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Efficacy of *Psidium guajava* (L.) Leaves Extract Against *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) Larvae

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ABSTRACT

Purpose: *Fall armyworm (FAW) Spodoptera frugiperda J.E Smith is an invasive pest of maize crops that has been recently reported in Pakistan (2019). Synthetic insecticides are the main choice of local farmers to control this deadly pest, but with continuous use of these pesticides health and environmental issues have arisen, which demands an alternative control measure. Considering this pest's significance, this study was conducted to determine the potentiality of Guava (Psidium guajava L.) against the third instar larvae of S. frugiperda; which is considered an effective repellent effect.*

Research Method: *The P. guajava leaves were extracted with methanol, ethyl acetate, n-hexane, and chloroform; each extract was prepared at different concentrations of 125, 250, 500, and 1000 ppm. Later, the larval mortality was recorded at different intervals of 24, 48, 72, and 96 hrs, and antifeedant activity at 24 hrs.*

Findings: *The highest mortality of 92.86% was observed on methanol extract at 1000ppm after 96 hrs. The mortality on all crude extracts and at every concentration increased at different intervals of the observation and reached the maximum at 96 hrs. However, the LC_{50} and LC_{90} values of 154.77 and 865.3ppm after 96 hrs were recorded in methanol. Similarly, the highest antifeedant activity was 87.5% recorded in methanol at 1000ppm, and the lowest antifeedant activity of 22.58% was noted in chloroform extracts at 125ppm concentration. It showed that the antifeedant activity relied on a concentrated dose; meanwhile, the P. guajava with methanol crude extracts successfully stopped the feeding activity of S. frugiperda.*

Originality/ Value: *Therefore, based on the obtained results, the P. guajava leaf extracts are highly recommended, particularly for their extraction with methanol and n-hexane which showed the highest larval mortality and antifeedant activity of S. frugiperda.*

Keywords: *Antifeedant activity, Larvicidal activity, Psidium guajava, Spodoptera frugiperda*

INTRODUCTION

Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is an invasive pest of maize crop in Sindh, Pakistan (Bhatti *et al.*, 2020). Although the pest is native to American regions of tropical and subtropical it has spread to several countries in Asia and Africa (Prasanna *et al.*, 2018). It is a polyphagous insect pest affecting more than 353 host plant species from 76 plant families (particularly Fabaceae, Asteraceae, and Poaceae) with maize being its prime host plant (Montezano *et al.*, 2018). Fall armyworm is a heavy foliage feeder that can cause 100% losses of cereal crops (Sisay *et al.*, 2019b). It prefers all growth stages of corn but usually in the young plant whorls for up to 45 days.

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The young larvae generally consume a large amount of foliage and sometimes they damage the growth point of the plant (Goergen *et al.*, 2016; Abrahams e*et al.*., 2017) which is more critical to overcoming. Older larvae can turn into cutworms because of their strong ability to cut edges, which enables them to feed on solid parts of the crop (Goergen *et al.*., 2016). Presently, FAW management mainly relies on synthetic insecticides which has resulted in widespread insect resistance to most insecticides and presented adverse effects on human health, wildlife, and beneficial insects all around the world (Kumar *et al.*, 2021).

Besides, the research has shown several types of pesticidal plant species, ensuring that their use can give better results to control insect pests including FAW without polluting the environment (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017). For pest management, secondary metabolites have not only been the best alternatives to compete with synthetic agrochemicals in terms of plant growth and protection but are generally seen as target-attacking, eco-friendly, biodegradable, and cost-effective (Mrid *et al.*, 2021). Because their best strengths are their specificity against the targeted pests and have been found non-toxic and non-pathogenic to humans and animals (Miresmailli and Isman, 2014; Stevenson *et al.*, 2017). These secondary metabolites include cyanogenic, glucosides, alkaloids, terpenes, quinones, phenolics, amines, glucosinolates, peptides, and polyacetylenes (Jamwal *et al.*, 2018) which are naturally synthesized by plants and are non-nutrient compounds derived from various plant's parts and act as a defensive shield to protect themselves from phytophagous herbivores (Khare *et al.*, 2020) either in term of antifeedant or produce mortality when directly come in contact with insects (Chauhan and Mishra, 2016; Aliyu *et al.*, 2016; Chauhan and Mishra, 2016).

These secondary metabolites are likely to avoid or defer the development of insect resistance (War *et al.*, 2012) and in response, herbivores have been noticed to act in distinct adaptive responses, including detoxification, excretion, or sequestration of plant secondary metabolites (War *et al.*, 2020). Adverse effects on the survival of the peach potato aphid *Myzus persicae* Sulzer and *Heliothis virescens* F were reported after jasmoic acid and salicylic acid application (Schweiger *et al.*, 2014) both are efficient phytohormones. Furthermore, species, like Jabuticaba (Brazilian grape tree), *Myrciaria cauliflora* M. (Alves *et al.*, 2014), Neem (*Azadirachta indica* A. Juss), Boldo (*Peumus boldus* M.) (Silva *et al.*, 2015) and Mexican poppy (*Argemone ochroleuca* S.) (Martinez *et al.*, 2017) have been addressed with good insecticidal properties against FAW. The secondary metabolites of these plants cause different physiological changes and produce various biochemical effects (i.e. nervous breakdown, antifeedant, stomach poison, and respiratory blockage) on insects more particularly against Noctuidae and Pyralidae insect families (Simmonds and Blaney, 1996; Mordue, 2004; Sengottayan, 2013). Guava, *Psidium guajava* (L.) belongs to the family Myrtaceae and the local name is Amrood, which is considered to have originated in tropical South America. It is grown in subtropical and tropical areas of the world like Asia, Egypt, Hawaii, Palestine, Florida, and other countries (Mani *et al.*, 2011). *P. guajava* contains active compounds like flavonoids, saponins, tannins, alkaloids, glycosides, and oxalates. Flavonoids are commonly spread in plants and perform many functions to grow in flowers, leaves, and seeds and protect from insect and microbial attacks; while saponins are known to have antimicrobial activities, prevent molds, and protect plants from insect attacks (Khanna and Kannabiran, 2007). The availability of the plant and its constituents make it a reliable substitute for synthetic insecticides if found to be effective against *S. frugiperda*. Therefore, the goal of the present study was to examine the antifeedant, larvicidal activities, and defoliation of leaf extracts of *P. guajava* against the third larval instar of *S. frugiperda*.

MATERIALS AND METHODS

Experimental Site

All the experiments were conducted at the Insect Molecular Laboratory, Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tando Jam in 2021.

Insect Collection and Rearing

The larvae were collected from the maize fields in the surrounding of Tando Jam (25° 25' 37.85" N: 68° 32' 10.28" E). The FAW larvae were reared on fresh and pesticide-free maize leaves and adults were reared on an artificial diet solution of 20% honey and 80% water at laboratory conditions of 27±2 ^{o}C with 60-70% relative humidity.

Experimental Design

The layout of the experiment was a Completely Randomized Design (CRD) with five treatments and each treatment was replicated three times. The extracts of P. *guajava* were treatments used in this experiment such as T1- *P. guajava* leaves extract with methanol, T2- *P. guajava* leaves extract with ethyl acetate, T3- *P.*

guajava leaves extract with n-Hexane, T4- *P. guajava* leaves extract with chloroform and T5- Control (treated leaves with distilled water).

Preparation of Extracts

P. *guajava* leaves were collected from a home garden in Tando Jam, Pakistan, and brought to the laboratory. The leaves were washed with tap water, shade-dried, and homogenized to a fine powder with an electronic grinder. Later, different samples of 1mg of fined ground leaves powder were soaked in 10 mL of methanol, ethyl acetate, n-hexane, and chloroform separately. After seven days, the solution was filtered with fine mesh, and the filtrate was dried by a rotary evaporator; later the crude extracts were stored at 4ºC until used (Figure 01).

Larvicidal Activity

FAW larval mortality was conducted using the no-choice leaf disc method. The crude extracts of P. *guajava*leaves at different concentrations of 125ppm (0.019g), 250ppm (0.038g), 500ppm (0.075g) and 1000ppm (0.15g) were prepared. All pre-starved (12 hrs) the third instar larvae individually were transferred into each plastic cup (2.8 x 5.15cm^2). A wet filter paper was placed inside each plastic cup to avoid early drying of leaves and then FAW larvae were fed on treated maize leaves (leaf discs). After 24 hrs of treatment, the larvae were shifted from plastic cups and placed in a Petri dish having fresh and untreated maize leaves. Each treatment contained three replications with five larvae per replication (n=15). Next, the larval corrected mortality was recorded at different intervals (24, 48, 72, and 96 hrs) using Abbott's (1925) formula.

$$\text{Corrected mortality (\%)} = \text{(1-treatment)} \times 100 \quad (1)$$

Where: n = Insect population, T = treated, Co = control

Antifeedant Activity

Antifeedant bioassay was carried out using a no-choice leaf disc method. A similar concentration of crude extracts of P. *guajava* leaves as in larvicidal activity was used for this experiment and dissolved in 150 mL of distilled water. Fresh maize leaf discs of 4 cm diameter were dipped in each crude extract and concentration, however, the other procedure remained the same as performed in larvicidal activity. Subsequently, after 24 hrs of treatment, the uneaten area of the leaf disc was measured by using the Leaf area meter and compared with the eaten area by FAW larvae in both the treated and control leaves. The area of leaf eaten by larvae in treatment was corrected from the control that shows the antifeedant activity and was calculated through the formula given by Bentley *et al.* (1984).

$$\text{Antifeedant activity} = \text{treatment leaf} \times 100 \quad (2)$$

Defoliation (%) of Maize Leaf

The defoliation experiment was conducted by using the no-choice leaf discs method as shown in (Figure 02), meanwhile, the other experimental design remained the same. The consumed leaf area (cm^2) of treated and control leaves was recorded by using the Bio leaf mobile application (Machado *et al.* 2016) after 24 hrs and compared.

Statistical Analysis

The data were analyzed through ANOVA using 8.1 Statistix software and the significant differences between the means of treatments were compared by LSD at $P \leq 0.05$. However, for bioassay, LC_{50} and LC_{90} probability response tests were used by using Polo Plus software.

RESULTS AND DISCUSSION

Larval Mortality

The results in Table 01 show that all the crude extracts were effective against FAW. Among four crude extracts, the highest mortality was found in methanol. Mortality increased as concentration was increased and found maximum at 1000ppm in all crude extracts.

In time intervals, the effect of all crude extracts against larvae was found to slow during the 24 hrs, but it increased with time and reached a maximum of 96 hrs of the experimental period. The best results were with methanol at 1000ppm during 96 hrs with the highest mortality of 92.86%. However, with methanol and n-hexane, the extracted crude helped to kill 50% larval population within 48 hrs at 500 ppm. These trends showed that the mortality was dose-dependent, but similarly, it showed the efficacy of methanol was for a shorter period. The bioassay results are given in Table 02 related to LC_{50} and LC_{90} of extracts against FAW larvae.

It showed to achieve 50% mortality, a minimum dose of 154.77 ppm with a maximum exposure of 96 hrs was required for methanol, and similarly 90% mortality with a dose of 865.3 ppm with a slope of 1.71±0.57;

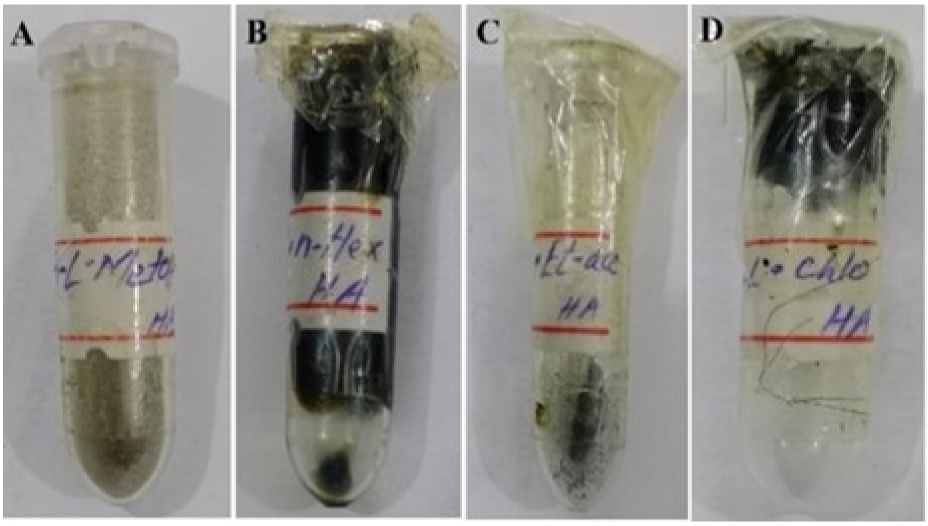

Figure 1: Showing different leaf extracts of *P. guajava* A methanol, B n-hexane, C ethyl acetate, and D chloroform.

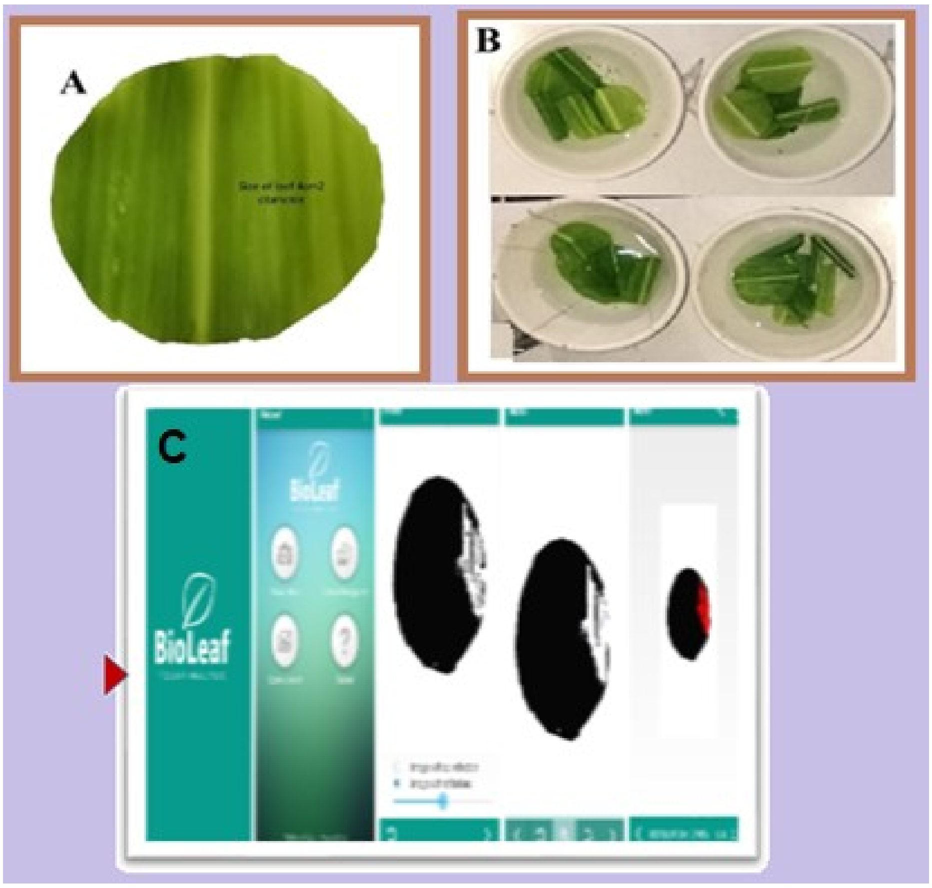

Figure 2: Picture showing A, Healthy maize leaves cut in diameter according to experimental selected plastic bowl B, Leaf Dipping method in different extracts C, Used software for experimentation.

x^2=0.21 during a similar time. We also recorded better results on n-Hexane after methanol, particularly with maximum exposure of 96 hrs which was achieved with a dose of 197.12 ppm (LC_{50}) and 1360.8 ppm (LC_{90}) with slope 1.53±0.54; x^2=0.08.

Antifeedant Activity

The antifeedant activity of *P. guajava* leaf extracts at different concentrations against the third larval instar of *S. frugiperda* showed a significant difference ($P<0.05$) as presented in Table 03. The highest antifeedant activity of 87.5% (0.31±0.01) with methanol followed by n-Hexane 80.51% (0.49 ± 0.03) at 1000ppm was recorded. Their antifeedant effect remained higher at each concentration than ethyl acetate and chloroform. Overall, the lowest antifeedant activity of 22.58% (1.95±0.03) was recorded at 125 ppm in chloroform extracts. It showed that the antifeedant activity relied

Table 1: Mortality percentage of the third instar larvae *S. frugiperda* on *P. guajava* crude extracts.

Crude Extracts	Concentration (ppm)	Mortality (%) at different time intervals (hrs.)			
		24 hrs	48 hrs	72 hrs	96 hrs
Methanol	125	14.29%	28.57%	35.71%	42.86%
	250	21.43%	35.71%	50.00%	57.14%
	500	28.57%	50.00%	64.29%	78.57%
	1000	42.86%	64.29%	78.57%	92.86%
Ethyl acetate	125	7.14%	14.29%	21.43%	28.57%
	250	14.29%	21.43%	35.71%	42.86%
	500	14.29%	28.57%	42.86%	50.00%
	1000	21.43%	42.86%	57.14%	64.29%
n-Hexane	125	14.29%	21.43%	35.71%	42.86%
	250	14.29%	28.57%	42.86%	50.00%
	500	28.57%	50.00%	64.29%	71.43%
	1000	35.71%	57.14%	71.43%	85.71%
Chloroform	125	7.14%	14.29%	21.43%	21.43%
	250	7.14%	21.43%	28.57%	35.71%
	500	14.29%	28.57%	35.71%	42.86%
	1000	21.43%	35.71%	50.00%	57.14%

Corrected mortality % was calculated according to the Abbott formula (Abbott, 1925).

Table 2: LC_{50} and LC_{90} of *P. guajava* crude extract against the third larval instar of *S. frugiperda* at different time intervals.

Crude Extracts	Time (hrs.)	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Chi -square
			Lower	Upper		Lower	Upper		
Methanol	24	477.9	327.3	738.1	3710.8	2813.8	5876.4	1.44±0.52	0.04
	48	329.1	252.7	535.8	2421.9	1587.2	3562.1	1.48±0.52	0.36
	72	261.7	98.7	467.6	2031.2	1364.7	3209.7	1.44±0.52	0.04
	96	154.77	78.8	251.8	865.3	682.9	1376.8	1.71 ± 0.57	0.21
Ethyl acetate	24	807.6	596.3	1301.8	4515.2	3247.9	6493.2	1.72±0.58	0.21
	48	674.3	514.4	1014.5	3677.8	2617.2	4978.3	1.53±0.54	0.08
	72	487.8	267.8	809.1	2510.8	1713.8	3987.4	1.44±0.52	0.04
	96	261.57	105.7	407.6	2011.6	1475.5	3119.7	1.44 ± 0.52	0.04
n-Hexane	24	634.1	479.4	949.5	4137.8	3057.8	6138.3	1.53±0.54	0.08
	48	498.8	387.5	811.1	2912.8	1989.8	4189.4	1.44±0.52	0.04
	72	329.4	178.7	589.6	2228.6	1426.3	3424.5	1.623±0.53	0.10
	96	197.12	157.1	329.4	1360.8	976.9	1994.4	1.53 ± 0.54	0.08
Chloroform	24	950.3	615.7	1433.4	5142.7	3756.3	7821.9	2.3±0.7	0.99
	48	827.6	634.5	1348.1	4115.2	3247.9	5469.2	1.76±0.6	0.21
	72	643.1	459.4	1004.5	3377.8	2517.5	4677.3	1.53±0.54	0.08
	96	491.7	357.3	802.1	3010.8	2113.8	4332	1.44 ± 0.52	0.04

LC_{50} and LC_{90} probability response tests were used by using Polo Plus software.
LC_{50} = lethal concentration 50%, LC_{90} = lethal concentration 90%

on the dose of concentration; meanwhile, the *P. guajava* with methanol crude extracts successfully stopped the feeding activity of *S. frugiperda* effectively.

Defoliation (%) of Maize Leaf after Consumption by S. frugiperda

The results in Table 04 and Figure 03 showed the defoliation of maize leaves by the third larval instar of *S. frugiperda* after being treated with different crude extracts at different concentrations. The defoliation percentage was low when maize leaves were treated

Table 3: Antifeedant activity (%) of *P. guajava* crude extracts at different concentrations against *S. frugiperda*.

Concentrations (ppm)	Crude Extract							
	Methanol		Ethyl acetate		n-Hexane		Chloroform	
	Mean ± SE (cm^2)	AI (%)	Mean ± SE (cm^2)	AI (%)	Mean ± SE (cm^2)	AI (%)	Mean ± SE (cm^2)	AI (%)
125	1.57 ± 0.03b	37.31	1.85 ± 0.06b	26.32	1.64 ± 0.052b	34.74	1.95 ± 0.03b	22.58
250	1.20 ± 0.05c	52.12	1.56 ± 0.14c	37.77	1.31 ± 0.03c	47.79	1.65 ± 0.08c	34.20
500	0.74 ± 0.02d	70.44	1.17 ± 0.05d	53.60	0.89 ± 0.07d	64.61	1.25 ± 0.04d	50.09
1000	0.31 ± 0.01e	87.5	0.89 ± 0.03e	64.55	0.49 ± 0.03e	80.51	1.0 ± 0.04e	60.21
Control	2.51 ± 0.06a		2.51 ± 0.06a		2.51 ± 0.06a		2.51 ± 0.06a	

Mean ± SE in each column followed by the same letter do not differ significantly using the LSD test ($p < 0.05$); AI (%): treatment antifeedant activity % calculated according to Bentley formula (Bentley *et al.*, 1984).

with *P. guajava* leaf extracted through methanol at each concentration. However, the lowest 0.98% defoliation was recorded at methanol on 1000ppm with a leaf area index of 6.99 in comparison to the control treatment which showed 24.02% leaf defoliation. The leaf defoliation in each extract was reduced when the dose was increased with methanol followed by n-Hexane produced more significant results than Ethyl acetate and Chloroform.

The present study regarding larval mortality, antifeedant activity, and defoliation percentage of *P. guajava* through crude leaf extracts with methanol, ethyl acetate, n-hexane, and chloroform at different concentrations against the third larval instar of *S. frugiperda* was studied. The review linked with this study suggested that sources of plant extracts have been used for thousands of years for bioactive and therapeutic purposes (Farzaei *et al*., 2013) for different pest control properties including antifeedant, toxicity, repellent, and fumigation (Niroumand *et al.*, 2016). Similarly, we found maximum larval mortality and antifeedant activity with the highest defoliation percentage at a maximum concentration of each crude extract. Magrini *et al.* (2015) also evaluated that all extracts of *Cabralea canjerana* (Vell.) showed the highest larval mortality and antifeedant activity at maximum concentration against *S. frugiperda*. However, this work found that in methanol and three other crude extracts of *P. guajava*, the highest mortality percentage of 92.86% was observed on methanol at 1000 ppm at 96 hrs. Likewise, Phambala *et al.* (2020) evaluated that the *Azadirachta indica* (A. Juss) and other six plant species were more effective than *Nicotiana tabacum* (L.) which showed the highest percentage of mortality with 75–98% at increased concentration/dose. Elanchezhiyan *et al.* (2015) observed five solvent extracts of *Tinospora crispa* (L.) and *P. guajava* against *S. litura* (F), the methanol extract was responsible for strong lethal activity. Most of the results are the findings of the present study. Meanwhile, the lowest larval mortality was 7.14-14.29% noted at a minimum concentration (125ppm) in chloroform and ethyl acetate and so as reported by Muthusamy *et al*. (2015). According to these, the highest larvicidal activity was observed, and the lowest larvicidal activity was at 125 mg/L in ethyl acetate extract in comparison to ethanol. The mortality on all crude extracts and at every concentration increased with time and reached a maximum of 96 hrs. Further, methanol and n-hexane almost killed 50% larval population of FAW after 48 hrs at 500-1000ppm which is considered a significant achievement in killing the targeted insect. Sisay *et al*. (2019a) evaluated the botanicals in Ethiopia against FAW, they found Nicotiana tabacum caused 50% mortality of the third instars after 72 hrs when extracted by methanol.

The bioassay results similarly illustrated with minimum LC_{50} and LC_{90} values of 154.77ppm and 865.3ppm were found at methanol extract leaves of *P. guajava* and the maximum 491.7 and 3010.8 in chloroform after 96 hrs. Elanchezhiyan *et al*. (2015) noted the lowest LC_{50} and LC_{90} values of 121.86 ppm and 237.92 ppm were recorded in methanol extract of *P. guajava* leaves and the highest LC_{50} and LC_{90} values of 164.22 and 259.53ppm at diethyl ether against *S. litura* larvae. These findings suggested that the effect of *P. guajava* leaves extracted through ethanol produced impressive results against lepidopteran. Duraipandiyan *et al*. (2011) reported the larval mortality of isolated Rhein from Cassia fistula flower against *Helicoverpa armigera* and *S. litura* with LC_{50} values of 606.50ppm and 1192.55ppm, respectively. The other part of our study was to confirm the antifeedant activity of different extracts which showed the highest antifeedant activity of 87.5% in methanol at 1000ppm. It means that the botanical pesticide did not allow the pest to consume the major portion of the offered leaves when treated. These results are to the previous findings of

Table 4: Defoliation (%) of treated maize leaves by *P. guajava* crude extracts against *S. frugiperda*.

Concentrations (ppm)	Methanol		n-Hexane		Ethyl acetate		Chloroform	
	Defoliation (%)	LAI cm^2	Defoliation (%)	LAI cm^2	Defoliation (%)	LAI cm^2	Defoliation (%)	LAI cm^2
125	10.5	2.46	12.43	2.11	14.67	1.78	15.9	1.7
250	4.6	4.08	5.3	3.8	7.4	3.18	8.6	2.9
500	2.8	5.8	3.12	4.84	5.4	3.82	5.9	3.42
1000	0.98	6.99	1.82	5.83	2.8	5.14	3.9	4.42
Control	24.02	0.86	24.02	0.86	24.02	0.86	24.02	0.86

Leaf ($4cm^2$ diameter); Defoliation % and Leaf area index (LAI) calculated by Bio leaf mobile application

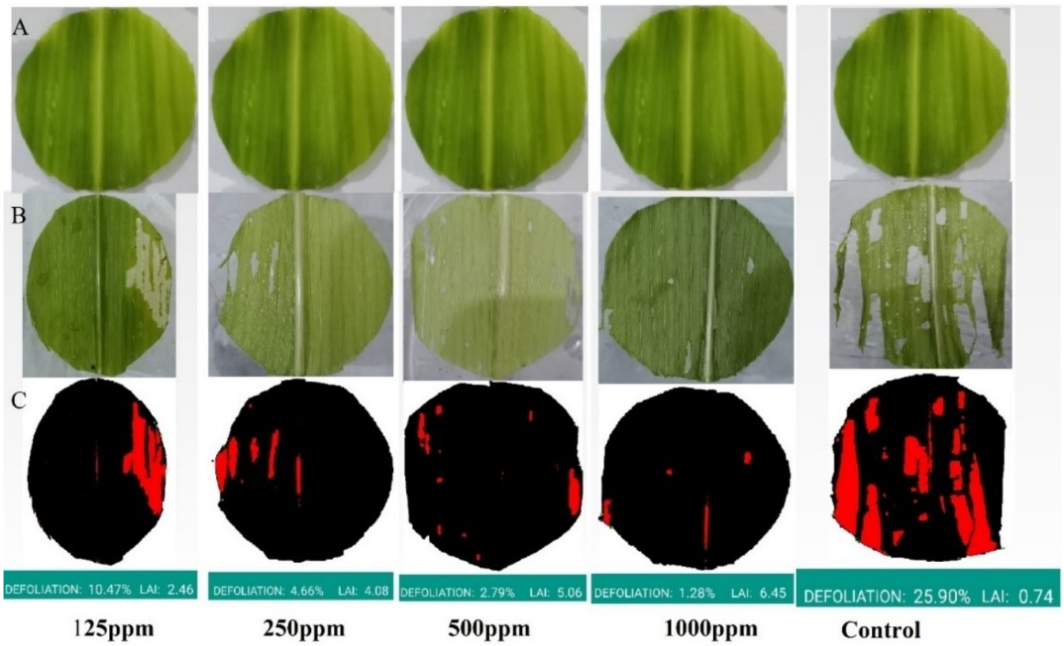

Figure 3: Showing results of defoliated maize leaves using bio leaf application A Healthy leaves, B Defoliated leaves, and C calculated leaves.

Elanchezhiyan *et al*. (2015), who reported that the methanol extract of *P. guajava* and *Tinospora crispa* showed 98.38% and 100% antifeedant activity against *S. litura* at the maximum concentration of 500ppm. Krishnappa *et al*. (2013) also reported the most antifeedant activity of 85.56% recorded in *Sanguisorba officinalis* at 1000ppm against *S. litura* . On the other hand, chloroform produced the lowest results 22.58% at the lowest concentration of 125ppm. This study found all the extracts performed reasonably well in antifeedant activity, though the minimum antifeedant activity was observed in the methanol extract of *P. guajava* leaves.

CONCLUSION

It was concluded that the *P. guajava* leaves extracts with methanol were found to be most effective against *S. frugiperda* . The maximum larval mortality was observed on methanol at 1000ppm after 96 hrs. The mortality increased with increased concentration of extracts and exposure time. Besides lethal effects, the *P. guajava* with methanol crude extracts also successfully stopped the feeding activity of *S. frugiperda* when introduced on treated leave.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

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