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PHYTO-TOXICITY OF INDIAN MEDICINAL PLANTS TINOSPORA CRISPA (MENISPERMACEAE) AND PSIDIUM GUAJAVA (MYRTACEAE) AGAINST TOBACCO CUTWORM, HELICOVERPA ARMIGERA (HUB.) (LEPIDOPTERA: NOCTUIDAE)

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A B S T R A C T

Helicoverpa armigera is highly polyphagous pest, infest more than 500 plant species and is a serious pest in India. The greatest damage is caused to cotton, tomatoes, maize, chick peas, alfalfa and tobacco etc. This notorious pest initially feeds on vegetative parts and subsequently on immature pods and ultimately causes severe loss of production. However, many chemicals available for treatment of insect pest are also toxic to natural enemies and gradually the pest will develop resistance to it. The objective of the present study was to evaluate larvicidal, ovicidal and oviposition deterrent activities of benzene, diethyl ether, ethyl acetate and methanol leaf extract of Tinospora crispa and Psidium guajava against Helicoverpa armigera. Twenty five early fourth instar larvae of Helicoverpa armigera was exposed to various concentrations and was assayed in the laboratory by using the protocol of Abbott's formula (1925).; the 24h LC50 values of the Tinospora crispa and Psidium guajava leaf extract was determined by probit analysis. The ovicidal activity was determined against Helicoverpa armigera to various concentrations ranging from 75-450 ppm under laboratory conditions. The hatch rates were assessed 48hrs post treatment. For oviposition deterrent activity, ten pairs of (adult moths) H. armigera were introduced in individual cage. 10% (w/v) sucrose solution with multivitamin drops was provided for adult feeding to increasing fecundity, five replicates were maintained for control and treatments. After 48h, the numbers of eggs laid on treated and control leaves were recorded and the percentage of oviposition deterrence was calculated. The LC 50 value of benzene, diethyl ether, ethyl acetate and methanol leaf extracts of Tinospora crispa were 86.26, 89.55, 77.64 and 62.63ppm, respectively. Psidium guajava shows the LC50 values of 128.51, 132.64, 124.97 and 118.65ppm, respectively. Among two plant extracts tested, Tinospora crispa extracts were found to be most significant ovicidal activity 100% egg mortality (zero hatchability) observed at 300ppm and 375ppm for Psidium guajava. Methanol extract of Tinospora crispa and Psidium guajava showed 100% oviposition deterrency against the gravid moths of H.armigera at 200 and 240ppm concentration. In general, the oviposition deterrency is directly proportional to the increase in the concentration and also among the various extracts tested, methanol extract was found to have significant activity than the other solvent extracts. From the results it can be concluded the crude extract of Tinospora crispa and Psidium guajava were an excellent potential for controlling agricultural pest Helicoverpa armigera.

INTRODUCTION

The development of integrated pest control programs in controlling the economically important pest, *Helicoverpa armigera* (Hub) has gained increased attention in many parts of the world. *H. armigera* is highly polyphagous pest, infest more than 500 plant species and is a serious pest in India. The greatest damage is caused to cotton, tomatoes, maize, chick peas, alfalfa and tobacco etc. The economic threshold of harmfulness in central Asia is three to five larvae per hundred plants of long-staple cotton and eight to 12 larvae per hundred plants on medium-staple cotton. In cotton crops, blooms that have been attacked may open prematurely and stay fruitless. When the bolls are damaged, some will fall off and others will fail to produce lint or produce lint of an inferior quality. Secondary infections by fungi and bacteria are common

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and may lead to rotting of fruits. Injury to the growing tips of plants may disturb their development, maturity may be delayed and the fruits may be dropped. This pest is reflected in the wide taxonomic range of wild and cultivated plants acceptable for oviposition by adults and feeding by larvae. This notorious pest initially feeds on vegetative parts and subsequently on immature pods and ultimately causes severe loss of production. However, many chemicals available for treatment of insect pest are also toxic to natural enemies and gradually the pest will develop resistance to it (Elumalai *et al*, 2008; Krishnappa *et al*, 2010a; Elumalai *et al*, 2010a,b; Anandan *et al*, 2010 and 2011; Pavela, 2010; Gokulakrishnan *et al*, 2012a). Nowadays, synthetic insecticides have been widely used for controlling this pest on different crops, but undesirable side effects of synthetic insecticides, including development of resistance, have necessitated a shift to more eco-friendly approaches for controlling this pest. In recent years, the use of synthetic organic insecticides in crop insect pest management programmes around the world has resulted in damage to the environment, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms. Due to these impacts of chemical insecticides prompted search for alternate techniques for insect pest management arises (Madhua *et al*, 2010; Kaushik *et al*, 2009). One possible way to reduce the high consumption of synthetic insecticides is through the application of botanical insecticides, generally considered to be environmentally and medically safe (Dayan *et al*, 2009).Plant derivatives are highly toxic to many insect species and more than 2000 plant species are known to possess some insecticidal properties (Krishnappa *et al*, 2010b). Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages (Elumalai *et al*, 2010a). Essential oils and their constituents have been reported to be an effective source of botanical pesticides (Tewary *et al*, 2005; Krishnappa *et al*, 2011a, b).The growing awareness of the hazards of excessive use of pesticides globally has led researchers to search for safer and more environment friendly alternative methods for insect pest control. Therefore, extensive studies are carried out to screen plants as insect growth control agents. Over the last two to three decades, greater attention has been focused on the bioactivity of phytochemicals for their potential as Pesticides against phytophagous insects (Padmaja and Rao, 2000). Research on natural products, that could be alternatives to synthetic pesticides and fungicides, for example, plant extracts and essential oils, has greatly increased during recent years (Anandan *et al*, 2010; Cohen *et al*, 2006).The neonate larvae initially attacking the foliage of the plants and the later stage feed on developing seeds in the pod. This pest is considered as the serious pests of various economically important crops such as, cotton, groundnut, chilly, tobacco, castor, banana, cabbage, crown of thorns, macadamia, mustard, poinsettia, rose, sugarcane and tomato as well as some legumes, teaes, etc., and also they have developed resistance in almost all commercially available chemical pesticides. Furthermore, literatures pertaining to the control of these two pests using phytopesticides are scanty. Hence, the present study the bioefficacy of *Tinospora crispa* and *Psidium guajava* were evaluated against *H. armigera* for its larvicidal, ovicidal and Oviposition deterrent activity.

MATERIALS AND METHOD

Plant material

The selected plants leaves were collected during growing season month of August 2013 from the Poompuhar Village, Sirkali Taluk, Nagapattinm District, Tamilnadu, India. Bulk samples were air-dried in the shade and after drying. These were ground to fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared and identified with the help of Plant Taxonomist, Department of Botany, Poompuhar College, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction method

The dried leaves (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with benzene, chloroform, hexane and methanol (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22–26 mm Hg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4^0C.

Rearing of test organism

Helicoverpa armigera (Hub).(Noctuidae: Lepidoptera) were cultured and maintained in the laboratory on castor leaves. Insects were cultured in plastic vials individually to avoid cannibalistic activity and larvae were provided with cotton leaves and maintained in the laboratory. Rearing conditions were a 12 h photo regime at 28±2°C and 75±5% relative humidity. An insect culture was continuously refreshed with wild moths captured by a light trap in the vicinity of the agricultural farm of Poompuhar Village, Nagapattinam District, Tamilnadu. Generally hale and healthy and uniform sized fourth instar larvae and newly (0-6 hrs old) eggs were used for the experiments.

Larvicidal activity

For the evaluation of larvicidal activity, the selected plant extracts tested is based on the wide range and narrow range tests, it was tested at 60-280ppm and they were tested against the freshly moulted (0-6 hrs) fourth instar larvae of selected lepidopteran agricultural field pest. Petioles of the leaves were tied with wet cotton plug to avoid early drying and placed in plastic trough (29cm x 8cm) 20 pre starved (4h) fourth instar larvae of test organisms were introduced individually and covered with muslin cloth. Five replicates were maintained and the number of larvae dead after 24h was recorded and the percentage of larval mortality was calculated using Abbott's formula (1925). All moribund pest larvae were considered as dead.

Ovicidal activity

For ovicidal activity, scales from the egg masses of

H.armigera were carefully removed using fine camel brush. 500 eggs from three selected lepidopterans were separated into five lots each having 100 eggs and dipped in 75-450ppm concentrations of plant different solvent extracts. Controls as mentioned above. Number of eggs hatched in control and treatments were recorded and the percentage of ovicidal activity was calculated using Abbott's formula (1925). For each experiment, five replicates and the hatch rate was assessed 120 h post treatment.

Oviposition deterrent activity

For oviposition deterrent activity 40- 240ppm concentration of selected plants extract were sprayed on respective fresh host plant and similar controls as mentioned above were also used here. The petioles of the treated leaves were inserted into a conical flask (cotton plugged) containing dechlorinated water to avoid early drying and placed inside the cage (60cm x 45cm x 45cm). Ten pairs of (adult moths) *H.armigera* were introduced in individual cage. 10% (w/v) sucrose solution with multivitamin drops was provided for adult feeding to increasing fecundity, five replicates were maintained for control and treatments. After 48h, the numbers of eggs (*H.armigera*) laid on treated and control leaves were recorded and the percentage of oviposition deterrence was calculated by using the methods of Williams *et al*., (1986).

Determination of lethal concentrations

Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of the test (target and non- target) organisms within the specified period of exposure, and it was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, LC_{50} and $LC_{90}$was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package 12.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

RESULTS

Results of the present study reflected spectrum of activity of selected plants extracts against the lepidopteran pest *Helicoverpa armigera* larvae and eggs. The toxicity of different solvent crude extract of *Tinospora crispa* and *Psidium guajava* were tested against larvae of *Helicoverpa armigera*. The data were recorded and statistical data ranging LC_{50}, LC_{90}, LCL, UCL, slope, Regression and *chi*-square value were calculated. Generally, as the concentration increases the rate of larval mortality are also increases. The LC_{50} value of benzene, diethyl ether, ethyl acetate and methanol leaf extracts of *Tinospora crispa* were 86.26, 89.55, 77.64 and 62.63ppm, respectively. *Psidium guajava* shows the LC_{50} values of 128.51, 132.64, 124.97 and 118.65ppm, respectively(table 1). Among two plant extracts tested, *Tinospora crispa* extracts were found to be most significant ovicidal activity 100% egg mortality (zero hatchability) observed at 300ppm and 375ppm for *Psidium guajava* (table 2).

Table 1 Larvicidal activity of *Tinospora crispa* and *Psidium guajava* leaf extract against larvae of *Helicoverpa armigera*

Solvent tested	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	Slope	Chi-square
		LCL	UCL			
Tinospora crispa						
Benzene	86.26	71.85	108.32	148.79	4.5601	11.206
Diethyl ether	89.55	74.61	112.94	153.72	4.1292	13.567
Ethyl acetate	77.64	61.32	96.88	142.61	3.9711	12.168
Methanol	62.63	52.75	89.64	136.85	4.2083	13.612
Psidium guajava						
Benzene	128.51	88.63	146.81	248.26	5.9014	14.964
Diethyl ether	132.64	93.54	152.33	256.81	4.6632	13.528
Ethyl acetate	124.97	76.66	132.69	227.32	4.2760	14.721
Methanol	118.65	69.25	125.28	206.94	3.1685	12.102

Each value mean± S.D represents mean of five values. Statistically significantly different at $P < 0.05$. LC_{50}; LC_{90}; LCL-Lower confidence limit; UCL-Upper confidence limit; Slope; Chi-square.

Table 2 Ovicidal activity of *Tinospora crispa* and *Psidium guajava* extracts against eggs of *Helicoverpa armigera*

Solvent tested	Percentage of egg hatch ability						
	Concentration (ppm)						
	Control	75	150	225	300	375	450
Tinospora crispa							
Benzene	100±.0.0	92.31±4.53	75.21±4.45	64.74±3.90	48.38±3.42	NH	NH
Diethyl ether	100±.0.0	85.41±4.26	66.67±3.48	48.96±3.51	33.42±3.37	NH	NH
Ethyl acetate	100±.0.0	77.30±3.18	54.13±3.74	44.33±3.79	29.40±3.72	NH	NH
Methanol	100±.0.0	51.43±3.71	30.50±2.66	22.97±3.75	NH	NH	NH
Psidium guajava							
Benzene	100±.0.0	95.61±4.49	82.70±4.22	66.25±3.18	54.53±3.97	31.50±3.29	NH
Diethyl ether	100±.0.0	85.53±4.91	73.54±3.60	59.36 ±3.57	42.16±3.80	28.33±3.85	NH
Ethyl acetate	100±.0.0	75.64±3.49	59.20±3.75	38.31 ±2.76	23.57±2.84	NH	NH
Methanol	100±.0.0	58.72±3.64	40.39±2.45	26.49±2.46	18.60±2.95	NH	NH

Values represent mean ± S.D. of five replications.
Eggs in control groups were sprayed with no phytochemicals.
NH - No hatchability (100% mortality)

Table 3 Oviposition deterrent activity of *Tinospora crispa* and *Psidium guajava* against the gravid moths of *Helicoverpa armiger*

Solvent tested	Concentrations tested (ppm), Oviposition deterrent activity %					
	40	80	120	160	200	240
			Tinospora crispa			
Benzene	10.45±2.78[b]	26.45±2.74[b]	53.43±3.45[b]	74.67±3.56[b]	86.19±4.58[b]	94.65±4.65[b]
Diethyl ether	15.67±2.41[c]	32.46±2.63[c]	56.18±4.86[c]	78.78±4.45[c]	90.78±4.49[c]	97.39±4.58[c]
Ethyl acetate	18.24±2.57[d]	34.23±2.89[d]	68.29±4.27[d]	81.46±3.78[d]	93.67±4.28[d]	100.00±0.00[d]
Methanol	22.56±2.60[e]	48.67±3.00[e]	75.65±3.51[e]	84.59±4.89[e]	100.00±0.00[e]	100.00±0.00[d]
Control	2.86±1.20[a]	2.86±1.20[a]	2.86±1.20[a]	2.86±1.20[a]	2.86±1.20[a]	2.86±1.20[a]
			Psidium guajava			
Benzene	9.61±2.39[b]	18.51±2.33[b]	48.60±2.34[b]	69.56±3.00[b]	82.55±3.25[b]	91.44±4.13[b]
Diethyl ether	12.50±2.47[c]	21.78±2.64[c]	51.85±3.46[c]	71.69±4.14[c]	86.65±3.80[c]	95.65±4.22[c]
Ethyl acetate	15.67±2.78[d]	27.12±2.15[d]	55.63±3.61[d]	74.52±4.25[d]	90.66±4.61[d]	100.00±0.00[d]
Methanol	20.85±2.80[e]	33.16±2.97[e]	61.55±3.57[e]	81.33±4.27[e]	92.46±4.65[e]	100.00±0.00[e]
Control	2.97±1.24[a]	2.97±1.24[a]	2.97±1.24[a]	2.97±1.24[a]	2.97±1.24[a]	2.97±1.24[a]

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at p<0.05. (MANOVA; LSD -Tukey's Test). Control groups were allowed to lay eggs on host plant sprayed with no phytochemicals.

Methanol extract of *Tinospora crispa* and *Psidium guajava* showed 100% oviposition deterrency against the gravid moths of *H.armigera* at 200 and 240ppm concentration (table 3). In general, the oviposition deterrency is directly proportional to the increase in the concentration and also among the various extracts tested, methanol extract was found to have significant activity than the other solvent extracts.Results of this study show that the selected Indian medicinal plants *Tinospora crispa* and *Psidium guajava* may be a potent source of natural larvicidal, ovicidal and oviposition deterrent activities against selected important agricultural lepidopteran field pest *H.armigera*.

DISCUSSION

In our results showed that, *Tinospora crispa* and *Psidium guajava* plants extracts have significant larvicidal and ovicidal activity against selected important agricultural lepidopteran field pest *H. armigera.* The results are comparable with an earlier report by Krishnappa *et al*, (2010a) they have been reported that *Tagetes patula* volatile oil contained 10 compounds and they were tested against the fourth instar larvae of *S. litura* for their antifeedant activity by leaf disc bioassay. Among the compounds tested Terpinolene was the most effective feeding deterrent agent against *S. litura.* Jeyasankar *et al*, (2010) who observed that high larval mortality indicates potential insecticidal properties present in the *Syzygium lineare* (*S. lineare*) plant extract and the isolated crystal compound. Jeyasankar *et al*, (2011) reported that a new crystal compound 2, 5-diacetoxy-2benzyl-4,4,6,6-tetramethyl-1,3-cyclohexanedione was isolated from the leaves of *S. lineare*. The insecticidal activity of the compound was assessed against fourth instar larvae of *S.litura*. Its activity was better than the positive control azadirachtin. The compound was responsible for growth inhibition on *S. litura*. It induced larval, pupal and adult deformities even at low concentration. Baskar *et al*, (2009) who observed pupicidal activity in different crude extract of *Atalantia monophylla* against *H. armigera*. Malarvannan *et al*, (2008) observed that *Argemone Mexicana* extracts reduced adult emergence and increased pupal mortality of *S. litura*.

Baskar *et al*, (2011) they have been reported that bioefficacy of leaf and root extracts of *Aristolochia tagala* against *S. litura* Effects on feeding, larvicidal and pupicidal activities and larval–pupal duration were studied. Higher antifeedant activity (56.06%), lethal concentration for feeding inhibition (3.69%), larvicidal (40.66%), pupicidal (28%), total mortality (68.66%) and prolonged larval–pupal duration (12.04–13.08 days) were observed in ethyl acetate leaf extract at 5.0% concentration. bioassay. Earlier, Anandan *et al*, (2011) reported that efficacy of ethyl acetate, methanol and aqueous extracts of *Acrous Calamus, Corchorus aestauaus, Cammelina bengalinsis, Emblica ficus religiosa* and *Lantena Camera* were tested at 1000 ppm for their antifeedant activity against fourth instar larvae of *H. armigera* using leaf disc (no-choice) method. The aqueous extract of *C. collinus* was found to have maximum antifeedant activity followed by *E. fisheri, F. religiosa* and *C. aestauaus.* Jayasankar *et al*, (2002) reported that mentha oil showed minimum ovicidal activity at 0.25% concentration 18.33 ± 3.15 and maximum ovicidal activity at highest concentration tested (2.0% - 28.99 ± 7.11). Ovicidal activity recorded from 0.50 and 1.0% were less significant (23.25 ± 4.66 and 24.74 ± 5.47 respectively). Neem oil showed maximum ovicidal activity at 2.0% concentration. Oil of *Origanum creticum* was significantly less toxic.

Pavela, (2005) who reported that twenty essential oils applied by fumigation were highly toxic to the third instar of *Spodoptera littoralis* larvae. Two essential oils *Nepeta cataria* and *Thuja occidentalis* were highly toxic with LC_{50}≤10.0 mL/m3 (5.5 and 6.5 mL/m3, respectively). Recently Duraipandiyan *et al*, (2011) they have been reported that larvicidal activities of rhein isolated from *Cassia fistula* flower against lepidopteron pests *S. litura* and *H. armigera* and the LC_{50} values was 606.50 ppm for *H. armigera* and 1192.55 ppm for *S. litura*. The survived larvae produced malformed aduts. Elumalai *et al*, (2010b) they have been reported that maximum ovicidal activity was found in *Ocimum basilicum* and *Zingiber officinale. S. litura* eggs were 100% of mortality (No hatchability) recorded in 300 ppm, respectively. Anandan *et al*, (2010) they have been reported that crude extracts of *Hyptis*

suaveolens and *Melochia chorcorifolia* against *S. litura*, four fractions obtained from *H. suaveolens*, fraction III was found to inhibit the feeding ratio of the *S. litura* and it is apparent from the table. While in *M. corchorifolia* only three fractions have been obtained, among them fraction II was found to induced more feeding deterrent activity at 2000 ppm concentration. Krishnappa *et al*, (2010c) reported that The *Clausena dentate* leaves essential oil against Armyworm, *S. litura* it produce significant larvicidal activity, with 24 hrs LC_{50} 111.54 ppm and LC_{90} 205.38 ppm, respectively. The major chemical compositions larvicidal activities were also tested. The LC_{50} and LC_{90} values of sabinene LC_{50} 21.42 ppm and LC_{90} 40.39 ppm, respectively. This was closely followed by biofloratriene LC_{50} 23.31 ppm and LC_{90} 43.62 ppm.

Earlier, Elumalai *et al*, (2010c) reported that the maximum larval mortality was found in the essential oil of *Zingiber officinales, Citrus limonum , Acorus calamus, Rosmarinus officinalis, Ocimum basilicum, Cuminum cyminum* and *Coriandrum sativum* tested againat armyworm, *S. litura* agricultural important lepidopteron pest with the LC_{50} values were 15, 34.55, 36.13, 38.2, 57.55, 63.99 and 65.07 ppm respectively. Baskar *et al*, (2010) reported that the ethyl acetate extract of *Couroupita guianensis* exhibited 69.7% against *H. armigera* at 5% concentration. The antifeedant activity was due to the presence of steroids, phenols, flavonoids and alkaloids in the ethyl acetate extract of leaf. Gokulakrishnan *et al*, (2012b) reported that the line of experiment was attempted with Plant Oil Formulation (POF), showed maximum percentage of ovipositional repellent activity against the gravid moths of *H. armigera* followed by *S. litura* and *E. vitella* were 84.75, 79.90 and 76.55 respectively. Gokulakrishnan *et al*, (2012a) reported that the most significant antifeedant activity was observed *Achaea janata* at 1000 ppm concentration *S. officinalis* (85.56) *S. litura, M. spicata* (82.85) *H. armigera* and *M. spicata* (90.55) *A. janata*. Thirugnanasampandan *et al*, (2008) reported these phytochemical compounds do not cause any harmful effects on human or environment since these phytochemicals have shown effective antioxidant and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potential. The present investigation lead the path of exploration of *Tinospora crispa* and *Psidium guajava* for eradication of selected important agricultural field pest, thereby, gaining a real momentum to include this plant product for intense integrated pest control programme.

CONCLUSION

An attempt has been made to evaluate the role of *Tinospora crispa* and *Psidium guajava* different extracts for their larvicidal and ovicidal bioassay against *Helicoverpa armigera*. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal, ovicidal and oviposition deterrent properties of natural product extracts as a potential agent for combating agricultural field pest.

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