Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading

Formulation of A Novel Phytopesticide PONNEEM and its Potentiality to control generalist Herbivorous Lepidopteran insect pests, *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

Susaimanickam Maria Packiam *, Veeramuthu Anbalagan, Savrimuthu Ignacimuthu, Subramanian Ezhil Vendan

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

ARTICLE INFO

Article history:
Received 5 August 2012
Received in revised from 18 September 2012
Accepted 19 November 2012
Available online 28 December 2012

Keywords:
Phytopesticide
PONNEEM
Lepidopteran
Ovipositon activity
Karanjin
Azadirachtin

ABSTRACT

Objective: To evaluate the deterrence of oviposition potentiality of a novel phytopesticide *PONNEEM* against the generalist herbivorous lepidopteran insect pests *Helicoverpa armigera* and *Spodoptera litura*. **Methods:** Different phytopesticidal formulations were prepared at different ratio to evaluate the deterrence of oviposition activity against *S. litura* and *H. armigera* at 5, 10, 15, and 20 μL/L concentrations. **Results:** The newly formulated different phytopesticides exhibited good results of oviposition deterrent activity against these two polyphagous insect pests. At 20 μL/L concentration of *PONNEEM*, 77.48% of the maximum deterrence of oviposition activity was recorded, followed by formulation A (49.23%). And 68.12% was observed against *H. armigera* followed by A (49.52%). *PONNEEM* exhibited statistically significant oviposition deterrent activity compared to all other treatments. **Conclusions:** The newly formulated *PONNEEM* was found to be effective phytopesticidal formulation to control the adult of *S. litura* and *H. armigera* due to the synergistic effect of biomolecules such as azadirachtin and karanjin. This is the first report of *PONNEEM* which was patented under the government of India. The potential use of this novel phytopesticide could be an agent of controlling the adults of lepidopteran insect pests which can be applied in the integrated pest management programme.

1. Introduction

The over use of synthetic pesticides for controlling insect pests in the agricultural fields have polluted not only agro-ecosystem but also the water we drink, the soil that we use and all natural resources that we use for our livelihood. This dreadful situation demands the human community to find an alternative and safe practices to control insect pests without causing ill-effects to non-target organisms, environment and so on. Now there is a conscious effort taken by the modern humanity to practice methods that are eco-friendly, economically viable, and easily available. As a result, there is an increasing interest in developing phytopesticides for controlling insect pests due to their effectiveness at low concentrations and low impacts on non-target organisms[1-3]. The biomolecules present in phytopesticides act as feeding deterrent, bioinsecticide, ovicide, oviposition deterrent and growth inhibition against field insect pests[4-6]. Klun c[7] reported that mosquitoes were differently repelled by isomers of piperidines. Pongam and neem oils or neem based commercial pesticides showed antioviposition effects on the adults of greenhouse whitefly[8]. *H. armigera*[9] and *S. litura* (Lepidoptera : Noctuidae) are major insect pests[10,11]. They attack a wide range of industrial, ornamental cereal, legume and vegetable crops throughout the world[12] especially in Asia, Africa, Austrialia. *S. litura* alone damages more than 180 crops causing 69 per cent reduction in yield[13,14]. Field insect pests like *H. armigera* and *S.litura* cause great damage to the agricultural crops and reduce their productivity[15]. By keeping this background we focussed out research to formulate a novel phytopesticidal formulation[16] known as *PONNEEM* at Entomology Research Institute (ERI) to find oviposition deterrent effect of PONNEEM, a newly formulated phytopesticide against *H. armigera* and *S. litura*. Due to its high efficacy in controlling field insect pests, *PONNEEM* was patented in India (Indian Patent No. 204381 by the Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India).

*Corresponding author: Susaimanickam Maria Packiam, Entomology Research Institute, Loyola College, Chennai, India - 600 034.
Tel: +91-44-2817 4644
Fax: 091-44-2817 5566
E-mail: mariaento@hotmail.com

2. Materials and methods

2.1. Formulation of Phytopesticide

Five phytopesticidal formulations were prepared using pungam, neem oils at different combinations. These 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 to the oils and again it was stirred at 120 r/min for 10 min. At last 0.123% Azadirachtin and 2% isopropyl alcohol were added and again it was mixed thoroughly by using a stirrer at 120 r/min for 10 min. Then the final formulations were obtained[11,16].

2.2. Insect culture

2.2.1. H. armigera

H. armigera larvae were collected from bhendi field at Mangadu, Kancheepuram district. The collected larvae were reared individually in a plastic container (vials) and regularly fed with bhendi till the larvae attained the pupal stage under laboratory conditions (28 ± 2℃ and 80 ± 5% RH). 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 mixed with few drops of multivitamin were provided for adult feeding to increase the rate of fecundity. The newly hatched adults were used for the present investigation.

2.2.2. S. litura

Egg masses of *S. litura* were collected from groundnut field at Vellavedu village near Poonamallee, Chennai. The eggs were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching the neonate larvae were reared on castor leaves till pre pupal stage and sterilized soil was provided for pupation. The pupae were collected from the soil and kept in oviposition chambers (40 cm×25 cm×25 cm). After adult emergence, cotton soaked with 10% (w/v) sugar solution with multivitamin drops was provided for adult moths to increase the rate of fecundity. The newly hatched adults were used for this study.

2.3. Deterrence of Oviposition against S. litura and H. armigera

Oviposition deterrent activities of different phytopesticidal formulations were studied at different concentrations (5, 10, 15 and 20 μL/L). The concentrations of different oil formulations were sprayed on fresh castor leaves for *S. litura* and cotton leaves for *H. armigera* along with selected controls nimbicidine and emulsifier with water and placed inside the cage (60 cm×45 cm×45 cm) covered with mosquito net. Ten pairs of *S. litura* moths and ten pairs *H. armigera* were introduced in separate cages and 10% (w/v) sucrose solution with multivitamin drops was provided for adult feeding. Five replicates were maintained for control and treatments. After 48 h the number of egg masses laid on treated and control leaves were recorded and the percentage of oviposition deterrence was calculated using the formula of Williams *et al*.[17].

$$\text{Oviposition deterrence (\%)} = \frac{\text{No. of egg masses on control} - \text{No. of egg masses on treated}}{\text{No. of egg masses on control}} \times 100$$

2.4. Statistical analysis

The deterrence of oviposition activity was evaluated using one way ANOVA. Significant differences between treatments were determined using Duncan's multiple range (DMRT) (P=0.05).

3. Results

PONNEEM has showed good results against insect pests due to the presence of bioactive molecules like azadirachtin (Fig.1) and karanjin (Fig.2)[28,29]. The combination of these two oils at 1:1 ratio gives synergistic effect in controlling lepidopteran pests.

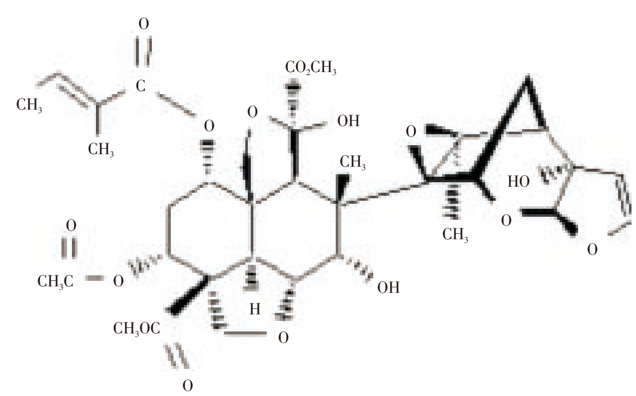

Figure 1. Structure of Azadirachtin.

The castor leaves treated with different concentrations (5, 10, 15 and 20 μL/L) of phytopesticidal formulations were provided for oviposition of gravid female moths of *S. litura*. The numbers of eggs laid by a female moth on treated and

Table 1.
Per cent oviposition deterrent activity of phytopesticidal formulations against S. litura

Treatments	Concentration tested			
	5 μL/L	10 μL/L	15 μL/L	20 μL/L
Formulation A(Pungam oil + Neem oil – 3:7)	34.94±8.15a	39.25±8.05a	41.99±5.98b	49.23±8.13c
Formulation B(Pungam oil + Neem oil – 7:3)	33.77±7.63a	33.92±8.04a	31.34±10.25a	32.36±8.45ab
Formulation C (PONNEEM)(Pungam oil + Neem oil – 1:1)	63.65±4.08b	71.55±4.44b	75.77±2.90c	77.48±4.15d
Formulation D(Pungam oil)	30.29±8.43a	28.73±7.90a	34.64±8.33ab	30.36±8.84a
Formulation E(Neem oil)	29.13±7.22a	35.08±8.22a	34.56±6.77ab	33.96±7.73ab
Formulation F(Nimbicidine)	27.78±9.12a	34.52±8.55a	38.38±6.96ab	42.71±7.92bc
Formulation G(Emulsifier control)	2.66±1.98a	2.66±1.98a	2.66±1.98a	2.66±1.98a

Values are mean of five replications. Means ± SD followed by same letter(s) in a column are not significantly different (P=0.05) by DMRT.

Table 2.
Per cent oviposition deterrent activity of phytopesticidal formulations against H. armigera

Treatments	Concentration tested			
	5 μL/L	10 μL/L	15 μL/L	20 μL/L
Formulation A(Pungam oil + Neem oil – 3:7)	28.81±5.78a	35.29±2.88a	36.90±3.81a	40.26±3.11a
Formulation B(Pungam oil + Neem oil – 7:3)	29.98±6.03a	34.21±4.70a	36.53±3.31a	46.29±5.11b
Formulation C (PONNEEM)(Pungam oil + Neem oil – 1:1)	45.59±3.39b	45.14±2.22b	57.54±1.99b	68.12±1.24c
Formulation D(Pungam oil)	27.40±3.58a	34.70±4.40a	35.78±3.62a	41.39±5.37a
Formulation E(Neem oil)	30.53±5.71a	37.11±3.83a	38.64±5.51a	49.51±2.93b
Formulation F(Nimbicidine)	30.26±6.66a	36.18±3.64a	38.11±6.92a	49.52±2.07b
Formulation G(Emulsifier control)	1.06±0.14a	1.06±0.14a	1.06±0.14a	1.06±0.14a

Values are mean of five replications. Means ± SD followed by same letter(s) in a column are not significantly different (*P*=0.05) by DMRT.

control leaves of castor are presented in Table 1. The per cent oviposition was greatly decreased with increasing concentrations of the treatments. The maximum oviposition deterrent activity against *S. litura* was seen in formulation C *PONNEEM* treated leaves followed by formulation A at 20μL/L compared to all other formulations (Figure 3).

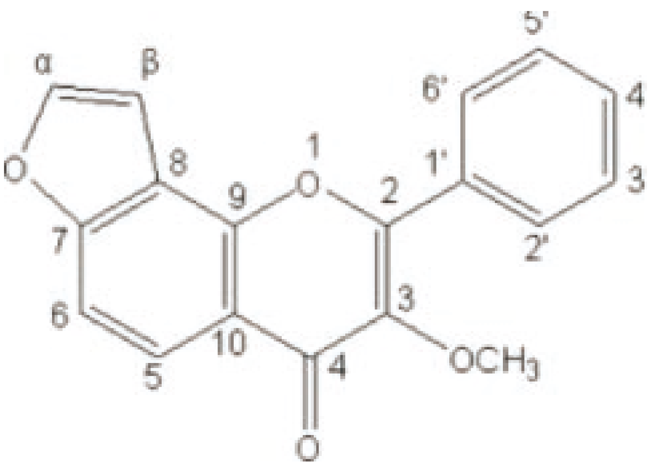

Figure 2. Structure of Karanjin.

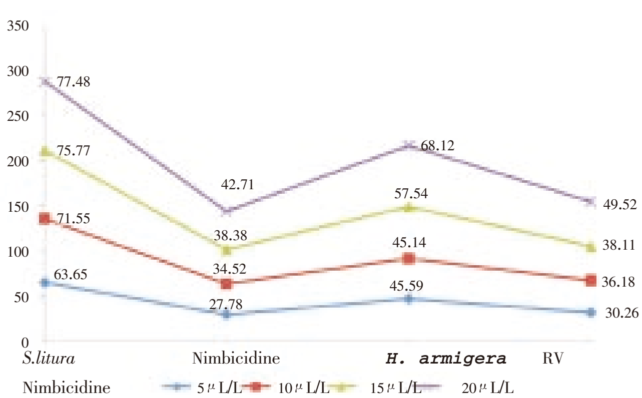

Figure 3. Impact of PONNEEM on the deterrence of oviposition of *S. litura* and *H. armigera* at 20μL/L compared to nimbicidine

Oviposition deterrent activity of phytopesticidal formulations against *H. armigera* was evaluated at different concentrations (5, 10, 15 and 20 μL/L). And the results are presented in Table 2. Oviposition deterrent activity of these phytopesticidal formulations was calculated based on the number of egg batches laid by the adult moths on treated and control castor leaves. High oviposition deterrent activity normally indicates potential deterrent activity of plant extracts. In the present study depending on the concentrations the deterrence of oviposition activity varied significantly. Data pertaining to the deterrent activity revealed that maximum oviposition deterrent activity was recorded in *PONNEEM* (Pungam oil and neem oil, 1:1 ratio) followed by formulation F (Neem oil) and formulation E (Nimbicidine) compared to control at 20μL/L (Figure 3).

4. Discussion

4.1. Deterrence of Oviposition activity against S. litura and H. armigera

In this present investigation, different phytopesticidal formulations exhibited deterrence of oviposition activity against *H. armigera* and *S. litura* depending on the concentrations. This finding coincides with finding of Dethier[18] who noticed that plant characteristics, such as chemicals, color, trichomes, and architecture, in concert with the insect's internal milieu, form the basis for discrimination between acceptable and unacceptable plants for feeding or oviposition by various species of phytophagous insects. Feeding and oviposition were deterred by exposing insects to substrates treated with compounds that are bitter tasting[19]. Female moths could have sensory receptors sensitive to host plant biochemical compositions in which contact chemoreceptors on their tarsi and ovipositor would be useful in assessing the suitability of host for oviposition[20,21]. The per cent oviposition of *S. litura* and *H. armigera* was greatly decreased with increasing concentrations of *PONNEEM*. Similary Packiam and Ignacimuthu[11] observed that *PONNEEM* treated larvae of *S. litura* became malformed pupae and reduced the laid egg hatchability of the emerged adult. *PONNEEM* was found to be effective in controlling mosquitoes vector[22] and ovicidal activity[16]. *PONNEEM* has shown good results against insect pests due to the presence of bioactive molecules like azadirachtin and karanjin (1:1,V/V). The bioactive molecules present in the plant based pesticides have a significant role in regulating the growth of insect pests. As a result the larvae are unable to continue to prolong the larval duration due to biomolecules of plants[23].

Pavunraj *et al*.[24] reported that effective fraction from *Melochia corchorifolia* with 1:1 ratio of neem and pongam showed antifeedant activity against four lepidopteran pests. Earlier Srinivasan and Sundarababu[25] reported that neem seed kernel extract deterred the egg laying capacity of *Leucinodes orbonalis*. Several investigators reported the reduction in the egg laying capacity of *S. litura* due to the treatment with the plant extracts[26]. Elumalai *et al*.[27] reported that fraction from diethyl ether extract of *Hyptis* *suaveolens* and *Melochia chorcorifolia* showed significant oviposition deterrent activity against *H. armigera*.

The novel phytopesticide *PONNEEM* exhibited statistically significant deterrence of oviposition activity against *H. armigera* and *S. litura* at all the concentrations when compared to all other treatments. At 20 μL/L concentration of *PONNEEM*, the maximum oviposition deterrent activity was observed against these two lepidopteran insect pests. Due to its high level efficacy, *PONNEEM* which was patented under the government of India could be used as a good phytopesticide for insect pest management. The efficacy of *PONNEEM* is the first report on deterrence of oviposition against lepidopteran pests.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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

References

[1] Wawan H, Ritsuko T, Shuhei N, Kenji F, Fusao. Oviposition deterrent activity of andrographolide against the diamondback moth (DBM), Plutella xylostella (Lepidoptera: Yponomeutidae). *Appl Entomol Zool* 1998; **33** (2): 239-241.

[2] Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Ann Rev Entomol* 2006; **51**:45-66.

[3] Rosell G, Quero C, Coll J, Guerrero A. Biorational insecticides in pest management. *J Pest Sci* 2008; **33**:103-121.

[4] Baskar K, Maheshwaran R, Kingsley S, Ignacimuthu *S. Bioefficacy* of plant extracts against Asian army worm *Spodoptera litura* Fab. (Lepiodoptera: Noctuidae). *J Agri Technol* 2011; **7**:123-131.

[5] Baskar K, Ignacimuthu S. Ovicidal activity of Atalantia monophylla (L) Correa against *Helicoverpa armiger*a Hubner (Lepidoptera: Noctuidae). *J Agric Technol* 2012a; **8**: 861-868.

[6] Baskar K, Ignacimuthu S. Antifeedant, larvicidal and growth inhibitory effect of ononitol monohydrate isolated from Cassia tora L. against *Helicoverpa armigera* (Hub) and *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Chemosphere* 2012b; **88**:384-388.

[7] Klun JA, Ma D, Gupta R. Optically active arthropod repellents for use against disease vectors. *J Med Entomol* 2000; **37**(1): 182-187.

[8] Roman P, Gerhard H. Repellent effects of pongam oil on settlement and oviposition of the common greenhouse whitefly Trialeurodes vaporariorum on chrysanthemum. *Insect Sci* 2007; **14**(3):219-224.

[9] Kumar S, Saini RK, Ram P. Natural Mortality of *Helicoverpa armiger*a (Hubner) Eggs in the cotton ecosytem. *J Agric Sci Technol* 2009; **11**:17-25.

[10] Desmukhe PV, Hooli AA, Holihosur SN. Bioefficacy of cold ethyl alcohol extract of Annona squamosa against *Spodoptera litura* Fabricius. *J Biopesticides* 2010; **3**(special issue): 271-274.

[11] Packiam SM, Ignacimuthu S. Effect of *PONNEEM* on *Spodoptera litura* (Fab.) and Its Compatibility with Trichogramma chilonis Ishii. *Braz Arch Biol Technol* 2012; **22**:291-298.

[12] Yu FL, Wu G, Liu TJ, Zhai BP, Chen FJ. Effects of Irrigation on the Performance of Cotton Bollworm *Helicoverpa armiger*a (Hubner) during different Pupal Stages. *Int J Pest Manag* 2008; **54**(2): 137-142.

[13] Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Ann Rev Entomol* 2006; **51**: 45-66.

[14] Gokulakrishnan J, Krishnappa K, Elumalai K. Effect of plant oil formulation against armyworm, *Spodoptera litura* (Fab.), Cotton Bolloworm, *Helicoverpa armiger*a (Hub.) and fruit borer, Earias vitella (Fab.) (Lepidoptera:Noctuidae). *Int J Current Life Sci* 2012; **2**(1): 1-4.

[15] Ignacimuthu S. Insect Pest Control: Using Plant Resources. Narosa Publishing House, New Delhi, India 2012.

[16] Packiam SM, Baskar K, Ignacimuthu S. Ovicidal activity of botanical oil formulations against *Helicoverpa armiger*a Hubner and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Asian J Tropical Biomed 2012 (In press).

[17] Williams AL, Mitchell ER, Heath RR, Barfield CS. Oviposition deterrents for fall armyworm (Lepidoptera: Noctuidae) from larval frass, corn leaves and artificial diet. *Environ Entomol* 1986; **15**:327-330.

[18] Dethier VG. Mechanism of host plant recognition. *Entomol Exp Appl* 1982; **31**:49-56.

[19] Ramaswamy SB, Cohen NE, Hanson FE. Deterrence of feeding and oviposition responses of adult Heliothis virescens by some compounds bitter-tasting to humans. *Entomol Exp Appl* 1992; **65**:81-93.

[20] Chen C, Dong CYF, Cheng LL, Hou RF. Deterrent effect of neem seed kernel extract on oviposition of the oriental fruit fly (Diptera: Tephritidae) in guava. *J Econ Entomol* 1996; **89**:162-166.

[21] Sharma PD, Jalan MS. Relative efficacy and persistence of different insecticides for the control of *Helicoverpa armiger* on cotton. *Pestol* 1997; **21**:12-15.

[22] Maheswaran R, Ignacimuthu S. A novel herbal formulation against dengue vector mosquitoes Aedes aegypti and Aedes albopictus. *Parasitol Res* 2012; **110**:1801-1813.

[23] Packiam SM, Baskar, K, Ignacimuthu S. Feeding deterrent and Growth inhibitory activites of *PONNEEM*, a newly developed phytopesticidal formulation against *Helicoverpa armiger*a (Hubner). *Asian J Tropical Biomed* 2012(In press).

[24] Pavunraj M, Baskar K, Ignacimuthu S. Efficacy of Melochia corchorifolia L. (Sterculiaceae) on Feeding Behavior of Four Lepidopteran Pests. *Int J Agric Res* 2012; **7**: 58-68.

[25] Srinivasan G, Sundarababu PC. Ovicidal and ovipositional deterrent effect of neem products on brinjal shoot and fruit borer, Lucinodes arbonalis Guenee (Lepidoptera: Pyralidae). In: Ignacimuthu S, Sen A, editors. Biopesticides in insect pest management. 1999; pp. 56-58.

[26] Sundararaju D, Sundara BPC. Oviposition and feeding deterrency in the matured shoots of cashew and neem against neem mosquito bug, Helopeltis antonii Signoret (Heteroptera: Miridae). *J Ent Res* 2000; **24**(2):103-107.

[27] Elumalai K, Jeyasankar A, Jayakumar M, Raja N, Ignacimuthu S. Effect of isolated fractions of *Hyptis suaveolen*s and *Melochia chorcorifolia* against the gram pod borer *Helicoverpa armiger*a (Hubner). In: Ignacimuthu S, Jayaraj S. editors. Sustainable Insect Pests Management. New Delhi: Narosa Publishing House; 2005: 181-187.

[28] Butterworth JH, Morgan ED. Isolation of a substance that suppresses feeding of locusts. Chemical Commun 1968; 23-24.

[29] Vismaya, Sapna Eipeson W, Manjunatha JR, Sriniva P, Sindhu Kanya TC. Extraction and recovery of karanjin: A value addition to karanja (Pongamia pinnata) seed oil. *Industrial Crops and Products.* 2010; **32**(2): 118-122.