanoic acid (2.89%) was the major component, followed by 7-Tetradecenal, (Z)- (1.41%), Bicyclo[4.1.0] hept-3-ene, 7,7-dimethyl-3-vinyl- (1.34%), Octadecanoic acid (1.19%). Many of these major phytoconstituents have been reported with insecticidal, antimicrobial, anticancer, and anti-inflammatory properties.

Table 4 Qualitative phytochemical screening of acetone, methanol, and water extracts of *Chromolaena odorata* and *Leonotis nepetifolia*.

Plant species	Solvent	Alkaloids	Flavanoids	Tannins	Terpenoids	Phenols	Saponins
C. odorata	Acetone	+	+	+	+	+	-
	Methanol	+	+	+	+	+	-
	Water	+	+	+	-	+	+
L. nepetifolia	Acetone	+	+	+	+	+	-
	Methanol	+	+	+	+	+	-
	Water	+	+	+	-	+	-

Key: + = present, -= absent.

Table 5 Quantitative phytochemical screening of acetone, methanol, and water extracts of *Chromolaena odorata* and *Leonotis nepetifolia*.

Plant species	Solvent	Total alkaloids[1]	Total phenolics[2]	Total flavonoids[3]	Total tannins[3]	Total terpenoids[4]
C. odorata	Acetone	4.06 ± 0.86^{b}	8.36 ± 0.01^{c}	2.04 ± 0.08^{bc}	3.35 ± 0.30^{bc}	2.04 ± 0.08^{cd}
	Methanol	3.44 ± 0.04^{cd}	11.95 ± 0.19^{a}	2.69 ± 0.0^{bc}	1.96 ± 0.05^{e}	1.64 ± 0.17^{e}
	Water	0.88 ± 0.03^{e}	9.34 ± 0.21^{b}	0.20 ± 0.03^{d}	8.29 ± 0.24^{a}	4.09 ± 0.24^{a}
L. nepetifolia	Acetone	5.51 ± 0.16^{a}	7.98 ± 0.02^{d}	0.26 ± 0.00^{d}	3.83 ± 0.13^{bc}	3.34 ± 0.19^{b}
	Methanol	3.64 ± 0.21^{cd}	9.40 ± 0.01^{b}	2.31 ± 0.01^{bc}	2.59 ± 0.15^{d}	2.12 ± 0.34^{cd}
	Water	0.88 ± 0.12^{e}	9.10 ± 0.02^{c}	4.24 ± 0.03^{a}	2.89 ± 0.19^{d}	1.17 ± 0.12^{f}

[1]mg Galanthamine equivalent (GEE) /g DW, [2]mg Tannic acid equivalent (TAE) /g DW, [3]mg Catechin equivalent (CE) /g DW, [4]mg Ursolic acid equivalent (UAE) /g DW. Values are the means of three replicates ± Standard Error (SE). Mean values with different alphabets in the column were significantly different ($p<0.05$) according to Tukey's test.

Discussion

The ovicidal activity of plant extracts effectively controls the pest at the egg stage itself, thus preventing the damage caused by other stages. The hatchability of *S. litura* eggs was directly proportional to the concentration of plant extract (Jeyasankar *et al*., 2013). This result is in agreement with the findings of Malarvannan *et al*. (2009) who reported ovicidal activity from petroleum ether, chloroform, hexane, acetone, and water extracts of the *Cipadessa baccifera* Miq., *Melia dubia* (Cav.) (Meliaceae), *Clausena dentate* (Willd.) M. Roem. (Rutaceae) and *Dodonaea angustifolia* L.f. (Sapindaceae) against *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae). Similarly, Sagha *et al*. (2017) reported ovicidal activity from various plants *viz. Artemisia abrotanum* L. (Asteraceae), *Abies balsamea* L. (Pinaceae), *Piper nigrum* L. (Piperaceae), *Eucalyptus polybractea* (Baker)

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(Myrtaceae), *Allium sativum* L. (Amaryllidaceae), rosewood (a blend of different oil constituents), *Tanacetum vulgare* L. (Asteraceae), and *Thymus zygis* L. (Lamiaceae) which reduced the egg hatchability against the lepidopteran pest *Plutella xylostella* L. (Sangha *et al.*, 2017). The methanol extract of *Gnidia glauca* (Fres.) Gilg at 50 mg/ml demonstrated the highest antifeedant (64%) and larvicidal (75%) activity against *S. litura* (Shiragave, 2018a, b). Crude extracts of *Atalantia monophylla* (L.) leaf were studied for ovicidal activity against *H. armigera* with different concentrations of acetone, methanol, and water solvents. Among all the solvents, methanol leaf extract at 1.0% showed the highest percentage of ovicidal (44%) activity.

Table 6 Phytoconstituents identified in the methanol extracts of *Chromolaena odorata* by GC-MS.

Sr. No	Name of the compound	Molecular formula	Mol. Wt	Activity	References
1	Methyl8-oxooctanoate	$C_9H_{16}O_3$	172.22	Pheromones of insects and their analogs /Antibacterial	Evanjaline and Mohan (2018)
2	Caprinicacid/n-Decanoicacid/ Aceticacid,3-methyl hept-3-yl ester	$C_{10}H_{20}O_2$	172.26	Pesticide, Fungicide	Ramya *et al.* (2015)
3	DL-Proline,5-oxo-,methyl ester	$C_6H_9NO_3$	143.14	Antibacterial and antifungal	Ravi *et al.* (2018)
4	4-Hydroxy-2-methoxy benaldehyde	$C_8H_8O_3$	152.15	Insect attractants, Repellants and antimicrobial agent. Antifungal	Jenkins and Erraguntla (2014)
5	4-Methoxy benzoic acid	$C_8H_8O_3$	152.15	Antifungal activity	Kim *et al.* (2011)
6	Methyl 9-oxononanoate	$C_{10}H_{18}O_3$	186.25	Potent antifungal, Antioxidant, Potent Antimicrobial	Syeda *et al.* (2011)
7	Octanedioic acid,dimethyl ester	$C_{10}H_{18}O_4$	202.25	-	-
8	3-Ethyl-2-pentanol	$C_7H_{16}O$	116.2	-	-
9	Suberic acid monomethyl ester/Azelaic acid	$C_9H_{16}O_4$	188.22	Antimicrobial	Leong and Oh (2018)
10	Nonanedioic acid,dimethyl ester/Dimethylazelaate	$C_{11}H_{20}O_4$	216.27	-	-
11	Dodecanoic acid/Lauric acid	$C_{12}H_{24}O_2$	200.32	Insecticidal, Antimicrobial	Sarip *et al.* (2016) Arora and Meena (2017)
12	Nonanedioic acid,monomethyl ester/Methyl hydrogen azelate	$C_{10}H_{18}O_4$	202.24	-	-
13	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	-	-
14	Adamantane-1-carboxamide, N-(4-pyridyl)-	$C_{16}H_{20}N_2O$	256.34	-	-
15	6-Phenyl hexanoic acid	$C_{12}H_{16}O_2$	192.25	Anticancer	
16	1,3-di-iso-propyl naphthalene	$C_{16}H_{20}$	212.33	Biochemical pesticide	EPA (2006)
17	1,7-di-iso-propyl naphthalene	$C_{16}H_{20}$	212.33	Biochemical pesticide	EPA (2006)
18	2,6-Diisopropyl naphthalene	$C_{16}H_{20}$	212.33	Biochemical pesticide	EPA (2006)
19	Tetra decanoic acid/Myristic acid	$C_{14}H_{28}O_2$	228.37	Larvicidal Antioxidant, Nematicidal	Gomathy and Rathinam (2017) Arora and Meena (2017)
20	Octacosane	$C_{28}H_{58}$	394.8	Insecticidal	
21	8-Phenyl octanoic acid	$C_{14}H_{20}O_2$	220.31	-	-
22	Phthalic acid,butyl undecyl ester	$C_{23}H_{36}O_4$	376.5	Antimicrobial lactivity, Antibacterial Activity,	Hameed *et al.* (2018)
23	Cyclooctane-1,4-diol,cis	$C_6H_{12}O_2$	116.16	Anticancer	
24	n-Hexa decanoic acid/Palmitic Acid	$C_{16}H_{32}O_2$	256.42	Antioxidant,nematicide,insecticidal	Beulah *et al.* (2018)
25	2-Pentyl-cyclo hexane-1,4-diol	$C_{11}H_{22}O_2$	186.29	-	-
26	Undec-10-ynoic acid,dodecyl ester	$C_{23}H_{42}O_2$	350.6	-	-
27	Octadecanoic acid/(Stearic acid)	$C_{18}H_{36}O_2$	284.5	Insecticidal activity, Antibacterial action,	Gomathy and Rathinam (2017)
28	Nopyl acetate	$C_{13}H_{20}O_2$	208.3	-	
29	3H-Cyclo deca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3, 10-dimethyl ene-3a,4,7,8,9,10,11,11a-octahydro-	$C_{15}H_{20}O_4$	264.32	-	-
30	Cyclononasiloxane, octa decamethyl-	$C_{18}H_{54}O_9Si_9$	667.4	Antioxidant, insecticidal	Ramli *et al.* (2017)
31	Tetracosamethyl-cyclo dodecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8	Insecticidal, activity,	Kumar *et al.*(2018)

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Table 7 Phytoconstituents identified in the methanol extracts of *Leonotis nepetifolia* by GC-MS.

Sr. No	RT	Name of the compound	Molecular formula	Mol. Wt	Peak Area%	Activity	Reference
1	5.769	L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143.1406	1.07	-	-
2	6.302	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186.25	0.2	Antifungal Antimicrobil	Karthick *et al.* (2015)
3	6.582	Cyclopropane, 1-bromo-2-(1,1-dimethylethyl)- Or1-Bromo-2-tert-butylcyclopropane	$C_7H_{13}Br$	177.08	0.74	-	-
4	7.444	Nonanedioic acid, dimethyl ester OrDimethyl azelate	$C_{11}H_{20}O_4$	216.27	0.26	-	-
5	7.596	Dodecanoic acid Orlauric acid	$C_{12}H_{24}O_2$	200.32	0.19	Insecticides, Antimicrobial	Sarip *et al.* (2016) Arora and Meena (2017)
6	8.315	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.2	0.19	Antimicroial	Huda *et al.* (2015)
7	9.124	L-Phenylalanine, N-acetyl-, methyl ester	$C_{12}H_{15}NO_3$	221.25	0.12	-	-
8	9.699	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	0.26	Nematicidal Larvicidal	Arora and Meena (2017) Gomathy and Rathinam (2017)
9	10.537	Pentacosane	$C_{25}H_{52}$	352.7	0.74	antibacterial	Mihailovi *et al.* (2011)
10	10.63	8-Phenyloctanoic acid	$C_{14}H_{20}O_2$	220.31	0.4	-	-
11	11.742	n-Hexadecanoic acid orPalmitic Acid	$C_{16}H_{32}O_2$	256.42	2.89	Antioxidant, nematicidal, insecticidal activity,	Eugin *et al*. (2014) Gomathy and Rathinam (2017) Rency *et al.* (2015)
12	13.01	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	$C_6H_{28}O_2$	252.39	0.18	Pheromone	Tumlinson *et al.* (1981)
13	13.063	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- Or Linolenic acid, methyl ester	$C_{19}H_{32}O_2$		0.37	Nematicidal, Insectifuge,	Rehana and Nagarajan (2013) Rency *et al.* (2015)
14	13.455	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210.36	1.41	-	-
15	13.692	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	1.19	Insecticidal activity, Antibacterial action,	Gomathy and Rathinam (2017)
16	13.952	Bicyclo[4.1.0]hept-3-ene, 7,7-dimethyl-3-vinyl-	$C_{11}H_{16}$	148.24	1.34	-	-

The results of the present study indicated that higher concentrations of both plants could act as a potent oral toxicant and feeding deterrent against *S. litura*. Chinnamani and Jeyasankar (2018) reported that the chloroform, ethyl acetate, and hexane extracts of *Pseudocalymma alliaceum* (Lam.), *Barleria buxifolia* L., *Solanum pseudocapsicum* L. were found to be effective against the 4th instar larvae of *S. litura* and *H. armigera*. Elanchezhian *et al*. (2019) reported that hexane, dichloromethane, diethyl ether, ethyl acetate, and methanol extracts of *T. malabarica* (Menispermaceae) had antifeedant activity against *S. litura*.

Our results showed that both plant extracts have considerable larvicidal activity against selected important agricultural lepidopteran field pest *S. litura.* According to Vetal and Pardeshi (2019) the ethanol solvent extract of *Argemone mexicana* L. showed the highest larvicidal property against third-instar larvae of *S. litura.* Sharma *et al. (*2016) evaluated the effect of *A. mexicana* leaves extract of different solvents on the gut of *Heliothis armigera* (Hub.). Shiragave (2017) reported that *Citrus limon* (L.) Burm., extract has significant natural ovicidal and larvicidal properties against the lepidopteran pest *H. armigera*. Insecticidal potentiality of *Exacum pendunculatum* L. was revealed against *S. litura* in which it was observed that methanol extract at 50 mg/ml showed the highest larvicidal activity (Shiragave, 2020). Gorawade *et al. (*2021) studied crude leaf extracts of *C. odorata* and *L. nepetifolia* for their ovicidal, antifeedant, and larvicidal activity with different acetone and methanol concentrations and aqueous extracts against the third instar larvae of *H. armigera*. The highest ovicidal (61.33 ± 0.57% and 63.45 ± 0.77%), antifeedant (62.45 ± 1.26% and 63.17 ± 0.66%), and larvicidal (65.33 ± 3.05% and 68.33 ± 0.57%) activities were recorded in methanol extract (5%) of *C. odorata* and *L. nepetifolia* respectively. Further TLC analysis was carried out with four different solvent systems to screen phenolics from methanol extracts. The solvent system benzene: ethyl acetate: formic acid (6:3:1) showed the highest five spots in both plant extracts compared to other solvent systems.

The identified major compounds possess some important biological potential for future development. There is a growing awareness of correlating the phytochemical compounds and their various biological activities (Sarip *et al.*, 2016).

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The present study coincided with Gomathy and Rathinam (2017) who reported that *Terminalia arjuna* (Roxb.) Wight & Arn bark extract consists of dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, and octadecanoic acid, among which octadecanoic acid shows insecticidal activity. It also possesses anti-inflammatory, cancer preventive, nematicidal, and insectifuge properties. Additionally, 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- identified in the present investigation were formerly determined by GC-MS determination of bioactive components of *Wedelia chinensis* (L.) Pruski by Banu and Nagarajan (2013).

Arora and Saini (2017) reported that methanol and ethyl acetate extracts of the root and stem of *Gisekia pharnaceoides* (Molluginaceae) consist of octadecanoic acid, which has antimicrobial activity. Ahmad *et al.* (2013) reported that myristic acid, hexadecanoic acid, octadecanoic acid, phthalic acid, and diethyl ester constituents were observed in Green Tea (*Camellia sinensis* (L.) Kuntze), which exhibit antioxidant, cancer-preventive, hypercholesterolemic, lubricant, nematicide, pesticide, anti-androgenic, and carcinogenic activity. The methanolic leaf extract obtained from *C. odorata* and *L. nepetifolia* were subjected to chemical analysis by GC-MS method, confirming the presence of phytocompounds responsible for insecticidal activities.

Conclusion

The methanol extract of *C. odorata* and *L. nepetifolia* at $P < 0.05$ showed maximum antifeedant, ovicidal and larvicidal activities and extended the larval and pupal duration. Thirty-one and sixteen compounds were identified from the methanol extract of *C. odorata* and *L. nepetifolia*, respectively, using GC–MS analysis. The presence of various bioactive compounds justifies the use of these plants as phytopesticides. Some of the bioactive secondary metabolites identified may become commercially important phytopharmaceuticals. However, further studies are needed to ascertain their biological and insecticidal activity.

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Disclosure statement

The authors reported no potential conflict of interest

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شناسایی ترکیبات عصاره برگ *Chromolaena odorata* و *Leonotis nepetifolia* و بررسی اثرات حشره‌کشی آن‌ها روی لارو برگ‌خوار توتون *Spodoptera litura*

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چکیده: این مطالعه با هدف بررسی خواص حشره‌کش عصاره برگ *Chromolaena odorata* و *Leonotis nepetifolia* روی لاروهای سن سوم برگ‌خوار توتون *Spodoptera litura* (F.) انجام شد. برگ هر دو گیاه با سه حلال استون، متانول و آب استخراج شدند و سپس خواص تخم‌کشی، ضدتغذیه و لاروکشی عصاره‌ها در غلظت‌های ۰/۵، ۱، ۲/۵، ۵ و ۷ درصد آزمایش شدند. عصاره متانولی (۵٪) گیاه *C. odorata* اثرات تخم‌کشی (۰/۵۷ ± ۷۳/۳۳ درصد)، ضدتغذیه (۰/۱٦ ± ۸۲/٤۵ درصد) و لاروکشی (۰/۰۵ ± ٦۸/۳۳ درصد) روی برگ‌خوار توتون نشان داد. نتایج مشابهی از عصاره متانولی (۵ درصد) *L. nepetifolia* برای اثر تخم‌کشی (۰/٤۱ ± ۷۱/۳۳ درصد)، ضدتغذیه (۰/۷۳ ± ۷۷/۷۱ درصد) و لاروکشی (۰/۰۸ ± ۷۳/۳۳ درصد) مشاهده شد. غربال‌گری فیتوشیمیایی مقدار قابل‌توجهی از آلکالوئیدها و فنولیک‌ها را در عصاره متانولی برگ هر دو گیاه نشان داد. علاوه براین، سی و یک ترکیب فعال زیستی از عصاره متانولی *C. odorata* و شانزده ترکیب از *L. nepetifolia* توسط GC-MS شناسایی شدند. ترکیبات شناسایی شده شامل فنل‌ها، اسیدهای چرب، استرها و اسانس‌ها بودند. ترکیبات حشره‌کش شناسایی شده ممکن است به پتانسیل خواص تخم‌کشی، ضدتغذیه و لاروکشی این دو گونه گیاه نسبت داده شود. بنابراین تحقیقات گسترده‌ای در مورد *C. odorata* و *L. nepetifolia* در توسعه آفت‌کش‌ها علیه لارو برگ‌خوار توتون مورد نیاز است.

واژگان کلیدی: *Chromolaena odorata*، *Leonotis nepetifolia*، *Spodoptera litura*، حشره‌کش، ترکیبات گیاهی

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