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RESEARCH ARTICLE

Antifeedant and Insecticidal Effect of Hexane Botanical Extracts on Diamondback moth (DBM), *Plutella xylostella* L.. (Lepidoptera: Plutellidae)

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ABSTRACT

Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is one of the nefarious pests of cruciferous crops. Crude extracts from six botanicals obtained using hexane by continuous hot percolation process in Soxhlet apparatus were evaluated for their effect on larval mortality, antifeedant, growth and development of second instar larvae of DBM. The results revealed that the antifeedant index of *Sesbania grandiflora* 5% was 20.82% followed by *Swietenia macrophylla* 5%, which had 15.61%. The larval mortality and adult emergence exhibited by *S. grandiflora* was (66.67% and 33.33% respectively) after 72 h of feeding on treated leaf. It was statistically on par with *S. macrophylla*, which had 63.33% larval mortality and 36.67% adult emergence. With regard to the developmental period of life stages, no significant difference was observed among the treatments. However, all the treatments were significantly superior over untreated check in prolonging the developmental period of DBM. It was concluded that the *S. grandiflora* and *S. macrophylla* hexane leaf extract 5% are promising botanicals against *P. xylostella*, as they possess insecticidal, antifeedant and growth inhibitory activity. These results open up the scope for further isolation of bioactive compounds and validation under field conditions, which would lead to formulation

development, ultimately it can be incorporated as ecofriendly component in the integrated pest management strategies.

KEYWORDS: Diamondback moth; Hexane; Botanicals; Soxhlet extraction; Antifeedant, Toxicity, Growth inhibitory

INTRODUCTION

India is the second largest producer of cruciferous vegetables in the world. They are prone to infestation by several insect pests; among them, the Diamondback moth (DBM) is the most destructive pest (Fletcher, 1914). The heavy population of this insect can even inflict more than 90% crop loss. Management of DBM costs US$0.77 billion annually (Li *et al*., 2016). Majority of the cabbage farmers use insecticides like quinalphos, chlorpyriphos, profenophos, cypermethrin, lambda-cyhalothrin, chlorantraniliprole and flubendiamide either by calendar-based spraying or routine spraying at an interval of 6 to 10 days for 6 to 8 times (Deore *et al*., 2017). It is well known that DBM has developed resistance to synthetic insecticides, which led to insecticide resistance, pest outbreaks, undesirable environmental effects (Negahban *et al*., 2006). Botanical-based products are environmentally safe alternatives in the place of harmful chemicals. They are an inexhaustible source of structurally diverse biologically active substances and approximately 1800 plants with insecticidal properties were reported by Grainge *et al*. (1984). Bioactive compounds in plants have complex combination of behavioural and physiological effects and make the insects difficult to evolve their resistance. *Sesbania grandiflora* 10% aqueous leaf extract was reported to possess insecticidal activity against *P. xylostella* (Sangavi and Edward, 2017). With this background, the laboratory screening of hexane extracts of six different botanicals was done against *P. xylostella* larva, to evaluate their effect on larval mortality, antifeedant index, growth and development.

MATERIAL AND METHODS

The laboratory experiment was conducted to evaluate the effect of six botanicals against *P. xyllostella* at Natural Pesticides Laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute (AC&RI), Tamil Nadu Agricultural University (TNAU), Madurai during February 2021. The botanicals selected for the evaluation were unripen fruits of neem, *Azadirachta indica* (Meliaceae); oleander leaves, *Nerium oleander* (Apocynaceae); plumeria leaves, *Plumeria rubra,* (Apocynaceae); humming bird tree leaves, *Sesbania grandiflora* (Fabaceae); mahogany leaves, *Swietenia macrophylla* (Meliaceae); marigold leaves, *Tagetus erecta* (Asteraceae).

Preparation of botanical extracts

The botanicals were collected from the fields of AC & RI, Madurai, Tamil Nadu. The collected plant samples were shade dried for 15 days and powdered using mechanical blender; Sieve no. 40. was used to achieve uniform size fine powder, which was stored in amber colored bottles to prevent sunlight exposure. The hexane extract was obtained by continuous hot percolation process in the Soxhlet apparatus. Powdered botanical samples (10g) were packed in cellulose thimble and subjected to extraction with hexane, a non-polar solvent, at 45^{o}C for 8 hours, i.e., until the solvent extracts of all the bioactive compounds became completely and visibly clear. The temperature was adjusted below the boiling point of hexane. The solvent extract was filtered through Buchner funnel using Whatman No.1 filter paper and condensed in Rotary Flash Evaporator at 45^{o}C under reduced pressure to obtain the crude extract, which was weighed, to estimate the recovery percentage of each botanical (Larkem *et al.*, 2021).

Evaluation of insecticidal, antifeedant and growth inhibitory effect of botanicals

A laboratory bioassay was carried out under no-choice condition by the standard leaf disc dip method. Fresh leaves were collected from cauliflower plants grown without any insecticides spray, under controlled conditions. The leaves were washed with distilled water and leaf discs were cut (5 cm dia) followed by air drying for 30min. The leaf discs were dipped in 5% test solution for about 30 seconds, to facilitate uniform treatment of active ingredients. The leaf discs were slantly placed for about 2 minutes in a tray containing blotting paper to remove excess solution and was air dried about 30 min. at room temperature (Ingle *et al*., 2017). Second instar larvae (10 nos. Pre-starved) were released on each treated disc in a plastic container lined with moist filter paper. There were nine treatments including solvent check, standard check (Azadirachtin 10,000ppm @ 2mL/lt.) and untreated check which was replicated thrice. The larvae were allowed to feed on the treated leaf disc daily and observations were recorded until adult emergence.

Antifeedant index (AI) was estimated using the formula, AFI = [(C-T)/(C+T)]*100 Where, C= Leaf area consumed in control; T= Leaf area consumed in treatment (Sadek, 2003) by the observations taken on leaf area fed after 24, 48 and 72 hours of treatment. At every 24hrs interval larvae were observed for any

mortality and malformations until adult emergence. Larval and pupal developmental period, adult life span number of pupa and adults who emerged were monitored and recorded. Per cent larval mortality and adult emergence were estimated.

Data analysis

Recorded data was subjected to arc sine and square root transformation and was statistically analyzed using SPSS 22 version software. Grouping was done by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The recovery yields (%) of the test botanicals ranged from 6.20 to 8.92 per cent. The recovery per cent is presented in descending order *viz*., *P. rubra* (8.92%), *A. indica* (8.42%), *S. macrophylla* (7.90%), *N. oleander* (7.85%), *T. erecta* (7.34%) and *S. grandiflora* (6.20%). Rohmah *et al*., (2020) reported that the n-hexane extracts of *S. grandiflora* leaves yield 3.43% of dry weight.

Antifeedant effect of hexane extracts from botanicals

The result of leaf disc dip bioassay on antifeedant activity is presented in Fig. 1. When the larvae were exposed to *S. grandiflora* 5% treated leaf, the area of leaf fed by the larva was minimum, with antifeedant index of 20.82% after 72hrs. At the same time, Azadirachtin 10000 ppm (treated check) at 2mL/lt. registered 34.97% antifeedant index. The antifeedant activity declined over time from 24 h to 72 h. Sangavi and Edward (2017) also reported that *S. grandiflora* 10% aqueous leaf extracts showed 52.31% antifeedant activity on *P. xylostella* after two days of treatment. The next best treatment was *S. macrophylla* which exhibited moderate antifeedant activity (15.61%). Moghadamtousi *et al*. (2013) reported that *S. macrophylla* ethyl acetate extracts of seeds showed good antifeedant activity against fourth instar larvae of *Spodoptera frugiperda.*

The class of compounds which impede insect feeding are antifeedants. They are not intended for direct killing of the insects, but when the insect does not feed, ultimately it results in the death of insects. The secondary metabolites exhibiting antifeedant activity are grouped into four major classes *viz*., sesquiterpene lactones, heterogeneous flavonoids, quassins, and limonoids. They degrade rapidly after application; hence they do not / cause little impact on the environment (Li *et al*., 2005). Bahera *et al*. (2012) and Wagh *et al*. (2009) reported the phytochemical profile of *S. grandiflora* as alkaloids, flavonoids, saponins, glycosides, cardiac glycosides, tannins and phenols. Reed (1994) reported that the high content of condensed tannins affects palatability; hence the presence of condensed tannins in *S. grandiflora* might have been responsible for their antifeedant activity. According to Akhtar *et al*. (2008), the insecticidal activity of plant derived extracts showed the antifeedant activity against most of the lepidopteran insects.

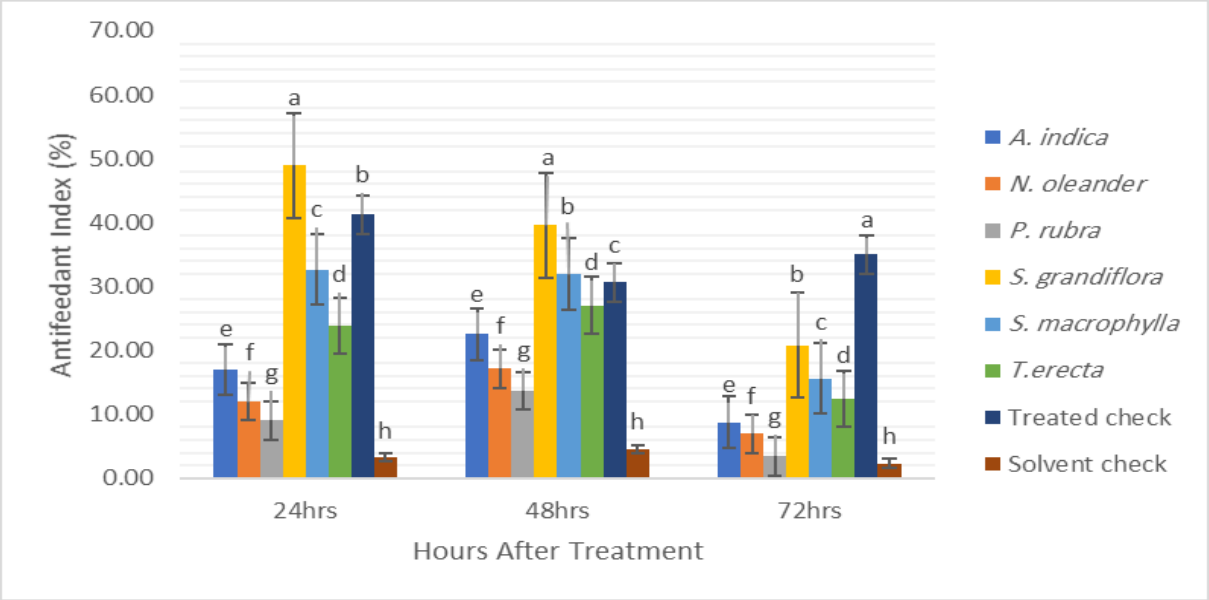

Fig1. Antifeedant effect of hexane extracts from botanicals against *P. xylostella*

Insecticidal and growth inhibitory effect from hexane extracts of botanicals

The developmental period of life stages, larval mortality and adult emergence percentage of *P. xylostella* was recorded and presented in table 1. The treated check with azadirachtin 10000ppm at 2mL/lt. left no larvae alive. The highest larval mortality percentage was observed in *S. grandiflora* (66.67%)

and *S. macrophylla* (63.33%). These findings are supported by the report of Elango *et.al.,* (2011). Wagh *et al*. (2009) reported that *S. grandiflora* contains plenty of secondary metabolites viz., sterols, saponins and tannins, responsible for its insecticidal property. Ravikumar (2010) also reported that the phytotoxin, alpha terthienyl from marigold exhibited extreme insecticidal property against mosquitoes but not on non-target organisms. Further, the hexane extracts of *T. erecta* showed better insecticidal properties against aphids and Fall Army worm, *S. frugiperda*.

Hussain and Kumaresan (2014) reported the GC-MS analysis results as the methanolic leaf extract of *S. grandiflora* were mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. Palmitic acid (11.8%), 9-hexadecenol (9.0%) and Octadecanoic acid were the major compounds in *S. grandiflora* that possessed pesticidal activities (Gopalakrishnan & Vadivel, 2011; Geetha *et al.*, 2013).

Table 1. Impact of plant extracts obtained using hexane on developmental period, larval mortality and adult emergence percentage in *P. xylostella.*

Treatments	Mean developmental period (days)*			Cumulative larval mortality (%)$	Adult emergence (%)$
	Larva	Pupa	Adult life span		
T_1-*A. indica*	12.33+0.11 (3.58)^{a}	4.67±0.11 (2.27)^{bc}	6.33±0.11 (2.61)^{c}	43.33±0.11 (41.15)^{f}	56.67±0.11 (48.85)^{f}
T_2-*N. oleander*	12.67±0.11 (3.63)^{a}	4.67±0.11 (2.27)^{bc}	5.33±0.11 (2.41)^{a}	53.33±0.11 (46.92)^{de}	46.67±0.11 (43.08)^{de}
T_3-*P. rubra*	12.33±0.11 (3.58)^{a}	4.00±0.00 (2.12)^{cd}	6.67±0.11 (2.68)^{c}	46.67±0.11 (43.08)^{ef}	53.33±0.11 (46.92)^{ef}
T_4-*S. grandiflora*	13.33±0.11 (3.72)^{a}	5.67±0.11 (2.48)^{a}	5.33±0.11 (2.41)^{a}	66.67±0.11 (54.78)^{b}	33.33±0.11 (35.22)^{b}
T_5-*S. macrophylla*	13.33±0.11 (3.72)^{a}	5.00±0.00 (2.35)^{ab}	5.67±0.11 (2.48)^{b}	63.33±0.11 (52.78)^{bc}	36.67±0.11 (37.22)^{bc}
T_6-*T. erecta*	12.33±0.11 (3.58)^{a}	5.33±0.11 (2.41)^{ab}	6.33±0.11 (2.61)^{c}	56.67±0.11 (48.85)^{cd}	43.33±0.11 (41.15)^{cd}
T_7-Treated check# (Azadirachtin 1%)	-	-	-	100.00±0.00 (89.09)^{a}	0.00±0.00 (0.91)^{a}
T_8-Solvent check	10.67±0.11 (3.34)^{b}	3.67±0.11 (2.04)^{d}	8.33±0.11 (2.97)^{d}	0.00±0.00 (0.91)^{g}	100.00±0.00 (89.09)^{g}
T_9-Untreated check	10.33±0.11 (3.29)^{b}	3.67±0.11 (2.04)^{d}	8.33±0.11 (2.97)^{d}	0.00±0.00 (0.91)^{g}	100.00±0.00 (89.09)^{g}
SEd	0.062	0.087	0.019	2.253	2.253

 # 100% mortality of larvae observed after 7 days after treatment, hence developmental period was not presented

Mean values of three replications are represented as mean ± standard deviation; *Figures in the parentheses are square root transformed values ($\sqrt{x} + 0.5$); $Figures in the parentheses are arc sine transformed values (x+0.5); In a column, the mean followed by the same letter are not significantly different from each other, DMRT ($p \leq 0.05$); SEd: Standard Error of the difference.

Adult emergence was observed in all the treatments with no pupal mortality and malformations. There was no significant difference among the treatments regarding the developmental period (days) of different life stages but all the treatments were significantly superior over the untreated check. There is evidence for the reduction in growth and fecundity when the insect feeds on the dietary tannins. Normally tannins bind with the proteins, hence, they affect insect growth and development, thereby reducing the nutrient absorption efficiency resulting in growth retardation. As *S. grandiflora* and *S. macrophylla* are evidenced to possess tannins, this might be the reason for the growth inhibitory effect, which would have resulted in prolongation of the developmental period and mortality.

CONCLUSION

Knowledge of this study provides information about plants having anti-insect activity. It is concluded that *S. grandiflora* and *S. macrophylla* plant hexane extracts have the potential to develop new and safe control products for *P. xylostella.* As naturally occurring insecticides, it could be used as an alternative for synthetic pesticides, since it is an eco-friendly and sustainable insecticide.

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