Antifeedant effect of emulsifiable concentrate formulation of *Ageratum conyzoides* (L.) (Asteraceae) extracts against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Abstract

The diamondback moth (DBM), Plutella xylostella, is a highly destructive cosmopolitan pest of cruciferous crops, causing severe economic losses worldwide. The research work aimed to evaluate the antifeedant activity of methanolic extracts of Ageratum conyzoides leaves and its emulsifiable concentrate (EC) formulations against diamondback moth larvae in both no-choice and two-choice bioassays under standard laboratory conditions. In both no-choice and two-choice bioassays, a concentration-dependent reduction in feeding was observed when cauliflower leaf discs were treated with methanolic extract and its EC formulation was offered to the fourth-instar DBM larvae. The antifeedant index (AFI) values were positively correlated with different concentrations of crude extracts (R^2 = 0.992) and EC formulations (R^2 = 0.941).

Moreover, the EC formulation of foliar extracts showed a substantial deterrent effect on the feeding behaviour of diamondback larvae compared to crude extracts. Our results provide environment-friendly substitutes to synthetic chemical-based insecticides for the management of diamondback moth.

Keywords: *Plutella xylostella*, *Ageratum conyzoides*, Foliar extracts, Emulsifiable concentrates, Antifeedant index.

Introduction

Plutella xylostella (L.) (Lepidoptera: Plutellidae), also known as the diamondback moth (DBM), is an oligophagous insect pest of worldwide distribution infesting economically important plants belonging to the *Cruciferae* family[56,58]. The diamondback moth is a devastating pest of *Crucifer* crops as it significantly damages their production[13,59]. The worldwide management cost of diamondback moth including an economic loss in agricultural production due to this insect, was estimated to be approximately US $4-$5 billion annually [24,58].The management of the diamondback moth is mainly reliant on chemical-based synthetic pesticides which led to the development of resistance against all major classes of pesticides and *Bacillus thuringiensis* (*Bt*) formulations in the agricultural fields[45].

In addition, the indiscriminate use of synthetic chemical pesticides has led to detrimental effects on the non-targeted organisms and the environment[11,43]. Hence, there is a compulsive demand to switch from conventional pesticides to an alternative sustainable approach for the management of diamondback moth. As a result, current pest control strategies for the management of diamondback moth have shifted the focus on the use of 'botanical insecticides' which provide an intriguing alternative to their chemical counterparts[17] as they have diverse modes of action and are safe for non-target organisms, humans and the environment.

Plants are rich in bioactive compounds and many of them have been demonstrated to possess insecticidal properties[8,14,16,19]. Many reports are available on the insecticidal properties of plant extracts against *Plutella xylostella*, *Dysdercus cingulatus*, *Rhodnius prolixus* and other insects[12,15,46]. Recently, certain weed plants have been reported to possess secondary compounds having repellent and insecticidal activity, which can be utilised in pest management programs as bio-insecticides[20,55]. *Ageratum conyzoides* (L.) (Family: Asteraceae), also known as "goatweed", is a potential allelopathic weed which has been reported for various insecticidal activities[23,54].

A. conyzoides has been found to exhibit multiple biological activities against insect-pest such as antifeedant and repellent activity against *Helicoverpa armigera*[38], precocious metamorphosis and prolonged development in *Heliothis zea*[9], antijuvenile activity against mosquito, *Anopheles stephensi* and *Culex quinquefasciatus*[40], larvicidal activity against *Aedes albopictus*[26], insecticidal and histopathological effect against dengue vector, *Aedes aegypti*[34] and acute toxicity against cowpea weevil, *Callosobruchus maculatus* (F.)[7]. This plant contains several secondary metabolites i.e. flavonoids, chromenes, benzofurans and terpenoids[21,57]. Moreover, the goatweed, *A. conyzoides*, is rich in precocene I, precocene II and coumarin compounds[35]. Precocene I and precocene II have been utilized as insect growth regulators through the induction of

juvenile deficiency hormones in insects[3,5,36,52]. Precocene II is reported to have cytotoxic effects on corpus allatum (CA) in insects, thereby eliminating the production of juvenile hormone[10,41,47]. Furthermore, precocene II is also reported to show a significant antifeedant effect against stored grain pests such as *Trogoderma granarium, Leptinotarsa decemlineata, Sitophilus granaries, Tribolium confusum, Myzus*[51] and *Spodoptera litura*[50]. Recently, the aqueous, hexane and methanol extracts of the leaves of *A. conyzoides*, have been observed to deter feeding by *P. xylostella* larvae[54]. Thus, the antifeedant effect of *A. conyzoides* leaves can be exploited for the management of insect pests.

Although the use of plant extracts as a replacement for chemical pesticides may seem quite fascinating, several factors limit their usage in the agricultural field such as poor shelf life and easy degradation in the presence of sunlight[48]. Nevertheless, these drawbacks can be overcome by converting plant extracts into stable formulations. Pesticide formulations are crucial in delivering insecticides to their intended target, thereby enhancing their efficacy. The use of water-based 'green pesticide' formulations including micro- and/or nano-emulsions, has drawn considerable attention in recent years[44]. Emulsifiable concentrate (EC) is highly prevalent formulation due to its numerous advantages such as excellent storage stability, easy applicability, significant biological activity etc.[1,22,44]

In a recent report, EC formulation prolonged the persistence of *Sophora alopecuroides* (L.) extracts under laboratory and field conditions for management of Asian citrus psyllid, *Diaphorina citri*[39]. Lina et al[25] reported the insecticidal activity of EC formulation of *Tephrosia vogelii* and *Piper aduncum* fruit leaf extract against *P. xylostella*. Our research work aimed to assess the effect of an EC formulation developed from the methanol extract of *A. conyzoides* using a biodegradable solvent (methyl oleate) and a blend of non-ionic emulsifiers. The bio-efficacy of crude methanol extract of *A. conyzoides* foliage and that of EC formulation on the feeding behaviour of *P. xylostella* has been evaluated.

Material and Methods

Material: Nonyl phenol ethoxylate (Emulsol PC25A and emulsol PC25N) was purchased from Kaiser Industries Ltd., Delhi. Methyl oleate was purchased from Mohini Organic Pvt. Ltd., Mumbai. Methanol was purchased from S D Fine Chem Ltd., Mumbai.

Insect Culture: The laboratory culture of *Plutella xylostella* (L.) was maintained in the laboratory inside a BOD incubator under standard conditions (25 ± 2^0 C temperature, 65 ± 5% relative humidity and 14D: 10L photoperiod).

Host Plant: All experiments were conducted using the cauliflower leaves cultivated in the field plots of the Department of Zoology, University of Delhi, Delhi (India). The seeds of cauliflower (*Brassica oleraceae*) (var. Poosi Special) were procured from National Seeds Bank, IARI, Pusa, New Delhi. The plants were raised regularly under pesticide-free conditions following standard farming practices.

Test Plants: Fresh *A. conyzoides* leaves were procured from Rishikesh, Uttrakhand (India) during the flowering season in April. The collected leaves were washed thoroughly, air-dried and pulverised to a fine powder using an electric grinder (Philips 600W). The obtained powder was weighed and kept in airtight containers till further use.

Preparation of Methanol Extract: The methanolic extract was prepared as described by Vats et al[54]. Briefly, 100 grams of powdered leaves were weighed and dipped in 500 mL of methanol in a 1-litre conical flask and kept undisturbed for 24 hours. The same procedure was repeated thrice and the pooled supernatant was filtered using Whatmann filter paper no. 1. The obtained extract was reduced on a rotary evaporator at 40-41 ºC under reduced pressure to remove the excess solvent and was further air-dried for 24 hours and refrigerated till further use. The phytochemical profiling of methanolic extract of *A. conyzoides* leaves using GC-MS analysis has been reported by Prajapati et al[35].

Development of Emulsifiable Concentrate (EC) formulation: The details of the methods for developing EC formulation are reported earlier by Prajapati et al[35]. 5% (w/w) emulsifiable concentrate (EC) formulation from methanolic extract of *A. conyzoides* was done by using a biodegradable solvent, methyl oleate and emulsifiers Emulsol PC25A Emulsol PC 25 N. Briefly, a mixture of blended emulsifiers and biodegradable solvents was mixed in a methanolic *A. conyzoides* extract to obtain a clear solution of EC. The developed EC was dispersed in hard water (342 ppm) to check the blooming.

Physico-chemical parameters like stability (cold stability and ATS stability), pH, emulsion stability and flash point of developed EC were studied and results showed that the developed EC passes all standard parameters. Finally, the obtained EC formulation was further tested for its antifeedant effect against the larvae of *P. xylostella*.

Preparation of Extract Concentrations: To obtain a 10% stock solution, 10 mL of control solution (which was prepared by combining 1 mL of methanol, 9 mL of distilled water and a drop of emulsifier Triton X) was used to dissolve 1g of crude extract. Using a control solution, all extract concentrations (5%, 4%, 3%, 2% and 1%) were prepared by serial dilution.

Preparation of Emulsion from developed EC: 0.5%, 0.4%, 0.3%, 0.2% and 0.1% emulsions were prepared by serial dilutions from 5% stock solution of EC using distilled water.

Feeding Bioassays: Antifeedant bioassays were performed in no-choice and choice test conditions to study the feeding

responses of larvae of *P. xylostella* as described by Vats et al[54].

No-Choice Bioassays: Cauliflower leaves, *Brassica oleraceae* var *botrytis* (Poosi spl.) were cut into circular discs (2.5 cm diameter), dipped into methanol extract and EC formulation respectively and air dried at room temperature. A single cauliflower leaf disc, treated or control was placed in the centre of a plastic Petri dish (9 cm diameter x 4 cm height) aligned with a moist filter paper to maintain the turgidity of the leaves. Five pre-starved fourth instar *P. xylostella* larvae were introduced on the leaf disc for feeding. All the Petri dishes were transferred to BOD incubator maintained at standard conditions (25 ± 2^0 C temperature, 65 ± 5% relative humidity and 14D: 10L photoperiod). The leaf discs were taken out after 18 hours of feeding and the consumption of the leaf area by the larvae was quantified using graph paper. Five replicates of each experiment were conducted with crude extract concentration, EC formulation and the control leaf disc (without extract).

Two-Choice Bioassays: Two-choice bioassays were conducted in a manner similar to no-choice bioassays, with the exception that both treated and control leaf discs were kept in the same Petri dish simultaneously. The antifeedant index was determined using the two-choice tests which are regarded as more sensitive than no-choice tests[3].

The Antifeedant Index (AFI) was determined using following formula[18]:

$$AFI = (C-T / C+T) \times 100$$

where C and T denote the control and treated leaf areas larvae consumed respectively.

Statistical Analysis: The experimental data obtained from feeding bioassays were analyzed using Sigma Stat 2.0. The raw data was subjected to one-way analysis of variance (ANOVA) and means were separated using Tukey's pairwise multiple comparison test so as to test the significant differences between concentrations of a particular treatment type and/or control. Student's t test was performed for antifeedant test to compare significant difference between control and respective treatments. R^2(square of Pearson correlation coefficient) was computed to establish linear relationship between different extract and EC formulation concentrations with antifeedant Index values using MS Excel program.

Results and Discussion

Application of plant extracts and semiochemicals mediating the behaviour of insects offers a promising opportunity for the control of notorious pests in agricultural fields and storage conditions. In lepidopteran insect pests, the larval feeding on leaves and fruiting structures causes maximum damage and hence, natural plant extracts and various formulations affecting the feeding behaviour of larvae are gaining wide attention as they offer a safer alternative to harmful insecticides in the pest management programs. In our previous report, foliar extracts of 'goatweed', *Ageratum conyzoides*, have been observed to cause significant antifeedant effect against the larvae of *Plutella xylostella*[54]. In the present laboratory studies, the methanol extracts of *A. conyzoides* leaves and its emulsifiable concentrate (EC), both showed significant deterrent effect on the feeding behaviour of *P. xylostella* larvae.

In no-choice bioassay, the quantity of leaf area fed by *P. xylostella* larvae decreased with increasing concentrations of the crude methanol extract ($F_{5, 29} = 48.64$, $p<0.05$) and EC formulations ($F_{5, 29} = 47.33$, $p<0.05$) (Table 1). In both cases, the mean leaf area consumption was significantly reduced ($p < 0.05$) on all the treated leaf discs as compared to the control leaf disc. Similar results were evident in two-choice bioassays, as the larvae consumed a significantly higher ($P<0.05$) percentage of leaf area on control discs in comparison to leaf discs treated with different concentrations of crude methanol extracts and EC formulations.

Even at lower tested concentrations of 1% methanol extract and 0.1% EC formulation, the mean leaf area consumed was nearly half as compared to the respective controls i.e. 97 mm^2 and 48.4 mm^2 and the feeding reduced further at higher doses (Table 2). A positive correlation between the antifeedant index (AFI) values and different concentrations of crude extracts (R^2 = 0.9921; $F_{4, 24}$: 25.92) and EC formulations (R^2 = 0.9415; $F_{4, 24}$**:** 36.76) of *A. conyzoides* was obtained (Table 3, Fig. 1 A and 1 B).

Table 1
Feeding response of *P. xylostella* larvae in no-choice conditions

	Leaf Area Consumed (mm^2)					
Crude Extract Concentration	Control	1%	2%	3%	4%	5%
Mean ± S.E[1].	122.2 ± 5.3^{a}	83.0 ± 2.6^{b}	80.8 ± 3.1^{b}	67.4 ± 2.5^{bc}	53.0 ± 3.5^{cd}	50.8 ± 4.5^{d}
EC Formulation	Control	0.1%	0.2%	0.3%	0.4%	0.5%
Mean ± S.E[1].	135.0 ± 6.9^{a}	92.6 ± 4.7^{b}	84.8 ± 6.9^{b}	72.2 ± 7.5^{bc}	47.0 ± 3.8^{c}	17.8 ± 3.4^{d}

Note: [1]Means followed by different lowercase superscripts differ significantly ($p<0.05$) between control and treatments for Crude extract and EC formulation. Data was statistically analyzed by one-way ANOVA and means were separated by Tukey's pairwise multiple comparison test

Table 2
Feeding response of *P. xylostella* larvae in choice conditions

Leaf Area Consumed (mm^2)			
Crude Extract Concentration	**Mean ± S.E[1]**	**EC Formulation Concentration**	**Mean ± S.E[1]**
Control (C) 1%	97.4 ± 6.2[a] 51.8 ± 2.2[b]	C 0.1%	82.0 ± 6.0[a] 48.4 ± 5.9[b]
C 2%	108.0 ± 7.9[a] 43.0 ± 3.3[b]	C 0.2%	102.4 ± 6.5[a] 33.6 ± 2.7[b]
C 3%	111.8 ± 6.5[a] 34.2 ± 4.1[b]	C 0.3%	100.2 ± 3.4[a] 33.4 ± 2.1[b]
C 4%	122.2 ± 7.4[a] 23.0 ± 3.6[b]	C 0.4%	115.2 ± 9.0[a] 12.8 ± 3.2[b]
C 5%	130.4 ± 4.1[a] 11.6 ± 1.4[b]	C 0.5%	118.6 ± 8.1[a] 5.8 ± 1.6[b]

Note: [1]Means followed by different lowercase superscripts differ significantly ($p<0.05$) between control and treatments for Crude extract and EC formulation. Data was statistically analyzed by student's t-test

Table 3
Antifeedant Index for crude methanol extract and EC Formulations of *A. conyzoides* foliage against fourth instar larvae of *P. xylostella*

Antifeedant Index					
Crude Extract Concentration	1%	2%	3%	4%	5%
Mean ± S.E[1].	31.41± 2.6[a]	42.64 ± 4.3[ab]	52.93 ± 5.8[bc]	68.85 ± 4.1[cd]	83.45 ± 2.3[d]
EC Formulation Concentration	0.1%	0.2%	0.3%	0.4%	0.5%
Mean ± S.E[1].	26.29 ± 6.2[a]	50.31 ± 3.8[b]	51.30 ± 3.1[b]	82.0 ± 4.3[c]	90.14 ± 3.0[c]

Note: Means followed by different lowercase superscripts differ significantly ($P<0.05$) between concentrations of each treatment. Data was statistically analyzed by one-way ANOVA and means were separated by Tukey's pairwise multiple comparison test

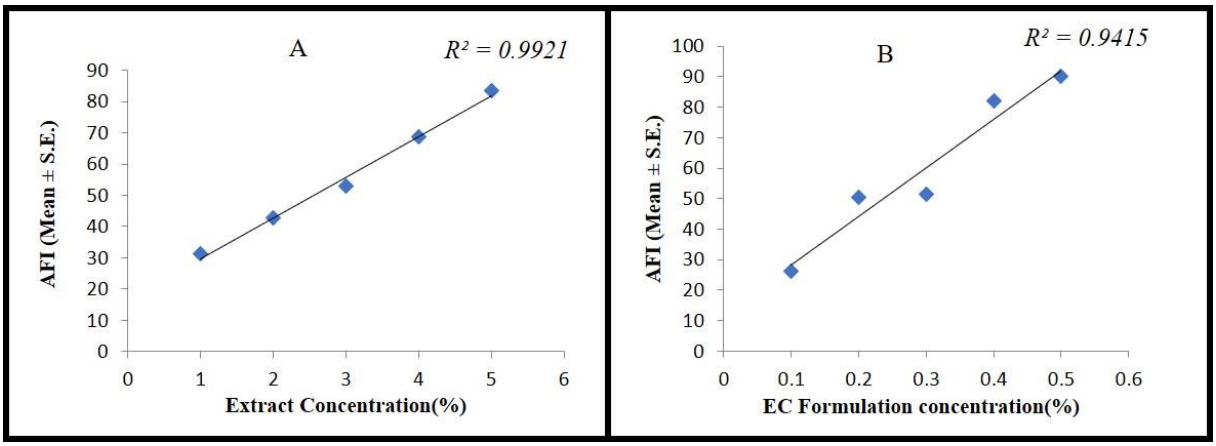

Figure 1: Correlation between (A) the Antifeedant Index (AFI) for crude methanol extract and (B) EC Formulations of *A. conyzoides* foliage

P. xylostella larvae showed lower consumption of treated leaf discs in both choice and no-choice bioassays as compared to control leaf discs of cauliflower. The feeding of larvae on cauliflower leaf discs could be due to presence of bioactive compounds acting as feeding stimulant such as glucosinolates and flavonoids[53] and/or lack of feeding deterrents against *P. xylostella*. On the other hand, reduced consumption of extract and EC formulation smeared leaf discs by the larvae could be due to masking effect on the feeding stimulants or due to contact phagodeterrent effect with increasing doses of different extracts as well as EC formulations. The obtained antifeedant index values suggest that feeding inhibition is positively dose-dependent. Such effect might be due to presence of different feeding deterrent compounds in methanol extract and EC formulation, which might have acted either individually or synergistically to deter the larval feeding[29,30,32], by interfering with the insect's central nervous system[28] or damaging the digestive system[6]. The primary mechanisms of functioning of antifeedant compounds are through their effect on gustatory receptors (GR). Lepidopteran insects have diverse form of GR proteins involved in processes like chemosensation, host

selection and adaptation[2]. Binding of GR to ligands leads to generation of multiple downstream cellular changes that cause change in feeding behaviour of insects[2]. Thus, perception of feeding deterrent compounds by taste sensilla such as medial and lateral sensillum of the larval maxillary glands may cause reduced feeding and consumption and ultimately may retard growth and disrupt development[31,37,42]. Although the antifeedant compounds act directly on the GR proteins expressed at gustatory receptor neurons (GRNs) of taste sensilla in insects, a few of them act through antagonizing the action of γ-amino butyric acid (GABA) at the neuronal ends and thus induce feeding deterrence.

Messchendorp et al[27] observed that the sensory input received by a medial deterrent cell of gustatory system directly caused antifeedant effect in *Pieris brassicae*. In a recent report, the bioactive compound Precocene I isolated in chloroform extracts of *Desmosstachya pinnata* (L.) not only deterred feeding by *Spodoptera litura* larvae, but also caused damage to their gut tissue[50]. Precocene II was reported to have antifeedant effect in no-choice bioassay with Mexican beetle, *Epilachna varivestis*[49]. *Ageratum conyzoides* is reported to have a feeding inhibitory effect against stored grain insect pests *Tribolium castaneum* and *Sitophilus oryzae*[4].

In the present study, the results of feeding bioassays with EC formulation indicate that feeding deterrence increased and thus provided better bio-efficacy than crude extract. Moreover, significant deterrent effect of EC formulation on larval feeding is evident comparatively at very low doses. The insecticidal activity of EC formulation of *Piper aduncum* fruit and *Tephrosia vogelii* leaf extract was reported against *P. xylostella*[25], but no such report on the antifeedant effect is available. The foliar extracts of *A. conyzoides* and its formulations exhibit greater potential for management of *P. xylostella* and thus, these could be further investigated to develop eco-friendly biopesticides for green agriculture[33]. Also, field studies are required to determine stability and safety of EC formulation for use by the farmers.

Conclusion

The present study suggested that the weed plant extracts and their EC formulation have significant inhibitory effect on the feeding behaviour of *P. xylostella* larvae under laboratory conditions. The emulsifiable concentrate formulations appear to be promising eco-friendly and safer alternatives to synthetic pesticides for the management of diamondback moth. However, further studies are required to test their efficacy and safety under field conditions.

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References

1. Abdelmaksoud N.M., El-Bakry A.M., Abdel-Aziz N.F., Sammour E.A. and Salem H.A.N., Potency of Emulsifiable Concentrate and Nanoemulsion Formulations as Green Insecticides against Two Insects, *Aphis craccivora* and *Liriomyza trifolii*, *Ind. Crops Prod.*, **208**, 117854 **(2024)**

2. Agnihotri A.R., Roy A.A. and Joshi R.S., Gustatory Receptors in Lepidoptera: Chemosensation and Beyond, *Insect Mol. Biol.*, **25(5)**, 519-529 **(2016)**

3. Akhtar Y. and Isman M.B., Comparative Growth Inhibitory and Antifeedant Effects of Plant Extracts and Pure Allelochemicals on Four Phytophagous Insect Species, *Journal of Applied Entomology*, **128**, 32-38 **(2004)**

4. Amelot M.E.A., Avendaño M., Aubert L. and Jorge Luis Avila J.L., Repellency and Feeding Deterrence Activity of *Ageratum conyzoides* against the Stored Grain Pests *Tribolium castaneum* and *Sitophilus oryzae*. Active plant parts and composition, *CIENCIA*, **11(1)**, 61-76 **(2003)**

5. Amsalem E., Teal P., Grozinger C.M. and Hefetz A., Precocene-I inhibits Juvenile Hormone Biosynthesis, Ovarian Activation, Aggression and alters Sterility Signal Production in Bumble Bee (*Bombus terrestris*) Workers, *J. Exp. Biol.*, **217**, 3178-3185 **(2014)**

6. Barbouche N., Hajem B., Lognay G. and Ammar M., Contribution under Investigation of the Biological Activity of Extracts of Sheets of *Cestrum parqui* Hérit. (Solanaceae) on the Locust Pilgrim *Schistocerca gregaria* (Forsk.), *Biotechnol. Agron.*, **5**, 85-90 **(2001)**

7. Bouda H., Tapondjou L.A., Fontem D.A. and Gumedzoe M.Y.D., Effect of Essential Oils from the Leaves of *Ageratum conyzoides*, *Lantana camara* and *Chromolaena odorata* on the Mortality of *Sitophilus zeamais* (Coleoptera: Curculionidae), *J. Stored Prod. Res.*, **37(2)**, 103–109 **(2001)**

8. Boulogne I. and Petit P., Insecticidal and Antifungal Chemicals produced by Plants: A Review, *Environ. Chem. Lett.*, **10(4)**, 325-347 **(2012)**

9. Bowers W.S. and Feldlaufer M.F., *In Vitro* Inactivation of *Tenebrio molitor* Corpora Allata by a Synthetic Precocene Analog, *Gen. Comp. Endocrinol*., **47(1)**, 120–124 **(1982)**

10. Bowers W.S. and Martinez-Pardo R., Antiallatotropins: Inhibition of Corpus Allatum Development, *Science*, **197**, 1369-1371 **(1977)**

11. Cox C. and Surgan M., Unidentified Inert Ingredients in Pesticides: Implications for Human and Environmental Health, *Environ. Health Perspect*., **114(12)**, 1803-1806 **(2006)**

12. Escobar-Garcia H.A., Nascimento V.F., De Melo M.A., Ramalho D.G. and De Bortoli S.A., Aqueous Botanical Extracts, via Different Extraction Methods, for Control of the Diamondback Moth, *Plutella xylostella* (Lepidoptera: Plutellidae), *J. Plant Dis. Prot.*, **131**, 255–263 **(2024)**

13. Furlong M.J., Wright D.J. and Dosdall L.M., Diamondback Moth Ecology and Management: Problems, Progress and Prospects, *Annu. Rev. Entomol*., **58**, 517–541 **(2013)**

14. Gonzalez-Coloma A., Reina M., Diaz C.E., Fraga B.M. and Santana-Meridas O., Natural Product-Based Biopesticides for Insect Control. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, https://doi.org/10.1016/b978-0-12-409547-2.02770-0 **(2013)**

15. Grainge M. and Ahmed S., Handbook of Plants with Pest-Control Properties, John Wiley & Sons Limited **(1988)**

16. Grieneisen M.L. and Isman M.B., Recent Developments in the Registration and Usage of Botanical Pesticides in California. Managing and Analyzing Pesticide Use Data for Pest Management, Environmental Monitoring, Public Health and Public Policy, 149–169, https://doi.org/10.1021/bk-2018-1283.ch008 **(2018)**

17. Ibrahim M.A., Oksanen E.J. and Holopainen J.K., Effects of Limonene on Growth and Physiology of Cabbage and Carrot Plants, *J. Sci. Food Agric*., **84(11)**, 1319–1326 **(2004)**

18. Isman M.B., A Physiological Perspective, In Roitberg B.D. and Isman M.B., eds., Insect Chemical Ecology: An Evolutionary Approach. Chapman and Hall, New York, 156–176 **(1992)**

19. Isman M.B., Bioinsecticides based on Plant Essential Oils: A Short Overview, *Zeitschrift für Naturforschung*, Section C, **75(7–8)**, 179–182 **(2020)**

20. Kaur A., Batish D.R., Kaur S. and Chauhan B.S., An Overview of the Characteristics and Potential of *Calotropis procera* from Botanical, Ecological and Economic Perspectives, *Front. Plant Sci.*, **12**, 690806 **(2021)**

21. Kong C., Hu F., Xu T. and Lu Y., Allelopathic Potential and Chemical Constituents of Volatile Oil from *Ageratum conyzoides*, *J. Chem. Ecol.*, **25**, 2347–2356 **(1999)**

22. Kozuki Y., Ohtsubo T., Zollinger R., Rhode A. and Dean S., A Predictive Solubility Tool for Pesticide Emulsifiable Concentrate Formulations, *J. ASTM Int.*, **6(9)**, 1–9 **(2009)**

23. Kumar S., Sharma A. K., Kumar B., Shakya M., Patel J. A., Kumar B., Bisht N., Chigure G. M., Singh K., Kumar R., Kumar S., Srivastava S., Rawat P. and Ghosh S., Characterization of Deltamethrin, Cypermethrin, Coumaphos and Ivermectin Resistance in Populations of *Rhipicephalus microplus* in India and Efficacy of An Antitick Natural Formulation Prepared from *Ageratum conyzoides*, *Ticks Tick-Borne Dis.*, **12**, 101818 **(2021)**

24. Li Z., Zalucki M.P., Yonow T. and Kriticos D.J., Population Dynamics and Management of Diamondback Moth (*Plutella xylostella*) in China: the Relative Contributions of Climate, Natural Enemies and Cropping Patterns, *Bull. Entom. Res*., **102(2)**, 197–214 **(2016)**

25. Lina E.C., Widhianingrum I., Putri M.E., Evalia N.F. and Makky M., Insecticidal Activity of *Piper aduncum* fruit and *Tephrosia vogelii* Leaf Mixed Formulations against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *JBiopest.*, **11(1)**, 69–75 **(2018)**

26. Liu X.C. and Liu Z.L., Evaluation of Larvicidal Activity of the Essential Oil of *Ageratum conyzoides* L. Aerial Parts and its Major Constituents against *Aedes albopictus*, *J. Entomol. Zool. Stud.*, **2(4)**, 345-350 **(2014)**

27. Messchendorp L., VAN Loon J.J.A. and Gols G.J.Z., Behavioural and Sensory Responses to Drimane Antifeedants in *Pieris brassicae* larvae, *Entomol. Exp. Appl.*, **79**, 195-202 **(1996)**

28. Mordue (Luntz) A.J. and Blackwell A., Azadirachtin: an update, *J. Insect Physiol*., **39(11)**, 903–924 **(1993)**

29. Moreira M.D., Picanço M.C., Barbosa L.C.A., Guedes R.N.C., Barros E.C. and Campos M.R., Compounds from *Ageratum conyzoides*: Isolation, Structural Elucidation and Insecticidal Activity, *Pest Manag. Sci*., **63(6)**, 615–621 **(2007a)**

30. Moreira M.D., Picanço M.C., Barbosa L.C. de A., Guedes R.N.C., Campos M.R.de., Silva G.A. and Martins J.C., Plant Compounds Insecticide Activity against Coleoptera Pests of Stored Products, *Pesqui. Agropecu. Bras*., **42(7)**, 909-915 **(2007b)**

31. Mullin C.A., Chyb S., Eichenseer H., Hollister B. and Frazier J.L., Neuroreceptor Mechanisms in Insect Gustation: A Pharmacological Approach, *J. Insect Physiol*., **40(11)**, 913-931 **(1994)**

32. Pari K., Rao P.J., Subrahmanyam B., Rasthogi J.N. and Devakumar C., Benzofuran and Other Constituents of the Essential Oil of *Ageratum conyzoides*, *Phytochemistry*, **49(5)**, 1385–1388 **(1998)**

33. Paul S., Datta B.K., Ratnaparkhe M.B. and Dholakia B.B., Turning Waste into Beneficial Resource: Implication of *Ageratum conyzoides* L. in Sustainable Agriculture, Environment and Biopharma Sectors, *Mol. Biotechnol*., **64**, 221–244 **(2022)**

34. Pintong A., Ampawong S., Komalamisra N., Sriwichai P., Popruk S. and Ruangsittichai J., Insecticidal and Histopathological Effects of *Ageratum conyzoides* Weed Extracts against Dengue Vector, *Aedes aegypti*, *Insects*, **11(4)**, 224 **(2020)**

35. Prajapati R., Roy S., Mishra S., Raza S.K. and Thakur L.K., Formulation Development, Standardization and Antimicrobial Activity of *Ageratum Conyzoides* Extracts and their Formulation, *Int. J. Pharm. Sci.*, **6(2)**, 369-374 **(2014)**

36. Pratt G.E. and Bowers W.S., Precocene II inhibits Juvenile Hormone Biosynthesis by Cockroach Corpora Allata *in vitro*, *Nature*, **265**, 548-50 **(1977)**

37. Qin D., Zhang P., Zhou Y., Liu B., Xiao C., Chen W. and Zhang Z., Antifeeding Effects of Azadirachtin on the Fifth Instar *Spodoptera litura* Larvae and the Analysis of Azadirachtin on Target Sensilla around Mouthparts, *Arch. Insect Biochem. Physiol*., **103(4)**, e21646 **(2020)**

38. Ragesh P.R., Bhutia T.N., Ganta S. and Singh A.K., Repellent, Antifeedant and Toxic Effects of *Ageratum conyzoides* (Linnaeus) (Asteraceae) Extract against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *Arch. Phytopathol. Pflanzenschutz*, **49**, 19–30 **(2016)**

39. Rizvi S.A.H., Xie F., Ling S. and Zeng X., Development and Evaluation of Emulsifiable Concentrate Formulation containing *Sophora alopecuroides* L. Extract for the Novel Management of Asian Citrus Psyllid, *Environ. Sci. Pollut. Res. Int.*, **26**, 21871-21881 **(2019)**

40. Saxena R.C., Jayashree S., Padma S. and Dixit O.P., Evaluation of Growth Disrupting Activity of *Ageratum conyzoides* Crude Extract on *Culex quinquefasciatus*, *J. Environ. Biol*., **15(1)**, 67–74 **(1994)**

41. Schooneveld H., Precocene-Induced Necrosis and Haemocyte-Mediated Breakdown of Corpora Allata in Nymph of the locust *Locusta migratoria*, *Cell Tissue Res*., **203(1)**, 25-33 **(1979)**

42. Seada M.A., Ignell R., Al Assiuty A.N. and Anderson P., Functional Characterization of the Gustatory Sensilla of Tarsi of the Female Polyphagous Moth *Spodoptera littoralis*, *Front. Physiol.*, **9**, 1606 **(2018)**

43. Shakeel M., Farooq M., Nasim W., Akram W., Khan F., Jaleel W., Zhu X., Yin H., Li S., Fahad S., Hussain S., Chauhan B.S. and Jin F., Environment Polluting Conventional Chemical Control Compared to an Environmentally Friendly IPM Approach for Control of Diamondback Moth, *Plutella xylostella* (L.), in China: A Review, *Environ. Sci. Pollution Res. Int*., **24(17)**, 14537–14550 **(2017)**

44. Shao H., Xi N. and Zhang Y., Microemulsion Formulation of a New Biopesticide to Control the Diamondback Moth (Lepidoptera: Plutellidae), *Sci. Rep*., **8(1)**, 10565 **(2018)**

45. Shehzad M., Bodlah I., Siddiqui J.A., Bodlah M.A., Fareen A.G. and Islam W., Recent Insights into Pesticide Resistance Mechanisms in *Plutella xylostella* and Possible Management Strategies, *Environ. Sci. Pollut. R*., https://doi.org/10.1007/s11356-023-29271-5 **(2023)**

46. Silva A.B., Montagnini D.L., Katchborian-Neto A., Andrade J.V., Manuquian H.A., Cavallari P.S.S.R., Almeida S.G., Barbosa E.P., Cunha W.R., Soares M.G., Santos M.F.C., Silva M.L.A. and Vacari A.M., Insecticidal Activity of Extracts of *Handroanthus impetiginosus* on *Plutella xylostella* (Lepidoptera: plutellidae) Larvae, *Nat. Prod. Res*., https://doi.org/10.1080/14786419.2023.2260069 **(2023)**

47. Soderlund D.M., Messeguer A. and Bowers W.S., Precocene II Metabolism in Insects: Synthesis of Potential Metabolites and Identification of Initial in Vitro Biotransformation Products, *J. Agric. Food. Chem.*, **28(4)**, 724–731 **(1980)**

48. Souto A.L., Sylvestre M., Tölke E.D., Tavares J.F., Barbosa-Filho J.M. and Cebrián-Torrejón G., Plant-Derived Pesticides as an Alternative to Pest Management and Sustainable Agricultural Production: Prospects, Applications and Challenges, *Molecules*, **26**, 4835 **(2021)**

49. Srivastava R.P. and Proksch P., Toxicity and Feeding Deterrence of Natural Chromene and Benzofuran Derivatives to *Epilachna varivestis*, *Naturwissenschaften*, **77**, 438–439 **(1990)**

50. Sundar N.S., Karthi S., Sivanesh H., Stanley-Raja V., Chanthini K.M., Ramasubramanian R., Ramkumar G., Ponsankar A., Narayanan K.R., Vasantha-Srinivasan P., Alkahtani J., Alwahibi M.S., Hunter W.B., Senthil-Nathan S., Patcharin K., Abdel-Megeed A., Shawer R. and Ghaith A., Efficacy of Precocene I from *Desmosstachya bipinnata* as an Effective Bioactive Molecules against the *Spodoptera litura* Fab. and Its Impact on *Eisenia fetida* Savigny, *Molecules*, **26(21)**, 6384 **(2021)**

51. Szczepanik M., Obara R., Szumny A., Gabryś B., Halarewicz-Pacan A., Nawrot J. and Wawrzeńczyk C., Synthesis and Insect Antifeedant Activity of Precocene Derivatives with Lactone Moiety, *J. Agric. Food Chem.*, **53(15)**, 5905–5910 **(2005)**

52. Unnithan G.C., Nair K.K. and Bowers W.S., Precocene-Induced Degeneration of the Corpus Allatum of Adult Females of the Bug, *Oncopeltus fasciatus*, *J. of Insect Physiol*., **23(9)**, 1081-1094 **(1977)**

53. Van Loon J.J.A., Wang C.Z., Nielsen J.K., Gols R. and Qiu Y.T., Flavonoids from Cabbage are Feeding Stimulants for Diamondback Moth Larvae Additional to Glucosinolates: Chemoreception and Behaviour, In Nielsen J.K., Kjær C. and Schoonhoven L.M., eds., Proceedings of the 11th International Symposium on Insect-Plant Relationships, Series Entomologica, **57**, https://doi.org/10.1007/978-94-017-2776-1_3 **(2002)**

54. Vats T.K., Rawal V., Mullick S., Devi M.R., Singh P. and Singh A.K., Bioactivity of *Ageratum conyzoides* (L.) (Asteraceae) on Feeding and Oviposition Behaviour of Diamondback Moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Int. J. Trop. Insect Sci*., **39**, 311-318 **(2019)**

55. Vats T.K., Rawal V., Singh A.K. and Mullick S., Deterrent Effect of *Calotropis procera* R. Br. on Feeding, Oviposition and Egg Hatchability of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *J. Asia Pac. Entomol*., **26(2)**, 102081 **(2023)**

56. Wang M., Zhu B., Zhang L., Xiao Y., Liang P. and Wu K., Influence of Seasonal Migration on Evolution of Insecticide Resistance in *Plutella xylostella*, *Insect Sci*., **29(2)**, 496–504 **(2021)**

57. Yadav N., Ganie S.A., Singh B., Chhillar A.K. and Yadav S.S., Phytochemical Constituents and Ethnopharmacological Properties of *Ageratum conyzoides* L., *Phytother. Res*., **33(9)**, 2163-2178 **(2019)**

58. Zalucki M.P., Shabbir A., Silva R., Adamson D., Shu-Sheng L. and Furlong M.J., Estimating the Economic Cost of One of the World's Major Insect Pests, *Plutella xylostella* (Lepidoptera: Plutellidae): Just How Long is a Piece of String?, *J. Econ. Ent*., **105(4)**, 1115–1129 **(2012)**

59. Zhu L., Li Z., Zhang S., Xu B., Zhang Y., Zalucki M.P., Wu Q. and Yin X., Population Dynamics of the Diamondback Moth, *Plutella xylostella* (L.), in Northern China: the Effects of Migration, Cropping Patterns and Climate, *Pest Manag. Sci*., **74(8)**, 1845–1853 **(2018)**.

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