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Insecticidal potential and thin layer chromatographic profiling of *Chromolaena odorata* L. and *Leonotis nepetifolia* (L) R.Br. leaf extracts against *Helicoverpa armigera* (Hubner)

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Abstract
In the present study crude extracts of *C. odorata* and *L. nepetifolia* plants were screened for their ovicidal, antifeedant and larvicidal activity with different concentrations of acetone, methanol 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 highest 5 spots in both plant extracts compare to other solvent systems.

Keywords: antifeedant, *Chromolaena odorata*, *Helicoverpa armigera*, *Leonotis nepetifolia*, thin layer chromatography

Introduction
The intensification of agriculture to fulfill food needs has been increased. Besides that loss in agricultural production has been also increased due to sevear insect pest attack. In the past decade, chemical pesticides have played a major role in agriculture crop protection programmes and have extremely benefited to mankind in respect of agriculture production but causes serious threat to human, animal health and to the environment.

In a tropical country like India, owing to continuous changing in climate conditions along with insect pest attack causes sevear losses in agriculture production. Considering the agro-ecosystems with an increase in population and dwindling land resources, there is worldwide demand for natural insecticides to increase the agriculture production. Therefore, screening of plant resources has been continuously going on to discover novel natural insecticides and to develop effective pest management strategy (Jose *et al*., 2018) [19].

Helicoverpa armigera (Hubner) is a pest of global importance with a broad geographical distribution and polyphagous habits (Chen *et al*.,) [8]. It is the major pests that show aggression to the agricultural crops and causing considerable harm to the economy (Gabriel *et al*., 2020) [11]. It has a wide host range of 360 species of wild and cultivated crops. Tomato, sorghum, cowpea, cotton, pigeonpea, chickpea, okra and range of vegetables suffer severely by *H. armigera* damage. It is able to adapt to various cropping systems due to its high polyphagy, wide geographical range, mobility, migratory potential, facultative diapause and high fecundity. (Honnakerappa and Udikeri, 2018)[15]. Its distribution is expanding and includes at least 145 countries and territories (51 in Africa, 42 in Asia, 29 in Europe, 20 in Oceania and 3 in South America) (Singh *et al*.,2018) [32].

Pest management in the developing countries like India is mainly depends on the use of chemical pesticides as they are the mainly dependable and economical but unsystematic apply of them resulted in a series of harms in the agro-ecosystem *viz*. resistance, resurgence and residue. The *H. armigera* was reported to have developed resistance against organophosphates and carbamates in many countries of Asia. Several insecticides and pesticides are used to control *H. armigera*. However, harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives (Prabhu *et al*., 2018) [25]. Hence Natural pesticides are good alternative to synthetic pesticides because they are safe to environment, natural enemies, humans and other animals, e.g. most botanical pesticides have low to moderate mammalian toxicity (Jawad *et al*., 2013) [17]

The plants are the only producers in the world who produce phytochemicals for their defense mechanism against plant eating insects (Thushimenan *et al*., 2016) [34]. These phytochemicals are ecologically sound, economically practical, socially acceptable and environmentally sustainable to develop natural insecticides for effective control of insect pest. In addition these phytochemicals are selective in their activity which suggesting that their application would be environmentally acceptable and compatible with integrated pest management (IPM) programs and effective in countering insect resistance.(Yankanchi *et al*., 2009) [37].

Phytopesticides are plant based pesticides which offer an eco-friendly approach to insect pest management than chemical pesticides. The biological compounds present in these formulations have several biological activities against insect pests. More than 2000 plant species have insecticidal properties against many insect species (Jose *et al*., 2017) [18]. At present, many researchers concentrate on naturally available materials to control the various agricultural pests. The plant based products showing insecticidal potentical by

acting as antifeedant, larvicidal, pupicidal, growth inhibitors, repellent, ovicidal, oviposition deterrent, prolong larval–pupal durations and produce larval–pupal intermediates and pupa–adult intermediates. In addition, they also reduced the protein content, detoxifying enzyme reduction, interfere with moulting and digestive enzymes of various agricultural pests (Kathirvelu *et al*., 2015) [21].

Chromolaena odorata (L.) is one of the world's worst tropical weed. It is a member of Asteraceae and one of the plants that has been associated with pesticidal and medicinal value in many areas. It is not habitat specific; however it grows commonly in wastelands (Udebuani *et al*., 2015) [35]. The weed goes by many common names including Siam weed, devil weed, French weed, communist weed etc. The native range of *C. odorata* is in the Americas, extending from Florida (USA) to northern Argentina. Away from its native range, *C. odorata* is an important weed in tropical and subtropical areas extending from west, central and southern Africa, India, Srilanka, Bangladesh, Laos, Cambodia, Thailand, southern China, Taiwan, Indonesia etc. *C. odorata* is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties. (Vaisakh and Pandey, 2012) [36]. The leaf of *C. odorata* contains grandular dots that emit a strong insecticidal smell (Akobundu and Agyakwa 1987) [4]. The phytochemical studies have revealed the presence of a wide range of chemical entities in the plant. These studies underline the significance of treating the widely occurring flora on this planet as potential sources of new drug entities and not only as harmful weeds. Theses plant contains essential oils, flavonones and chalcolnes with cytotoxicity and anticancer properties, antimicrobial flavonoids, antioxidant phenolic compounds, antiinflammatory fatty acids, potent α-glucosidase inhibitory and antibacterial active kaurane-type diterpenoids and alkaloids. (lawal *et al*., 2015) [22].

Leonotis nepetifolia (L.) (Family-Lamiaceae) is a tall annual herb growing in plains, roadsides, waste places near villages and is often cultivated throughout India. The plant is identified by its finely pubescent obtusely quadrangular stems, long internodes and spinous whorls of orange scarlet flowers with densely wooly upper lip. It is native to tropical Africa but it was introduced and naturalized throughout hotter parts of India but it is nowhere common. Common names of the plant are Knod grass (English), Lion's ear (English), Matijer (Gujrati) Gathivan (Hindi), Dipmal (Marathi), Murandai or Thenthumbai (Tamil) and Ranabheri (Telugu). Medicinal uses of the plant are reported in Madagascar, Brazil, Canada, Kenya and many African countries to treat kidney diseases, rheumatism, dysmenorrhoea, bronchial asthma, fever and diarrhea (Pushpan *et al*., 2012) [27]. The drug is reported to have antibacterial, antiviral, fungicidal, anti-inflammatory, antinociceptive, pesticides, anticancer, antidiarrheal, anticonvulsant, antiplasmodial and antidiabetic activities of different solvent extracts of the various plant parts of *L. nepetifolia* (Almeida *et al*., 2018) [5].

This study therefore sought to determine the insecticidal activities of *C. odorata* and *L. nepetifolia* leaf extracts against *H. armigera* and their thin layer chromatographic studies at laboratory conditions.

Materials and Methods

Plant collection and extract preparation

Fresh leaves of *C. odorata* and *L. nepetifolia* were collected around Devchand College, Arjunnagar, Maharashtra, India (16.405876 N latitude and 74.363426 E longitudes) The plant material was identified and a specimen was deposited at Herbarium of Department of Botany, Shivaji University, Kolhapur, Maharashtra, India (Voucher specimen No. VBG01 and VBG02 respectively). The leaves were shade-dried, powdered using electric grinder and extracted separately with acetone, methanol and water. The crude extracts were collected in clean borosil vials and stored in the refrigerator at 4°C for subsequent bioassay against *H. armigera* (Baskar *et al*., 2010) [6]. The extract were further diluted with respective solvents following serial dilution method to obtain desired concentration *viz*., 10, 20, 30, 40 and 50 mg/ml concentrations for subsequent use in the different experiments.

Rearing of *H. armigera*

Larvae of *H. armigera* were collected from infested chickpea fields around Nippani area. The larvae were fed with fresh tomato leaves in the laboratory at 26 ± 1°C, 13 ± 1 hr photophase, 11 ± 1 hr scotophase, and 75 ± 5% relative humidity. After emergence, adults were released into the oviposition chambers for egg laying and provided with 20% honey solution. Fresh, tender tomato leaves were kept inside the chambers to stimulate oviposition. The eggs were kept in hatching chambers at 75 ± 5% relative humidity. Newly-hatched larvae (neonates) were maintained on the natural diet. Third instar larvae (weighing 30– 40 mg) were used for bioassay.

Ovicidal activity

Twenty eggs of *H. armigera* were dipped in crude extracts with concentrations 10, 20, 30, 40 and 50 mg/ml. From each concentration 0.5 ml was used for analysis. Azadirachtin was used as positive control. For each tested dose, three replicates were done. The percentage of egg hatched was calculated by number of larvae that emerged after treatment. After 4 days, the number of eggs hatched was noted. Petri plates were placed in an incubator at 28 ± 2 °C, 75 ± 5% R.H., and 12:12 h (L:D).

Antifeedant activity

Antifeedant activity of plant extracts was studied using leaf disc no choice method (Isman *et al*., 1990) [16]. Fresh tomato leaf discs of 4 cm in diameter were punched using cork borer and dipped in 10, 20, 30, 40 and 50 mg/ml concentrations all extracts separately. Leaf discs treated with acetone, methanol and water were considered as control. After air dried, treated leaf discs were kept inside the each petridish (15mm × 90 mm diameter) separately containing wet filter paper to avoid early drying of the leaf disc and a single 2 hrs pre-starved fourth instar larva was introduced into each petridish. A progressive consumption of leaf area by larvae after 24 hr feeding was recorded from control and treated leaf disc using Image J Software. Five replicates were maintained for each concentration. 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

For the evaluation of larvicidal activity, crude extracts with concentrations 10, 20, 30, 40 and 50 mg/ml with selected solvents were prepared. The topical application method described by Akhtar *et al*, (2012) [3], with slight modification was used to evaluate larvicidal potential. Each larva of third instar applied the dosage of 3 µL of the above mentioned concentrations solution respectively on the dorsum of the thorax and abdominal regions by using micro-pipette. Petri plates were placed in an incubator at 28 ± 2 °C, 75 ± 5% R.H., and 12:12 h (L:D). Three replicates were done for each concentration, and the number of live larvae was counted after 24 h. larval mortality was observed and results were recorded. Mortality data was corrected by using the Abott's, (1925) [1] formula and then used for statistical analysis.

Thin layer chromatographic studies of phenolics

Thin layer chromatography was performed using standard methods (Harborne, 1998) [13]. Small quantities of samples (2 mg/ml) were dissolved in their respective solvents. TLC is performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F254 (Merck). Six phenolic standards and samples prepared with methanol solvent were spotted onto the TLC plate as a single spot with capillary tubes. All plates were dried and visualized by spraying 3% $FeCl_3$ (prepared in ethanol). The used mobile phase systems chloroform: ethyl acetate: formic acid (5:4:1) (Solvent system I), Benzene: ethyl acetate: formic acid (6:3:1) (Solvent system II), Toulene: ethyl acetate: formic acid (12:6:1) (Solvent system III) and chloroform: ethanol: acetic acid (60:4:6) (Solvent system IV). The R_f values of the observed spots were calculated and recorded.

Results and Discussion

Ovicidal Activity

The ovicidal activities of different crude extracts of *C. odorata* and *L. nepetifolia* were studied using the dipping method. The methanol extracts of both plant sample was showed highest ovicidal activity (61.33±0.57% and 63.45±0.77%) against *H. armigera* (Table 1) and which was significantly high as compared to other extracts and azadirachtin. The lowest ovicidal activities were recorded in water extract 14.16±0.22% and 24.56±0.15% respectively. The results were in confirmation with Mahla *et al*., (2002) [23] where ovicidal action on hatching was highest in methanolic extract of *Melia azedarach*. It was evident from the results obtained in the earlier work that the methanol leaf extract of *Tinospora cardifolia* could be used effectively in ovicidal activity of agricultural field pest *Spodoptera litura* and *Helicoverpa armigera*. (Selvam and Ramakrishnan, 2014) [30]

Table 1: Percent ovicidal activity of *C. odorata* and *L. nepetifolia* leaf extracts against *H. armigera*.

Sr.No	Solvent	Treatment (%)	Ovicidal Activity (%)	
			C. odorata	*L. nepetifolia*
1.	Acetone	0.5	18.62±0.11	20.55±1.11
		1.0	21.34±1.08	25.66±1.24
		2.5	24.33±1.24	30.54±1.52
		5.0	41.45±0.44	36.22±0.54
2.	Methanol	0.5	31.98±0.44	30.85±1.51
		1.0	44.54±0.44	36.22±2.62
		2.5	46.33±2.11	44.88±1.25
		5.0	61.33±0.57	63.45±0.77
3.	Water	0.5	14.16±0.22	24.56±0.15
		1.0	19.44±2.11	32.61±1.08
		2.5	22.33±2.30	44.66±0.57
		5.0	25.33±1.12	54.76±1.22
4.	Azadiractin	0.1	39.64±1.61	40.16±1.31
5.	Negative control	-	6.55±2.22	5.86±1.12

Values were the means of three replicates ± standard error.

Antifeedant activity

The percentage antifeedant activity of all solvent extracts of *C. odorata* and *L. nepetifolia* were evaluated and results pertaining to different concentration were presented in (Table 2).The efficiency of all solvent extracts against *H. armigera* were assayed by comparing the average leaf area consumed in the treated and control leaf discs. Higher antifeedant percentage indicated the decreased rate of feeding. In the present investigation, antifeedant activity is varied significantly based on the solvents used and concentrations of the solvent extracts. Among the tested solvent extracts of both plant samples, methanol extract was exhibited highest antifeedant activity 62.45±1.26% and 63.17±0.66% at 5 % concentration followed by acetone extract 50.11±1.57% and 46.13±1.41% respectively. The least antifeedant activity was noted in water extract 4.30±2.49% and 5.74±0.11% at 0.5 % concentration followed by acetone extract 10.77±0.44% and 11.31±1.26% respectively. It is noted that, as the concentration of extract was increased, the antifeedant activity was also increased. The study noted considerable feeding deterrent activity in all tested solvent extracts with special emphasis of methanol extract and acetone extract. In addition, present study noted the dose dependant antifeedant activity in tested solvent extracts against the third instar larvae of *H. armigera*. Our results are agreed with Chen zetan [9] who reported the significant antifeeding action and poisoning of the crude extract of *C. odorata* L against the *Helicoverpa armigera*. Similalry, methanol extract of *Gliricidium sepium* exhibited significant antifeedant activity at a higher concenteration against *Helicoverpa armigera*. (Jose *et al*., 2017) [18]. several authors have reported that plant extracts possess similar type of antifeedant activity against *Helicoverpa armigera* (Akhtar and Isman, 2004; Raja *et al*., 2005 Baskar *et al*., 2009) [2, 29, 7].

Table 2: Percent of antifeedant activity of *C. odorata* and *L. nepetifolia* leaf extracts against *H. armigera*.

Sr.No	Solvent	Treatment (%)	Antifeedant activity (%)	
			C. odorata	*L. nepetifolia*
1.	Acetone	0.5	10.77±0.44	11.31±1.26
		1.0	38.25±1.33	13.48±0.24
		2.5	45.14±2.32	37.88±5.12
		5.0	50.11±1.57	46.13±1.41
2.	Methanol	0.5	5.10±1.59	5.50±0.14
		1.0	10.12±1.88	12.01±1.08
		2.5	48.81±3.14	17.87±0.41
		5.0	62.45±1.26	63.17±0.66
3.	Water	0.5	4.30±2.49	5.74±0.11
		1.0	11.54±1.11	13.21±4.09
		2.5	45.71±4.34	33.89±2.46
		5.0	53.66±2.16	48.33±8.62
4.	Azadiractin	0.1	51.25±0.34	45.66±0.53
5.	Negative control	-	4.26±1.08	2.75±2.31

Values were the means of three replicates ± standard error.

Larvicidal activity

The larvicidal activity of different crude leaf extracts of *C. odorata* and *L. nepetifolia* were tested against third instar larvae of an *H.armigera*. The perusal of the data clearly revealed that methanol extract at 5% concentration showed potential larvicidal effect (65.33±3.05% and 68.33±0.57%) respectively (Table.3) followed by acetone extract (48.33±0.47% and 49.66±0.14%). Whereas, very poor larvicidal effect (21.66±1.15% and 16.66±0.52%) was noted with water extract at 0.5% concentration respectively. The positive control azadirachtin showed 41.66±1.15% mortality which was comparable to methanolic extract of *C. odorata* and *L. nepetifolia.* As evidenced from the table, generally increased larval mortality was observed with increased concentration of the extracts tested against *H.armigera*. Among all tests, methanolic extract showed significant mortality rate as compared to other. All the concentration of methanol showed statistically superior activity. Concentration dependent activity was noticed in all the three extracts. The methanol extract of *R. nasutus* was reported to have a pesticidal effect against the larvae of *S. litura* (Kamaraj *et al*. 2008) [20]. Similarly insecticidal potentiality is revealed by various studies (Gorawade *et al*., 2019; Shiragave, 2020,) [12, 31]

Table 3. Percent larvicidal activity of *C. odorata* and *L. nepetifolia* leaf extracts against *H. armigera.*

Sr.No	Solvent	Treatment (%)	Larvicidal activity (%)	
			C. odorata	*L. nepetifolia*
1.	Acetone	0.5	30.22±1.77	29.66±0.57
		1.0	35.55±0.55	31.33±0.57
		2.5	38.13±1.66	39.66±0.44
		5.0	48.33±0.47	49.66±0.14
2.	Methanol	0.5	28.66±2.51	33.63±0.57
		1.0	32.33±3.21	46.88±1.52
		2.5	45.66±1.15	48.65±3.78
		5.0	65.33±3.05	68.33±0.57
3.	Water	0.5	21.66±1.15	16.66±0.52
		1.0	28.66±2.08	21.66±3.78
		2.5	32.66±0.57	26.33±2.30
		5.0	36.66±0.57	38.33±1.41
4.	Azadiractin	0.1	41.66±1.15	40.16±0.75
5.	Negative control	-	6.33±1.08	7.41±1.10

Values were the means of three replicates ± standard error.

Table 4: Thin layer chromatographic studies of phenolics from *C. odorata* and *L. nepetifolia* methanolic leaf extracts.

Sr. No	Plant sample	No. of Spots	R_f values (Solvent System-I)
1.	Standard phenolics	6	0.07,0.02,0.66,0.76,0.81,0.87
2.	*C. odorata*	3	0.23,0.30,0.66
3.	*L. nepetifolia*	3	0.23,0.30,0.66
		No. of Spots	R_f values (Solvent System-II)
1.	Standard phenolics	6	0.04,0.14,0.37,0.59,0.67,0.82
2.	*C. odorata*	5	0.04,0.07,0.10,0.37,0.59
3.	*L. nepetifolia*	5	0.04,0.07,0.10,0.37,0.59
		No. of Spots	R_f values (Solvent System-III)
1.	Standard phenolics	6	0.04,0.11,0.26,0.42,0.52,0.63
2.	*C. odorata*	2	0.26,0.42
3.	*L. nepetifolia*	2	0.26,0.42
		No. of Spots	R_f values (Solvent System-IV)
1.	Standard phenolics	6	0.08,0.32,0.37,0.69,0.77,0.91
2.	*C. odorata*	1	0.91
3.	*L. nepetifolia*	1	0.91

Thin layer chromatography of phenolics

In the present investigation both plants extracts was exhibited spots on TLC plates which confirmed the presence phenolics. The four different solvent systems were used to screened phenolics. All the four solvent systems showed six spots on TLC for tested standard phenolics compounds like quircetin, catechol, gallic acid, Vanillic acid chlorogenic acid, ferulic acid with greenish, demish blue, blackish, brownish, bluish and chocolate colours respectively.

Solvent system I show three spots in both *C. odorata* and *L.*

nepetifolia samples and two were matches with standard phenolics and may be identified as catechol and quircetin and one spot was unknown. Solvent system II represented the five spots in *C. odorata* and *L. nepetifolia* samples respectively. Among which three spots were matches with standards and may be identified as quircetin, gallic acid and vanillic acid and two were unknown. Solvent system III exhibit only two spots in *C. odorata* extract and matched with standards and might be identified as gallic acid and vanillic acid, where no spots detected in *L. nepetifolia* sample. Solvent system IV represented one spot in both samples and matched with standards and mightay be identified as ferulic acid. These results were matches with Radomir *et al*., (2004) [28] which confirm the phenolic standards. The presence of phenolic group in plants is to protect them from microbial, insect and herbivores damage (Hassan, 2015) [14]. Many of these active compounds also possess other functional attributes like anti-inflammatory, antimutagenic, hypocholestemic and antiplatelet aggregation properties (Praveena *et al*., 2012) [26]. The range of R_f values as reported in previous studies are 0.3 to 0.9 for terpenes, 0.05-0.85 for phenolic compounds, and 0.5 to 0.9 for flavonoids in *C. odorata* extracts (Fecka and Cisowski 1999; Muchuweti *et al*. 2005; Talukdar *et al*. 2010) [10, 24, 33].

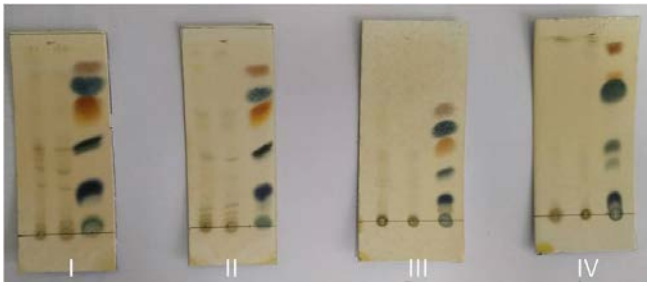

Fig 1: Thin layer chromatograpichc studies of phenolics from *C. odorato* and *L.* nepetifolia methanolic leaf extracts. I) chloro form: ethy. I acetate: formic acid (5:4:1), II) Benzene: ethyl acetate: formic acid (6:3:1), III) Toulene: ethylacetate: formic acid (12:6:1), IV) chloroform: ethanol: acetic acid (60:4:6).

Conclusion

From our findings, it can be concluded that 5% methanol extracts of both plants contains significant insecticidal activity (Ovicidal, Antifeedant, Larvicidal) against *H. armigera* followed by acetone and water. The phenolic compounds were spotted in TLC have insecticidal properties. However there is the need to conduct further intense study on *C. odorata* and *L. nepetifolia* to develop novel phytopesticides for the effective management of *H. armigera*.

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