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Evaluation of Biological Activity of *Hyptis suaveolens* (Lamiaceae) Against Certain Behavioural and Physiological Aspects of *Plutella xylostella* (Lepidoptera: Plutellidae)

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Abstract: *Plutella xylostella* (L.) (diamond back moth) is considered as cosmopolitan serious insect pest of cruciferous plants. In this study, crude extract of *Hyptis suaveolens* (Lamiaceae) was used to evaluate the larvicidal activity, Oviposition detterent and ovicidal activities against *Plutella xylostella* (Lepidoptera, Plutellidae). The 24 h LC_{50} and 96 h LC_{50} of aqueous extract of *H. suaveolens* was 0.0081 mg/l and 0.009 mg/l against *P. Xylostella*, respectively. There was a significant negative correlation between the LC_{50} and exposure time of corresponding treatments. The maximum toxicity appears in Petroleum ether extract (96 h 0.004 mg/l) followed by methanol extract (96 h 0.009 mg/l). The egg laying on the cauliflower leaf surface treated with aqueous, methanol and petroleum ether extract was significantly reduced in the concentration dependent manner, as compared to the control in both no- choice and choice methods. However, the petroleum ether extract did not affect egg laying of female diamond back moths. The egg hatchability reduced significantly at higher concentration (5%) of methanol and petroleum ether but not at any concentration of aqueous extract. These results indicates that the crude extracts of *Hyptis suaveolens* could potentially be used for the management of diamond back moth, *Plutella xylostella.*

Keywords: *Plutella xylostella*, *Hyptis suaveolens*, Toxicity, Oviposition deterrent, Ovicidal activity

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Introduction

The diamond back moth (DBM), *Plutella xylostella* (L) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of crucifers worldwide. *Plutella xylostella* larvae significantly lower the quantity and quality of food by feeding on the foliage of cruciferous plants from seedling stage to harvest (Talekar and Shelton, 2003; Shelton *et al*., 2000). Global DBM management is projected to cost US$ 3 billion per year (Rajput and Meena, 2020). DBM has demonstrated strong resistance

to practically all insecticides used in the field, including biopesticides such *B. thuringiensis* crystal toxins and *Saccharopolyspora spinosa* spinosyns (Desai *et al*., 2012) There have been reports of insecticide resistance related crop loss in Australia, South- East Asia, Japan, the United States, and Central America (Anonymous, 2018-19; Ahuja *et al*., 2010). For the control of susceptible *P. xylostella,* a wide variety of insecticides with various modes of action are available, nevertheless resistance has been noted to all, including the newest mode of action in one or more places. Botanical insecticides can influence the behaviour and development of the herbivorous insect, which uses the plant for their reproduction. Also, alternative sources of potentially suitable insecticides include botanical insecticides, antifeedants and insect growth regulators of their natural origin having non-neurotoxic modes of action, and low environmental persistence (Goswami. *et al*., 2010; Ali *et al*., 2019; Kapinder *et al*., 2022).

The plants possess properties like repellency, anti-feeding, fast knock down, flushing action, bio-degradability and broad-spectrum activity for resistance reduction (Mochiah et al., 2011). Many plants such as *Melia azedarach, Azadirachta indica, Azeratum conyzoides, Lantana camra, Cyperous rotundus* and several more have shown effectiveness against *Plutella xylostella*. Several limonoids, alkaloids, terpenoides, flavanoids of plants are known to exhibit insect antifeedant, oviposition, repellent, growth regulatory effect and toxicity effect on insect pest with no harm to environment and non-target organisms (Facey *et al*., 2005). The Lamiaceae family includes the plant *Hyptis suaveolens*, also referred to as Wilayati tulsi. It is a medicinal plant with ethnobotanical importance and is regarded as an unpleasant weed. The essential oils of *Helianthus spicigera* and *H. suaveolens* have been reported to be a viable alternative to conventional pesticides for the control of several pests associated with preserved food (Raja *et al*., 2005; Sanon *et al*., 2006a; Othira *et al*., 2009), indicating the need for additional research. This plant's potential has been evaluated for lethality against *Artemia salina* L. nauplii, brine shrimp, and insecticidal and repellent action against *Tribolium castaneum* (Herbst), the red flour beetle (Sanon *et al*., 2006b; Noudjou *et al*., 2007; Kouninki *et al*., 2007; Kossou *et al*., 2007). In this study, plant extracts of *Hyptis suaveolens* were used to investigate its efficacy for the management of *Plutella xylostella* insect population in the laboratory condition.

Materials and Methods

Plant collection:

Whole plants of *Hyptis suaveolens* (Lamiaceae) was collected from Deen Dayal Upadhaya Gorakhpur University campus. All plant parts of *Hyptis suaveolens*was were washed with the tap water and dried under shade for a week and were pulverized by using grinder.

Rearing of Larvae and Adult:

In the laboratory, the collected larvae of *Plutella xylostella* from agriculture field were kept in 500 ml plastic containers, with cauliflower leaf as a food material for *P. xylostella* larvae. The containers were lined with tissue paper to absorb excess moisture, and closed with a cap slightly loose to facilitate ventilation and also to avoid escape of larvae. The larvae were reared at 25±1˚C temperatures under 65 ± 5% R.H. and 14L: 10D photoperiod. Newly emerged third instar larvae were used in bioassay studies.

Preparation of plant extract:

(A) *Aqueous extract*:

The maceration technique (Michel, 1934) was used to prepare the aqueous extract, in which 10 g of plant powder were soaked in 200 ml of distilled water and after manual shaking, they were left for 24 h at room temperature in order to extract the hydro soluble compounds which were then strained through a voile fabric to obtain the aqueous extract solutions.

(B) *Methanol and Petroleum ether extract preparation*:

In order to prepare methanol or petroleum ether

extracts, whole plant powder *(Hyptis suaveolens)* was weighed (10 g) and soaked with 200 ml of the solvent. The mixture was stirred for 1 h with magnetic stirrer and then kept for 24 h at room temperature. The solvent was decanted after 24 h and the process was repeated three times with solvent and decanted in the jar to extract maximum of the active compounds from the plant. The pooled extract was then filtered using a Whatman filter paper No. 1. The extract was then concentrated in rotary evaporator at 40°C under reduced pressure to near dryness. The extracts were refrigerated for further use.

Preparation of treatment solutions:

Five concentrations were made (1%, 2%, 3%, 4% and 5%). The percentage solution was prepared using following formula:

$$\%\frac{W}{V} = \frac{Mass\ of\ solute(g)}{Volume\ of\ solution(ml)} \times 100$$

For 5% solution, 5 g of solute was added with 100 ml of water (stock solution). For 4%, 3%, 2%, and 1% solutions,8 ml, 6 ml, 4 ml, and 2 ml of stock solutions were added with 2, 4, 6, and 8 ml of water, respectively. For control solution, 0.5 ml of either solvent and 1 drop of triton-x were added to 9.5 ml of distilled water.

Toxicity Bioassay:

The experiment of toxicity bioassay was conducted by leaf disc method. The cauliflower leaf discs of 3 cm diameter were taken and dipped in different concentrations of *Hyptis suaveolens* crude extracts for 30 sec and further air dried for 3 min. The late second instar larvae of *P. xylostella* (10- Larvae) were introduced into each petri dish under no choice condition. In order to prevent larva from escaping, these petri-dishes were sealed with Para film. Each treatment was replicated 5 times and each replicate consisted of 10 larvae. Mortality was assessed after 24 h intervals for 4 days (24, 48, 72, and 96 h). The obtained data is tabulated and the mortality was corrected using Abbot's formula (Abbott, 1925). After every 24 h, fresh leaf discs of their respective treatments were replaced with the previous leaf disc of cauliflower. Abbot's formula to find the corrected mortality percentage is given as:

$$Corrected\ mortality = \frac{\%\ of\ larvae\ alive - \%\ of\ larvae\ alive\ in\ treated}{\%\ of\ larvae\ alive\ in\ control} \times 100$$

Oviposition Bioassay:

In this experiment, both no-choice and choice bioassays were performed with freshly emerged male and female moths. These moths were released in a mating cage and number of eggs laid by females was calculated after 24 h. The oviposition cage was made of a rectangular net with wooden frame (40 cm x 20cm x 20cm) with windows (10 cm x 10 cm) on side walls, covered with muslin cloth. The bottom of cage was lined with tissue paper. The oviposition cage was marked in 3 equal parts, i.e. two sides and middle. Two diet cups having 10% sucrose solution, soaked in cotton swabs were placed on cage floor for feeding of adult moths. Freshly excised tender cauliflower leaves of same size were painted on both sides using a fine hair brush with the plant extract of desired concentration and air dried at room temperature for 3 min.

Similarly, the leaf surfaces painted with control solution and served as control. Bouquet of leaf, applied with either extract or control solution, was prepared by dipping its petiole into water, filled in a reagent bottle to prevent wilting. The mouth of bottle was plugged with cotton. In the choice bioassays, both control and treated leaf bouquets were placed inside the oviposition cage at opposite end sectors. Ten pairs of mated male and female moths were released in the cage just before the onset of dark phase. After 24 h, bouquets of treated or control leaf were removed and eggs laid were counted separately. The experiments were replicated five times, each replicate with fresh 10 pairs of male and females from different batches of stock culture. The sides of the treated and control leaf bouquets were randomized in the oviposition cage during the experiments to avoid the side effects. The Oviposition Deterrent Index (ODI) was calculated by Huang and Renwick (1994) methods:

$$ODI = (Cn - Tn / Cn + Tn) \times 100$$

Where, Cn and Tn represents number of eggs laid on control and treated leaves, respectively.

Similar procedure was adopted for no- choice bioassays, except the single leaf bouquet was placed, either control or treated at centre of the oviposition cage.

Egg hatchability bioassays:

In the egg hatchability bioassays, the leaf containing 24 h laid eggs was removed and cut into pieces with sharp edged razor, each piece having 20 eggs. The leaf pieces were dipped separately into respective extract concentration or control solution for 30 sec and air dried at room temperature for 3 min. Each leaf piece with eggs was placed in a plastic container (10 cm x 10 cm) lined with a moist blotting paper. The containers were inspected twice daily at 10 a.m. and 5 p.m. for 5 days to record egg hatching. Each experiment was replicated five times with different batches of eggs.

Statistical analysis:

Larvicidal activities of the different solvent extract (Aqueous, Methanol and Petroleum ether) of *Hyptis suaveolens* (Lamiaceae) were performed. The LC values, lower and upper confidence limits (LCL and UCL), slope values, t- ratio, g- values and heterogeneity factors were calculated by using POLO computer software (Robertson *et al.,* 1977). The regression coefficients for the observed data were estimated using linear and non-linear curve fitting, further the significance of regression coefficient was calculated using the method of Sokal and Rohlf (1973). The experimental data were subjected to one way analysis of variance (one way-ANOVA) followed by Tukey's pair-wise multiple comparison test, if ANOVA indicated a significant effect. In choice bioassays, paired t-test was used to compare control and treatment groups (Oviposition).

Results

The toxicity of crude plant extracts of *Hyptis suaveolens* against *Plutella xylostella* was time and concentration dependent. The 24 h LC_{50} and 96 h LC_{50} of aqueous extract of *H. suaveolens* was 0.0081 mg/l and 0.009 mg/L against *P. Xylostella*, respectively (Table 1). There was a significant negative correlation between the LC_{50} and exposure time of corresponding treatments. The maximum toxicity appears in Petroleum ether extract (96- h 0.004 mg/l) followed by methanol extract (96-h 0.009 mg/l). The negative slope values for different solvent-based extracts were found to be 0.00097, 0.00102, and 0.00111 for aqueous, methanol, and petroleum ether extracts, respectively. The variation in the slope values was found to be very minute for all the solvent extracts, with steepest slope for petroleum ether extract showed the highest effectiveness on the DBM larvae. The intercepts for these linearly fitted curves were found to be 0.096, 0.093, and 0.103 for aqueous, methanol, and petroleum ether extracts, respectively. The testing of significance of the regression coefficient (Ts) was done by using the method discussed by Sokal and Rohlf (1973). A significant negative regression was observed between the exposure time and the LC_{50} values. The testing significance (Ts) values were found to be 4.52, 3.99, and 4.77 for aqueous, methanol, and petroleum ether extracts, respectively.

In case of all three solvents, the LC_{50} values are linearly varying with the exposure time. As exposure time increases the LC_{50} values decrease linearly. The variation of LC_{50} values with time is fitted by using linear equation of following form:

$$y = mx + c$$

Where, y corresponds to LC_{50} values and x corresponds to the exposure time, m represents the slope of the linear curve and c is a constant. The linearly fitted curves for aqueous, methanol, and petroleum ether extracts are shown in Figures 1a, b, c.

It was observed that the variation in LC_{50} values with respect to time was linear in nature with adjusted R^2 values were found to be 0.868, 0.834, and 0.880 for aqueous, methanol, and petroleum ether extracts, respectively (Fig. 1). A

Table 1: Toxicity of *H. suaveolens* extracts against *P. xylostella* larvae at different exposure periods

Exposure Period	Tested material	LC_{50}	LCL- UCL	Slope value	t- ratio	Chi- square	Heterogeneity
24 h	Aqueous extract	0.081	0.048- 0.827	1.003 ± 0.355	2.904	4.456	0.19
	Methanol extract	0.079	0.050- 0.328	1.278 ± 0.372	3.437	5.071	0.22
	Petroleum ether	0.085	0.053- 0. 391	1.297 ± 0.379	3.421	5.496	0.24
48 h	Aqueous extract	0.038	0.023- 0.042	1.405 ± 0.337	4.165	3.236	0.14
	Methanol extract	0.031	0.028- 0.072	1.131 ± 0.336	3.362	3.109	0.18
	Petroleum ether	0.039	0.028- 0.074	1.178 ± 0.337	3.489	4.514	0.21
72 h	Aqueous extract	0.021	0.009- 0.017	1.950 ± 0.351	5.561	5.411	0.24
	Methanol extract	0.014	0.012- 0.029	1.124 ± 0.329	3.414	3.773	0.16
	Petroleum ether	0.015	0.005- 0.021	1.021 ± 0.328	3.106	3.833	0.67
96 h	Aqueous extract	0.009	0.001- 0.006	2.283 ± 0.744	3.069	7.257	0.32
	Methanol extract	0.002	0.002- 2.621	1.065 ± 0.337	2.162	5.901	0.16
	Petroleum ether	0.004	0.001- 0.006	1.451 ± 0.465	3.121	14.234	0.61

Mortality was determined at every 24 h up to 96 h. Each set of experiment was replicated five times. Abbreviation: LCL= lower confidence limit; UCL= upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. Ts = testing significance of the regression coefficient of *H. suaveolens* extract in aqueous (4.52^{+}), methanol (3.99^{+}), and petroleum ether (4.77^{+}). (+) Linear regression between x and y

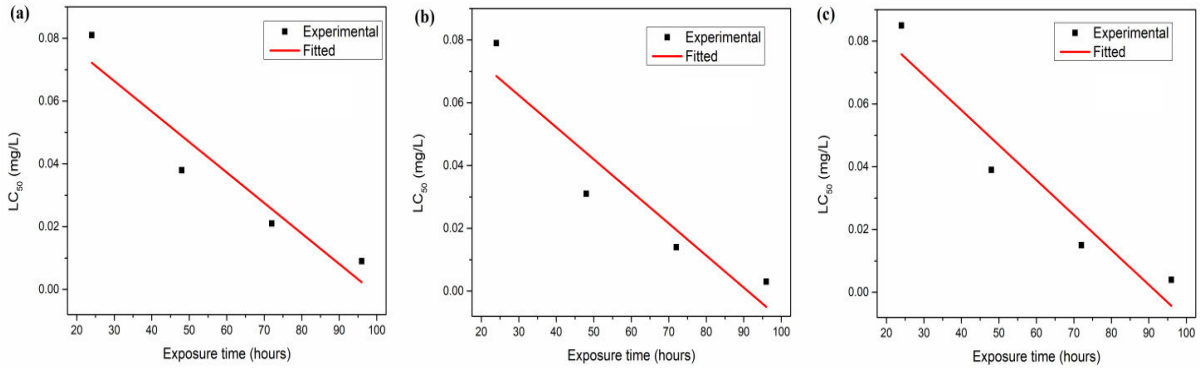

Fig. 1: Variation in the LC_{50} values with respect to exposure time for (a) aqueous, (b) methanol, and (c) petroleum ether extracts.

linear regression was suited for the exposure time and the LC_{50} values.

In the oviposition bioassay, concentration dependent oviposition deterrent effect of the aqueous, methanol and petroleum ether extracts was observed in both no-choice and choice bioassays as compared to control ($P<0.05$) (Table 3). The number of eggs laid by female moths was significantly lower at 5% aqueous extract (110.6 eggs, Table 3) when compared to control (136.0 eggs), however, no significant effect was observed between control and other tested concentration of aqueous plant extract (i.e., at 1%, 2%, 3% 4% eggs laid 126, 120.8, 120.4, 121.1, respectively; Table 3). Similar result was also found in methanol extract where 1%, 2% and 3% concentration did

Table 2: Ovipositional responses of *P. xylostella* females to different extracts of *H. suaveolens* in no- choice and choice bioassay

Concentration (%)	Number of Eggs Laid (Mean± S.E.M.)						
	No- choice			Concentration (%)	Two-choice		
	HA	HM	HPE		HA	HM	HPE
Control	136.0 ± 6.01^{a}	139.2±6.31^{a}	140.2±8.30^{a}	Control	89.6 ± 8.05^{ns}	102.6±2.76***	76.6 ± 7.18^{ns}
				1%	72.0 ± 9.26	62.0±2.91	77.2 ± 6.68
1%	126.0 ± 3.16^{ac}	136.0± 6.56^{a}	118.6±6.11^{ac}	Control	95.6*** ± 4.61	92.4 ± 6.29***	93.2 ± 10.9^{ns}
				2%	61.6 ± 3.65	48.6 ±1.96	79.4 ± 6.15
2%	120.8 ± 5.93^{ac}	122.8±7.10^{ac}	114.4±2.58^{bc}	Control	94.8 ± 21.33^{ns}	104.2±12.28***	80.4± 6.73^{ns}
				3%	81.2 ± 7.75	36.6 ± 2.60	71.6 ± 7.83
3%	120.4 ±3. 14^{ac}	120.0±6.46^{ac}	107.2±7.55^{bc}	Control	103.6±10.24***	105.6 ± 5.24***	108.4 ± 9.64^{ns}
				4%	39.8 ± 3.02	22.4 ± 0.92	87.2 ± 8.95
4%	121.1 ± 4.42^{ac}	107.2±6.58^{bc}	104.6±4.28^{bc}	Control	102.8 ± 8.35***	132.4±11.35***	105.2 ± 5.08**
				5%	22.8±1.42	15.6 ± 1.50^{a}	70.8± 5.00^{a}
5%	110.6± 4.98^{bc}	103.0±7.40^{bc}	90.6±3.15^{b}				

Means followed by different lower-case superscripts differ significantly between control and treatments in no-choice tests, and at each concentration in choice tests. HA, HM, HPE: Aqueous, Methanol and Petroleum ether extract of *Hyptis suaveolens*. **, *** Response significant at $p<0.01$ and $p<0.001$, respectively. ^{ns} Difference not significant statistically.

Table 3: Oviposition Deterrent Index (ODI) of *H. suaveolens* extracts against *P. xylostella*

Concentration (%)	Oviposition Deterrent Index (ODI)		
	Mean ± S.E.M.		
	HA	HM	HPE
1%	11.5^{a} ± 2.80	24.7^{a} ± 1.98	7.6^{a} ± 2.81
2%	21.6^{a} ± 2.96	30.7^{a} ± 3.72	12.2^{a} ± 4.06
3%	18.0^{a} ± 3.77	47.6^{b} ± 3.56	8.9^{a} ± 4.76
4%	41.9^{b} ± 4.40	66.7^{c} ± 1.17	9.62^{a} ± 3.25
5%	63.3^{c} ± 1.84	78.1^{c} ± 3.20	19.6^{a} ± 4.34

Means followed by different lower-case superscripts differ significantly between control and treatments.

Table 4: Egg hatchability of *P. xylostella* to different extracts aqueous, methanol and petroleum ether extract of *H. suaveolens*

Concentration (%)	Mean ± S.E.		
	HA	HM	HPE
Control	101.2^{a} ± 1.77	99.4^{a} ± 2.82	101.0^{a} ± 1.41
1%	90.4^{b} ± 1.86	83.6^{b} ± 2.06	88.0^{b} ± 1.78
2%	86.0^{b} ± 2.07	77.2^{b} ± 2.15	77.0^{c} ± 2.19
3%	76.4^{c} ± 2.31	61.6^{c} ± 2.83	67.0^{d} ± 2.30
4%	59.2^{d} ± 2.57	42.6^{d} ± 2.37	48.6^{d} ± 2.50
5%	52.8^{d} ± 2.35	37.2^{d} ± 1.82	43.0^{e} ± 1.48

Means followed by different lower-case superscripts differ significantly between control and treatments.

not exhibit significant difference with control (136, 122.8, 120 and 139.2 respectively). However, 4% and 5% concentration effectively suppressed the egg laying of female diamond back moths (107.2 and 103, respectively). In contrast, petroleum ether extract exhibited more effectiveness than aqueous and methnol extracts (Table 2). The oviposition was significantly suppressed even at 2% concentration (114.4), however, no difference was observed among 2%, 3%, 4% and 5% concentrations (114.4, 107.2, 104.6 and 90.6 eggs, respectively) of petroleum ether extract. The ODI value of aqueous and methanol extracts were found less effective however, the ODI values of petroleum ether extract was found very effective in decreasing the egg laying by female DBM (Table 3).

The egg hatchability was significantly decreased at all the concentration of aqueous, methanol and petroleum ether extract as compared to control (Table 4). However, the ovicidal effect of aqueous and methanol extracts at 4% and 5% concentration was not significant among themselves (59.2 and 52.8 for aqueous and 42.6 and 37.2 for methanol, respectively). The effect of all extracts of *H. suaveolens* on hatching of *P. xylostella* eggs indicates the presence of inhibiting compounds in the extracts that adversely affect the egg development.

Discussion

In case of toxicity bioassay, the calculated values of t-ratio for aqueous extract was found to be 2.904, 4.165, 5.561, and 3.069 for 24 h, 48 h, 72 h, and 96 h of exposure duration, respectively. For methanol solvent, these values were found to be 3.437, 3.362, 3.414, and 3.162 for 24 h, 48 h, 72 h, and 96 h of exposure duration, respectively. Similarly, for petroleum ether, the values of t-ratio were 3.421, 3.489, 3.106, and 3.121 for 24 h, 48 h, 72 h, and 96 h of exposure duration, respectively. In all the cases, the calculated values of heterogeneity factor were found to be less than 1.0 which indicates that in the replicate tests of the random samples, the concentration response line falls within 95 % of the confidence limit, indicating adequate fit of the data by the given model. The testing of significance of regression coefficient showed a significant negative regression ($p< 0.05$) between the exposure time and the LC_{50} values of different treatments (Tarkeswar *et al.*, 2014).

Reduced egg laying by *Plutella xylostella* females on host cauliflower leaf surface treated with all the tested extract, i.e., aqueous, methanol and petroleum ether of *Hyptis suaveolens* extract were observed. This may be either due to the presence of repellent in the extract, restricting arrival of female moths on oviposition substances, or due to the presence of deterrent chemicals in the extract that inhibit egg laying by gravid females on arrival or both. Such reduction in egg laying by *Artemia salina* and *Tribolium castaneum* female was observed, when the volatile oil from dried plant of *Hyptis suaveolens* applied on (Kamrul *et al.*, 2018).

Oviposition deterrent effects (ODE) of *Ageratum conyzoides* crude aqueous and methanolic extract were reported on *Plutella xylostella* (Vagisha *et al.*, 2023). Such ODE on oviposition of diamond back moth has also been recorded from plant extracts of Chinaberry (Chen *et al.*, 1996), Neem (Charleston *et al.*, 2005) and Yam bean (Basukriadi and Wilkins, 2014). The inhibition occurs through chemoreceptors. In insect, receptors are located on the antennae, proboscis, ovipositor and tarsi, transmitting signals to the central nervous system for information processing (Schoonhover *et al.*, 2005). Present study clearly indicates that *Hyptis suaveolens* possesses chemicals that inhibit larvae and oviposition of gravid *Plutella xylostella.*

Conclusion

The finding of the present study are most important and may be very helpful in the management of *Plutella xylostella.* The present study suggests that *Hyptis suaveolens* has tremendous potential for the management of *P. xylostella* in the fields. Moreover, complex mixtures of active constituents, as found in

botanical insecticides, may also be advantageous in terms of pest resistance and behavioral desensitization. This system of management will not only be environmental friendly but also sustainable.

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