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IMPACT OF SOLVENTS AND EXTRACTION METHODS ON THE INSECTICIDAL ACTIVITY OF *SESBANIA GRANDIFLORA* L. LEAF EXTRACTS AGAINST DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA*

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ABSTRACT

Diamond back moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a notorious pest of cruciferous crops causing extensive damage. The present study compares the insecticidal activity of *Sesbania grandiflora* extracts in hexane, ethyl acetate, and ethanol obtained from two extraction methods: Soxhlet and cold maceration against *P. xylostella*. Soxhlet extraction by all the solvents showed a distinct effect on larval mortality, adult emergence, and the growth and development of second-instar larvae than cold maceration extraction. Soxhlet ethanolic extract at 5% had significantly better insecticidal activity than cold maceration, with complete larval mortality. The antifeedant index was also higher in ethanol extract, followed by ethyl acetate and hexane extract. However, there was no significant variation in the developmental period. Thus, Soxhlet ethanolic extract of *S. grandiflora* is concluded to have a promising insecticidal activity on *P. xylostella.*

Key words: *Sesbania grandiflora*, *Plutella xylostella*, insecticidal activity, larval mortality, adult emergence, ethanol, antifeedant index, developmental period, Soxhlet, cold maceration, ethanol, ethyl acetate, hexane.

The consumption of crops by pests is sufficient to feed an additional one billion people worldwide (Birch et al., 2011); hence, effective pest management is vital to assure enhanced crop productivity. The management of the diamond back moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), an important pest of brassicas, is an intimidating task globally (Sarfraz and Keddie, 2005; Charleston et al., 2006a; Furlong et al., 2013; Li et al., 2016). The primary strategy for controlling *P. xylostella* to date is the extensive application of broad-spectrum chemical pesticides, however over and misuse of insecticides, led to insecticide resistance development in *P. xylostella* (Sparks et al., 2020). The rapid development of resistance to nearly all groups of insecticides, including organochlorines, organophosphates, carbamates, pyrethroids, insect growth regulators, abamectins, pyrazoles, oxadiazines, neonicotinoids, spinosad and indoxacarb has made chemical control less effective (Abdel-Razek et al., 2006; Charleston et al., 2005; Qian et al., 2008). Therefore, developing a more effective and alternate strategy is crucial for managing *P. xylostella*. Considerable efforts have been directed toward screening and exploring the bioactivity of various plants against insect pests for the last two decades. Natural products such as plant extracts containing secondary metabolites are eco-friendly, less toxic to non-target organisms than synthetic insecticides, and vital for discovering and developing new pesticides (Bhatta et al., 2019). Botanical insecticides derived from plants are natural compounds and can be exploited as an alternative to chemical insecticides in delaying resistance development and bringing about effective control against *P. xylostella* (Isman and Grieneisen, 2014; Mayanglambam et al., 2021).

Sesbania grandiflora (L.) Poir. (Fabaceae), also known as West Indian-pea, is a fast-growing tree and is widely distributed in India, Indonesia, Myanmar, Philippines, and Thailand. The leaves taste bitter and are rich in vitamin C, calcium, sterols, saponin, quercetin, myricetin, and kaempferol (Mustafa et al., 2010). Earlier research reported the insecticidal activity of leaf extracts of *S. grandiflora* against *P. xylostella* extracted using aqueous (Sangavi and Edward, 2017) and ethyl acetate (Susmitha et al., 2022) solvents. *S. grandiflora* leaves showed insecticidal, antifeedant, and growth inhibitory activities (Susmitha et al., 2022).

Soxhlet and cold maceration extraction are the most commonly adopted methods to obtain the desired crude. Soxhlet extraction is a laborious process with relatively more significant quantities of solvents and has been followed for many decades. Cold maceration involves low extraction temperature similar to cold pressing, resulting in an odor similar to that in the original plant material without causing degradation of the thermolabile compounds present in the fraction. Although various methods have been described in the literature for extracting crude material, a scientific comparative study to know which method is superior is needed for *S. grandiflora* extract. With this background, laboratory studies were done to explore the insecticidal effects of *S. grandiflora* in various extracts in the increasing order of polarity, namely, hexane, ethyl acetate, and ethanol, to evaluate the larval mortality, antifeedant index, and growth inhibition effects.

MATERIALS AND METHODS

Sesbania grandiflora leaves were collected from the Agricultural College and Research Institute (AC&RI), Tamil Nadu Agricultural University, Madurai, India (9°58'N, 78°12'E). Laboratory experiments were conducted to study the efficacy of *S. grandiflora* at the Insectary in the Department of Agricultural Entomology, AC&RI, Madurai during November 2022. *S. grandiflora* extract was prepared using two different methods, which are cold maceration and the Soxhlet method. The collected *S. grandiflora* leaves were thoroughly washed and dried in the shade for 15-20 days until brittle and ground to a coarse powder in a blender. In cold maceration extraction, *S. grandiflora* leaf powder was mixed with the respective solvent in a ratio of (1:10 w/v) in a conical flask. The mixture was stirred thoroughly with a glass rod and kept in a mechanical shaker with intermittent shaking for 72 hrs. The mixture was filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C, and the resultant residue was kept in a refrigerator till further use. In the Soxhlet method of extraction, the *S. grandliflora* leaf powder was packed in a thimble and placed in the sample holder of the Soxhlet extractor with the respective solvent poured in the round-bottom flask in the ratio (1:10 w/v). A continuous hot percolation technique was done until the solvent in the sample holder appeared colorless. The final extract was filtered using Whatman No. 1 filter paper and concentrated to dryness using a rotary evaporator at 40°C. The content of extractable matter was calculated in mg/g using a digital weighing balance for recovery estimation and stored in the refrigerator until further use. *Laboratory rearing of P. xylostella* was initiated with the larvae from infested cauliflower and maintained until pupation. The pupae were transferred to adult emergence cages and mustard seedlings were provided for oviposition of adults. First instar larvae were reared on mustard leaves and the later instars were transferred to fresh cauliflower leaves and utilized for further analysis (Nithya et al., 2021).

A standard leaf disc dip method was done in laboratory conditions under no-choice conditions to evaluate the insecticidal activity of crude extracts of *S. grandiflora* against the second instar larvae of *P. xylostella* (Ingle et al., 2017). The cauliflower leaf discs (5 cm dia) were treated with 5% of the test solutions for 30 seconds, solvents were allowed to dry and provided as feed for the larvae until pupation, to study the effect on growth and development. Three replications for each treatment, with 10 larvae/ replicate, were maintained. The antifeedant index was estimated by measuring the leaf area consumed after 24, 48, and 72 HAT (Hours after treatment). In the treatments, the mean development period, including larval mortality, adult emergence, and malformation, if any, was also observed (Susmitha et al., 2022). Preliminary qualitative phytochemical evaluation of the extracts was carried out on the group of Alkaloids, Flavonoids, Phenols, Saponins, Triterpenoids, Steroids, and Tannins using standard procedures (Apoorva et al., 2021). The experiment was conducted in completely randomized design (CRD), and the data subjected to arc sine and square root transformation. Statistical analysis was carried out using SPSS software (version 22) for ANOVA, and grouping of data was done by Tukey's post hoc test (Tukey 1977).

RESULTS AND DISCUSSION

The % recovery yields revealed that the polar solvent, ethanol, recorded a higher extraction yield than the mid-polar solvent, ethyl acetate, and non-polar solvent, hexane. Among the two different extraction methods used, the Soxhlet method of extraction showed higher yields of 33.32% in ethanol, followed by 10.08% and 9.65% in ethyl acetate and hexane, respectively. In contrast, the cold maceration method yielded 27.02% in ethanol followed by 7.10% and 6.05% in ethyl acetate and hexane, respectively (Fig. 1). The results indicate that the Soxhlet method of extraction is capable of yielding higher recovery. This finding agrees with Alara et al. (2018), who reported

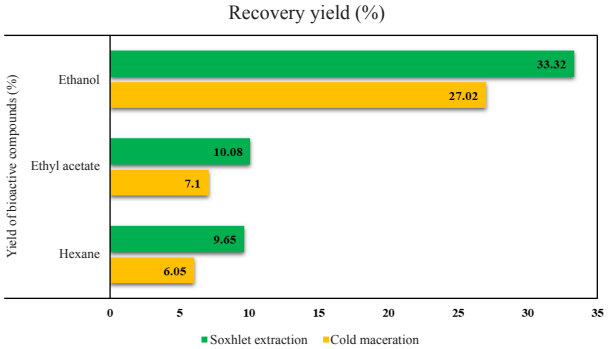

Fig. 1. Recovery (%) of *S. grandiflora* crude extracts using different solvents

that enhanced yield is obtained in Soxhlet extraction compared to maceration-based extraction techniques, which is quite efficient. However, this method risks the degradation of thermolabile compounds because of higher temperatures. The solvent ethanol is polar, which results in the leaching of more active ingredients during extraction compared to other solvents (Sankeshwari et al., 2018). In a study conducted by Omanakuttan et al. (2023) to comparatively analyze the extraction methods for *Cassia fistula* L. roots, it was concluded that the maximum extraction yield was obtained for the Soxhlet method compared to the maceration method.

The absolute antifeedant index was higher in extracts obtained by Soxhlet extraction than in cold maceration. The Soxhlet extracts at all hours of treatment indicated that the leaf area fed by the *P. xylostella* larvae was higher and statistically equivalent in ethanol extract and treated check with an activity of 78.06% and 79.31%, 77.67% and 77.38%, 74.39% and 75.53% at 72, 48 and 24 HAT, respectively, followed by ethyl acetate and hexane extract with an activity of 58.61% and 54.02% at 72 HAT, 56.44% and 52.29% at 48 HAT, 50.11% and 49.35% at 24 HAT respectively. The cold maceration extracts of ethanol, ethyl acetate, hexane, and aqueous recorded a comparatively lesser antifeedant activity of 48.39%, 28.83%, 27.52%, and 21.92% at 72 HAT, following a similar trend at 48 and 24 HAT. The absolute antifeedant index increased with exposure time in all the treatments and was higher in Soxhlet extraction. Complete larval mortality was observed in the ethanol (Soxhlet) and treated check. In contrast, it was 73.33% in ethyl acetate (Soxhlet), followed by hexane (Soxhlet) and ethanol (cold maceration), with 63.33% compared to the untreated check. Notably, the aqueous extracts recorded a lower mortality of 26.67% compared to the other treatments. The developmental period did not exhibit significant differences. However, prolonged larval and pupal periods with a reduced adult period were observed in the treatments compared to untreated check (Table 1).

The present findings corroborate with the finding of Susmitha et al. (2022) that the ethyl acetate extracts of *Sesbania grandiflora* at 5% exhibited maximum antifeedant index (34.27%) with larval mortality of 76.67%, adult emergence of 23.33% and no significant difference in the developmental period when compared to control. Similar results were given by Sangavi and Edward (2017) that 10% of aqueous extracts of *S. grandiflora* recorded a larval mortality of 73.33% on 5 DAT (days after treatment), and an antifeedant effect of 52.31% was recorded on 2 DAT (52.31 %). 10% aqueous extract of *S. grandiflora* caused the highest mortality of 94.43% of *Tetranychus urticae* at 72 HAT as reported by Premalatha et al. (2018). However, Meenambigai (2019) has documented 100% mortality of third instar *P. xylostella* larvae on 5 DAT at 0.6% concentration of hexane extract of *S. grandiflora* and 85.00 and 40.00% mortality at 0.6 and 0.4% concentrations of ethyl acetate and ethanol extracts, respectively.

In a study conducted by Tulashie et al. (2021) to characterize and assess the toxicity of neem extracts obtained by two different methods to manage fall armyworm (FAW) invasion. The neem seed oil extract (NSOE) and methanolic neem leaf extract (MNLE) were obtained from neem seeds and leaves by Soxhlet extraction and cold maceration, respectively. The yields after extraction for NSOE and MNLE were 23.92% and 17.05%, respectively. The LC_{50} of 1.78%, 0.97%, and 0.68% was estimated for NSOE and LC_{50} of 2.67%, 2.62% and 1.64% for MNLE after 2, 6 and 12 hr, respectively. It indicated that NSOE had high pesticidal activity compared to MNLE. The qualitative phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, terpenoids, saponins, steroids, and tannins in ethanolic extracts obtained from both extraction techniques without glycosides. The absence of glycosides and saponins was detected in all the other extracts except the aqueous extract, wherein only the presence of alkaloids was qualitatively confirmed, as shown in Table 2. Similar findings were reported by Padmalochana and Dhana Rajan (2014) that the ethanolic extract of *S. grandiflora* showed the presence of alkaloids, tannins, saponins, and steroids. The insecticidal activity could be due to the presence of alkaloids, flavonoids, steroids, tannins, terpenes, and terpenoids from different plants, which have been reported (Shaalan et al., 2005). The development of insects can be harmed by ingesting both flavonoids

Table 1. Effect of solvent extracts of *S. grandiflora* on *P. xylostella*

	Treatment	Absolute antifeedant index (%) [#$]			Mean developmental period (days)[#@]			Cumulative larval mortality (%) [#$]	Adult emergence (%) [#$]
		24 HAT	48 HAT	72 HAT	Larva	Pupa	Adult longevity		
Soxhlet	T1 – Hexane extract (5%)	49.35± 0.88 (44.63)^{b}	52.29± 0.66 (46.32)^{c}	54.02± 0.79 (47.31)^{c}	13.67± 0.58 (3.76)^{a}	6.33± 0.58 (2.61)^{a}	5.67± 0.58 (2.48)^{b}	63.33± 0.58 (15.15)^{bc}	36.67± 0.58 (11.76)^{bc}
	T2 – Ethyl acetate extract (5%)	50.11± 0.60 (45.07)^{b}	56.44± 0.46 (48.70)^{b}	58.61± 0.86 (49.96)^{b}	13.33± 0.58 (3.72)^{a}	5.33± 0.58 (2.41)^{ab}	5.33± 0.58 (2.41)^{b}	73.33± 0.58 (16.25)^{b}	26.67± 0.58 (10.22)^{b}
	T3 – Ethanol extract (5%)	74.39± 0.75 (59.60)^{a}	77.67± 0.59 (61.80)^{a}	78.06± 0.65 (62.07)^{a}	0.00± 0.00 (0.71)^{c}	0.00± 0.00 (0.71)^{c}	0.00± 0.00 (0.71)^{c}	100.00± 0.00 (18.91)^{a}	0.00± 0.00 (4.05)^{a}
	T4 – Hexane extract (5%)	21.38± 0.83 (27.54)^{e}	21.47± 0.53 (27.60)^{f}	27.52± 0.64 (31.64)^{e}	13.33± 0.58 (3.72)^{a}	6.00± 0.00 (2.55)^{a}	5.33± 0.58 (2.41)^{b}	53.33± 0.58 (13.97)^{c}	46.67± 0.58 (13.12)^{c}
	T5 – Ethyl acetate extract (5%)	25.57± 0.44 (30.37)^{d}	28.07± 0.97 (31.99)^{e}	28.83± 0.89 (32.47)^{e}	13.33± 0.58 (3.72)^{a}	5.67± 0.58 (2.48)^{ab}	5.00± 0.00 (2.35)^{b}	56.67± 0.58 (14.37)^{c}	43.33± 0.58 (12.69)^{c}
Cold	T6 – Ethanol extract (5%)	42.10± 0.94 (40.45)^{c}	45.75± 0.34 (42.56)^{d}	48.39± 0.23 (44.08)^{d}	13.00± 0.00 (3.67)^{a}	5.33± 0.58 (2.41)^{ab}	5.33± 0.58 (2.41)^{b}	63.33± 0.58 (15.15)^{bc}	36.67± 0.58 (11.76)^{bc}
	T7 – Aqueous extract (5%)	20.43± 0.67 (26.87)^{e}	21.86± 0.84 (27.87)^{f}	21.92± 0.57 (27.91)^{f}	13.37± 0.58 (3.76)^{a}	5.67± 0.58 (2.48)^{ab}	5.67± 0.58 (2.48)^{b}	26.67± 0.58 (10.22)^{d}	73.33± 0.58 (16.25)^{d}
	T8 – Azadirachtin 1 EC @ 2 ml/ lit	75.53± 0.65 (60.35)^{a}	77.38± 0.85 (61.60)^{a}	79.31± 0.86 (62.94)^{a}	0.00± 0.00 (0.71)^{c}	0.00± 0.00 (0.71)^{c}	0.00± 0.00 (0.71)^{c}	100.00± 0.00 (18.91)^{a}	0.00± 0.00 (4.05)^{a}
	T9 – Untreated check	-	-	-	10.67± 0.58 (3.34)^{b}	4.67± 0.58 (2.27)^{b}	7.33± 0.58 (2.80)^{a}	0.00± 0.00 (4.05)^{e}	100.00± 0.00 (18.91)^{e}
	SEd	0.38	0.37	0.38	0.06	0.08	0.08	0.48	0.54
	F value	2438.25	2796.54	2569.45	1155.98	197.14	218.27	182.57	168.04
	P value	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**

[#]Mean values of three replications represented as mean± standard deviation; [$]Figures in the parentheses arc sine transformed values (x+0.5)
[@]Figures in parentheses square root transformed values√x+0.5; Means followed by same letter not significantly different from each other by Tukey's test ($p \leq 0.05$); SEd: Standard Error of the difference; **Highly Significant.

Table 2. Qualitative phytochemical screening of solvent extracts of *S. grandiflora*

S. No.	Phytochemical	Test	Cold maceration				Soxhlet extraction			Observation
			Hexane	Ethyl acetate	Ethanol	Aqueous	Hexane	Ethyl acetate	Ethanol	
1	Alkaloids	Wagners	+	+	+	+	+	+	+	Brown precipitate
2	Phenols	Ferric chloride	+	+	+	-	+	+	+	Intense colour
3	Flavanoids	Ferric chloride	+	+	+	-	+	+	+	Intense green colour
4	Terpenoids	Salkowaski	+	+	+	-	+	+	+	Lower layer yellow
5	Saponins	Saponins	-	-	+	-	-	-	+	Honeycomb froth formation
6	Steroids	Sulfur	+	+	+	-	+	+	+	Sinks to the bottom
7	Tannins	Ferric chloride	+	+	+	-	+	+	+	Intense green colour
8	Glycosides	Keller-Kiliani	-	-	-	-	-	-	-	Two layers - brown and bluish-green

+ Presence, - Absence

and tannins. Free radical species can be generated by flavonoids, resulting in cellular toxicity. In contrast, tannins reduce enzyme movement and protein existence (Padin et al., 2013), creating an obstruction in nutrition. Therefore, the digestibility in the larval stages is affected, leading to death over time (Padial et al., 2023). Therefore, the choice of the extraction technique is crucial to achieve better yields without compromising biological activities. Therefore, *S. grandiflora* leaves extracted using the Soxhlet extraction method with ethanol have promising insecticidal activity against *P. xylostella*, which can be attributed to secondary metabolites that may be extremely bitter and deter insect herbivores.

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AUTHOR CONTRIBUTION STATEMENT

AY: Conducted the experiment, collected, curated and interpreted the data, and drafting of the original manuscript. MS: conceptualised, framed the research proposal, analyzed the results and involved in correction of the manuscript. MM: guided in planning of experiments, analyzed the results and involved in correction of the manuscript. SV and MLM: Guided and involved in the phytochemical analysis. RN: Advisory committee member for the research. All the authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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