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Efficacy of hexane and ethyl acetate extracts of six plant species on growth and development of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Abstract
The diamondback moth, *Plutella xylostella* (L.), is one of the most economically damaging pests of cruciferous crops worldwide, and its widespread resistance to synthetic insecticides has intensified interest in plant-derived alternatives. In the present study, hexane and ethyl acetate extracts of six plant species *viz*., *Azadirachta indica*, *Nerium oleander*, *Plumeria rubra*, *Sesbania grandiflora*, *Swietenia macrophylla* and *Tagetes erecta* was obtained by Soxhlet extraction and evaluated at 5% concentration for their effects on larval weight gain, pupal weight and adult weight of *P. xylostella* under laboratory conditions, with azadirachtin 1% EC (2 ml/l) as a treated check and solvent and untreated checks for comparison. Both the extracts of six plant species significantly reduced larval, pupal and adult weights relative to the untreated and solvent checks ($p < 0.001$). Among the botanicals, *S. grandiflora* and *S. macrophylla* were consistently the most active comparable to the azadirachtin treated check, whereas their hexane and ethyl acetate extracts produced strong but sub-lethal growth suppression. Ethyl acetate extracts generally produced greater sub-lethal growth suppression than the corresponding hexane extracts for most species. These results indicate that solvent polarity strongly influences the growth- and development-inhibiting potency of these botanical extracts against *P. xylostella*, with ethyl acetate extracts of *S. grandiflora* and *S. macrophylla* emerging as the most promising candidates, approaching the potency of azadirachtin, for further evaluation as components of botanical-based integrated pest management programmes.

Keywords: *Plutella xylostella*, hexane extract, ethyl acetate extract, weight gain, growth inhibition

Introduction
The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is regarded as the most destructive insect pest of cruciferous vegetables globally, causing 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 capacity to develop resistance to virtually every class of synthetic insecticide, including *Bacillus thuringiensis* toxins toxins (Zhao et al. 2006; Pu et al. 2010) [11, 17], have made its management increasingly difficult and have driven 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, 15], 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 determines the class of secondary metabolites recovered: hexane selectively isolates non-polar constituents such as terpenoids, fatty acid derivatives and other lipophilic compounds; 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 and compare the effect of hexane and ethyl acetate extracts of six plant species on larval weight gain, pupal weight and adult weight 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 candidates and solvent systems for future formulation as botanical insecticides.

Materials and Methods
1. 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 and ethyl acetate as extraction solvents. For each solvent, 10 g of powdered plant material was placed in a cellulose thimble and subjected to continuous hot extraction using a Soxhlet apparatus. For hexane extraction, the sample was extracted with hexane for 1–2 days, maintaining the extraction temperature below the boiling point of the solvent, until the solvent in the extraction chamber became visibly clear. Similarly, a separate 10 g portion of the same powdered plant material was extracted independently with ethyl acetate under the same conditions for 1–2 days. 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 and ethyl acetate 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.

2. Preparation of test concentrations and bioassay method

Test solutions of the hexane and ethyl acetate extracts were each 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 onto 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 and ethyl acetate extracts were evaluated as separate trials, each following an identical experimental design in a Completely Randomized Design (CRD) replicated three times.

3. Data recording

Larval weight gain was recorded as the difference between initial larval weight (at treatment) and weight just prior to pupation. Pupal weight was recorded within 24 hours of pupation, and adult weight was recorded within 24 hours of adult emergence, using an electronic balance.

4. Statistical analysis

Data on larval weight gain, pupal weight and adult weight, for the hexane and ethyl acetate trials, 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. Effect of hexane extract on larval, pupal and adult weight

Hexane extracts of the six test plant species, applied at 5% concentration, significantly influenced larval weight gain, pupal weight and adult weight of *P. xylostella* relative to the solvent and untreated checks ($F = 1034.83$, 1516.79 and 468.19 respectively; $p < 0.001$ for all three parameters) (Table 1).

Larval, pupal and adult weights were significantly reduced by all botanical extracts compared with the untreated and solvent checks. For larval weight, the untreated (4.97 mg) and solvent checks (4.69 mg) recorded the highest values and were statistically similar (group f). Among the extracts, *A. indica* (4.02 mg) and *P. rubra* (3.85 mg) recorded higher larval weights (group e), followed by *N. oleander* (3.26 mg) and *T. erecta* (3.27 mg) (group d). *S. macrophylla* (2.10 mg) and *S. grandiflora* (1.23 mg) recorded significantly lower values (groups c and b, respectively). Azadirachtin recorded the lowest larval weight (0.52 mg; group a) and caused 100% mortality within seven days.

A similar trend was observed for pupal weight. The untreated (4.40 mg) and solvent checks (4.32 mg) recorded the highest values (group e), followed by *A. indica* (3.76 mg), *P. rubra* (3.77 mg) and *N. oleander* (3.72 mg). *T. erecta* (3.59 mg) recorded an intermediate value, whereas *S. macrophylla* (2.65 mg) and *S. grandiflora* (2.12 mg) significantly reduced pupal weight (groups b and a, respectively). No pupal weight was recorded for azadirachtin due to complete larval mortality.

Adult weight was highest in the untreated (2.36 mg) and solvent checks (2.25 mg) (group d). *A. indica* (1.79 mg), *P. rubra* (1.87 mg) and *T. erecta* (1.84 mg) formed the intermediate group (c), while *N. oleander* (1.64 mg) and *S. macrophylla* (1.54 mg) recorded lower weights (group b). *S. grandiflora* produced the lowest adult weight among the botanical treatments (1.35 mg; group a). No adult weight was recorded in the azadirachtin treatment due to complete larval mortality.

Table 1: Effect of hexane extracts of six plant species on larval weight gain, pupal weight and adult weight of Plutella xylostella

Treatments	Dose (%)	Larval weight gain* (mg)	Pupal weight* (mg)	Adult weight* (mg)
T1 - A. indica	5	4.02±0.02 (2.13)e	3.76±0.02 (2.06)d	1.79±0.01 (1.51)c
T2 - N. oleander	5	3.26±0.03 (1.94)d	3.72±0.03 (2.05)cd	1.64±0.01 (1.46)b
T3 - P. rubra	5	3.85±0.02 (2.09)e	3.77±0.02 (2.07)d	1.87±0.01 (1.54)c
T4 - S. grandiflora	5	1.23±0.01 (1.32)b	2.12±0.01 (1.62)a	1.35±0.01 (1.36)a
T5 - S. macrophylla	5	2.10±0.02 (1.61)c	2.65±0.01 (1.78)b	1.54±0.02 (1.43)b
T6 - T. erecta	5	3.27±0.01 (1.94)d	3.59±0.01 (2.02)c	1.84±0.02 (1.53)c
T7 - Treated check	2 ml/l	0.52±0.01 (1.01)a	-	-

(Azadirachtin 1%)				
T8 - Solvent check	-	4.69±0.02 (2.28)f	4.32±0.01 (2.19)e	2.25±0.02 (1.66)d
T9 - Untreated check	-	4.97±0.01 (2.34)f	4.40±0.01 (2.21)e	2.36±0.01 (1.69)d
SEd	-	0.019	0.017	0.019
F-value	-	1034.831	1516.790	468.191
p (significance)	-	0.000	0.000	0.000

*Mean values of three replications are represented as mean ± standard deviation; figures in parentheses are square root transformed values; #100% mortality of larvae was observed within 7 days of treatment, hence pupal and adult weights could not be recorded for T7; within a column, means followed by the same letter are not significantly different from each other (DMRT; F-test; $p \leq 0.05$; n = 10); SEd: Standard error of the difference.

2. Effect of ethyl acetate extract on larval, pupal and adult weight

Ethyl acetate extracts of the same six plant species, applied at 5% concentration, also significantly influenced larval weight gain, pupal weight and adult weight of *P. xylostella* relative to the solvent and untreated checks (F = 1023.45, 1392.64 and 583.02 respectively; $p < 0.001$ for all three parameters) (Table 2).

Larval, pupal and adult weights were significantly reduced by all botanical extracts compared with the untreated and solvent checks. For larval weight, the untreated (4.81 mg) and solvent checks (4.79 mg) recorded the highest values (group g). Among the extracts, *A. indica* (3.39 mg) recorded the highest weight gain (group f), followed by *N. oleander* (3.19 mg) and *P. rubra* (3.17 mg) (group e). *T. erecta* (2.89 mg), *S. macrophylla* (1.55 mg) and *S. grandiflora* (1.24 mg) recorded progressively lower values (groups d, c and b, respectively). Azadirachtin recorded the lowest larval weight (0.53 mg; group a) and caused 100% mortality within seven days.

A similar trend was observed for pupal weight. The untreated (4.32 mg) and solvent checks (4.28 mg) recorded the highest values (group f), followed by *P. rubra* (3.67 mg) and *A. indica* (3.53 mg) (group e). *N. oleander* (3.32 mg) and *T. erecta* (2.95 mg) recorded intermediate values, whereas *S. macrophylla* (2.28 mg) and *S. grandiflora* (1.54 mg) produced the lowest pupal weights (groups b and a, respectively). No pupal weight was recorded for azadirachtin due to complete larval mortality.

Adult weight was highest in the untreated (2.35 mg) and solvent checks (2.29 mg) (group e). *A. indica* (1.74 mg) and *P. rubra* (1.80 mg) formed the next group (d), followed by *T. erecta* (1.56 mg) (group c). *N. oleander* and *S. macrophylla* recorded similar weights (1.46 mg), while *S. grandiflora* produced the lowest adult weight (1.22 mg; group a). No adult weight was recorded for azadirachtin owing to complete larval mortality.

Table 2: Effect of ethyl acetate extracts of six plant species on larval weight gain, pupal weight and adult weight of Plutella xylostella

Treatments	Dose (%)	Larval weight gain* (mg)	Pupal weight* (mg)	Adult weight* (mg)
T1 - A. indica	5	3.39±0.01 (1.97)f	3.53±0.02 (2.01)e	1.74±0.01 (1.50)d
T2 - N. oleander	5	3.19±0.02 (1.92)e	3.32±0.02 (1.96)d	1.46±0.02 (1.40)b
T3 - P. rubra	5	3.17±0.02 (1.91)e	3.67±0.02 (2.04)e	1.80±0.01 (1.52)d
T4 - S. grandiflora	5	1.24±0.01 (1.32)b	1.54±0.02 (1.43)a	1.22±0.01 (1.31)a
T5 - S. macrophylla	5	1.55±0.01 (1.43)c	2.28±0.01 (1.67)b	1.46±0.01 (1.40)bc
T6 - T. erecta	5	2.89±0.02 (1.84)d	2.95±0.02 (1.86)c	1.56±0.01 (1.44)c
T7 - Treated check (Azadirachtin 1%)	2 ml/l	0.53±0.01 (1.01)a	-	-
T8 - Solvent check	-	4.79±0.02 (2.30)g	4.28±0.01 (2.19)f	2.29±0.01 (1.67)e
T9 - Untreated check	-	4.81±0.02 (2.31)g	4.32±0.01 (2.20)f	2.35±0.01 (1.69)e
SEd	-	0.019	0.017	0.016
F-value	-	1023.45	1392.64	583.02
p (significance)	-	0.00	0.00	0.00

*Mean values of three replications are represented as mean ± standard deviation; figures in parentheses are square root transformed values; #100% mortality of larvae was observed within 7 days of treatment, hence pupal and adult weights could not be recorded for T7; within a column, means followed by the same letter are not significantly different from each other (DMRT; F-test; $p \leq 0.05$; n = 10); SEd: Standard error of the difference.

3. Comparison across the two solvent systems

Comparison of Tables 1 and 2 shows a clear overall trend of increasing growth-suppressive and, for the most active species, outright toxic potency with increasing solvent polarity: hexane (non-polar) < ethyl acetate (mid-polar). Ethyl acetate extracts generally produced greater suppression of larval, pupal and adult weight than the corresponding hexane extracts for most species, particularly *A. indica*, *N. oleander*, *S. macrophylla* and *T. erecta*, while *P. rubra* performed comparably in both solvents. This indicates that the most potent bioactive constituents of *S. grandiflora* and *S. macrophylla* are of relatively high polarity and are concentrated, or activated, primarily in the ethyl acetate fraction. *T. erecta* likewise showed its strongest activity in ethyl acetate. In contrast, *A. indica*, *N. oleander* and *P. rubra* showed comparatively modest differences among the two solvents.

Discussion

The significant reduction in larval weight gain, pupal weight and adult weight recorded across hexane and ethyl acetate extract treatments, relative to the solvent and untreated checks, indicates that bioactive fractions of these six plant species interfere with normal larval feeding, nutrient utilization and/or the endocrine processes governing growth and metamorphosis in *P. xylostella*. Reduced weight gain under sub-lethal botanical treatment is commonly attributed to antifeedant activity, digestive enzyme inhibition, or disruption of hormonal regulation of growth, whereas outright larval mortality, as observed with the methanol

extracts of *S. grandiflora* and *S. macrophylla*, points to a more direct toxic mode of action. The current findings are consistent with (Sangavi and Edward 2017) [12] and (Meenambigai 2019) [6], who found that *S. grandiflora* leaf extract had insecticidal effect against *P. xylostella*. *S. grandiflora* aqueous leaf extracts of 10 per cent concentration was found to be superior in acaricidal action on red spider mite, *Tetranychus urticae* with highest mortality (97.77%) at 72 HAT (Premalatha 2018) [10].

The escalating potency of *S. grandiflora* and *S. macrophylla* with increasing solvent polarity — from strong sub-lethal growth suppression in hexane to outright larval mortality in ethyl acetate is consistent with these species being reported sources of saponins and related glycosides, This pattern suggests that the principal insecticidal principles in these two species reside in a relatively polar fraction, and that hexane or ethyl acetate extraction alone, as used in many prior screening studies, may substantially underestimate their true bioactivity. The comparatively modest solvent-dependence of A. indica, *N. oleander* and *P. rubra* suggests that their principal bioactive constituents are more evenly distributed across the polarity range, or are already substantially captured in the non-polar and mid-polar fractions. The findings of our study accord with those of Hussain and Kumaresan (2014) [2] and Larkem *et al.*, (2021) [5].

Taken together, these findings support solvent polarity as a key determinant of the growth-regulatory and toxic potency of botanical extracts against *P. xylostella*, and indicate that a polarity-graded extraction and screening approach, as used in the present study, is valuable for identifying both the most promising plant species and the most effective solvent system for each. The consistent ranking of treatment effects across larval, pupal and adult weight parameters, and across all three solvent systems, strengthens confidence that the observed effects reflect genuine developmental disruption or lethality rather than measurement artefacts. Plant metabolites also reduce larval and pupae survival rates, as well as adult emergence (Koul *et al.*, 2008) [4]. Many botanical pesticides have been shown to affect the developmental period, growth, and adult emergence of insects (Shaalan *et al.*, 2005) [14].

Conclusion

Hexane and ethyl acetate extracts of all six plant species tested significantly reduced larval weight gain, pupal weight and adult weight of *P. xylostella* compared with untreated and solvent controls, confirming their potential as sources of botanical growth-disrupting and, in some cases, directly toxic agents. Potency generally increased with solvent polarity, and the ethyl acetate extracts of *S. grandiflora* and *S. macrophylla* stood out by causing complete larval growth inhibition comparable to the azadirachtin standard, while *T. erecta* showed its strongest sub-lethal activity. These results support further chemical characterization, particularly of the ethyl acetate fractions of *S. grandiflora*, *S. macrophylla* and *T. erecta* and field-level evaluation of these extracts as candidate components of integrated pest management programmes for *P. xylostella* on cruciferous crops.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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