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Emamectin benzoate: Potential larvicide and antifeedant agent against cotton Boll worm *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract
Helicoverpa armigera, a global polyphagous pest, attacks a wide variety of crops causing huge agricultural loss. Overuse of conventional insecticides for *Helicoverpa* control has made *Helicoverpa* resistant to insecticides leading to more severe attacks on crops diverting interest of researchers to explore alternate control agents. Present study investigates the cidal and antifeedant potential of Emamectin benzoate; a semi-synthetic avermectin derived from the soil actinomycetes, *Streptomyces avermitilis;* against early IV instars of *H. armigera*. Larvae were fed on the castor leaf discs (3.5 cm diameter) dipped in different concentrations of Emamectin benzoate; ranging from 0.05 µg/mL-1.5 µg/mL. The leaf disc areas were measured pre-and post-larval feeding to estimate the antifeedant potential of compound. The effect of feeding was also assessed on the survival of larvae by scoring the larval mortality till 96 h. Our investigations showed significant larvicidal potential of Emamectin benzoate against *H. armigera* revealing respective LC_{50} values of 0.26 µg/mL, 0.095 µg/mL, 0.043 µg/mL and 0.027 µg/mL after 24, 48, 72 and 96 h feeding. Furthermore, a remarkable decrease of 93.59% was observed in larval feeding potential indicating significant antifeedant efficacy of Emamectin benzoate. A strong correlation between antifeedant index and the Emamectin benzoate concentration resulted in 1.48-fold index reduction with a decrease in concentration. Our results demonstrated efficacy of Emamectin benzoate as an effectual larvicidal and antifeedant agent against *H. armigera*. Employing selective insecticide can tackle issues of pest resistance and pest resurgence after ascertaining in the fields as *Helicoverpa* control agent and negating impact on non-target organisms.

Keywords: Antifeedant, Emamectin benzoate, *Helicoverpa armigera*, Larvicidal, Leaf dip assay

INTRODUCTION

Helicoverpa armigera (Lepidoptera: Noctuidae) is one of the most devastating polyphagous pests of crop fields in Asia, South America, Australasia and Africa causing extensive crop loss each year. Regarding monetary value, Indian agriculture suffers an annual loss of about US$ 42.66 million due to insect pests (Sharma, 2001; Thakur *et al.,* 2006; Singh *et al*., 2014). Various conventional synthetic insecticides have been used for the control of this pest; such as organochlorines – endosulfan; organophosphates – chlorpyrifos; carbamates – carbaryl; pyrethroids – cypermethrin, lambda-cyhalothrin, deltamethrin; microbial – chlorfenapyr, neonicotinoids – imidacloprid; indoxacarb, etc. (Forrester *et al.,* 1993; Ishtiaq *et al.,* 2012; Carneiro *et al*., 2014). Almost 30% of the pesticides are targeted against *H. armigera* which results in the consumption of a major proportion of total insecticides in the market just to control this pest (Ahmad, 2007; Lammers and Macleod, 2007).

Over the years, the extensive use of chemical insecticides has led to the development of insecticide resistance in lepidopterans which not only increased insecticide cost due to frequent and recurrent applications with higher quanities, but has also contributed significantly to the environmental pollution. *H. armigera* has been shown to develop resistance against various conventional insecticides; organophosphates (Gunning *et al*., 1998), spinosad, carbamates and synthetic pyrethroids (Wang *et al*., 2009; Avilla and González-Zamora, 2010; Karaagaç *et al.,* 2013). Fourteen populations from Northern China have shown to

possess 43-fold to 830-fold resistance to fenvalerate (Yang *et al.,* 2013). Moreover, the tendency of insecticides to accumulate in lipids has increased the human health risks by their amplification in aquatic food chains (Schlechtriem *et al*., 2012). To alleviate the problem developed due to the overdependence on conventional insecticides in pest control programs, various groups of insecticides of natural origin have been synthesized with specific and selective action against desired species; and found to be safer for natural enemies and environment (Dhadialla *et al*., 1998; Thompson *et al*., 2000; Ignacimuthu and Jayaraj, 2003; Smagghe *et al*., 2003).

Among various bio-insecticides dominant in Indian market field with different modes of action, Emamectin benzoate, a second generation Avermectin, composed of abamectin extracted from *Streptomyces avermitilis* a soil microorganism, has been found to possess significant efficacy against pests (Burg *et al*., 1979, Birah *et al*., 2008; Temple *et al.,* 2009). Laboratory screening of Emamectin benzoate salts against *Spodoptera eridania* (Southern armyworm), *Heliothis virescens* (Tobacco budworm) and other lepidopterans advovcated its efficacy at quite low dosages (Mrozik, 1994; Vargus *et al*., 1997; Ishaaya *et al.,* 2002; Grafton-Cardwell *et al*., 2005). It has been reported that Emamectin benzoate acts as a chloride channel activator which results in influx of chloride ions into the membrane leading to nerve membrane depolarization and eventually blocking the nerve impulse conduction. Gradually it results in the muscle paralysis and feeding cessation ultimately leading to death (El-Sheikh and El-Sayed, 2015).

Despite of reports indicating potential efficacy of Emamectin benzoate against lepidopterans, only some degree of work has been carried out on its possible use as a control agent of *H. armigera.* Limited literature is available on the larvicidal and antifeedant potential of Emamectin benzoate against *H. armigera*, although extensive research has been conducted on several insecticides and bio-insecticides (Wise *et al.,* 2010; Rashwan *et al.,*2013; Khaliq *et al.,* 2014; Nikam *et al.,* 2015). Therefore, the present study is an attempt to explore the efficacy of bio-insecticide Emamectin benzoate as a potential larvicide and antifeedant against early fourth instars of *H. armigera*. The research outcomes may provide useful information to develop a strategy for *H. armigera* management which can be shared with researchers, farmers and workers across the globe to develop a safer product for *H. armigera* management.

MATERIALS AND METHODS

Rearing of *Helicoverpa armigera:* The culture of *H. armigera* was procured from NBAIR Live Insect Repository (Bengaluru) with Accession No. NBII-MP-NOC-01 to carry out the investigations and was maintained in a BOD incubator under controlled conditions of 27 ± 1°C and 80 ± 5% RH (Relative Humidity) with a photoperiod of 12h daylight/12h darkness. Larvae were reared on artificial (Mishra *et al*., 2015) as well as natural diet (castor leaves). However, in order to maintain hygiene, to avoid chances of infection and to rear healthy and disease-free larvae, artificial diet (Table 1) was preferred over natural diet. The larvae were reared individually in Petri plates (7 cm diameter) to avoid the cannibalistic behaviour of the larvae.

The pupae formed were transferred to the plastic cages (15cm x 15cm) for adult emergence after disinfection with 0.5% sodium hypochlorite (NaClO) solution (Singh and Rembold, 1992). Adults were fed with 10% sucrose solution kept in the cages in plastic vials. After two days, male and female adults were released (in 5:3 ratio) into 2000 mL glass jars. Each jar was covered with muslin cloth and adults were left undisturbed for mating and egg laying. The egg-laden muslin cloth was replaced every day with a fresh one, which was then kept in a tight-lid box to avoid larval escape.

Impact of Emamectin benzoate on the survival of *Helicoverpa armigera:* Larvicidal activity of Emamectin benzoate against *H. armigera* was studied by ingestion assay using leaf dip no-choice method (Ramya, 2008). The assay was conducted at a temperature of 27 ± 1°C, 80 ± 5% RH with a photoperiod of 12h daylight/12h darkness. Fresh and infection/disease-free castor leaves were procured from the surroundings and leaf discs of 3.5 cm diameter were cut with the help of a borer. The discs were dipped completely in different concentrations of Emamectin benzoate (0.05 µg/mL to 1.5 µg/mL) for 20 seconds and air dried. Single 4 h-starved early fourth instar larva of *H. armigera* was introduced in a Petri dish lined with moist filter paper and an experimental leaf disc was provided as the food. The control assays were run with leaf discs dipped in distilled water. Post 24 h-exposure, the larvae were maintained on fresh non-treated castor leaves which were replaced with fresh leaves every 24 h. Larval mortality was recorded every 24 h till 96 hours of treatment to assess the delayed toxicity of Emamectin benzoate and percent mortality was calculated. The assays at each concentration were carried out in three replicates, each with 10 larvae.

Antifeedant potential of Emamectin benzoate against *Helicoverpa armigera:* Antifeedant bioassay of Emamectin benzoate against *H. armigera* was conducted at laboratory conditions of 27 ± 1°C, 80 ± 5% RH and 12/12: L/D photoregime using leaf dip no-choice method (Mishra *et al*., 2015). Fresh castor leaf discs of 3.5

cm diameter were punched and scanned using Image J software. The discs were dipped in different concentration of Emamectin benzoate (0.05 µg/mL to 1.5 µg/mL) for 20 seconds separately and air-dried. Single 4 h-starved early fourth instar larva of *H. armigera* was introduced in a Petri dish lined with moist filter paper containing single experimental leaf disc for food. The control assays were conducted with leaf discs dipped in distilled water. After 24 h, both experimental and control leaves were rescanned using Image J software. The result was analyzed to assess the antifeedant potential of Emamectin benzoate. The experiment was conducted in triplicates for each concentration, with 10 larvae per replicate. The Antifeedant Index was calculated using formula as provided in Equation No. 1 below (Isman *et al*., 1990; Wheeler and Isman, 2001).

$$Antifeedant\ Index = \frac{C-T}{C+T} X\ 100 \quad \text{..Eqn(1)}$$

Where, C represents the consumed area of castor leaf discs in control assays and T value indicates the area of experimental castor leaf discs consumed by the early fourth instars of *H. armigera*.

Statistical analysis: The larvicidal tests with more than 20% larval mortality in controls were discarded and repeated again. If the control mortality ranged between 5-20%, it was corrected using Abbott's formula as presented in Equation No. 2 (Abbott, 1925).

$$Corrected\ Mortality\ (\%) = \frac{T-C}{100-C} X\ 100 \quad \text{..Eqn(2)}$$

Where, T is the per cent mortality in the treated larvae and C is the per cent mortality in control.

The data was subjected to dosage-mortality regression analysis using computerized SPSS Program (Version: 19.0) to understand the linear relationship between the concentration (dose) of insecticide and larval response. The LC_{50} and LC_{90} values with 95% fiducial limits were calculated to measure the difference between the test samples (Finney, 1971).

RESULTS

Present investigations were conducted to assess the larvicidal and antifeedant potential of an avermectin; Emamectin benzoate; against early fourth instars of *H. armigera.* Larvicidal bioassay with Emamectin benzoate performed at different concentrations, ranging from 0.05 µg/mL to 1.5 µg/mL, showed a dose-dependent efficacy, the cidal activity increasing with the rise in concentration. No larval mortality was observed in control assays during the exposure period. The cidal effects of Emamectin benzoate on the early fourth instars of *H. armigera* are presented in Table 2. The data obtained shows the respective LC_{50} values of 0.269 µg/mL, 0.095 µg/mL, 0.043 µg/mL and 0.027 µg/mL against *H. armigera* early fourth instars obtained after 24 h, 48 h, 72 h and 96 h of feeding on experimental discs. On the other hand, the LC_{90} values obtained after 24 h, 48 h, 72 h and 96 h of feeding were 2.738 µg/mL, 1.070 µg/mL, 0.942 µg/mL and 0.549 µg/mL, respectively. It clearly shows a positive correlation between the toxicity of Emamectin benzoate and time duration after feeding on experimental discs; the lethal effects of the Emamectin benzoate increased as the time lapsed after feeding on experimental discs increased. The LC_{50} values reduced by 2.83 and 2.21-fold after 2 and 3 days' post-ingestion of experimental discs; respectively. A significant 1.59-fold increase in the larval mortality was observed on continuing the assay till 96 h. These results indicate delayed toxicity of Emamectin benzoate against early fourth instars of *H. armigera*.

The antifeedant efficacy of Emamectin benzoate was evaluated by estimating and comparing the leaf area consumed by early fourth instars of *H. armigera* in experimental and control assays (Fig.1). The bioassay results presented in Table 3 clearly reveal the dose-dependent increase in the antifeedant activity of Emamectin benzoate. The provision of leaf discs dipped in different concentrations of Emamectin benzoate to the larvae results in 59.70% to 88.88% reduced food consumption as compared to consumption observed in control assay (Table 3). The highest feeding deterrence observed at 1.5 µg/mL caused only 4.48% leaf area consumption while the least feeding deterrence observed at 0.05 µg/mL results in consumption of 21.11% leaf area as compared to the 69.94% area consumed by larvae in control sets after 24 h (Fig. 2).

DISCUSSION

Over the years application of synthetic insecticides for the control of *H. armigera* has largely contributed to the development of insecticide resistance in pest populations due to increased detoxification activity of enzymes (Li *et al*., 2007). Present investigations were performed to assess the toxicity of a selective and eco-safe insecticide, Emamectin benzoate against *H. armigera.* Toxicity of Emamectin benzoate has been studied among different insect species and has been found to possess high toxicity against several lepidopterans, such as *H. armigera*, *H. virescens*, *Pseudoplusia includens, Plutella xylostella, Spodoptera exigua, Trichoplusia ni, S. frugiperda* and *S. littoralis* (Payne *et al*., 1999; Argentine *et al*., 2002; Firake and Pande, 2009).

Present study clearly reveals the appreciable larvicidal potential of Emamectin benzoate against *H. armigera* with respective LC_{50} values of 0.269 µg/mL and 0.043 µg/mL for 24 h and 72 h. Slightly higher LC_{90} value of 2.738 µg/mL obtained in the current study can be attributed to the variations and heterogeneity in the population. These results

Table 1. Components of artificial diet provided to *Helicoverpa armigera* during rearing.

S. No.	Ingredient	Make	Quantity
	Part I		
1	Gram flour	-	55.00 g
2	Casein	(Qualigens)	10.00 g
3	Yeast powder	(Merck)	5.00 g
4	Sorbic acid	(CDH chemicals)	0.25 g
5	Methyl Paraben	(Merck)	1.00 g
6	Formaldehyde	(Merck)	0.05 g
7	Water (double distilled)	-	195.00 mL
	Part II		
1	Agar	(Merck)	6.00 g
2	Water (double distilled)	-	170 mL
	Part III		
1	Wheat bran	-	5.00 g
2	Ascorbic acid	(Qualigens)	1.30 g
3	Salt mix	(Qualigens)	5.0 g
4	Streptomycin sulfate	(Qualigens)	0.057 g
5	Cholesterol	(Qualigens)	0.050 g
6	Multivitamin	(Merck)	0.50 g
7	Vitamin E	(Merck)	0.30 g
8	Water (double distilled)	-	45.00 mL
	Total		500.00 mL

Table 2. Larvicidal activity of Emamectin benzoate against early fourth instars of *Helicoverpa armigera.*

Feeding duration (h)	LC_{50} (µg/mL)	95% Fiducial limits	LC_{90} (µg/mL)	95% Fiducial limits	Regression coefficient	S.E.	χ^2	df
24	0.269	0.089 - 0.592	2.738	1.130 - 18.092	1.271	0.305 (1.617)*	6.822	3
48	0.095	0.028 - 0.250	1.070	0.378 - 10.985	1.221	0.307 (1.682)	1.021	2
72	0.043	0.006 - 0.134	0.942	0.271 - 27.588	0.957	0.281 (1.540)	1.026	2
96	0.027	0.002 - 0.085	0.549	0.162 - 15.252	0.978	0.299 (1.638)	0.606	2

*Figures in parentheses indicate the standard deviation;LC_{50} - lethal concentration that kills 50% of the exposed larvae, LC_{90} - lethal concentration that kills 90 % of the exposed larvae;S.E. = Standard error, $\chi 2$ = chi-square, df = degree of freedom;Test samples were transformed into log covariant (log_{10}), $p>0.05$, level of significance is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits

Table 3. Antifeedant potential of Emamectin benzoate against early IV instars of *Helicoverpa armigera* when fed on castor leaf discs during no-choice bioassay.

Concentration (µg/mL)	Initial mean leaf area (mm^2) ± SEM	Final mean leaf area (mm^2) ± SEM	Leaf area consumed (mm^2) ± SEM*	Antifeedant Index
Control	978.116 ± 1.020	294.003 ± 7.088	684.113 ± 8.108 a	-
0.05	817.512 ± 4.476	644.983 ± 85.016	172.589 ± 89.49 b	59.70
0.1	827.651 ± 3.549	728.493 ± 30.965	99.157 ± 27.41 c	74.68
0.5	845.368 ± 16.124	769.730 ± 20.736	75.638 ± 4.612 cd	80.08
1.0	897.395 ± 4.927	827.793 ± 12.513	69.602 ± 7.58 d	81.53
1.5	898.432 ± 8.518	858.163 ± 13.020	40.269 ± 4.501 e	88.88

Mean ± SEM, calculated for three replicates, each with 10 larvae; *Figures in each column followed by different letters are significantly different ($P< 0.05$, one way ANOVA followed by Tukey's all pair wise multiple comparison test)

are in conformity with the previously reported 100% larval mortality in *H. armigera* caused by Emamectin benzoate by residue contact method resulting in LC_{50} of 1.75 µg/mL and LC_{90} of 13.08 µg/mL (Parsaeyan *et al*., 2013). Almost similar results have been obtained by El-Sheikh and El-Sayed (2015) who reported respective LC_{50} values of 0.34 µg/mL and 0.09 µg/mL after 24 h and 72 h when 3rd instar of *Spodoptera litura* were fed with the diet treated with Emamectin benzoate. The comparable LC_{90} values of Emamectin benzoate against different lepidopteran pests in the range of 0.002-0.89 µg/mL have been obtained by Jansson and Dybas (1996). The LC_{50} values in the range of 0.005-0.021 µg/mL were revealed by Argentine *et al.* (2002) against six species of lepidopterans; *H. virescens*, *P. includens*, *P. xylostella*, *S. exigua*, *T. ni* and *S. frugiperda*. In contrast,

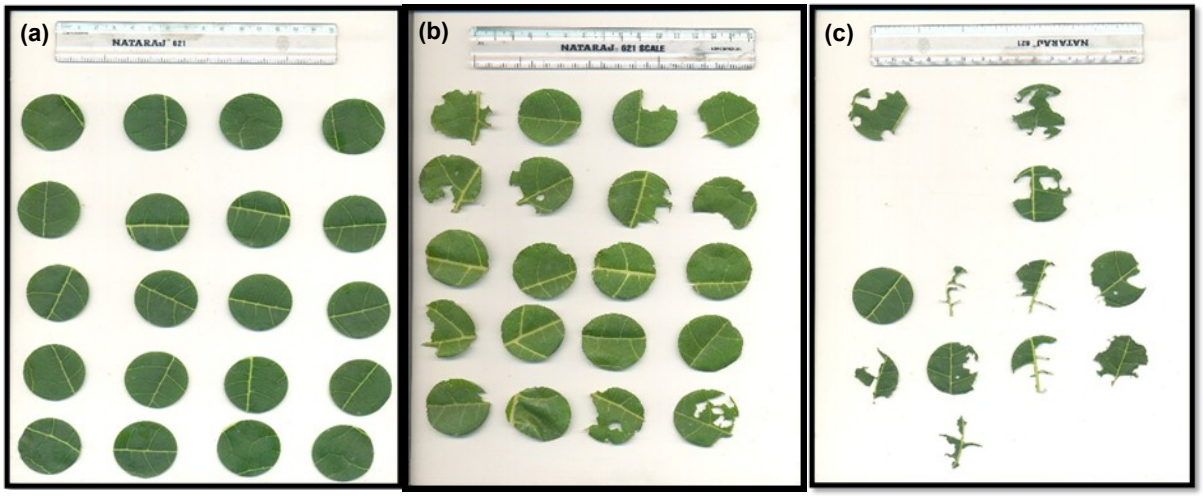

Fig. 1. *Leaf dip antifeedant bioassay against Helicoverpa armigera with castor leaf discs; (a) Leaf discs before feeding; (b) Post-feeding – experimental leaf discs dipped in 1.5 µg/mL Emamectin benzoate; (c) Post feeding – control leaf discs.*

Jansson *et al.* (1997b) reported that foliar spray with Emamectin benzoate at 0.003 µg/mL could kill 90% of the neonates of *H. virescens,* while 90% of *H. zea* were killed at 0.002 µg/mL. Studies have shown 875-fold to 2,975-fold higher potency of Emamectin benzoate than tebufenozide against *H. virescens* while 12.5-fold to 20-fold higher efficacy in comparison to λ-cyhalothrin and 175-fold to 400-fold more potency as compared to fenvalerate (Jansson *et al*., 1997a).

In 2012, Fanigliulo and Sacchetti field-tested Emamectin benzoate at a concentration of 1.5 Kg/ha and could significantly control *H. armigera* within two years. Earlier Dunbar *et al.* (1998) reported effective control of *H. virescens* and *H. zea* larvae through diet incorporated with Emamectin benzoate. As stated earlier, the effectiveness of Emamectin benzoate as a larvicide has been reported by Saeed *et al.* (2012) in Pakistan against *S. exigua* revealing LC_{50} value of 0.005 mg/L. Higher larvicidal efficiency of Emamectin benzoate against 2nd instar of *S. littoralis* as compared to spinosad has been evidenced by Korrat *et al.* (2012).

The present study also assessed the impact of Emamectin benzoate on the feeding behaviour of early fourth instars of *H. armigera* using no-choice bioassay. Our results revealed that larval feeding with castor leaves dipped in various concentrations of Emamectin benzoate, ranging from 0.05 µg/mL to 1.5 µg/mL, reduced consumption by 59.70% to 88.88%. Till date, the antifeedant properties of Emamectin benzoate has not been explored against insects. Nevertheless, Mishra *et al.* (2015) evidently showed comparatively lower antifeedant potential of stem and leaf methanol extracts of *Thevetia neriifolia* against *H. armigera* showing 20.8% to 73.31% reduction in consumption of leaves dipped in 0.5%, 1%, 3% and 5% extract. Arivoli and Tennyson (2013) screened antifeedant activity of 25 plants against III instars of *S. litura* using leaf disc no-choice method and reported antifeedant potential of 1.0% hexane leaf extracts of *Vitex negundo* and 1.0% hexane seed extracts of *Abrus precatorius* resulting in 86.41 and 78.61 antifeedant activities, respectively. Antifeedant properties of the extracts prepared from *Rhinacanthus nasutus*, *Ocimum canum*, *O. sanctum* and *Citrus sinensis* were shown by Kamaraj *et al.* (2008) against early fourth instars of *H. armigera*. The similar potential of around 25 different plants has been observed against sixth instars of *H. armigera* by Ramya and Jayakumararaj (2009). Likewise, the significant antifeedant effect of ethyl acetate leaf extract of *Syzygium lineare* has been reported by Jeyasankar and Ignacimuthu (2010) against larvae of *S. litura*.

Selvam and Ramakrishnan (2014) carried out investigations with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts prepared from the leaves of *Tinospora cardifolia* to assess the antifeedant activity against *H. armigera* IV instars using leaf discs assays. Higher antifeedant activity of methanol extracts was established over the other tested extracts revealing respective AFI_{50} values of 274.52, 240.76, 222.96, 218.2, 193.75 mg/L. Assays to screen 0.625%, 1.25%,

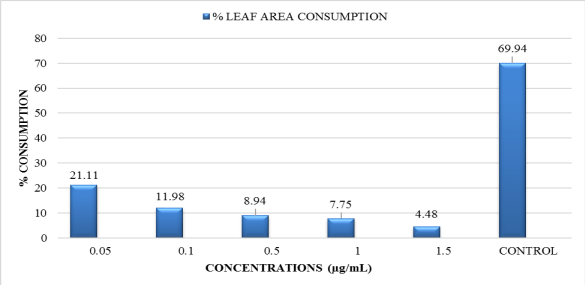

Fig. 2. *Percent area of castor leaf discs consumed by early fourth instars of Helicoverpa armigera dipped in different concentrations of Emamectin benzoate.*

2.5% and 5% methanol extracts of *Swertia chirata* whole plant for their antifeedant activity against *H. armigera* using leaf disc no-choice assay revealed the high antifeedant activity. The respective antifeedant index recorded was 37.13%, 47.72%, 56.64% and 68.53% against *H. armigera* (Balaraju *et al.,* 2011). Likewise, Muthusamy *et al*. (2015) also reported higher antifeedant efficacy of the methanol extract over non-polar extract when they conducted the investigations with the benzene, chloroform, hexane and methanol extracts of *Caesalpinia bonducella* leaves against *H. armigera,* the methanol extract displaying significant higher antifeedant activity (%) at 400 mg/L (86.87 ± 2.82) and 500 mg/L (96.73 ± 2.36) over other extracts.

Conclusion

Present investigations carried out with an objective to explore an environment-safe alternative to curb the harm caused by *H. armigera* larvae, explored the antifeedant and larvicidal potential of Emamectin benzoate against *H. armigera*. Our investigations demonstrated the significant larvicidal potential of Emamectin benzoate with maximum mortality at 1.5 µg/mL. The LC_{50} value of 0.269 µg/mL for 24-hour duration depicts the strong cidal efficacy of Emamectin benzoate. Increased efficacy of Emamectin after 48 h and 72 h of feeding established the delayed toxic effects. Feeding with 1.5 µg/mL of Emamectin benzoate also resulted in significant feeding deterrence effects resulting in only 4.48 % consumption with antifeedant index of 88.88. These results confirmed the efficacy of Emamectin benzoate as an effectual larvicide and antifeedant agent against *H. armigera*. The present investigation suggests the potential and effective use of Emamectin benzoate in integrated pest management programs implemented for the control of *H. armigera.*

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