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The Major Phyto-Compounds Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate Derived from *Indigofera tinctoria* Medicinal Flora Tested Against Various Target Medical and Agronomic Pests

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Abstract

Objectives: To determine the phytochemical screening and major phyto-compounds from *Indigofera tinctoria* leaf methanol extract as well as ovicidal and larvicidal toxicity towards medical and agronomic pests, *Aedes aegypti, Culex quinquefasciatus, Spodoptera litura and Helicoverpa armigera*. **Methods:** In this study, the phytochemical screening was done by prescribed method, finding of phyto-constituents were made by GC-MS analysis, ovicidal and larvicidal toxicity of selected pests were recorded after 24 hrs. post treatment at various concentrations. The mortality was assessed by using probit analysis to calculate LC_{50}/LC_{90}. **Findings:** By the phytochemical screening, the more numbers of phytochemicals were obtained from methanol extract as well as GC-MS analysis displayed sum of 10 phyto-compounds gained 100% besides two phyto-compounds were major constituents Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate. The phyto-products of *I. tinctoria* were produced maximum eggs toxicity around 100% at higher concentration. Similarly, *I. tinctoria* leaf methanol extract borne major phyto-compounds major constituents Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate were tested 3rd instar larvae of selected medicinal and agronomic pests with their LC_{50}/ LC_{90} value were 10.93/18.65 μg/ml, 10.87/18.77 μg/ml, 15.29/27.17 μg/ml, 16.84/29.41μg/ml and 11.16/19.38 μg/ml, 10.43/18.51 μg/ml, 14.57/26.58 μg/ml and 15.61/28.63 μg/ml were recorded on various pests of *Ae. aegypti, Cx. quinquefasciatus and S. litura and H. armigera*, respectively. **Novelty:** The statistical analysis of the data clearly indicates that phyto-compounds of *I. tinctoria* induced outstanding larval lethality were observed on selected pests. Particularly, the identified phyto-compounds showed multifold toxicity against the selected pests.

Keywords: Indigofera tinctoria; Phytocompound; Targetfauna; Ecosafety; Pesttoxicity

1 Introduction

Recently, medical and vector biology fields are facing plenty of challenges to protect public and cattle from infected bite of female mosquitoes[1]. In many countries, mosquitoes are vastly spreading many unsolved diseases to human and other blood yielding faunas as well as they making serious alarming to economic crises[2]. The Dipteran vector spreads many different pathogenic viruses as the results that severely threaten blood yielding many faunal lives[3].

Spodoptera litura is a voracious feeder and consuming varieties of flora (including vegetables, fruits, seeds, flower, rhizomes, etc.,). It is polyphagous lepidopteron pest which feeding above 200 floral hosts and it includes 40 different families[4]. Recently, it is vastly distributed in many terrains of Asian country and it has gradually emerged as vital pest in many revenue crops[5]. *Helicoverpa armigera* is a globally distributed polyphagous pest which vastly consuming many revenues agronomic crops[6]. It consuming above 180 floral species includes 47 families, at initially feeds on tender leaves then later stage consuming all parts of flora (Fruits, seeds, seed coat, flower, stems, rhizome, etc.,). Most of the Indian villagers nearly above 80% mainly depend on agronomy and its byproducts. Insect's pests significantly damaged plenty of crops as the results cause the productivity and revenue crises[7]. Recently (a half a century), most of the countries are enormously used unadvisable toxic pesticides in vector control and crop protection aspects as the result unpredicted damage caused on eco-system, pest resurgence, high-cost production and very hard to application, great loss of soil productivity, declining population size of beneficial microbes, pollinator, non-target fauna etc.,[8]. The continued as a long-term application of toxic pesticides in the pest control management (both medical and crops protection) to develops negative impact on biosphere and its related factors. Therefore, most of the research communities/ scientists were planned to rectify through organic pesticides especially by naturally available phyto-products[9]. *Indigofera tinctoria* is a greenish small herb and it can be used to treat as a medicine for diseases and infection on human and cattle[10,11]. However, the bio-toxic efficacy of *I. tinctoria* and its phytocompounds on insect pests were not explored intensively. Therefore, present examination, we planned to *I. tinctoria* derived phytoproducts were tested towards the eggs and larval toxicity of target pests species.

2 Methodology

2.1 Collection and processing of floral material

The fresh, neatly cleaned and diseases free *I. tinctoria* leaves (Figure 1 A-C) were collected during flowering season in the month of January-April, 2020 from Cuddalore and Mayiladuthurai District. The floral sample was identified by well skilled botanist Dr. R. Janagan, the fully shade dried leaf was ground into fine powder, followed by extraction and condensation done it through standard protocol[12]. The condensed floral materials were stored at -10^{0}C by using deep refrigeration for dimensional activities of various bioassays.

2.2 Phytochemical screening and GC-MS analysis

The various leaf extracts (Hexane, diethyl ether, dichloromethane, acetate and methanol extract) of *I. tinctoria* medicinal herb were subjected to qualitative analysis of various phyto-chemicals were described through proper methods [13]. Moreover, plants are holding hundreds of phyto-compounds it can be classified into major and minor phyto-compounds which based on the acquiring concentration phyto-compounds. The GC-MS analysis of leaf methanol extract was subjected to Agilent technologies and identified various phyto-compounds were compared with NIST/ WILEY library [14].

Fig 1. A fresh growing flora of *I. tinctoria* (A), Dried leaf powder (B) and Condensed leaf methanol extract (C)

2.3 Target pest rearing

Ae. aegypti and Cx. quinquefasciatus eggs, larvae and pupae were collected from sewage treatment plant near AVC College (Autonomous) and *S. litura and H. armigera* eggs and larvae were collected from *Arachis hypogaea* and *Abelmoschus esculentus* agronomic field near Cuddalore and Mayiladuthurai District., Tamilnadu, India. The collected medical pests' juvenile stage (eggs, larvae and pupae) was reared in separate container. The plastic container filled with drinking tap water which maintained appropriate temperature, humidity and photoperiod. The larvae allowed to the diet of yeast powder, biscuits crumble, algae bloom and natural honey in the ratio of 1:2:1:1, respectively. The juvenile stage of agronomic pests were reared in above mentioned condition, the *S. litura* larvae allowed to fed with Ricinus communis tender leaves and *H. armigera* larvae fed with *Abelmoschus esculentu* tender fruits.

2.4 Eggs toxicity of target pests

Eggs toxicity of medical pests: The eggs toxicity was assessed through standard methodology [15]. The freshly laid eggs (0-5 hrs aged) of selected ecto-parasitic vector were collected from mother culture. 25 Nos. eggs were exposed to various concentrations of *I. tinctoria* phyto-products in the glass container. The phyto-products were dissolved with 1ml of DMSO which prepared in to various concentrations (range between lower to higher concentration). After 24 hrs. post treatment eggs toxicity were concluded as well as in-between every 6 hrs. interval thoroughly observed death rate of eggs. As usual, control was maintained without phyto-products composites. Eggs toxicity of agronomic pests: The eggs of polyphagous pests were collected which setup into (One batch was 25 Nos. eggs) five batches for single concentration. The required phyto-products of *I. tinctoria* were sprayed which allowed into 24 hrs. post treatment and estimated the eggs mortality [16]. The experiment was done on under laboratory condition. As usual, control was maintained without phyto-products composites.

2.5 Larval toxicity of target pests

Larval toxicity of medical pests: The phyto-products of *I. tinctoria* were diluted with 1ml DMSO which mixed with 450 ml of chlorine free tap water and make into different concentration made into 500 ml beaker each experiment constantly replicated five times. Invariably 3rd instar larvae 25 count released into respective concentration in which control was maintained without phyto-products. Top of the beakers were tightly closed with muslin cloth which may give the protection and entry of any intruder. The larval toxicity carefully noticed every 6 hours interval which monitored up to 24 hours of post treatment. While conducting experiment, larvae were starved and it was followed by the standard protocol[15]. The total larval mortality was corrected by prescribed method[16]. Larval toxicity of agronomic pest: The 3rd instar larvae of *S. litura* were allowed to phyto-products of *I. tinctoria* different concentration treated with Ricinus communis leaf disk as well as same larval stage of *H. armigera* larvae allowed to phyto-products of *I. tinctoria* different concentration treated with *Abelmoschus esculentu* sliced disk of tender fruits. The larval toxic bioassay followed by[15]. Agronomic pests, each batch allowed 25 larvae which introduced in separate containers, the toxicity noticed and the percent mortality corrected by standard method[16].

2.6 Statistical Analysis

The recorded larval and eggs toxic raw data were assessed through Probit analysis[17] for examining for mean ± S.D, DMRT test, LC_{50} /LC_{90}, UCL, LCL, regression, chi-square, etc., were estimated with the help of IBM- SPSS 26.0v. The observation of $p \leq 0.05$ were considered to be statistically significant.

3 Results and Discussion

3.1 Phytochemical screening and GC-MS analysis

The selected medicinal herb, different leaf extracts of *I. tinctoria* (hexane, diethyl ether, dichloromethane, ethyl acetate and methanol) were assessed for phytochemical screening, the more numbers of phytochemicals (Alkaloids, anthraquinones, coumarins, flavonoids, glycosides, phenolics, saponins, steroids, tannins and triterpenes) were obtained from methanol extract which clearly enumerated in Table 1. The Indian medicinal flora, *I. tinctoria* leaf extract (methanol extract) was tested by GC-MS analysis for observing various phyto-compounds which apparently shown in Figure 2 and its peak, retention time, concentration, etc., were evidently noticed in Table 2. A total of 15 phyto-compounds gained 100% besides two phyto-compounds were major constituents Heptasiloxane, hexadecamethyl- (Peak 6; Retention Time 37.435; Composition Area 99326; Composition Area% 22.69, etc.,) and 1,1-Dimethylethyl 3-phenyl-2-propenoate (Peak 12; Retention Time 39.845; Composition Area 85954; Composition Area% 19.64, etc.,). The noticed major floral-bioactive compounds were obviously authenticated through MS analysis which presented in Figures 3, 4, 5 and 6. The different leaf extracts of *I. tinctoria* were evaluated for detecting the availability of phytochemical in the respective solvents and their results were compared with selected all extracts but a greater number of phytochemicals groups were identified from high polarity solvent of methanol extract followed by other solvent extracts. Previously, many investigations were evidently done on several herbal parts as well as they were well effective and target specificity on different life stages of pests[18,19].

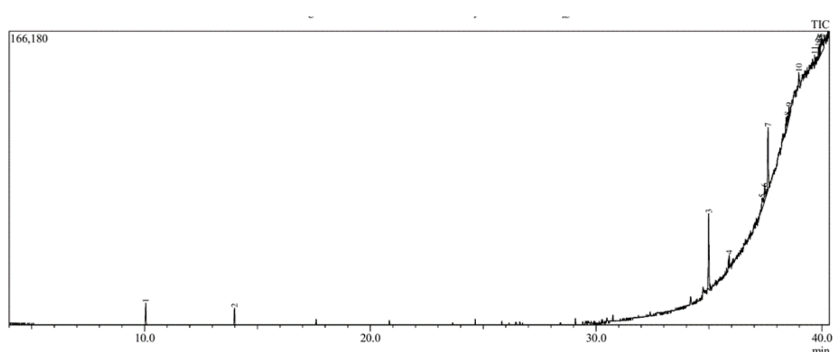

Fig 2. GC-MS chromatogram of *I. tinctoria* leaf methanol extract

Table 1. The various qualitativephyto-chemicals detected from leaf extracts of *I. tinctoria*

Sl. No.	Phytochemical screening	Indigofera tinctoria different leaf extracts				
		Hex	DEE	DCM	ETA	MET
1.	Carbohydrates	–	+	–	–	–
2.	Alkaloids	–	–	–	+	+
3.	Flavonoids	+	+	+	+	+
4.	Saponins	–	–	–	–	+

Continued on next page

Table 1 continued

5.	Tannins	–	+	+	–	+
6.	Triterpenes	–	+	+	–	+
7.	Resins	+	–	–	+	–
8.	Coumarins	–	–	–	+	+
9.	Anthraquinones	+	+	+	–	+
10.	Phenolics	–	–	–	–	+

HNE: Hexane; DEE: Diethyl ether; DCM: Dichloromethane; ETA: Ethyl acetate; MET: Methanol
+ = phytochemical group Zero
– = phytochemical group Appeared

Table 2. GC-MS analysis of *I. tinctoria* leaf methanol extract

PE	RT	ST	ET	AR	AR %	HE	HE %	A/H	CN
1	10.05	10.02	10.08	22270	5.09	12454	7	1.79	BENZOIC ACID, 2,5-BIS(TRIMETHYLSILOXY)-, TRIMETHYLSILYL ESTER
2	13.983	13.955	14.01	16304	3.72	9544	5.36	1.71	Cyclohexasiloxane, dodecamethyl-
3	34.988	34.94	35.045	31134	7.11	7808	4.39	2.32	1,2-BENZENEDICARBOXYLIC ACID
4	35.892	35.88	35.92	49613	11.33	7127	4	1.32	Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester
5	37.345	37.315	37.435	25597	5.85	6363	3.57	4.02	1,3-Dioxane, 4-(hexadecyloxy)-2-pentadecyl-
6	37.455	37.435	37.57	99326	22.69	42792	24.04	3.99	Heptasiloxane, hexadecamethyl-
7	37.612	37.57	37.66	9608	2.19	7295	4.1	2.42	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
8	38.47	38.4	38.5	29942	6.84	6186	3.47	4.84	1,4-METHANO-5,8,10-METHENOCYCLOBUTA [4,5] PYRROLO[2,1-A]PHTHALAZINE-5,10A(6H,7AH)-DICARBOXYLIC ACID, DECAHYDRO-9-(PHENYLTHIO)-, D
9	38.545	38.5	38.59	15759	3.6	7100	3.99	2.22	1,3,2-DIAZAPHOSPHOLIDINE, 2-CHLORO-1,3-DIMETHYL-
10	38.977	38.955	39.03	18318	4.18	7817	4.39	2.34	CYCLONONASILOXANE, OCTADECAMETHYL-
11	39.676	39.655	39.685	6995	1.6	6915	3.88	1.01	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
12	39.845	39.825	39.875	85954	19.64	35514	19.95	1.66	1,1-DIMETHYLETHYL 3-PHENYL-2-PROPENOATE
13	39.893	39.875	39.905	8781	2.01	7100	3.99	1.24	(1R,2R,3R)-4-(1-HYDROXY-2,3-EPOXY-3-TRIMETHYLSILYLPROPYL)PHENYL ACETATE
14	39.92	39.905	39.93	6412	1.46	6950	3.9	0.92	OCTADECANOIC ACID, TRIMETHYLSILYL ESTER
15	39.945	39.93	40.09	11734	2.68	7054	3.96	6.96	1,18,21-TRIAZA-3,4

PE (Peak); RT (Retention time); ST (Start time); ET (End Time); AR (Area); AR% (Area %); HE (Height); HE% (Height %); A/H (Area /Hight)

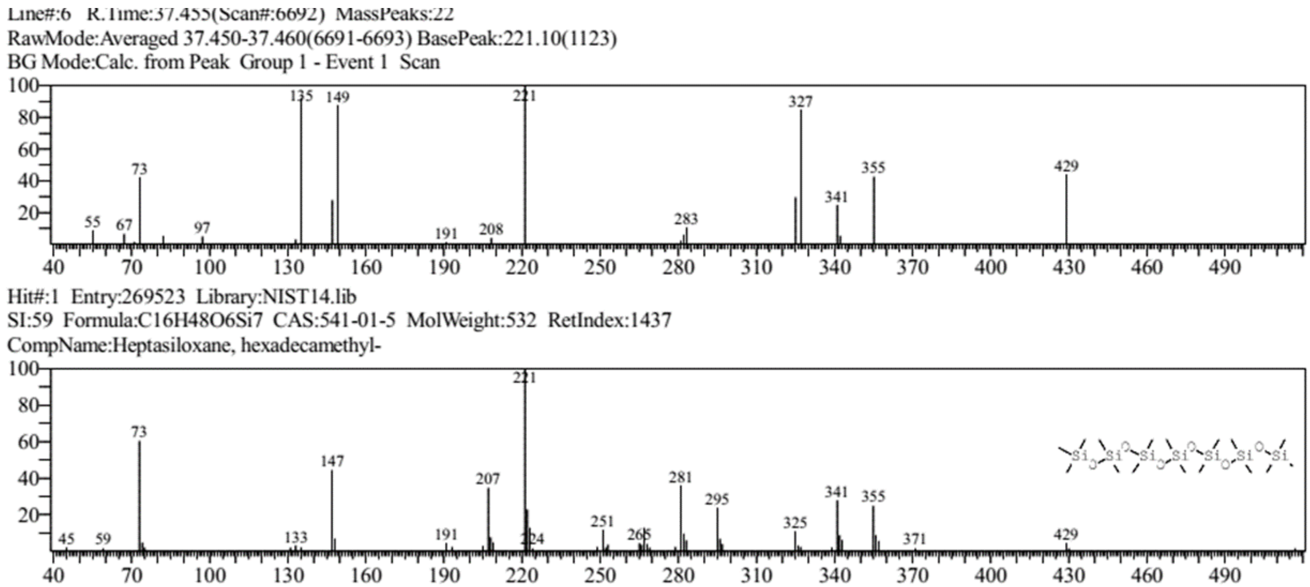

Fig 3. Mass spectrum and structure of Heptasiloxane, hexadecamethyl- phyto-compound

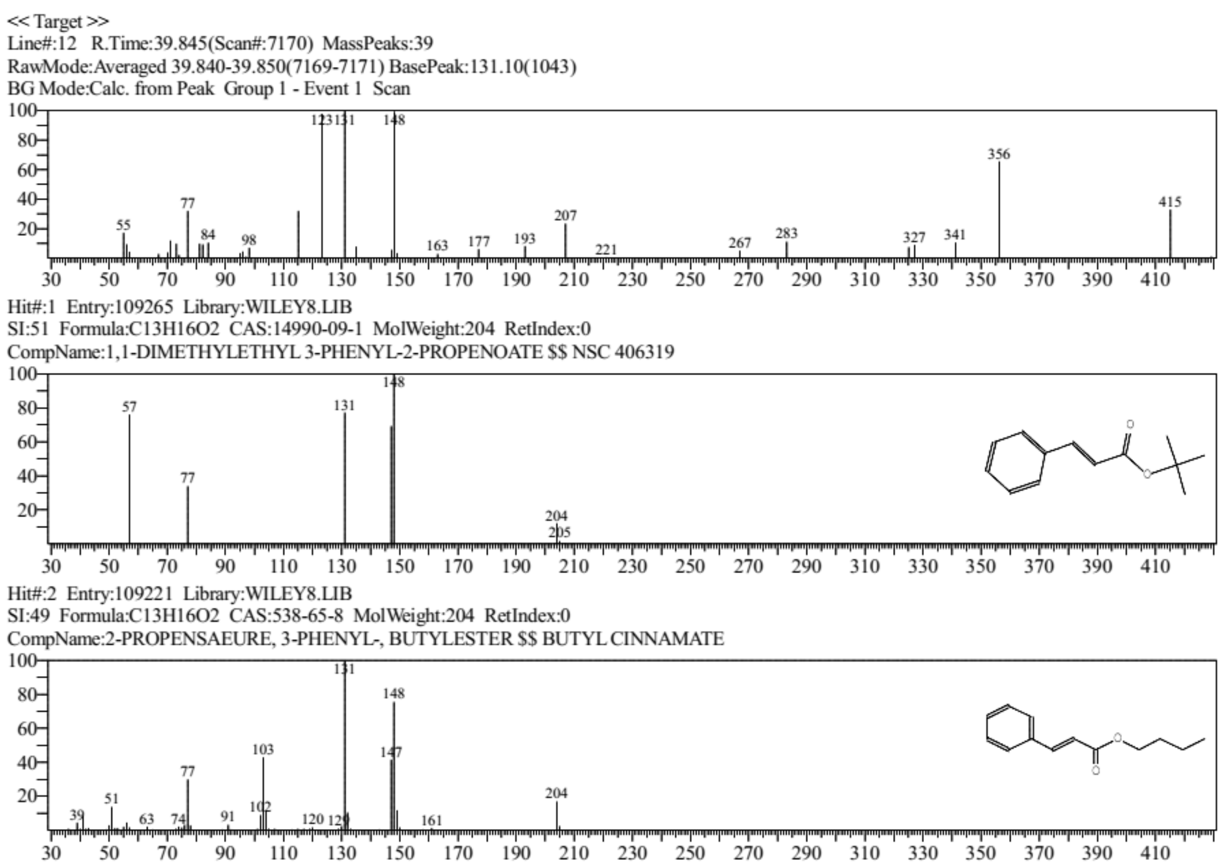

Fig 4. Mass spectrum and structure of 1,1-Dimethylethyl 3-Phenyl-2-Propenoate compound

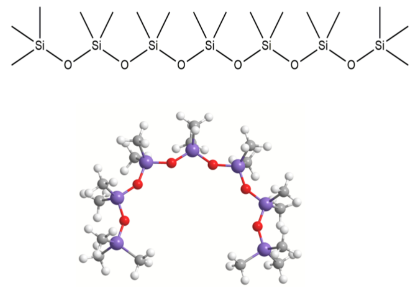

Fig 5. The 2D and 3D structure ofphyto-compound Heptasiloxane, hexadecamethyl

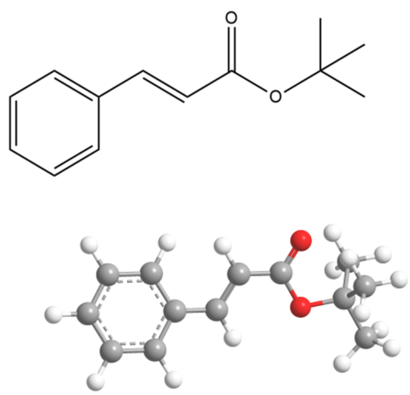

Fig 6. The 2D and 3D structure of phyto-compound1,1-Dimethylethyl 3-Phenyl-2-Propenoate

3.2 Ovicidal activity of major bio-active compounds

Egg toxic effects of *I. tinctoria* leaf methanol extract borne major phyto-compounds Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate tested on eggs of medicinal and agronomic pests are represented in Table 3. The phyto-compound Heptasiloxane, hexadecamethyl- showed statistically significant eggs toxicity on *Ae. aegypti, Cx. quinquefasciatus, S. litura and H. armigera* with their values were 28.4%, 49.4%, 79.7%, 97.5%, 24.6%, 48.5%, 76.7%, 98.4% and 20.8%, 44.5%, 74.8%, 95.4%, 22.2%, 43.6%, 76.7% and 92.4% recorded by 4, 8, 12 and 16μg/ml concentrations respectively. Similarly, the major phyto-compound 1,1-Dimethylethyl 3-Phenyl-2-Propenoate against eggs of medicinal and agronomic pests, the egg toxicity was observed against *Ae. aegypti, Cx. quinquefasciatus, S. litura and H. armigera* with their values were 21.6%, 42.8%, 72.7%, 93.5%, 21.6%, 42.5%, 79.7%, 91.4% and 21.6%, 42.2%, 75.6%, 94.4%, 23.2%, 47.7%,75.3% and 96.4% recorded by 4, 8, 12 and 16μg/ml concentrations respectively. The various phyto-products tested; the topmost toxicity was recorded by the selected phyto-compounds of *I. tinctoria* against selected pests species. The similar categories of many works were previously described against various pest species. Previously, the medicinal flora of *Cyathocline purpurea, Blumea lacera, Neanotis montholonii and Neanotis lancifolia* extract showed 70–90% eggs toxicity observed at higher concentrations against *Ae. aegypti*[(20)]. *Sophora alopecuroides* derived phyto-constituents Sophocarpin and Sophordine were provided maximum egg toxicity 88.5% 78% at 100 μg/ml against *Ae. albopictus* [(21)]. *Origanum vulgare* borne phytocompounds carvacrol, p-cymene, and γ-terpinene against *H. armigera* eggs were EC50 values of 33.48, 47.85, and 56.54 μg/ml, respectively[(22)].

3.2.1 Values of major bio-active compounds

The two different floral-bioactive compounds Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate of *I. tinctoria* tested on the 3rd instar larvae of vector mosquitoes and agronomic field pests *Ae. aegypti, Cx. quinquefasciatus and S. litura and H. armigera*. Lethal toxicity of major phyto-compound Heptasiloxane, hexadecamethyl-: The LC_{50}/ LC_{90} value of 10.93/18.65 μg/ml, 10.87/18.77 μg/ml, 15.29/27.17 μg/ml and 16.84/29.41μg/ml were recorded on various pests of *Ae. aegypti, Cx. quinquefasciatus and S. litura and H. armigera*, respectively. Lethal toxicity of major phyto-compound Dimethylethyl 3-

Table 3. Ovicidal activity of *I. tinctoria* phyto-products against eggs of medical and agronomic pests

Concentration	% Ovicidal activity of medical and agronomic pests, freshly laid (0-6 hours age old) eggs			
	Ae. aegypti	*Cx. quinquefasciatus*	*Ae. aegypti*	*Cx. quinquefasciatus*
Heptasiloxane, hexadecamethyl-			1,1-Dimethylethyl 3-Phenyl-2-Propenoate	
4 μg/ml	28.4±3.6	24.6±2.4	21.6±1.6	21.6±1.2
8 μg/ml	49.4±2.6	48.5±1.8	42.8±2.6	42.5±2.8
12 μg/ml	79.7±3.4	76.7±4.3	72.7±2.4	79.7±3.6
16 μg/ml	97.5±2.8	98.4±6.4	93.5±3.8	91.4±4.8
Heptasiloxane, hexadecamethyl-			1,1-Dimethylethyl 3-Phenyl-2-Propenoate	
Concentration	*S. litura*	*H. armigera*	*S. litura*	*H. armigera*
4 μg/ml	20.8±1.8	22.2±1.5	21.6±1.2	23.2±1.4
8 μg/ml	44.5±1.1	43.6±1.9	42.2±1.9	47.7±1.1
12 μg/ml	74.8±1.7	76.7±1.5	75.6±1.3	75.3±1.9
16 μg/ml	95.4±1.4	92.4±1.6	94.4±1.2	96.4±1.6

Value represents mean±S.D. of five replications. Values in a column with a different superscript alphabet are significantly different at P <0.05 level DMRT Test.

Phenyl-2-Propenoate: the LC_{50}/ LC_{90} value of 11.16/19.38 μg/ml, 10.43/18.51 μg/ml, 14.57/26.58 μg/ml and 15.61/28.63 μg/mlwere recorded on various pests of A*e. aegypti, Cx. quinquefasciatus and S. litura and H. armigera*, respectively (Table 4). The statistical analysis of the data clearly indicates that a phyto-compounds of *I. tinctoria* induced outstanding larval lethality were observed on selected pests. Similarly, many different works previously done and it has been evidently supported with present examination, Enhalus acoroides and Halophila ovalis showed above 50% larval death recorded at 500ppm against *Ae. aegypti and Cx. quinquefasciatus*[(23)]. The larval toxicity of various phyto-compounds showed predominant toxicity against agronomic pests[(24)]. The *I. tinctoria* derived phyto-compounds were showed appreciable safety towards environment and higher toxicity observed on selected pests.

Table 4. LC values of *I. tinctoria* phyto-products against larvae of medical and agronomic pests

Medical pests	LC50 (μg/ml)	LCL-UCL (μg/ml	LC90 (μg/ml)	LCL-UCL (μg/ml)	R-value	χ2
Heptasiloxane, hexadecamethyl-						
Ae. aegypti	10.93	8.54-13.10	18.65	15.88-24.46	y=1.9+0.18x	8.156
Cx. quinquefasciatus	10.87	10.06-11.65	18.77	17.53-20.37	y=1.81+0.17x	4.949
S. litura	15.29	14.04-16.47	27.17	25.35-29.53	y=1.63+0.11x	1.860
H. armigera	16.84	15.57-18.07	29.41	27.40-32.06	y=1.72+0.1x	1.332
1,1-Dimethylethyl 3-Phenyl-2-Propenoate						
Ae. aegypti	11.16	10.33-11.97	19.38	18.08-21.09	y=1.77+0.16x	5.451
Cx. quinquefasciatus	10.43	7.69-12.75	18.51	15.57-25.06	y=1.73+0.17x	8.861
S. litura	14.57	13.28-15.77	26.58	24.76-28.95	y=1.64+0.11x	1.262
H. armigera	15.61	14.26-16.87	28.63	26.60-31.31	y=1.56+0.1x	3.857

LC_{50}: Lethal Concentration showed 50% mortality; LC_{90}: Lethal Concentration showed 90% mortality; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit; R- value: Regression value; χ^2 value: Chi-square value

4 Conclusion

The *I. tinctoria* borne phyto-products showed efficiently succeed that selected medical and agronomic pests. The phyto-products relatively higher toxicity on harmful pests and it could be former friendlier, zero hazards to environment, at low concentration obtained undeniably eradicating target-fauna and it can be approached to pest management techniques.

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