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Comparative evaluation of six plant-derived hexane extracts against the diamondback moth, *Plutella xylostella* (L.)

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Abstract
The diamondback moth, *Plutella xylostella* (L.), is a major pest of cruciferous crops worldwide, and the widespread evolution of resistance to conventional insecticides has intensified interest in safer, plant-derived alternatives for its management. In the present study, hexane extracts of six plant species *viz.*, *Azadirachta indica*, *Nerium oleander*, *Plumeria rubra*, *Sesbania grandiflora*, *Swietenia macrophylla*, and *Tagetes erecta* obtained through Soxhlet extraction were evaluated at 5% concentration for larvicidal toxicity, effects on larval weight, and growth indices against *P. xylostella* under laboratory conditions, with azadirachtin 1% EC (2 ml/l) as a treated check and solvent and untreated checks for comparison. All extracts exhibited significant toxicity relative to the checks at 24 hours, with *S. grandiflora* and *S. macrophylla* recording the highest mortality among the extracts (46.67% and 43.33%, respectively) at 48 hours, on par with the treated check (43.33%) by 72 hours, *S. grandiflora* achieved 53.33% mortality, close to the treated check (63.33%). Larval weight gain was markedly suppressed in these two extracts, with larvae weighing only 0.85 mg (*S. grandiflora*) and 1.30 mg (*S. macrophylla*) on day 10, compared to 4.26 mg in the untreated control, and both treatments showed a one-day extension of larval duration, pupating on day 13 versus day 12 for the control. Growth index analysis further confirmed their superior bioefficacy, with larval growth indices of 2.51 (*S. grandiflora*) and 2.77 (*S. macrophylla*) against 9.39 in the untreated control, along with correspondingly lower suitability indices and higher antibiosis indices. Overall, the findings identify *S. grandiflora* and *S. macrophylla* hexane extracts as promising botanical candidates, with toxicity and growth-inhibitory effects approaching those of the standard azadirachtin check, warranting their further exploration as eco-friendly components of integrated pest management programs against *P. xylostella*.

Keywords: *Plutella xylostella*, hexane extract, toxicity, larval weight, growth index

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is widely regarded as the most destructive insect pest of cruciferous vegetables worldwide, inflicting annual management costs and yield losses estimated in the billions of US dollars (Sarfraz *et al*. 2005; Zalucki *et al*. 2012) [12, 15]. Its short generation time, high fecundity, and well-documented ability to develop resistance to virtually every class of synthetic insecticide, including *Bacillus thuringiensis* toxins (Zhao *et al*. 2006 [16]; Pu *et al*. 2010), have rendered its management increasingly challenging and have spurred the search for alternative, resistance-breaking control strategies.

Plant-derived bioactive compounds offer a promising alternative to synthetic insecticides owing to their generally lower mammalian toxicity, faster environmental degradation, and multiple modes of action (Bowers 1992; Mohan *et al*. 2005; Sharma and Srivastava 2005; Pavela 2016) [1, 7, 8], all of which reduce the likelihood of resistance development. Botanicals may act as antifeedants, growth regulators, oviposition deterrents, or direct toxicants, often disrupting larval nutrition and moulting rather than causing acute mortality alone. The polarity of the extraction solvent governs the class of secondary metabolites recovered: hexane selectively isolates non-polar constituents such as terpenoids, fatty acid derivatives, and other lipophilic compounds, whereas ethyl acetate, being of intermediate polarity, additionally recovers moderately polar constituents such as flavonoids and coumarins all of which may contribute to insect growth-regulatory or toxic activity.

The present study was therefore undertaken to evaluate the effects of hexane extracts of six plant species on the mortality, larval weight and growth indices of *P. xylostella* under laboratory conditions, in comparison with a standard botanical check (azadirachtin) and appropriate solvent and untreated controls, with the objective of identifying promising candidate plants and solvent systems for future formulation as botanical insecticides.

Materials and Methods

1. Insect culture

A laboratory culture of *P. xylostella* was maintained on fresh mustard seedlings under controlled laboratory conditions. Healthy second-instar larvae were used for all bioassays.

2. Plant material and extraction

Six botanicals, namely neem (*Azadirachta indica*), oleander (*Nerium oleander*), frangipani (*Plumeria rubra*), hummingbird tree (*Sesbania grandiflora*), Honduran

mahogany (*Swietenia macrophylla*) and marigold (*Tagetes erecta*), were selected based on the results of preliminary screening. Unripe fruits of each plant were collected separately (1 kg), shade-dried for 15–20 days until completely brittle and ground using a mechanical blender. The ground material was passed through a 40-mesh sieve to obtain a uniform fine powder. The powdered samples were stored in amber-coloured bottles under dry conditions and protected from direct light until further use.

The powdered plant materials were extracted separately using hexane. 10 g of powdered plant material was placed in a cellulose thimble and subjected to continuous hot extraction using a Soxhlet apparatus, maintaining the extraction temperature below the boiling point of the solvent, until the solvent in the extraction chamber became visibly clear. The extracts obtained with each solvent were separately filtered through a Büchner funnel using Whatman No. 1 filter paper. The respective solvents were removed under reduced pressure using a rotary evaporator at 40–45 °C. The resulting hexane crude extracts were collected separately, weighed and stored in amber-coloured vials under appropriate conditions until further bioassay and chemical analysis. The percentage recovery of each extract was calculated based on the initial weight of the powdered plant material.

3. Preparation of test concentrations and bioassay method

Test solutions of the hexane extracts were prepared at 5 per cent concentration using 0.1% Triton X-100 as a surfactant. Fresh, untreated cabbage leaves were washed with distilled water and air-dried for 30 minutes. Leaf discs of 5 cm diameter were cut and dipped in the respective 5 per cent test solution for approximately 30 seconds to ensure uniform deposition of the active ingredient (Ingle *et al*., 2017) [3]. Treated discs were held in a slanted position for about two minutes in a tray to drain excess solution, and then air-dried for approximately 30 minutes at room temperature before use.

Second-instar larvae of *P. xylostella*, pre-starved for 2 hours, were released on to each treated leaf disc at 10 larvae per replication, within a plastic container lined with moist filter paper to maintain humidity. The treated check consisted of azadirachtin 1% EC at 2 ml/l, applied by the same leaf-dip procedure; solvent check discs were dipped in the respective carrier solution (0.1% Triton X-100 in distilled water) without extract, and untreated check discs received no treatment. The hexane extracts were evaluated in a Completely Randomized Design (CRD) replicated three times. Mortality and Larval weight were recorded till pupation using an electronic balance.

4. Statistical analysis

Data on mortality, larval weight and growth index for the hexane trial were subjected to square root transformation prior to analysis of variance (ANOVA) to normalize variances and satisfy homogeneity assumptions. Treatment means within each trial were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Results

1. Toxicity

After 24 hours of exposure up to 72 hours, the insecticidal activity of hexane extracts of all the selected plants were recorded and presented (Fig.1). Toxicity tests found that after 24 hours of exposure, all of the treatments were statistically equal, but different from untreated and solvent check. After 48 h, there was no significant difference between *S. grandiflora* (46.67%), *S. macrophylla* (43.33%), and treated check (43.33%) and they all showed maximal mortality. *S. grandiflora* had a larval death rate of 53.33 per cent after 72 hours of treatment, which was lower than the treated check (63.33%).

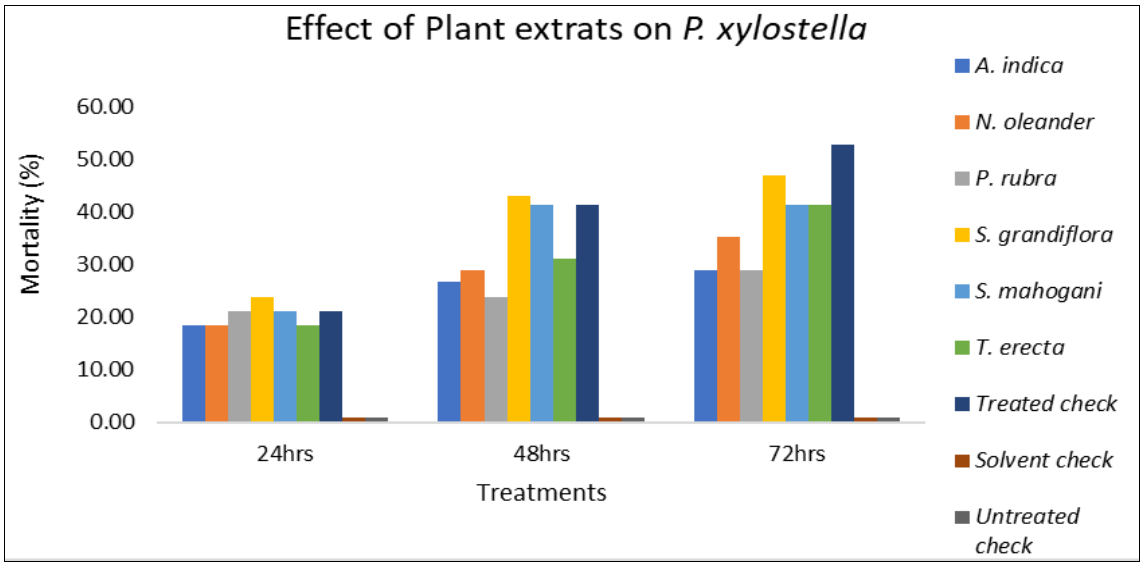

Fig 1: Toxicity of bioactive compounds isolated using hexane on *P. xylostella*

2. Effect on larval weight

The weight of 4-day old larvae fed hexane (5%) plant extract treated leaves until pupation was dramatically reduced by seven days after feeding. There was no variation in larval weight at day 4 because uniform weights of larvae were used for the investigation. On day 7, there was substantial reduction in larval weight in treated check (0.38 mg), it was followed by *S. grandiflora* (0.47 mg) and the next best treatment was *S. macrophylla* (0.73 mg). In *S. grandiflora*, the larval weight was only 0.63 and 0.80 mg on

days 8 and 9, whereas in *S. macrophylla*, it was 0.86 and 1.00 mg and found significantly lower than the untreated control (2.71 and 3.66 mg, respectively). On day 10, the larval weight in the untreated control was 4.26 mg, whereas it was much lower in all other treatments, especially in *S. grandiflora* and *S. macrophylla*, where it was 0.85 and 1.30 mg, respectively. On day 10, the treated check resulted in 100 per cent larval mortality. The larva in the untreated check achieved a maximum weight of 5.11 on day 11 and entered the pupal stage on day 12, whereas all of the treatments were still in the larval stage on day 12 and pupated on day 13. The two treatments, *S. grandiflora* and *S. macrophylla*, showed one-day extension of the larval stage (Fig. 2).

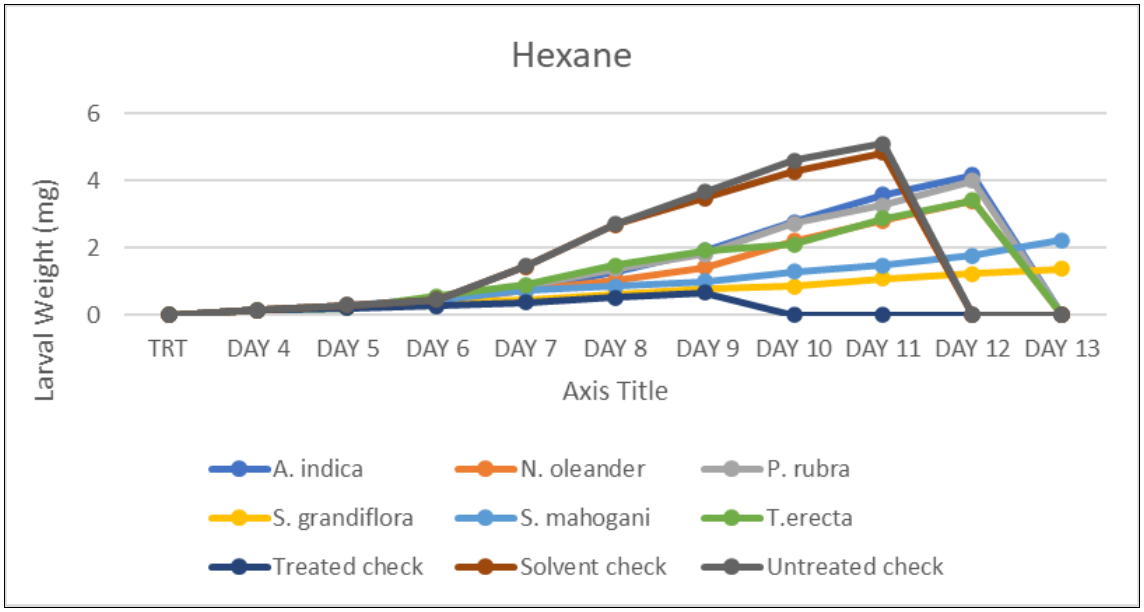

Fig 2: Effect of Hexane-Isolated Bioactive Compounds on the Larval Growth of *P. xylostella*

3. Effect on growth index

While evaluating the growth indices, the larval growth index in *S. grandiflora* (2.51) and *S. macrophylla* (2.77) was lower than the untreated check (9.39) (Table 1). For all treatments, the larval-pupal index varied from 0.92 to 0.98. The pupal weight index ranged from 0.48 to 0.98, while the adult weight index ranged from 0.57 to 0.95. The adult emergence index ranged from 0.33 to 1.00. Howe's growth index was 0.12 for the untreated control and 0.09 to 0.10 for the other treatments. Developmental index ranged from 1.02 to 1.08. *S. grandiflora* (0.86 and 5.99) and *S. macrophylla* (0.93 and 6.48) had lower suitability and antibiosis index values, respectively.

Table 1: Effect of bioactive compounds isolated using hexane on growth index of *P. xylostella*

Treatments	Larval Growth index	Larval-Pupal index	Pupal Weight index	Adult Emergence index	Adult Weight index	Howe's Growth index	Developmental index	Suitability index	Antibiosis index
T_1-*A. indica*	4.59	0.94	0.85	0.57	0.76	0.10	1.06	1.27	8.88
T_2-*N. oleander*	3.42	0.92	0.84	0.43	0.70	0.09	1.08	1.07	7.49
T_3-*P. rubra*	4.34	0.94	0.86	0.53	0.79	0.10	1.06	1.23	8.62
T_4-*S. grandiflora*	2.51	0.98	0.48	0.33	0.57	0.09	1.02	0.86	5.99
T_5-*S. macrophylla*	2.77	0.94	0.60	0.37	0.65	0.09	1.06	0.93	6.48
T_6-*T. erecta*	3.53	0.95	0.81	0.43	0.78	0.10	1.05	1.09	7.65
T_7-Solvent check	9.39	1.01	0.98	1.00	0.95	0.13	0.99	2.06	14.45
T_8-Untreated check	9.39	-	-	-	-	0.12	-	-	-

Discussion

The present findings, wherein *S. grandiflora* and *S. macrophylla* hexane extracts caused significant reduction in larval weight and growth indices along with extended larval duration, arein agreement with earlier reports establishing that botanical pesticides adversely affect insect growth and development. Talukder (2006) [14] reported that botanical pesticides diminish the weight of larvae, pupae, and adults while prolonging developmental phases, and Koul *et al.* (2008) [4] similarly observed that plant metabolites reduce larval and pupal survival as well as adult emergence. Shaalan *et al.* (2005) [13] further demonstrated that many botanical pesticides affect the developmental period, growth, and adult emergence of insects, findings that closely parallel the suppressed growth indices and delayed pupation recorded in the present study.

The efficacy of *S. grandiflora* observed here corroborates the findings of Sangavi and Edward (2017) [11], who

reported that ethyl acetate leaf extract of *S. grandiflora* at 0.6 per cent concentration reduced adult emergence to as low as 45 per cent compared to 100 per cent in the control. Likewise, the growth-inhibitory and developmental effects of *S. macrophylla* recorded in the present study are supported by Dinesh-Kumar *et al*. (2018) [2], who observed a reduction in adult longevity by 5.3, 3.2, and 2.7 days relative to the control at 15, 20, and 30 ppm, respectively, following treatment with methanolic leaf extract of *S. macrophylla*. Additionally, Safraz *et al*. (2005) reported an insect-static effect of *S. macrophylla* twig extracts on the metamorphosis of *Spodoptera frugiperda*, indicating a promising insect growth-regulatory potential of this species that aligns with the pronounced antibiosis and reduced suitability indices observed in the present investigation.

The growth-inhibitory activity of related botanicals has also been linked to specific fatty acid derivatives. Hexane, as employed in the present study, preferentially recovers such non-polar fatty acid derivatives and related lipophilic constituents, the presence of structurally similar compounds may plausibly account for the pronounced growth-inhibitory activity of *S. grandiflora* and *S. macrophylla* extracts against *P. xylostella*. The insecticidal potential of *S. grandiflora* against *P. xylostella* observed in the present study is further consistent with the reports of Sangavi and Edward (2017) and Meenambigai (2019) [5, 11], both of whom recorded insecticidal activity of *S. grandiflora* leaf extract against this pest. The efficacy of *S. grandiflora* extends beyond lepidopteran pests as well, as Premalatha (2018) [9] reported that a 10 per cent aqueous leaf extract of *S. grandiflora* exhibited superior acaricidal action against the red spider mite, *Tetranychus urticae*, with the highest mortality (97.77%) recorded at 72 hours after treatment. Taken together, these corroborating reports across different pest species and extract types reinforce the consistent bioactive potential of *S. grandiflora* and *S. macrophylla*, supporting their candidacy as effective botanical alternatives for the management of *P. xylostella*.

Conclusion

The study revealed that hexane extracts of the tested plants adversely affected the survival and development of *Plutella xylostella*, with *Sesbania grandiflora* and *Swietenia macrophylla* showing the most promising insecticidal and growth-disrupting effects, comparable to the standard azadirachtin check. Both extracts significantly reduced larval weight and growth indices while extending larval duration, indicating a predominantly antifeedant and growth-regulatory mode of action. These findings highlight *S. grandiflora* and *S. macrophylla* as potential eco-friendly candidates for inclusion in integrated management programmes against *P. xylostella*, warranting further studies on active compound identification and field-level validation.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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