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RESEARCH ARTICLE

BOTANICAL EXTRACTS OF *TINOSPORA CRISPA* (MENISPERMACEAE) AND *PSIDIUM GUAJAVA* (MYRTACEAE) AGAINST IMPORTANT AGRICULTURAL POLYPHAGOUS FIELD PEST ARMYWORM, *SPODOPTERA LITURA* (FAB.) (LEPIDOPTERA:NOCTUIDAE)

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ABSTRACT

The development of integrated pest control programs in controlling the economically important pest, *Spodoptera litura* (Fab.) has gained increased attention in many parts of the world. The objective of the present study was to evaluate Antifeedant, larvicidal and ovicidal activities of benzene, diethyl ether, ethyl acetate and methanol leaf extract of *Tinospora crispa* and *Psidium guajava* against *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae),Antifeedant activities of the selected plant extract were studied using leaf disc no-choice method as described by Isman *et al.* (1990), with slight modifications. Five replicates were maintained for each concentration and the progressive consumption of leaf area by the larvae after 24h was recorded in control and treated discs using leaf area. Twenty five early fourth instar larvae of *S. litura* was exposed to various concentrations and was assayed in the laboratory by using the protocol of Abbott's formula (1925); the 24h LC_{50} values of the *Tinospora crispa* and *Psidium guajava* leaf extracts were determined by probit analysis. The ovicidal activity was determined against *S. litura* to various concentrations were tested under laboratory conditions and the hatch rates were assessed 120 h post treatment. The antifeedant activity of *Tinospora crispa* and *Psidium guajava* tested against *S. litura* fourth instar larvae was determined during a 24 hours test period. All extracts are showed moderate antifeedant activitiy; however, very least antifeedant activity was noted in benzene and significant antifeedant activity was observed in methanol extract.Methanol extract of *Tinospora crispa* and *Psidium guajava* showed 100% and 98.38% feeding deterrency against the fourth instar larvae of *S. litura* at 500ppmconcentration. The LC_{50} value of benzene, diethyl ether, ethyl acetate and methanol leaf extracts of *Tinospora crispa* were 92.64, 96.25, 94.67 and 84.94ppm, respectively and *Psidium guajava* shows the LC_{50} values of 144.95, 164.22, 135.64 and 121.86ppm, respectively.The chi-square values are significant at $p \leq 0.05$ level. Among five solvent extracts, the methanol extract was responsible for strong lethal activity observed against selected pest species. Among two plant solvents tested, *Tinospora crispa* extracts were found to be most significant ovicidal activity 100% egg mortality (zero hatchability) observed at 160ppm and 200 ppm for *Psidium guajava*. From the results it can be concluded the crude extract of *Tinospora crispa* and *Psidium guajava* were an excellent potential for controlling agricultural pest *Spodoptera litura*.

INTRODUCTION

Spodoptera litura (Fab.) is highly polyphagous pest of economically important crops throughout tropical and subtropical Asia. In India, it feeds on 180 species of cultivated crops and few wild plants, which includes various economically important crops such as cotton, groundnut, chilly, tobacco, caster, bendy and pulses etc. (Niranjankumar and Regupathy, 2001; Elumalai *et al*, 2007; Krishnappa *et al*, 2010a). It is a strong flier and disperses long distances annually during the summer months. It is one of the most economically important insect pests of 51 counties including India, Japan, China, and other countries of Southeast Asia. Management of this pest using synthetic chemicals has failed due to the development of resistance against many insecticides (Raman *et al*, 2007; Kodandaram and Dhingra, 2007; Mushtaq Ahmad *et al*, 2007; Ranga Roa *et al*, 2008). The development of integrated pest control programs in controlling the economically important pest, *Spodoptera litura* has gained increased attention in many parts of the world (Elumalai *et al*, 2010b; Krishnappa *et al*, 2010b; Anandan *et al*, 2010). *S. litura* is highly polyphagous pest, and this is reflected in the wide taxonomic range of wild and cultivated plants acceptable for

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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*, 2010c).Gokulakrishnan *et al*, 2012a, and Baskaran *et al*, 2012a, reported that crude plant extracts have insecticidal activity against *S. litura* larvae. Recent studies are suggested that plant extracts can be used for the Integrated Pest Management. Plant and insects have co-evolved over millions of year, plant have accumulated specific secondary metabolites to counteract insect damage (Kanniyan, 2002). Insecticides of plant origin have been exploited from time immemorial in many Asian and African countries for the management of insect pests of crop plants and stored products. Especially, in India and china, experiences documented on the walls of tombs explicit the pesticidal value of many such plants (Karl Maramorsch, 1991; Krishnappa *et al*, 2011a). Though insecticides of plant origin exerted coherent management over insect pests for centuries, invention of synthetic organic insecticides in the latter half of twentieth century suddenly replaced botanicals from insect management scenario.

Chemical pesticides play a significant role in increasing agricultural production by controlling the insect pests. However, the chronic effects of chemical pesticides on living organism and the environment prompt us to restrict the use of many pesticides (Elumalai *et al*, 2010a; Krishnappa *et al*, 2011b; Anandan *et al*, 2011; Isman *et al*, 1990; Katyal and Satake, 1996). Moreover, synthetic Insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, form workers, consumer, fish, birds and other domestic and wildlife animals etc. (Wattanachari and Tintanon, 1999; National Research council, 2000; Rohani *et al*, 2001). The threats posed by chemical pesticides demand an urgent search for an environmentally safer alternative method of crop protection. Also the injudicious use of synthetic pesticides can lead to secondary out breaks of pests that are normally under natural control. There have also been cases of pests becoming tolerant to insecticides, resulting in the use of double and triple application rates (Elumalai *et al*, 2010b; Gokulakrishnan *et al*, 2012b). In addition, problems such as health hazards, undesirable side effects and environmental pollution are caused by the continuous use of synthetic chemical pesticides. The broad spectrum action of many synthetic pesticides may also cause adverse environmental effects by harming beneficial organism such as natural enemies and pollinators. These hazards associated with the use of synthetic insecticides have renewed interest in the application of botanical pesticides for crop protection (Nas, 2004). Attention is being paid to tap plant sources, which have evolved astonishingly diverse array of chemical constituents. The use of locally available plants in the control of pests is an ancient technology in many parts of the world (Roy *et al*, 2005). Mean while, the concept of pest management was radically revised towards advocating suppression of pest populations below levels capable of causing economic injury rather than total eradication. Therefore, reliance on synthetic insecticides alone was left behind and many alternative strategies were introduced in the pest management. Among them, botanicals offer ample scope because of their considerable effect on target pests and relative safety to non target organisms. However, large scale utilization of botanicals in pest management is obstructed by non-availability of formulations. From a scientific angle, it is not difficult to explain why plants should represent themselves as valuable sources of such phytochemicals. Having been subjected to constant attack by phytophagous insects, plants developed in defense, a wide array of phyto-chemicals which at higher quantities ensure continuity of plant species by preventing herbivore attack (Ramachandran and Subramanian, 1993). Most of the botanical pesticides are non selective poisons that target a broad range of pests (Leatemia and Isman, 2004; Gokulakrishnan *et al*, 2012b; Baskaran *et al*, 2012b, c, d). The present work is an attempt to investigate the antifeedant, larvicidal and ovicdal activity of *Tinospora crispa* and *Psidium guajava* plants extract against larvae and eggs of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae).

MATERIALS AND METHODS

Plant material

The selected plants leaves were collected during growing season month of January-2014 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 (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with benzene, diethyl ether, ethyl acetate 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

Armyworm, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) collected from nearby fields formed the initial source for continuous, disease free culture. The insect culture was maintained on castor (*Ricinus communis*) leaves under standard conditions of temperature (27±2°C) and relative humidity (70±5%) throughout the period of study.

Antifeedant activity

Antifeedant activities of the selected plants extract were studied using leaf disc no-choice method as described by Isman *et al*., 1990 with slight modifications. Fresh castor leaf discs (for *S. litura*) of 3cm diameter were used for the experiments. Selected plant extracts prepared different concentrations *viz*., 100-

500ppm were treated individually on the fresh leaf discs. One treatment with acetone alone was used as positive control and one treatment without solvent was considered as negative control (0 ppm). In each Petri discs (1.5 cm x 9cm) wet filler paper was placed to avoid early drying of the leaf disc single fourth instar larva of *S.litura* was introduced individually. Five replicates were maintained for each concentration and the progressive consumption of leaf area by the larvae after 24h was recorded in control and treated discs using leaf area.

Larvicidal assay

For the evaluation of larvicidal activity, the selected plants extract tested is based on the wide range and narrow range tests, it was tested at 60-300ppm 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 assay

For ovicidal activity, scales from the egg masses of *S. litura* 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 40-240ppm 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.

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 *Spodoptera litura* larvae and eggs. The toxicity of different extracts of *Tinospora crispa* and *Psidium guajava* were tested against *Spodoptera litura.* The antifeedant activity of *Tinospora crispa* and *Psidium guajava* tested against *S. litura* fourth instar larvae was determined during a 24 hours test period. All extracts are showed moderate antifeedant activitiy; however, very least antifeedant activity was noted in benzene and significant antifeedant activity was observed in methanol extract. Methanol extract of *Tinospora crispa* and *Psidium guajava* showed 100% and 98.38% feeding deterrency against the fourth instar larvae of *S. litura* at 500ppmconcentration (table 1). The LC_{50} value of benzene, diethyl ether, ethyl acetate and methanol leaf extracts of *Tinospora crispa* were 92.64, 96.25, 94.67 and 84.94ppm, respectively and *Psidium guajava* shows the LC_{50} values of 144.95, 164.22, 135.64 and 121.86ppm, respectively.The chi-square values are significant at $p \leq 0.05$ level. Among five solvent extracts, the methanol extract was responsible for strong lethal activity observed against selected pest species (table 2). Among two plant solvents tested, *Tinospora crispa* extracts were found to be most significant ovicidal activity 100% egg mortality (zero hatchability) observed at 160ppm and 200 ppm for *Psidium guajava* (table 3). These results suggest that selected plants extracts have the potential to be used as an ideal eco-friendly approach for the control of important agricultural pest *Spodoptera litura*.

DISCUSSION

In our results showed that, the selected medicinal plants extracts tested against fourth instar larvae and 0-6 hour's old eggs of *Spodoptera litura*. Larvicidal activity mainly depends on the presence of toxic materials present in plants extracts. Also the mortality may be due to reduction in the total protein content which is a major component for the metamorphosis of the larval instars, this was clear that the dead larvae showed the symptoms of improper metamorphosis from one instar to another instar. This result is also coinciding with the findings of Krishnayya and Rao (1995), who had reported that the application of Plumbagin greatly reduced the protein concentration of *H. armigera*. Earlier, Elumalai *et al*, (2010a) reported that the plant essential oils are currently studied more and more because of the possibility of their use in plant protection. Biological activities of 10 essential oils were studied using fourth instar larvae of armyworm, *S. litura.* During preliminary screening, the extracts were tested at 1,000 ppm concentration. All Essential oil are showed moderate larvicidal effects; however, the highest larval mortality was found in the essential oil of *Zingiber officinales, Citrus limonum, Acorus calamus, Rosmarinus officinalis, Ocimum basilicum, Cuminum cyminum* and *Coriandrum sativum* with LC_{50} values were15, 34.55, 36.13, 38.2, 57.55, 63.99 and 65.07 ppm respectively. 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. 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 *Spodoptera litura* in the laboratory condition. Pavela, (2005) reported that twenty essential oils applied by fumigation were highly toxic to the third instar of *S. littoralis* larvae. Two essential oils *Nepeta cataria* and *Thuja occidentalis* were highly toxic with $LC_{50} \leq 10.0$ ml/m^3 (5.5 and 6.5 mL/m3, respectively). Five essential oils *Salvia sclarea, Thymus mastichina, Origanum majorana, Pogostemon cablin* and

Mentha pulegium were toxic with LC_{50} between 10.1 and 20.0 ml/m^3 (11.9, 19.3, 19.6, 14.8 and 11.5 ml/m^3, respectively). 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 adults.

concentration. Krishnappa *et al*, (2010b) 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. LC_{50} and LC_{90} values of sabinene 21.42 ppm and 40.39 ppm, respectively.

Table 1 Antifeedant activity of *Tinospora crispa and Psidium guajava* extracts against the larvae of *Spodoptera litura*

Solvent tested	Concentrations tested (ppm), Antifeedant activity %				
	100	200	300	400	500
			Tinospora crispa		
Benzene	15.22±1.35[b]	26.27±1.73[b]	43.38±2.67[b]	74.22±2.46[b]	88.78±3.81[b]
Diethyl ether	21.82±1.47[c]	34.66±1.95[c]	56.62±3.59[c]	76.22±3.68[c]	91.57±4.74[c]
Ethyl acetate	24.34±2.79[d]	38.55±2.27[d]	62.93±3.70[d]	79.53±2.53[d]	94.60±4.55[d]
Methanol	28.44±2.22[e]	42.47±2.55[e]	76.45±2.73[e]	83.00±3.76[e]	100.00±0.00[e]
Control	2.26±1.30[a]	2.26±1.30[a]	2.26±1.30[a]	2.26±1.30[a]	2.26±1.30[a]
			Psidium guajava		
Benzene	13.25±1.14[b]	26.37±1.22[b]	40.26±2.77[b]	68.67±3.76[b]	90.26±3.29[b]
Diethyl ether	15.87±1.76[c]	28.48±1.24[c]	42.30±3.68[c]	70.54±2.67[c]	93.40±3.25[c]
Ethyl acetate	18.55±2.34[d]	31.00±2.26[d]	52.34±2.29[d]	75.55±3.00[d]	96.74±4.44[d]
Methanol	21.33±2.37[e]	34.06±2.34[e]	57.56±3.40[e]	81.64±3.14[e]	98.38±4.87[e]
Control	2.84±1.52[a]	2.84±1.52[a]	2.84±1.52[a]	2.84±1.52[a]	2.84±1.52[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 fed with tender host leaf disc with no phytochemicals.

Table 2 Larvicidal activity of *Tinospora crispa and Psidium guajava* leaf extracts against *Spodoptera litura*

Solvent tested	LC_{50} (mg/L)	95%Confidence Limits(mg/L)		LC_{90} (mg/L)	Slope	Chi-square*
		LCL	UCL			
			Tinospora crispa			
Benzene	92.64	75.23	106.29	166.48	3.1064	10.460
Diethyl ether	96.25	79.15	112.67	174.46	3.2546	12.304
Ethyl acetate	94.67	75.66	108.64	168.93	4. 0321	12.921
Methanol	84.94	68.14	95.57	135.46	4.2063	13.916
			Psidium guajava			
Benzene	144.95	92.57	163.94	245.32	3.6024	10.619
Diethyl ether	164.22	98.51	167.26	259.53	5. 0438	15.537
Ethyl acetate	135.64	95.13	154.37	248.61	4.7506	14.310
Methanol	121.86	82.34	134.46	237.92	3.5109	11.124

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 3 Ovicidal activity of *Tinospora crispa* and *Psidium guajava* extracts against *Spodoptera litura*

Name of the solvent	Percentage of egg hatch ability						
	Concentration (ppm)						
	Control	40	80	120	160	200	240
			Tinospora crispa				
Benzene	100±0.0	67.56±3.47	37.12±3.94	21.34±2.52	18.76±2.42	NH	NH
Diethyl ether	100±0.0	62.85±4.26	34.25±3.15	19.66±2.45	21.34±3.52	NH	NH
Ethyl acetate	100±0.0	59.66±3.98	28.81±2.64	17.20±2.91	21.34±3.52	NH	NH
Methanol	100±0.0	45.97±3.64	20.66±2.31	12.82±2.49	NH	NH	NH
			Psidium guajava				
Benzene	100±0.0	81.43±4.37	73.51±4.64	46.66±3.55	27.18±1.6	NH	NH
Diethyl ether	100±0.0	79.62±4.64	67.94±3.51	40.51±3.64	22.46±1.3	NH	NH
Ethyl acetate	100±0.0	68.14±3.76	48.30±3.40	31.27±2.61	19.65±1.5	NH	NH
Methanol	100±0.0	51.42±3.61	32.86±3.19	24.65±2.94	16.45±2.73	NH	NH

Each value mean± S.D represents the mean of six values.
Eggs in control groups were sprayed with no phytochemicals.
NH - No hatchability (100% mortality)

Anandan *et al*, (2010) they have been reported that crude extracts of *H. suaveolens* and *M. corchorifolia* 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

This was closely followed by biofloratriene LC_{50} 23.31 ppm and LC_{90} 43.62 ppm. Isman *et al*, (2001) have been reported that three of the essential oils were highly toxic to the cutworm *S. litura*: oils of *Satureia hortensis*, *Thymus serpyllum* and *Origanum creticum.* Oil of *Mentha arvensis* was the only other oil producing at least 50% mortality. An attempt has been made to evaluate the role of medicinal plants essential oils for their

larvicidal bioassay against *S. litura*. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal properties of natural product oils.

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. The extracts might inhibit the quantum of neurosecretory protein produced in the *Corpus cardiacum*. The reduction in the body protein content of treated insects might have been due to inhibition of further synthesis or protein degradation due to the stress. Ayyangar and Rao, (1990) have showed that the azadiractin injected into the final instar larvae of *S. litura* (µg/g of body weight) significantly reduced the protein content and had no effect on the electrophoretic pattern of haemolymph proteins and esterase. Reduction in the body protein content of *S. litura* was reported when the insects were treated with juvenoids like methoprene (Sundaramurthy *et al*, 1978; Kranthi, 1991), extracts of *Argemone mexicane*, *Nerium odorum* (1-5%) (Tirupati, 2003) and 25 aza- cholesterol (Suryakala, 1998). An attempt has been made to evaluate the role of medicinal plants extracts for their larvicidal and ovicidal bioassay against *Spodoptera litura*. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal and ovicidal properties of natural phyto-pesticides.

CONCLUSION

Tinospora crispa and *Psidium guajava* offers promise as potential bio control agent against *Spodoptera litura* particularly in its markedly antifeedant, larvicidal and ovicidal effect. The selected extracts could be used in laboratory as well as agricultural fields for the control of lepidopteran pests. However, further studies on the identification of the active principals involved and their mode of action and field trials are needed to recommend *Tinospora crispa* and *Psidium guajava* as an insecticidal product used to combat and protect from pests in a pest Control Program.

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