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Evaluation of botanical ethyl acetate extracts for the management of 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 widespread resistance to conventional insecticides has intensified interest in plant-derived alternatives for its management. In the present study, ethyl acetate 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, larval weight reduction 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. Among the six extracts, *S. grandiflora* and *S. macrophylla* exhibited the most notable insecticidal activity. At 24 and 48 h post-treatment, larval mortality caused by these two extracts and the treated check was statistically comparable. By 72 h, the treated check recorded the highest mortality (66.67%), followed by *S. grandiflora* (56.67%) and *S. macrophylla* (53.33%), with no significant difference among them. Larval weight did not differ significantly among ethyl acetate treatments until day 5, after which *S. macrophylla* (0.36 mg) and *S. grandiflora* (0.47 mg) recorded reduced larval weight compared to the untreated control, while the treated check showed the lowest weight (0.29 mg) and proved superior on days 6–7, with complete larval mortality by day 10. On day 10–11, *S. grandiflora* recorded the lowest larval weight (1.13 mg), even showing weight loss, and outperformed *S. macrophylla* (1.35 mg), against 4.96 mg in the untreated control. Pupation was delayed by one additional day in *S. grandiflora* relative to other treatments. The larval growth index was markedly lower in *S. grandiflora* (1.86) and *S. macrophylla* (2.09) than in the untreated check (9.39), with Howe's growth index similarly reduced (0.09–0.10 vs. 0.13). Larval-pupal, pupal weight, adult weight, emergence, and developmental indices ranged narrowly across treatments. Suitability and antibiosis indices were lower in *S. grandiflora* (0.72 and 5.06) than *S. macrophylla* (0.80 and 5.60), indicating stronger antifeedant and growth-disrupting activity. These findings confirm the potential of *S. grandiflora* and *S. macrophylla* ethyl acetate extracts as eco-friendly botanical alternatives for integrated management of *P. xylostella*.

Keywords: *Plutella xylostella*, ethyl acetate 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) [13, 16]. 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 [17]; 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, 8, 9], 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: ethyl acetate 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 ethyl acetate 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 ethyl acetate. 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 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 ethyl acetate crude extracts were collected separately, weighed and stored in amber-coloured vials under appropriate conditions until further bioassay and chemical analysis.

3. Preparation of test concentrations and bioassay method

Test solutions of the ethyl acetate 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 ethyl acetate 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 ethyl acetate 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 and 48 hours of treatment, ethyl acetate extracts of *S. grandiflora* (23.33 and 46.67%), *S. macrophylla* (20 and 43.33%), and treated check (26.67 and 46.67%) were statistically equal. The treated check placed highest after 72 h, with a death rate of 66.67 per cent, followed by *S. grandiflora* (56.67%) and *S. macrophylla* (53.33%), with no significant difference (Fig.1).

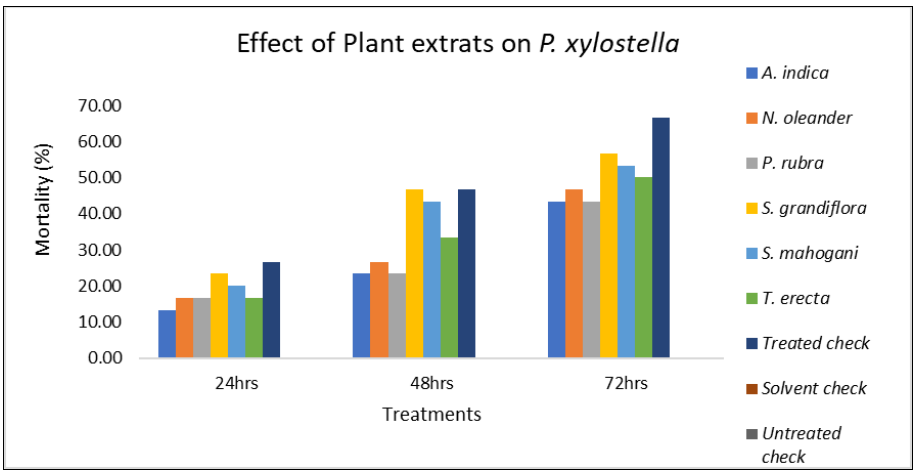

Fig 1: Toxicity of bioactive compounds isolated using ethyl acetate on *P. xylostella*

2. Effect on larval weight

There was no significant change between treatments in ethyl acetate extract until day 5. *S. macrophylla* (0.36 mg) had the lowest larval weight compared to *S. grandiflora* (0.47), but treated check had the least larval weight of 0.29 mg and was determined to be superior to all other treatments on the sixth and seventh day. Both *S. grandiflora* and *S. macrophylla* were statistically comparable on days 8 and 9. On day 10, *S. grandiflora* showed a 0.01 mg weight loss compared to the previous day, and was shown to be superior to *S. macrophylla*, which gained 0.14 mg weight compared to the untreated control (0.89 mg). On day 10, complete larval mortality was seen in the treated check. On day 11, the untreated check had the maximum larval weight (4.96 mg), whereas *S. grandiflora* had the lowest larval weight (1.13 mg), followed by *S. macrophylla* (1.35 mg). On day 12, the larvae entered the pupal stage, but in all other treatments, they were still in the

larval stage, with pupation occurred on day 13 except for *S. grandiflora*, which had a one-day delay in entering the pupal stage (Fig. 2).

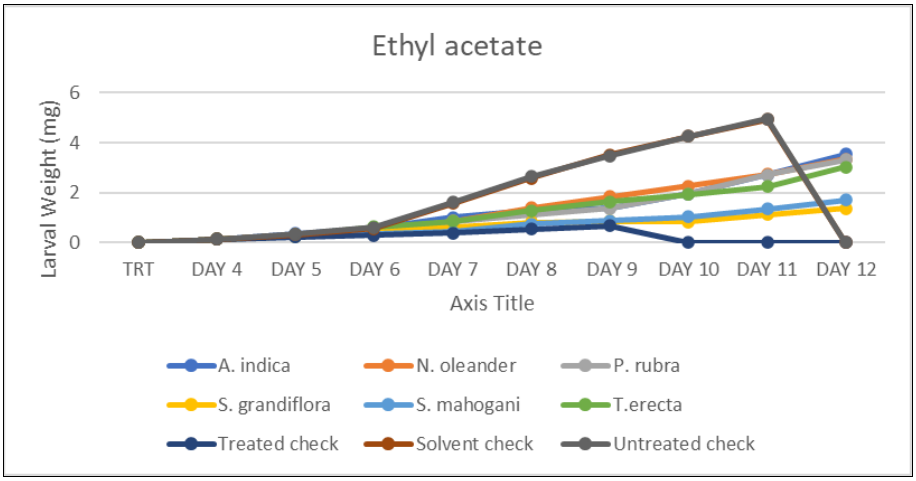

Fig 2: Effect of Ethyl acetate Isolated Bioactive Compounds on the Larval Growth of *P. xylostella*

3. Growth index

The larval growth index in *S. grandiflora* (1.86) and *S. macrophylla* (2.09) was lower than the untreated check when the growth indices were evaluated (9.39) (Table 1). The larval-pupal index ranged from 0.94 to 1.06 for all treatments. The weight index of the pupa ranged from 0.36 to 0.85, while the adult weight index was 0.52 to 0.77. The emergence index for adults ranged from 0.23 to 0.47. The untreated control had a Howe's growth index of 0.13, while the other treatments had a growth index of 0.09 to 0.10. Developmental index ranged from 0.95 to 1.06. Suitability and antibiosis index values were lower in *S. grandiflora* (0.72 and 5.06) and *S. macrophylla* (0.80 and 5.60), respectively.

Table 1: Effect of bioactive compounds isolated using ethyl acetate 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*	3.89	0.97	0.82	0.47	0.74	0.10	1.03	1.14	8.01
T_2-*N. oleander*	2.91	0.94	0.77	0.37	0.62	0.09	1.06	0.97	6.76
T_3-*P. rubra*	3.50	0.94	0.85	0.43	0.77	0.10	1.06	1.09	7.65
T_4-*S. grandiflora*	1.86	1.06	0.36	0.23	0.52	0.09	0.95	0.72	5.06
T_5-*S. macrophylla*	2.09	1.00	0.53	0.27	0.62	0.09	1.00	0.80	5.60
T_6-*T. erecta*	2.97	0.98	0.68	0.37	0.67	0.10	1.02	0.97	6.78
T_7-Solvent check	9.39	1.00	0.99	1.00	0.98	0.13	1.00	2.07	14.49
T_8-Untreated check	9.39	-	-	-	-	0.13	-	-	-

Discussion

The present findings, wherein *S. grandiflora* and *S. macrophylla* ethyl acetate 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) 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) [14] 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) [12], 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), 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. Ethyl acetate, as employed in the present study, preferentially recovers

such mid-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) [6, 12], 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) [10] 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 ethyl acetate 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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