Asian Pacific Journal of Tropical Biomedicine
journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014B546

Feeding deterrent and growth inhibitory activities of PONNEEM, a newly developed phytopesticidal formulation against *Helicoverpa armigera* (Hubner)

Soosaimanickam Maria Packiam*, Kathirvelu Baskar, Savarimuthu Ignacimuthu

Entomology Research Institute, Loyola College, Chennai, 600 034, Tamil Nadu, India

PEER REVIEW

Peer reviewer

Dr. Sebastian Raveendar, PhD, Post Doctoral Fellow, Animal Genomics & Bioinformatics Division, National Institute of Animal Science, Rural Development Administration, Suwon, 441-706 South Korea.
Tel: +82-10-4105-7178
E-mail: raveendars@gmail.com

Comments

The author has written the manuscript with standard language and the objective of the paper covered with standard methodology. The newly developed phytopesticidal formulation showed promising growth inhibitory and egg hatchability. The study revealed significant results which will help the researchers or the public private industries to develop eco-friendly pesticides for safer agriculture.
Details on Page S327

ABSTRACT

Objective: To assess the feeding deterrent, growth inhibitory and egg hatchability effects of PONNEEM on *Helicoverpa armigera* (*H. armigera*).
Methods: Five oil formulations were prepared at different ratios to assess the feeding deterrent, growth inhibitory and egg hatchability effects on *H. armigera*.
Results: Invariably all the newly formulated phytopesticidal oil formulations showed the feeding deterrent and growth inhibitory activities against *H. armigera*. The maximum feeding deterrent activity of 88.44% was observed at 15 μL/L concentration of PONNEEM followed by formulation A (74.54%). PONNEEM was found to be effective in growth inhibitory activities and egg hatchability at 10 μL/L concentration. It exhibited statistically significant feeding deterrent activity and growth inhibitory activity compared with all the other treatments.
Conclusions: PONNEEM was found to be effective phytopesticidal formulation to control the larval stage of *H. armigera*. This is the first report for the feeding deterrent activity of PONNEEM against *H. armigera*. This newly formulated phytopesticide was patented in India.

KEYWORDS
Feeding deterrent activity, *Helicoverpa armigera*, PONNEEM, Growth inhibitory, Phytopesticidal formulation, Egg hatchability

1. Introduction

In spite of the good production of food, the crop loss caused by various storage and field insect pests is enormous. Due to the field insect pests, there are heavy losses throughout the world[1]. Insect pests play a significant role in damaging the crops[2]. To safeguard the crops, the chemical pesticides are repeatedly used against insect pests. Over dependent and indiscriminate use of pesticides paved the way for the insect pests to develop pesticide resistance, and pest resurgence which aroused environmental pollution caused ill-effects to the beneficial insects and toxic effects to the crops and humans[3,4]. So these harmful effects have led the people to replace chemical pesticides with natural compounds of plant origin. Plant-based compounds or pesticides are found to be effective in controlling insect

*Corresponding author: Soosaimanickam Maria Packiam, Entomology Research Institute, Loyola College, Chennai, 600 034, India.
Tel: +91-44-2817 4644
Fax: 091-44-2817 5566
E-mail: mariaento@hotmail.com;
Foundation Project: Supported by Entomology Research Institute, Loyola College, Chennai, India (Grant No: ERI/MP/01/2006).

Article history:
Received 22 Jan 2014
Received in revised form 26 Jan, 2nd revised form 8 Feb, 3rd revised form 14 Feb 2014
Accepted 17 Mar 2014
Available online 5 Apr 2014

pests. They are eco-friendly and economically viable and easily available[5]. Today the whole world is convinced of following eco-friendly methods in agriculture.

Plant-based pesticides are potential agents to formulate commercial active ingredients for pest control. Feeding deterrent has been considered as an alternative to chemical pesticides. The biological compounds present in neem oil are found to be strong antifeedants and growth inhibitors against Lepidopteran pests[6]. The feeding deterrant activity against *Spodoptera litura* (*S. litura*) was reported by Rajasekaran and Kumaraswami[7]. *Pongamia pinnata* was reported to be effective against insect pests among stored grains, field and plantation crops as oviposition deterrents and larvicides[8].

There is no information about the feeding deterrent, growth regulation and hatchability activities with additive or synergistic effect of neem and karanj oils against *Helicoverpa armigera* (*H. armigera*). This study aimed at assessing the feeding deterrent, growth regulation and hatchability effects of PONNEEM, a newly prepared oil formulation consisting of pungam and neem oils in equal ratio, against the fourth instar larvae of *H. armigera*.

2. Materials and methods

2.1. Insect pest culture

H. armigera larvae were collected from bhendi field at Mangadu village of Kancheepuram district, Tamil Nadu, India. The collected larvae were reared individually in a plastic container (vial) and regularly fed with bhendi till the larvae attained the pupal stage under laboratory conditions [(27±2) °C and (80±5)% relative humidity]. Sterilized soil was provided for pupation. After pupation, the pupae were collected from the soil and placed inside the cage. Cotton swabs soaked with 10% honey solution which was mixed with few drops of multivitamin were provided for adult feeding to increase the rate of fecundity. Black muslin cloth was placed inside the oviposition cage for egg laying. The eggs were collected from the cloth and allowed to hatch. After hatching the newly emerged larvae were fed with artificial diet in separate vials. These laboratory reared larvae were used for feeding deterrent activity.

2.2. Formulation of phytopesticides

Different oils were taken at specified ratio in a stainless steel vessel with a stirrer and were stirred at 120 r/min for 10 min. Then 8% emulsifier and 1% stabilizer were added into the oils and again they were stirred at 120 r/min for 10 min. At last 0.123% azadirachtin and 2% isopropyl alcohol were added and again they were mixed thoroughly by using a stirrer at 120 r/min for 10 min. Then the final formulations were obtained[9,10].

2.3. Feeding deterrent activity

Different oil formulations were evaluated for their feeding deterrent activity, which used choice method in the following manner. A stock concentration of 500 μL/L of fresh oil formulations was prepared by mixing with dechlorinated water. From the stock, required concentrations such as 5, 10, and 15 μL/L were prepared and tested against the fourth instar larvae of *H. armigera*. Leaf discs of 3 cm in diameter from fresh cotton (*Gossypium* sp.) was punched by using a cork borer; the leaf discs were dipped in 5, 10, and 15 μL/L concentrations. Leaf discs treated with Nimbicidine® served as reference control; leaf discs dipped in emulsifier with water were considered as negative control. Treated leaf discs were placed inside the petridish with wet filter paper to avoid early drying of the treated leaf discs. In each petridish consisting of control and treated leaf discs, single 4th instar larvae of *H. armigera* was released individually for choice method. The experiment was repeated for 20 times. Progressive consumption of leaf area by the larvae after 24 h was recorded using leaf area meter (Delta-T Devices, Serial No. 15736 F96, UK). After treatment the larvae were reared continuously to assess the impact of phytopesticidal oil formulations during the development. The percentage of feeding deterrent index was calculated using the formula of Jannet, *et al*[11].

$$= \frac{\text{Area protected in control leaf} - \text{Area protected in treated leaf}}{\text{Area protected in control leaf} + \text{Area protected in treated leaf}} \times 100$$

2.4. Growth regulation

Growth regulation activities of selected oil formulations (pungam oil, neem oil and PONNEEM) were studied at 10 μL/L concentration against 4th instar larvae of *H. armigera*. Ten larvae were put in a Petri dish with cotton leaves treated with 10 μL/L. Leaves treated with emulsifier with water served as negative control and the leaves treated with Nimbicidine® as reference control. After 24 h feeding the larvae were transferred to normal cotton leaves for studying the developmental periods. For each treatment five replicates were maintained. During the developmental period deformed larvae, pupas, adults and successful adults emerged and the mortality of larvae, pupas and adults were recorded. In addition, time taken to pupation, pupal duration, pupal weight, adult longevity, fecundity and egg hatchability were recorded.

2.5. Fecundity and egg hatchability

The adult moths emerged from the treated and control categories were released inside the oviposition cages (30 cm×30 cm×30 cm) and castor leaves were kept inside the cages. The egg masses laid by the moths were separated daily until all the moths inside the cage died. The scales that covered the egg masses were removed carefully and the total numbers of eggs found in all the egg masses were counted. Finally, the total number of eggs laid by one female moth was calculated. All the egg masses were incubated at laboratory conditions [(27±2) °C; (80±5)% relative humidity; (11.0±0.5) h photoperiod] and the number of larvae, which emerged from the eggs were counted. Per cent hatchability of eggs was calculated.

2.6. Statistical analysis

The antifeedant and growth inhibitory activities were subjected to analysis of variance. Significant differences between treatments were determined by DMRT (P<0.05).

3. Results

3.1. Feeding deterrent activity

The oil formulations obtained from pungam and neem oils were tested for their feeding deterrent activity against 4th instar larvae of *H. armigera* at 5, 10 and 15 μL/L concentrations. The results are presented in Table 1. The maximum feeding deterrent activity of 88.44% was observed in formulation C (PONNEEM) at 15 μL/L followed by formulation A (74.59%). At 15 μL/L concentrations, all the treatments exhibited more than 70% antifeedant activity against *H. armigera* except formulation D (pungam alone). PONNEEM exhibited 83.01% of feeding deterrent activity at 5 μL/L concentration followed by Nimbicidine® (71.47%). The percent feeding deterrentt activity of oil formulations varied significantly. Even at lower concentration PONNEEM also showed statistically significant activity when comparing to other treatments and control.

3.2. Growth regulation

The fourth instar larvae of *H. armigera* were fed with cotton leaves treated with different oil formulations for one day and later transferred to normal cotton leaves to record the time that were taken for pupation (in days), pupal duration (in days), pupal weight (in mg), adult longevity (in days), fecundity of moths and eggs′ hatchability. The data was statistically analyzed and presented in Table 2. Within the different oil formulations, the maximum time taken for pupation was recorded in pungam oil formulation followed by neem oil and PONNEEM, and the minimum on Nimbicidine® compared to all other formulations at 10 μL/L. However, for all different oil formulations, treated larvae took longer time for pupation compared to emulsifier

Table 1
Per cent antifeedant activity of different oil formulations against 4th instar larvae of *H. armigera* using choice method.

Treatments	5 μL/L		10 μL/L		15 μL/L	
	Treated	Control	Treated	Control	Treated	Control
Formulation A (pungam oil+neem oil, 3:7)	63.27±4.26^{c}	13.68±0.58^{c}	74.54±1.66^{d}	12.09±1.14^{b}	74.59±7.54^{c}	15.48±1.21^{cd}
Formulation B (pungam oil+neem oil, 7:3)	61.58±2.12^{bc}	11.29±1.29^{b}	69.76±4.39^{c}	13.49±0.16^{c}	70.91±6.71^{bc}	14.52±0.56^{b}
Formulation C (PONNEEM) (pungam oil+neem oil, 1:1)	83.01±5.65^{e}	9.36±1.32^{a}	88.40±6.02^{e}	9.53±0.64^{a}	88.44±9.08^{d}	10.05±0.29^{a}
Formulation D (pungam oil)	59.11±3.89^{b}	13.68±0.58^{c}	65.23±6.14^{b}	12.09±1.14^{b}	66.34±6.75^{b}	15.48±1.21^{cd}
Formulation E (neem oil)	61.58±2.12^{bc}	11.29±1.29^{b}	69.76±4.39^{c}	13.49±0.16^{c}	70.91±6.71^{bc}	14.52±0.56^{b}
Formulation F (Nimbicidine®)	71.47±2.53^{d}	11.29±1.29^{b}	72.51±5.38^{cd}	13.10±0.78^{c}	70.34±4.20^{b}	14.84±0.94^{bc}
Formulation G (emulsifier control)	15.84±0.60^{a}	15.84±0.60^{d}	15.84±0.60^{a}	15.84±0.60^{d}	15.84±0.60^{a}	15.84±0.60^{d}

Values are mean of twenty replications, means±SD. Figures in parenthesis are arc sine transformed before ANOVA. Values followed by the same letter(s) in a column are not significantly different (P=0.05) by DMRT.

Table 2
Per cent reduction of growth in *H. armigera* larvae treated with different oil formulations for 24 h at 10 μL/L.

Treatments	Reduction of growth					
	Time taken to pupation (days)	Pupal duration (days)	Pupal weight (mg)	Adult longevity (days)	Fecundity eggs/moth	Hatchability
Pungam oil	15.60±0.54^{c}	12.00±1.22^{b}	103.80±5.76^{b}	4.60±0.89^{b}	291.60±7.76^{b}	67.60±4.39^{d}
Neem oil	14.60±1.34^{c}	14.20±0.83^{d}	79.00±2.00^{a}	4.40±0.54^{b}	251.60±4.82^{a}	37.80±2.28^{b}
PONNEEM	14.40±1.51^{bc}	10.60±1.14^{b}	74.80±3.03^{a}	3.40±0.54^{a}	262.00±3.24^{a}	30.20±2.68^{a}
Nimbicidine®	13.00±1.58^{b}	12.80±0.83^{cd}	125.00±2.23^{c}	5.60±0.89^{c}	570.00±8.27^{c}	63.20±3.63^{c}
Emulsifier control	8.20±0.83^{a}	9.00±1.22^{a}	200.00±2.12^{d}	6.40±0.54^{c}	1082.20±12.71^{d}	97.00±1.00^{e}

Means±SD followed by the same letter(s) in a column are not significantly different (P=0.05) by DMRT.

control. Generally pupal weight, adult longevity, and fecundity in PONNEEM were reduced compared to all the other formulations and control.

3.3 Egg hatchability

The fourth instar larva of *H. armigera* were treated at 10 μL/L concentrations for 24 h. After the treatments the larvae were transferred to normal diet and continuously reared up to pupa and adult emergence. A number of eggs were laid by the treated adults and the number of hatched eggs was observed. The maximum reduction in eggs' hatchability was observed in PONNEEM followed by the other treatments. PONNEEM exhibited 30.2% of eggs' hatchability followed by neem oil (Table 2).

4. Discussion

Feeding deterrent is a chemical agent that inhibits feeding without killing the insect directly while the insect remains near the treated foliage and dies through starvation[12]. Most potent insect's antifeedants are sesquiterpene lactones, diterpinoids, triterpinoids, quinoline and indole alkaloids, which are presented in plants. They enhance the feeding deterrent activity against pests[13]. In the present study PONNEEM exhibited 88.44% of feeding deterrent activity against the fourth instar larvae of *H. armigera* at 15 μL/L concentration. Formulation A exhibited 74.59% of antifeedant activity against *H. armigera*. Invariably the per cent in feeding deterrent activity of all the formulations was found to be above 70% at 15 μL/L concentration. This result denoted that the combination of pungam and neem oil showed significant results comparing to Nimbicidine® at all concentrations. The present investigation showed that PONNEEM was found to be an effective antifeedant agent against *H. armigera*. This indicates that the active principles such as karanjin and azadirachtin presented in PONNEEM inhibiting larval feeding or making the food unpalatable or the substances directly acted on the chemosensilla of the larvae, which resulted in feeding deterrence due to synergistic effect. According to earlier reports botanicals possess similar type of feeding deterrent activity against *S. litura*[14-16]. Chemical antifeedants play a major role in the non-host plants' unsuitability as food for insects. In the present investigation, the combination of pungam and neem oils (1:1) exhibited very good results in controlling Lepidopteran insect pests. The present finding coincides with the findings of Packiam and Ignacimuthu who reported that PONNEEM showed more than 80% antifeedant activity against *S. litura*[9]. Neem oil based formulation of active fraction of ethyl acetate extract of *Hydnocarpus alpine* showed antifeedant activity against *S. litura*[17]. Pavunraj, *et al*. reported that the effective fraction from *Melochia corchorifolia* with 1:1 ratio of neem and pongam showed antifeedant activity against four Lepidopteran pests[1].

Due to the toxic effect of plant-based pesticide, the treated larvae of *H. armigera* grew into deformed pupas and adults. The morphological deformities at larval, pupal and adult stages of Lepidopteran pests were noticed by earlier workers[18-21]. The biological compounds present in the plants have a vital role in regulating the growth of insect pests because they work on juvenile hormone of insect pests. The enzyme ecdysone plays a major role in shedding of old skin and this phenomenon is called ecdysis or moulting.Due to the presence of the plant compounds in the larvae of targeted insect pests the function of ecdysone was arrested. As a result the larvae were unable to moult, and continued to prolong the larval duration leading to death[22]. In the present study, PONNEEM treated larvae exhibited maximum percentage of deformed growth of larvae, pupae and adults. The toxic effects of azadirachtin from neem oil and karanjin from pungam oil caused the morphological deformities at different stages of larvae, pupas and adults. These compounds are found to interfere with the hormones of the insects. These results are consistent with the earlier reports on various Lepidopteran species[23]. Joseph reported that neem seed kernel extract was effective in inhibiting larval growth and inducing the pupal and adult deformities in *Earius narcissus indica*[24]. Jeevan triguard formulation based on *Azadirachta indica* produced growth related abnormalities in *S. litura*[25]. Many natural products inhibited the larval growth, increased the larval-pupal duration and multiplied the abnormalities of emerged adults of *H. armigera* and *S. litura*[26-28]. Decreased pupal weight, adult longevity, fecundity and percent hatchability of eggs were observed in the present study. It may be due to reduced feeding rate of the larvae treated with plant extracts affecting the juvenile hormone and ecdysone. When the feeding rate was decreased, pupal weight was also decreased and the development of oocyte was also reduced. The amount of eggs laid by adults was reduced or normal adult emergence was suppressed or sometimes adults laid eggs before copulation because of improper development of oocyte caused by PONNEEM treatment. Because of the infertile eggs, the hatchability was reduced. Several workers have reported that the reduced rate of pupal weight, hatchability and adult emergence was due to the influence

of plant substances[9,29,30].

Among the various phytopesticidal oil formulations evaluated, formulation C (PONNEEM) showed the maximum feeding deterrent activity, reduction in growth regulation and egg hatchability against *H. armigera* due to the synergistic action of neem and karanj oils. This newly developed phytopesticide PONNEEM is found to be good at controlling the Lepidopteran pests.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank the Entomology Research Institute, Loyola College, Chennai, India for financial support (No:ERI/MP/01/2006).

Comments

Background

Over the past 50 years, crop protection has relied heavily on synthetic chemical pesticides, but their availability is now declining as a result of new legislation and the evolution of resistance in pest populations. Therefore, alternative pest management tactics are needed. Plant-based pesticides known as phytopesticides are found as safer and alternative technology to chemical pesticides. Phytopesticides which are ecofriendly, economically viable and easily available to the farmers have multiple mode of action against insect pests. With this background the present study aimed to find out the feeding deterrent, growth inhibitory and egg's hatchability effects of different oil formulations against *H. armigera* which is seriously polyphagous in the world especially in Asia.

Research frontiers

The study deals with eco-friendly crop protection which is the need of the hour. The oil formulation known as PONNEEM showed significant growth inhibitory and egg's hatchability effects against *H. armigera*. PONNEEM, which revealed that the organic pesticides were alternatives to the synthetic pesticides in crop protection. The manuscript presents useful and commendable finding which will be greatly helpful for the development of eco-friendly organic pesticides for the sustainable agriculture.

Related reports

The study showed that it was conducted based on the previous reports of Rajasekaran and Kumaraswami (1985); Kumar, *et al.* (2002) and Koul, *et al.* (2004). The manuscript's results and methodology have been found to be standard methods which are discussed with other suitable and up to date reports.

Innovations and breakthroughs

The phytopesticidal formulations are highly essential in the protection of agricultural crops. The newly developed phytopesticidal oil formulation (PONNEEM) exhibited significant growth inhibitory and egg hatchability effects against *H. armigera*. As far as I concerned this is the first report on plant-based oil formulation at equal ratio for growth inhibitory and egg's hatchability against the *H. armigera* because of the effect of synergism.

Applications

The paper is essential, like a benchmark report to good agricultural practices. The present study helps to develop eco-friendly bio-pesticides to control economically important agricultural pests like *H. armigera*.

Peer review

The author has written the manuscript with standard language and the objective of paper covered with standard methodology. The newly developed phytopesticidal formulation showed promising growth inhibitory and egg hatchability. The study revealed significant results which will help the researchers or the public private industries to develop eco-friendly pesticides for safer agriculture.

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