Indian Journal of Entomology 84(3): 659-662 (2022) DoI No.: 10.55446/IJE.2021.115

EFFICACY OF ETHYL ACETATE EXTRACTS OF BOTANICALS ON DIAMOND BACK MOTH *PLUTELLA XYLOSTELLA* L.

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ABSTRACT

In the present study, ethyl acetate extracts of six botanicals were extracted by continuous hot percolation process in Soxhlet apparatus and evaluated to study the antifeedant, insecticidal and the growth and development inhibitory activity on second instar larvae of diamond back moth *Plutella xylostella* L. The results showed that the *Sesbania grandiflora* at 5% exhibited maximum antifeedant index (34.27%) followed by *Swietenia macrophylla* 5% (28.91%). Extracts of these plants were also found to be the most effective against larvae giving 76.67 and 23.33% mortality, respectively. The adult emergence was 23.33 and 26.67%, respectively with these, while developmental period did not reveal any significant differences.

Key words: *Plutella xylostella*, ethyl acetate, *Sesbania grandiflora, Swietenia macrophylla,* botanicals, soxhlet extraction, antifeedant, toxicity, growth inhibition, developmental period

Cruciferous vegetables are infested by a number of insect pests, of which the diamond back moth (DBM) *Plutella xylostella* L. is the most destructive (Tamilselvan et al., 2021). Prophylactic management of vegetable crop pests with insecticides is estimated to cost US$1.4 billion worldwide, if yield losses are included it rises to US$ 2.7 billion (Furlong et al., 2013). In India, 50 to 80% yield loss in the marketable yield was observed due to *P. xylostella* in cabbage (Sandur, 2004; Ayalew, 2006), and it is known for its ability to develop resistance to insecticides (APRD, 2020). Use of pesticides in agriculture has led to resistance, outbreaks, resurgence, and such undesirable environmental effects (Negahban et al., 2006). To prevent the development of resistance, a combination of insecticides having different mechanisms of action should be used in rotation. In field populations, *P. xylostella* has developed resistance against nearly 95 conventional insecticides (APRD, 2020), including new chemistry insecticides such as cyantraniliprole in Australia (Evans, 2008), Brazil (Ribeiro et al., 2017), China (Qin et al., 2018) and Japan (Jouraku et al., 2020). Hence, the effective natural plant products can be safely incorporated as ecofriendly alternative to insecticides (Naz et al., 2018). Plants are a virtually inexhaustible source of structurally diverse biologically active substances and approximately 1800 plants possess insecticidal properties (Grainge et al., 1984). The complex combination of behavioural and physiological actions contained in these plant compounds makes it difficult for insects to evolve resistance. Sangavi and Edward (2017) reported that 10% aqueous leaf extract of *S. grandiflora* showed larval mortality in *P. xylostella*. *Nerium oleander* L. (stem, leaves and flowers) 70% hydroethanolic extract decreased the larval and pupal weight of pink boll worm *Pectinophora gossypiella* (Moustafa et al., 2018). In the present study, screening of ethyl acetate extracts of botanicals was done to evaluate their efficacy as antifeedant, insecticidal and inhibitor of growth and development on *P. xylostella*. As ethyl acetate is a commercially available solvent and approved for botanical extraction, which helps in isolation of desired compounds and provided higher purity, it has been used (Pintac et al., 2018).

MATERIALS AND METHODS

The plant parts of six botanicals viz., unripen fruits, *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) were collected from fields of Agricultural College and Research Institute (AC&RI), Madurai, Tamil Nadu. The laboratory experiment was conducted at the Department of Agricultural Entomology, AC&RI, Madurai. The collected leaves and unripen fruits of the botanicals were shade dried (15 days), powdered in mechanical blender and passed through sieve (no. 40). The powder thus obtained was stored in amber-coloured bottles, to prevent the exposure to sunlight. The ethyl acetate extract was taken from 10 g powder by continuous hot percolation technique in Soxhlet apparatus (Larkem et al., 2021). The resultant extract was filtered (Buchner funnel using Whatman No.1 filter paper), condensed (Rotary Flash Evaporator (Evator) at 45°C) and weighed, to estimate the recovery.

The bioassay was carried out based on leaf dip method under no choice condition (Ingle et al., 2017). There were nine treatments including solvent and standard checks (azadirachtin 10,000 ppm @ 2ml/ l), and untreated check, and replicated thrice. The larvae were allowed to feed on 5% treated leaf disc until pupation, to understand the impact of botanicals on the growth and development of *P. xylostella* (Baskar et al., 2011). The leaf area consumed by the larvae was measured after 24, 48 and 72 hr of treatment and antifeedant index estimated (Sadek, 2003). Larvae were observed for mortality, if any, malformations and developmental period, pupal duration and mortality, adult emergence and lifespan. Data obtained was subjected to arc sine and square root transformation and then statistically analyzed using SPSS 22 version (IBM Crop, 2013) software. Grouping was done by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results on extraction yields of the plant samples using ethyl acetate revealed that it is in the descending order as: *A. indica* (9.81%), *N. oleander* (9.42%), *S. macrophylla* (8.24%), *S. grandiflora* (7.27%), *P. rubra* (6.13%) and *T. erecta* (5.54%). The antifeedant efficacy of given in Table 1 reveal that larvae fed with *S. grandiflora* treated leaves exhibited the least area of feeding- antifeedant index being 46.74, 42.98 and 34.27% at 24, 48 and 72 hr after treatment, respectively;

Table 1. Effect of ethyl acetate extracts of botanicals on *P. xylostella.*

Treatments	Antifeedant Index (AI)$			Mean developmental period (days)*			Cumulative larval mortality (%)$	Adult emergence (%)$
	24 h	48 h	72 h	Larva	Pupa	Adult life span		
T_1-*A. indica*	26.74± 0.09 (31.14)e	24.07± 0.04 (29.38)^{f}	14.11± 0.19 (22.06)^{f}	12.00± 0.00 (3.46)^{b}	4.33± 0.11 (2.08)^{bc}	6.00± 0.19 (2.45)^{b}	53.33± 0.64 (46.92)^{d}	46.67± 0.64 (43.08)^{d}
T_2-*N. oleander*	31.38± 0.05 (34.07)^{d}	27.70± 0.05 (31.76)^{e}	15.80± 0.08 (23.43)^{e}	12.67± 0.11 (3.61)^{b}	4.67± 0.11 (2.16)^{b}	5.67± 0.11 (2.38)^{b}	63.33± 0.67 (52.78)^{c}	36.67± 0.67 (37.22)^{c}
T_3-*P. rubra*	20.19± 0.05 (26.70)^{f}	18.67± 0.10 (25.61)^{g}	11.40± 0.06 (19.73)^{g}	12.33± 0.11 (3.46)^{b}	3.67± 0.11 (1.91)^{bc}	7.00± 0.19 (2.65)^{bc}	56.67± 0.64 (48.85)^{d}	43.33± 0.64 (41.15)^{d}
T_4-*S. grandiflora*	46.74± 0.19 (43.13)^{a}	42.98± 0.08 (40.96)^{b}	34.27± 0.06 (35.84)^{b}	12.67± 0.11 (3.61)^{b}	4.67± 0.11 (2.16)^{b}	5.67± 0.11 (2.38)^{b}	76.67± 0.74 (61.22)^{b}	23.33± 0.74 (28.78)^{b}
T_5-*S. macrophylla*	43.11± 0.10 (41.04)^{b}	35.74± 0.07 (36.72)^{c}	28.91± 0.05 (32.53)^{c}	12.67± 0.11 (3.61)^{b}	4.33± 0.11 (2.08)^{bc}	6.00± 0.19 (2.45)^{b}	73.33± 0.74 (59.00)^{b}	26.67± 0.74 (31.00)^{b}
T_6-*T. erecta*	40.28± 0.09 (39.40)^{c}	29.96± 0.07 (33.19)^{d}	24.96± 0.06 (29.98)^{d}	12.33± 0.11 (3.46)^{b}	3.67± 0.11 (1.91)^{bc}	6.33± 0.11 (2.52)^{bc}	66.67± 0.67 (54.78)^{c}	33.33± 0.67 (35.22)^{c}
T_7-Treated check# (Azadirachtin 1%)	44.37± 0.22 (41.77)^{b}	51.15± 0.09 (45.66)^{a}	42.32± 0.07 (40.59)^{a}	0.00± 0.00 (0.71)^{a}	0.00± 0.00 (0.71)^{a}	0.00± 0.00 (0.71)^{a}	100± 0.00 (89.09)^{a}	0.00± 0.00 (0.91)^{a}
T_8-Solvent check	3.03± 0.13 (9.99)^{g}	2.21± 0.12 (8.50)^{h}	3.11± 0.16 (10.10)^{h}	10.67± 0.11 (3.32)^{c}	3.33± 0.11 (1.83)^{c}	7.67± 0.11 (2.77)^{c}	0.00± 0.00 (0.91)^{e}	100± 0.00 (89.09)^{e}
T_9-Untreated check	-	-	-	10.67± 0.11 (3.32)^{c}	3.33± 0.11 (1.83)^{c}	7.67± 0.11 (2.77)^{c}	0.00± 0.00 (0.91)^{e}	100± 0.00 (89.09)^{e}
SEd	0.426	0.387	0.477	0.056	0.106	0.112	2.368	2.368
F-value	1344.57	1689.78	849.44	526.26	41.29	69.08	287.631	287.631
P (significance)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Mean values of three replications represented as mean ± standard deviation; # 100% mortality of larvae observed after 7 days of treatment, hence developmental period was not presented. *Figures in parentheses square root transformed values ($\sqrt{x+0.5}$); $Figures in parentheses arc sine transformed values (x+0.5); In a column, mean followed by same letter not significantly different from each other, DMRT (F-test; $p \leq 0.05$; n=10); SEd: Standard error of the difference.

while with *S. macrophylla* it was 43.11, 35.7 and 28.91%, respectively, and it was statistically on par with the treated check, azadirachtin 10000 ppm @ 2ml/ l after 24 hr. The antifeedant activity declined over time from 24 to 72 hr. This observation is in agreement with that of Sangavi and Edward (2017), who reported that *S. grandiflora* 10% aqueous extracts showed 52.31% antifeedant activity after two days on *P. xylostella*. Moghadamtousi et al. (2013) reported that ethyl acetate extracts of *S. macrophylla* seeds showed good antifeedant activity against fourth instar larvae of *Spodoptera frugiperda*. Phytochemical evaluation of *S. grandiflora* showed the presence of different secondary metabolites viz., alkaloids, flavonoids, saponins, glycosides, cardiac glycosides, tannins and phenols (Bahera et al., 2012). The presence of condensed tannins in the *S. grandiflora* extract may be responsible for unpalatability of treated surface (Reed, 1994).

Developmental period of life stages, larval mortality and adult emergence details given in Table 1 reveal complete larval mortality in the treated check, azadirachtin 10000 ppm @ 2 ml/ l, which did not exhibit any phytotoxicity symptoms; among the botanicals evaluated, maximum mortality was in *S. grandiflora* (76.67%), which was statistically on par with that of *S. macrophylla* (73.33%). Similar findings were reported by Elango et al. (2011) on *S. grandiflora* that ethyl acetate leaf extract 0.01% showed 34% larval mortality of *Anopheles subpictus*. Wagh et al. (2009) observed that *S. grandiflora* contains plenty of saponins, sterols and tannins, which might be responsible for its insecticidal property. Hussain and Kumaresan (2014) with GC-MS analysis of *S. grandiflora* leaves methanolic extracts revealed that these mainly composed of oxygenated hydrocarbons and phenolic hydrocarbons. Palmitic acid (11.8%), 9-hexadecenol (9.0%) and octadecanoic acid were the major compounds having pesticidal activities (Gopalakrishnan and Vadivel, 2011; Geetha et al., 2013; Mishra et al., 2021). No pupal mortality and malformations were recorded in these treatments. There was no significant difference among the treatments regarding the developmental period (days) of life stages but all the treatments were significantly superior over untreated check. It is concluded that *S. grandiflora* and *S. macrophylla* ethyl acetate plant extracts have potential against *P. xylostella.*

ACKNOWLEDGEMENTS

The authors thank the Department of Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu Agricultural University for providing facilities.

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Manuscript Received: August, 2021; Revised: October, 2021;
Accepted: October, 2021; Online Published: November, 2021)
Online published (Preview) in www.entosocindia.org Ref. No. e21190