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 oxicidal 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 LC95 values of the *Tinospora crispa* and *Psidium guajava* leaf extracts were determined by probit analysis. The oxicidal 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* was assessed against *S. litura* fourth instar larvae was determined during a 24 hours test period. All extracts are showed moderate antifeedant activity; 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 500ppm concentration. The LC50 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 LC50 values of 144.95, 164.22, 135.64 and 121.86ppm, respectively. The chi-square values are significant at p ≤ 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 oxicidal 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*.

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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. (Niranjanakumar 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 countries 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.
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 years. 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 ovicidal 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, Nagapattinam 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°C.

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 (LC₅₀) 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, LC₅₀ and LC₉₀ 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 activity; 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 detergency against the fourth instar larvae of S. litura at 500ppm concentration (table 1). The LC₅₀ 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 LC₅₀ values of 144.95, 164.22, 135.64 and 121.86ppm, respectively. The chi-square values are significant at p<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 officinale, Citrus limonum, Acorus calamus, Rosmarinus officinalis, Ocimum basilicum, Cumminium cymimun and Coriandrum sativum with LC₅₀ values were 15, 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 LC₅₀ ≤10.0 ml/m³ (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 LC50 between 10.1 and 20.0 ml/m3 (11.9, 19.3, 19.6, 14.8 and 11.5 ml/m3, respectively). Duraiyappan 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 LC50 values were 606.50 ppm for H. armigera and 1192.55 ppm for S. litura. The survived larvae produced malformed adults.

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

<table>
<thead>
<tr>
<th>Solvent Tested</th>
<th>Concentration (ppm)</th>
<th>Antifeedant Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Tinospora crispa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>15.22±3.58</td>
<td>26.27±1.73</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>21.82±1.47</td>
<td>34.66±1.95</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>24.64±2.79</td>
<td>38.55±2.27</td>
</tr>
<tr>
<td>Methanol</td>
<td>28.44±2.22</td>
<td>42.47±2.55</td>
</tr>
<tr>
<td>Control</td>
<td>2.26±1.30</td>
<td>2.26±1.30</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>13.25±1.46</td>
<td>26.37±1.22</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>15.87±1.76</td>
<td>28.48±1.24</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>18.55±2.34</td>
<td>31.00±2.26</td>
</tr>
<tr>
<td>Methanol</td>
<td>21.33±2.37</td>
<td>34.06±2.34</td>
</tr>
<tr>
<td>Control</td>
<td>2.84±1.52</td>
<td>2.84±1.52</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Solvent tested</th>
<th>LC50 (mg/L)</th>
<th>95% Confidence Limits (mg/L)</th>
<th>LC50 (mg/L)</th>
<th>95% Confidence Limits (mg/L)</th>
<th>Slope</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LCL</td>
<td>UCL</td>
<td>LCL</td>
<td>UCL</td>
<td></td>
</tr>
<tr>
<td>Tinospora crispa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>92.64</td>
<td>75.23</td>
<td>166.48</td>
<td>3.1064</td>
<td>10.460</td>
<td></td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>96.25</td>
<td>79.15</td>
<td>174.46</td>
<td>3.2546</td>
<td>12.304</td>
<td></td>
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<tr>
<td>Ethyl acetate</td>
<td>94.67</td>
<td>75.66</td>
<td>168.93</td>
<td>4.0321</td>
<td>12.921</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>84.94</td>
<td>68.14</td>
<td>135.46</td>
<td>4.2063</td>
<td>13.916</td>
<td></td>
</tr>
<tr>
<td>Psidium guajava</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>144.95</td>
<td>92.57</td>
<td>245.32</td>
<td>3.6024</td>
<td>10.619</td>
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<tr>
<td>Diethyl ether</td>
<td>164.22</td>
<td>98.51</td>
<td>259.53</td>
<td>5.0438</td>
<td>15.537</td>
<td></td>
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<tr>
<td>Ethyl acetate</td>
<td>135.64</td>
<td>95.13</td>
<td>248.61</td>
<td>4.7506</td>
<td>14.310</td>
<td></td>
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<tr>
<td>Methanol</td>
<td>121.86</td>
<td>82.34</td>
<td>237.92</td>
<td>3.5109</td>
<td>11.124</td>
<td></td>
</tr>
</tbody>
</table>

Each value mean±S.D represents mean of five values. Statistically significantly different at P < 0.05. LC50; LC90; 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

<table>
<thead>
<tr>
<th>Name of the solvent</th>
<th>Concentration (ppm)</th>
<th>Percentage of egg hatching ability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Tinospora crispa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>100±0.0</td>
<td>67.5±6.37</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>100±0.0</td>
<td>62.85±4.26</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>100±0.0</td>
<td>59.66±3.98</td>
</tr>
<tr>
<td>Methanol</td>
<td>100±0.0</td>
<td>45.79±3.64</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>100±0.0</td>
<td>81.43±4.37</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>100±0.0</td>
<td>76.92±4.64</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>100±0.0</td>
<td>68.14±3.76</td>
</tr>
<tr>
<td>Methanol</td>
<td>100±0.0</td>
<td>51.42±3.61</td>
</tr>
</tbody>
</table>

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 concentration. Krishnappa et al. (2010b) reported that The Clausen dentate leaves essential oil against armyworm, S. litura it produce significant larvicidal activity, with 24 hrs LC50 111.54 ppm and LC90 205.38 ppm, respectively. The major chemical compositions larvicidal activities were also tested. LC50 and LC90 values of sabine 21.42 ppm and 40.39 ppm, respectively.

This was closely followed by biofloratriene LC50 23.31 ppm and LC90 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 cardiaum. 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 azadirachtin 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 odoratum (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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