Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Withania somnifera acts as a potential insect growth regulator in the polyphagous pest, *Pericallia ricini*

Sandeep Kumar Gaur and Krishna Kumar*

Department of Zoology, University of Allahabad, Allahabad 211002, India

Vol. 57, No. 4: 379–388, 2017

DOI: 10.1515/jppr-2017-0052

Received: August 21, 2017
Accepted: November 14, 2017

*Corresponding address:
gaurs808@gmail.com;
kkumaruniv@rediffmail.com

Abstract

Both seed and root extracts of the medicinal plant, Ashwagandha, *Withania somnifera* exhibit insect growth regulatory activity against the polyphagous pest, *Pericallia ricini*. Topical administration of *W. somnifera* seed and root extracts to last instar larvae of *P. ricini* disrupted moulting and metamorphosis, leading to a number of developmental abnormalities such as delay in larval-pupal and pupal-adult ecdysis, formation of larval-pupal, pupal--adult and larval-pupal-adult mosaics/chimeras, ecdysial failure, suppression of pupation and adult emergence and formation of abnormal pupae and adultoids. The treatment with seed extracts was more severe than that of root extracts as it completely suppressed the pupation and adult emergence. The results clearly suggest that the medicinal plant, *W. somnifera* acts as a potential insect growth regulatory (IGR) disrupting the moulting and metamorphosis as a consequence of interference with the endocrine system.

Key words: adultoids, Ashwagandha, chimeras, ecdysial stasis, hairy caterpillar

Introduction

Conventional methods of insect pest management with chemical pesticides have resulted in severe problems of environmental pollution such as contamination of water, air and soil. Pollution adversely affects the ecosystem, destroys biodiversity such as wild life, changes soil microbial diversity, disrupts the dynamics of food chains in the community and changes system processes. Furthermore, there is an increase in resistant pest populations, a decline in non target organisms such as earthworms, mites, spiders, fish, aquatic organisms, amphibians, birds, predators, and pollinators (wild bees, bumble bees, honey bees, fruit flies, humming birds, honey eater birds and sun birds). All of these factors also pose dangers to human beings. Acute and chronic human illnesses are rapidly increasing due to polluted water, air and food as a result of biomagnification of the toxic compounds (Tunaz and Uygun 2004; Brittain *et al.* 2010; Damalas and Eleftherohorinos 2011; Zacharia 2011; Gill and Garg 2014; Ibrahim 2016; Mahmood *et al.* 2016). Considering the various harmful effects of chemical pesticides, appropriate alternative strategies must be developed and explored. For example, biopesticides are considered to be safe and convincing strategies for the management and control of various insect pests (Pant *et al.* 2016). These are generally derived from living organisms, microorganisms and other natural sources (Mehrotra *et al.* 2016). They have certain unique attributes such as target specificity and the absence of toxic residues. Furthermore, they are eco-friendly in nature, easy to apply, biodegradable and economically feasible. Additionally, they have slower/negligible development of pest resistance (Pant *et al.* 2016; Rajapakse *et al.* 2016) and fewer toxic effects on the environment and human health (El-Wakeil 2013; Mehrotra *et al.* 2016).

Pericallia ricini Fab. (Lepidoptera: Arctiidae) is a polyphagous pest of zinnia, balsam (Jain *et al.* 1972), castor, gingelly, cotton, country bean, drum stick, coccinia, banana, calotropis, sunflower, oleander, tea, sweet potato, pumpkin (David and Ananthakrishnan 2004), brinjal, maize (Singh and Gandhi 2012), and vanilla (Vanitha *et al.* 2011). Infestation by this polyphagous

pest causes defoliation of tender leaves and growing shoot tips of vanilla (Vanitha *et al.* 2011).Various methods have been used to control *P. ricini* such as chemical pesticide, diflubenzuron which inhibited the deposition of chitin in the cuticle (Mayuravalli *et al.* 1989), produced various deformities e.g. larval-pupal intermediates, abnormal pupae and abnormal adults (Nathan and Nathan 2011). It also affected the bioenergetics of the larvae of this pest (Krishnan and Chockalingam 1989). Administration of penfluron to *P. ricini* caused sterility in adults (Khan and Srivastava 1992). Moreover, application of botanicals such as flower extracts of *Delonix regia* caused ovicidal, larvicidal and pupicidal activities against *P. ricini* (Chockalingam *et al.* 1992). Salonine (triterpenoids) isolated from neem oil caused antifeedant and growth regulatory activities such as delayed moult, prolonged larval duration, larval and pupal mortality in *P. ricini* (Govindachari *et al.* 1996). Secondary metabolites of sponges were found to have potential larvicidal and pesticidal effects against *P. ricini* (Joseph *et al.* 2010). Botanical insecticide, azadirachtin disrupted physiological processes such as apolysis and ecdysis and induced morphological deformities at larval and pupal stages of *P. ricini* (Gnanamani and Dhanasekaran 2013). Ethyl acetate extracts of *Tragia involucrata* showed antifeedant, larvicidal and growth inhibitory effects against this agriculturally important pest, *P. ricini* (Jeyasankar *et al.* 2014). Biological control agents, e.g. entomopathogenic fungi, for instance, *Beauveria bassiana* (Shophiya *et al.* 2014), *Verticillium lecanii* and *Paecilomyces fumosoroseus* (Sahayaraj and Borgio 2012) and entomophagous predator, *Eocanthecona furcellata* (Shophiya and Sahayaraj 2014) have also been used for the control of *P. ricini*.

Ashwagandha, *Withania somnifera* (Family: Solanaceae) is an ancient and important medicinal plant used as an herb in the traditional Indian system of Ayurveda. In clinical studies this medicinal plant has been shown to possess anti-microbial, anti-tumor, anti-inflammatory, anti-stress, cardioprotective, neuroprotective, anti-diabetic (Dar *et al.* 2015), analgesic and chondroprotective properties (Ramakanth *et al.* 2016). This medicinal plant is used in the treatment of various diseases like epilepsy, stress, Parkinson's and Alzheimer's disease, arthritis, rheumatism and to improve health and longevity (Pratibha *et al.* 2013).

Whereas the medicinal properties of Ashwagandha, *W. somnifera,* are well documented, its insect growth regulatory effects have not been explored except in an earlier study which showed the insect growth regulatory (IGR) effect of leaf extracts on tobacco caterpillar, *Spodoptera litura* (Gaur and Kumar 2010). In the present communication, the IGR effects of seed and root extracts of *W. somnifera* on the polyphagous pest, *P. ricini* have been described.

Materials and Methods

Insect rearing

The wild eggs of *P. ricini* were collected from the castor plant, *Ricinus communis* and kept in sterilized glass beakers at 26±2°C, 70±5% relative humidity (RH) in a BOD incubator. The first instar larvae were transferred to glass troughs and fed fresh, soft and sterilized castor leaves. The fresh castor leaves were supplied daily to developing larvae which were shifted to sterilized glass troughs to prevent overcrowding and infection from excrement. The last instar larvae were transferred to sterilized glass troughs containing sawdust for pupation. The last instar larvae moulted into pupae inside the cocoons and after a pupation period of 10–11 days adult moths emerged from the cocoons. Moths were kept in acrylic net cages measuring about 30 × 30 × 30 cm and provided with 10% honey solution for feeding and fresh castor leaves for oviposition.

Plant material

Root and seeds of Ashwagandha, *W. somnifera* were purchased locally and online from Srivilliputtur, Tamil Nadu (India). The seeds and roots were washed thoroughly with distilled water and dried in sunshade for a week and separately ground with the help of a pestle and grinder to make a fine powder.

Extraction of plant material

125 g each of dried root and seed powder of *W. somnifera* were dissolved separately in 250 ml of acetone (boiling point 40–60°C) and heated at 50°C for two days by using a Soxhlet apparatus and filtered through Whatmann no. 1 filter paper. The filtrate was condensed in a rotatory evaporator under reduced pressure of 22–26 mm Hg at 45°C to allow complete evaporation of acetone. A dark brown, sticky, thick paste of crude extract of root and seeds of *W. somnifera* was obtained separately. The desired concentrations were obtained by dissolving 1 mg of crude extract into 1 ml of acetone and kept in a refrigerator at 4°C.

Experimental procedure

Freshly moulted seventh instar larvae of *P. ricini* were selected from the stock batch of the homogenous culture of a single laying of egg mass and divided into batches of 20 larvae each. Each larva of the experimental group was administered topically with the desired doses of 5, 10, 15 and 20 µg of root and seed extracts of *W. somnifera* separately on the dorsum of the posterior abdominal segments with the help of a microapplicator. The controls were either untreated or treated with similar concentrations of pure acetone

only. After topical administration, both treated and control larvae were transferred to sterilized glass troughs and provided with fresh castor leaves for feeding and sawdust for pupation. All the experiments were repeated thrice. Observations were recorded at regular intervals, beginning 24 h post treatment till emergence of adultoids/adults.

Fixation and preservation

All the specimens with morphological deformities were fixed in Bouin's fluid for 24 h and then the specimens were washed three times with 70% ethyl alcohol to remove Bouin's fluid from the specimens and preserved in 70% ethyl alcohol for morphological studies (Singh and Kumar 2011b).

Statistical analysis

All the data regarding larval-pupal and pupal-adult ecdysis duration were subjected to one-way ANOVA to determine significant differences between mean larval and pupal duration of the treated and control groups. The correlation coefficient was also calculated to determine the correlation between the doses administered and different deformities produced. All the calculations were done using Graph Pad Prism 5.01 software.

Results

Larval mortality

Topical application of seed extracts of *W. somnifera* to last instar larvae of *P. ricini* resulted in larval mortality and 10, 13, 20 and 25% of the larvae died within 24–48 h of treatment at 5, 10, 15 and 20 µg doses, respectively. There was a positive correlation between the doses administered and the mortality, showing a dose dependent response (r = +0.99) (Table 1). However, treatment of last instar larvae with root extracts of *W. somnifera* did not result in any larval mortality.

Larval-pupal ecdysis

Topical administration of seed extracts of *W. somnifera* to last instar larvae of *P. ricini* resulted in delayed larval-pupal ecdysis when compared to the controls. The larval-pupal ecdysis was prolonged up to 13.93±0.62, 15.04±0.51, 15.25±0.41, and 16.78±0.70 days at 5, 10, 15 and 20 µg doses, respectively, as compared to 6.85±0.06 days in controls ($F = 68.42$; df = 4, 254; $p \leq 0.001$) (Table 1). There was a positive correlation between the doses administered and the delay in larval-pupal ecdysis showing a dose dependent response (r = +0.86) (Table 1).

Topical application of different doses of root extracts of *W. somnifera* to last instar larvae resulted in postponement of larval-pupal ecdysis. The larval-pupal ecdysis was prolonged up to 7.54±0.14, 7.55±0.15, 7.70±0.11 and 8.21±0.42 days at 5, 10, 15 and 20 µg doses, respectively, as compared to 6.75±0.05 days in controls ($F = 12.92$; df = 4, 176; $p \leq 0.001$) (Table 2). There was a positive correlation between the doses applied and the delay in larval-pupal ecdysis showing a dose dependent response (r= +0.93) (Table 2).

Pupal-adult ecdysis

Pupal-adult ecdysis was also significantly delayed when last instar larvae were treated with root extracts of *W. somnifera*. It increased from 10.70±0.07 in controls to 11.57±0.14, 11.93±0.18, 12.00±0.23 and 11.80±0.20 days at 5, 10, 15 and 20 µg doses, respectively ($F = 19.41$; df = 4, 151; $p \leq 0.001$). There was a positive correlation between the doses administered and the mean pupal-adult ecdysis duration, showing a dose dependent response (r= +0.68) (Table 2).

Ecdysial failure

Topical administration of seed and root extracts of *W. somnifera* to last instar larvae of *P. ricini* caused ecdysial failure. The effect was more pronounced with seed extract treatment where 90, 87, 80 and 75% cases

Table 1. Effect of topical administration of seed extracts of *Withania somnifera* to last instar larvae of *Pericalia ricini* (n = 20, 3 replicates)

Dose [µg]	Larval mortality [%]	L-P ecdysis [mean ± SE]	Ecdysial failure [%]	Pupation [%]	Adult emergence [%]
0	0	6.85 ± 0.06	0	100	100
5	10	13.93 ± 0.62*	90	0	0
10	13	15.04 ± 0.51*	87	0	0
15	20	15.25 ± 0.41*	80	0	0
20	25	16.78 ± 0.70*	75	0	0
	(r = +0.99)	(r = +0.86)	(r = +0.59)	(r = –0.71)	(r = –0.71)

*significant at $p \leq 0.001$; one-way ANOVA; r = correlation coefficient; L-P = larval-pupal

Table 2. Effect of topical administration of root extracts of *Withania somnifera* to last instar larvae of *Pericallia ricini* (n = 20, 3 replicates)

Dose [µg]	L-P ecdysis [mean ± SE]	P-A ecdysis [mean ± SE]	Ecdysial failure [%]	L-P mosaics [%]	Pupation [%]	Abnormal pupae [%]	P-A mosaics [%]	Adult emergence [%]	L-P-A chimeras [%]	Adultoids [%]	Normal adults [%]
0	6.75 ± 0.05	10.70 ± 0.07	0	0	100	0	0	100	0	0	100
5	7.54 ± 0 .14*	11.57 ± 0.14*	27	8	65	2	5	58	5	45	8
10	7.55 ± 0.15*	11.93 ± 0.18*	33	12	55	2	8	45	2	36	7
15	7.70 ±0 .11*	12.00 ± 0.23*	37	13	50	2	16	32	0	22	10
20	8.21 ± 0.42*	11.80 ± 0.20*	63	5	32	3	3	26	2	19	5
	(r = +0.93)	(r = +0.68)	(r = +0.95)	(r = +0.45)	(r = –0.95)	(r = +0.87)	(r = +0.44)	(r = –0.93)	(r = –0.08)	(r = +0.14)	(r = –0.72)

*significant at $p \leq 0.001$; one-way ANOVA; r = correlation coefficient; L-P = larval-pupal; P-A = pupal-adult; L-P-A = larval-pupal-adult

of ecdysial failure occurred at 5, 10, 15 µg and 20 µg respectively (r = +0.59) (Table 1). With root extracts only 27, 33, 37 and 63% larvae suffered from ecdysial failure at 5, 10, 15 and 20 µg, respectively, thus showing a dose dependent response. There was a positive correlation between the doses administered and the percentage of ecdysial stasis (r = +0.95); (Table 2).

All the last instar larvae treated with seed extracts of *W. somnifera* suffered from complete ecdysial failure and there was a complete suppression of pupation and adult emergence (Plate 1.B; Table 1).

Depending upon the degree of abnormality the ecdysial stasis caused by the root extracts of *W. somnifera* could be divided into the following types:

(1) The larva unable to shed larval exuviae from all over the body; loss of hairs from the body; unusually tanned thoracic legs (Plate 1.C).
(2) The larval body was extremely contracted and shrunken, thereby exhibiting ecdysial stasis (Plate 1.D).
(3) The larva showing ecdysial failure with rectal prolapse; loss of hairs from the body (Plate 1.E).
(4) Pupa with larval exuviae attached all over the body except at the head and anterior and middle region of the thorax (Plate 1.F and G) and pupa with larval exuviae attached to the ventral surface of the head, thorax and abdomen (Plate 1.H and I).
(5) Pupa with larval exuviae attached to the posterior extremity of the abdomen (Plate 1.J).
(6) Pupa covered with larval exuviae but ruptured on the head, thorax and middle abdomen, the abdomen still pupal and without tanning (Plate 1.K). In some specimens the head, thorax and abdomen were still pupal and without tanning (Plate 1.L).

Larval-pupal mosaics

Topical treatment of root extracts of *W. somnifera* to last instar larvae of *P. ricini* resulted in the formation of larval-pupal mosaics at all the doses.

These larval-pupal mosaics could be broadly classified into the following types:

(1) Larval-pupal mosaic consisting of the anterior half of the larval body whereas the posterior half of the body was pupal in appearance (Plate 2.A and B).
(2) Larval-pupal mosaic with larval head, larval thoracic legs and prolegs present, whereas the abdomen was pupal in appearance but still had fragments of larval exuviae attached to different parts of the abdomen (Plate 2.C–E).
(3) Larval-pupal mosaics showing complete pupal body but with larval prolegs (Plate 2.F and G); in some cases, larval exuviae were attached to the posterior extremity of the body (Plate 2.H).

Pupation

When the last instar larvae of *P. ricini* were treated with seed extracts of *W. somnifera* the pupation was completely suppressed and none of the treated larvae formed pupae (Table 1) whereas treatment with root extracts resulted in a significant reduction of the percentage pupation. The percentage pupation decreased from 100% in controls to 65, 55, 50 and 32% at 5, 10, 15 and 20 µg doses, respectively, showing a negative correlation between the doses administered and the percentage pupation (r = –0.95) (Table 2).

Abnormal pupae

Topical treatment of root extracts of *W. somnifera* to last instar larvae of *P. ricini* resulted in the formation of abnormal pupae with reduced left wings and untanned metathoracic legs (Plate 3.B).

Pupal-adult mosaics

Treatment of last instar larvae with root extracts of *W. somnifera* resulted in the formation of pupal-adult mosaics (Table 2). Pupal-adult mosaics consisted of antennae, labial palpi, proboscis, legs and left wing

which were pupal in appearance. The legs of the left side were not fully developed; the eyes had characteristic adult pigmentation but were not fully developed; posterior extremities of the abdomen towards ventral surface were pupal in appearance and genitalia were in the form of a scar and not developed; the dorsal surface of the body showed patches of white pupal cuticle and adult cuticle with a few scales at several places (Plate 3.C and D).

Adult emergence

Administration of seed extracts of *W. somnifera* to last instar larvae of *P. ricini* caused complete suppression of adult emergence whereas treatment of larvae with root extracts resulted in a significant reduction in the emergence of adults. There was a negative correlation between the doses administered and the incidence of adult emergence (r = –0.93) (Table 2).

Larval-pupal-adult chimeras

The larval-pupal-adult chimeras, resulting from treatment with root extract of *W. somnifera*, contained larval prolegs and bifurcated pupal proboscis. The rest of the body had adult characteristics but with few scales, rudimentary mesothoracic legs and crumpled wings (Plate 3.E).

Adultoids

Topical administration of root extracts of *W. somnifera* to last instar larvae of *P. ricini* resulted in the production of adultoids with bifurcated proboscis, crumpled wings and highly deformed legs, less tanned abdomen and few scales (Plate 4.C and D). In some adultoids pupal exuviae remained attached to the head region (Plate 4.E and F).

Discussion

Administration of seed and root extracts of Ashwagandha, *W. somnifera* to last instar larvae of *P. ricini* clearly demonstrated its insect growth regulatory effects against this polyphagous pest. This was evident by the interference with moulting and metamorphosis. The effect of the administration of seed extracts was more severe since it caused absolute toxicity to treated larvae which suffered from ecdysial stasis and inhibition of development resulting in complete suppression of pupation and adult emergence. This clearly demonstrates that plant extracts act as insect growth regulators. The insecticidal properties of several plant extracts such as

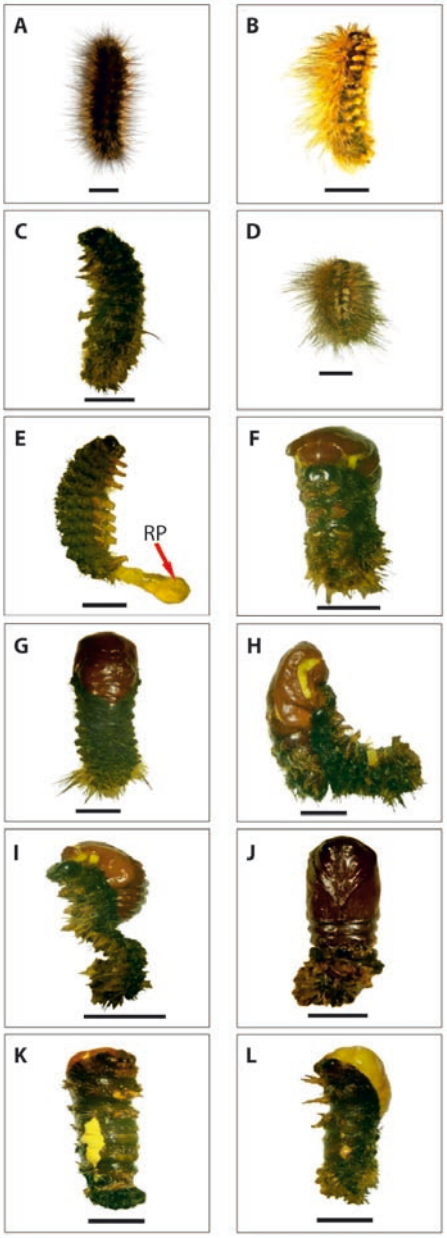

Plate 1. (A) Normal 7th instar day 1 larva of *Pericallia ricini*; (B) larva with complete ecdysial failure (5 µg, seed extracts of *Withania somnifera*); (C) larva with complete ecdysial failure unable to cast off larval exuviae from all over the body (10 µg, root extracts of *W. somnifera*); (D) extremely contracted and shrunken larva (15 µg, root extracts of *W. somnifera*); (E) larva with rectal prolapse (20 µg, root extracts of *W. somnifera*); (F and G) pupa with larval exuviae ruptured on dorsal surface of head and anterior and middle region of the thorax (15 µg, root extracts of *W. somnifera*); (H and I) lateral view of pupa with larval exuviae attached to ventral surface of head, thorax and abdomen (10 and 20 µg, root extracts of *W. somnifera*); (J) ventral view of pupa with larval exuviae attached to the posterior extremity of the abdomen (5 µg, root extracts of *W. somnifera*); (K) ventral view of pupa with ruptured larval exuviae on head, thorax and middle abdomen (20 µg, root extracts of *W. somnifera*); (L) lateral view of pupa with untanned pupal head, thorax and abdomen with larval exuviae attached to ventral surface (15 µg, root extracts of *W. somnifera*); bar = 5 mm; the arrow points out the deformities (RP – rectal prolapse)

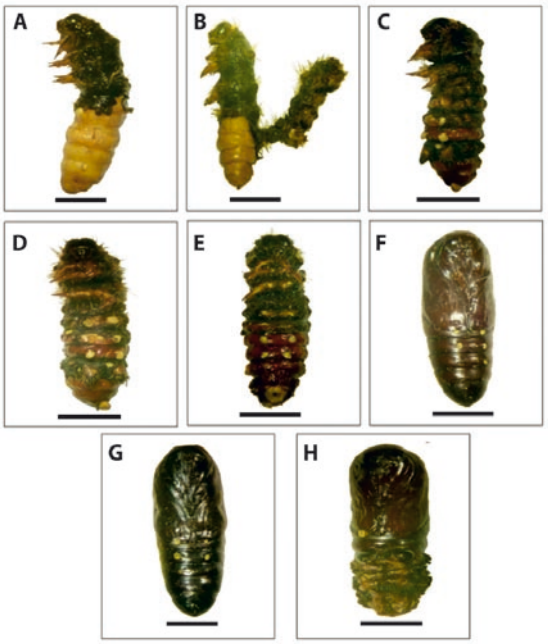

Plate 2. (A and B) Larval-pupal mosaic of *Pericallia ricini*; with anterior half larval and posterior half pupal in appearance (10 and 5 µg, root extracts of *Withania somnifera*); (C–E) larval-pupal mosaic with larval head, thoracic legs and prolegs whereas abdomen is pupal in appearance (15 µg, root extracts of *W. somnifera*); (F and G) ventral view of larval-pupal mosaics with larval prolegs (5 and 10 µg, root extracts of *W. somnifera*); (H) ventral view of larval-pupal mosaic with larval proleg and larval exuviae (20 µg, root extracts of *W. somnifera*); bar = 5 mm

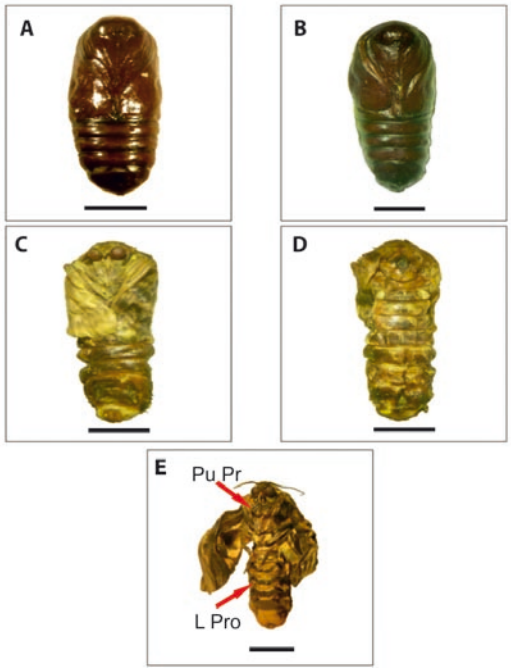

Plate 3. (A) Normal pupa of *Pericallia ricini*; (B) ventral view of abnormal pupa with reduced left wing (20 µg, root extracts of *Withania somnifera*); (C and D) ventral and dorsal view of pupal-adult mosaic(15 µg, root extracts of *W. somnifera*); (E) ventral viewof larval-pupal-adult chimera (20 µg, root extracts of *W. somnifera*) bar = 1 mm; the arrows point out the deformities. L Pro – larval prolegs; Pu Pr – pupal proboscis

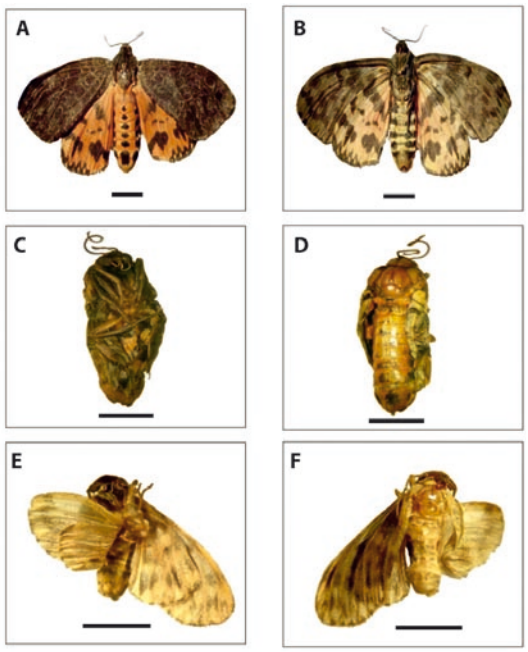

Plate 4. (A and B) Dorsal and ventral view of normal adult of *Pericallia ricini*; (C and D) ventral and dorsal view of adultoids with bifurcated proboscis, deformed wings and legs (15 µg, root extracts of *Withania somnifera*); (E and F) ventral and dorsal view of adultoid with pupal exuviae attached to head region; bar = 5 mm

seeds of *Cercis siliquastrum, Calendula officinalis, Peganum harmala* and fruits of *Melia azedarach* against flower thrips *Frankliniella occidentalis* have been demonstrated (Razavi and Ahmadi 2016). Toxicological effects of Ashwagandha have also been observed against the larvae/adults of various insects, for instance, leaf extracts against larvae of *Culex quinquefasciatus* (Karmegam *et al.* 1997), leaf, stem, fruit and root extracts against adults of *Callosobruchus chinensis* (Gupta and Srivastava 2008), leaf, fruit and root extracts against adult *Sitophilus oryzae* (Yankanchi and Gadache 2010; Suvanthini *et al.* 2012), green and red fruit, seed, fruit without seeds, leaf and root extracts against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* (Sakthivadivel and Daniel 2008; Bansal *et al.* 2011), root, stem and leaf extracts against larvae of *Tribolium castaneum* (Arora *et al.* 2011), and leaf extracts against larvae and adults of *Oryzaephilus surinamensis* (Madkaur *et al.* 2013). Furthermore, acaricidal activity by the leaves of this medicinal plant against the larvae of deltamethrin resistant *Hyalomma anatolicum* (Singh *et al.* 2014a) and fully engorged females of synthetic pyrethroid resistant *Rhipicephalus microplus* (Singh *et al.* 2014b) have been shown. Moreover, antifeedant properties of the Ashwagandha have also been observed against the larvae of *Epilachna varivestis* (Ascher *et al.* 1981) and it has been suggested that the effect might be due to the presence of withanolide E (Isman 2002). It has also been observed that *W. somnifera* possesses antitermite properties (Ahmed *et al.* 2007). The various toxicological and IGR effects

of *W. somnifera* could be due to the presence of certain chemically active compounds like withanolides, withaferins, saponins and alkaloids (isopelletierine, anaferine) in the roots and seeds (Pratibha *et al.* 2013; Dar *et al.* 2015). Withanolides and withaferins are steroids and their action can be compared with another phytoecdysteroid, azadirachtin, derived from *Azadirachta indica* which acts as an antifeedant and moult inhibitor.

Administration of seed and root extracts of *W. somnifera* to last instar larvae of *P. ricini* significantly delayed larval-pupal ecdysis. It also delayed pupal-adult ecdysis (when treated with root extracts) as compared to controls. The essential oil of the *Pogostemon cablin* plant, containing an active ingredient, pogostone, has been reported to cause prolongation of larval and pupal duration in the lepidopteran insect, *Spodoptera exigua* (Huang *et al.* 2013). Extracts from different plants have been observed to increase larval, pupal and adult duration in several lepidopteran insects. For instance, methanolic extracts of leaves and seeds of the chinaberry tree, *Melia azedarach* caused increased larval and pupal duration in teak defoliator, *Hyblaea puera* (Nathan and Sehoon 2006). Seed extracts of *Caesalpinia crista* caused prolongation of larval and pupal duration in *Helicoverpa armigera* (Nathala and Dhingra 2006). Leaf, root and fruit extracts of *Pedalium murex* caused extension of larval, pupal and adult duration in tobacco cutworm, *Spodoptera litura* (Sahayaraj and Sathyamoorthi 2010) and mahlab oil extracted from kernels of *Prunus mahaleb* caused prolongation of larval duration in *Spodoptera littoralis* (Mead *et al.* 2016). Similar results have also been observed with administration of juvenile hormone analogue (JHA), pyriproxyfen to larvae of Indian meal moth, *Plodia interpunctella* (Ghasemi *et al.* 2010). Such postponement of larval-pupal or pupal-adult ecdysis or extension of the larval or pupal period may certainly be due to inhibition of the moulting process caused by an increase in the juvenile hormone titre in the insect body (Hangartner and Masner 1973; Lapcharoen *et al.* 2005) which is responsible for direct inhibition of prothoracic glands or modification of the prothoracicotropic brain activity (Ciemior *et al.* 1979). There are two juvenile hormone (JH) sensitive periods during last larval instar. The first one controls the larval-pupal development and the second one controls the pupal-adult development. If JH is present during the JH sensitive period, the current developmental state is maintained (Nijhout 1998; Gilbert 2006).

Ecdysial stasis was another conspicuous effect produced by treatment with both seed and root extracts of *W. somnifera*. Treated larvae suffered from complete ecdysial failure when treated with seed extracts. However, the extent of ecdysial failure was highly variable when treated with root extracts and ranged from complete ecdysial failure to partial failure where larval exuviae remained attached to different parts of the body to varying degrees. These effects were similar to those produced by the topical administration of IGR (lufenuron) to lepidopteran insects, *H. armigera* (Butter *et al.* 2003) and juvenoids (diofenolan and pyriproxyfen) to *Papilio demoleus* (Singh and Kumar 2011a, b) and *S. litura* (Singh and Kumar 2015). During development, moulting and metamorphosis are triggered by brain prothoracicotropic hormone which determines the ecdysteroids titre, as well as the activity and responsiveness of prothoracic glands (Thyagaraja *et al.* 1992). The high JH titre inhibits the secretion of prothoracicotropic hormone (PTTH) in the last larval instar (Rountree and Bollenbacher 1986) and this has an indirect inhibitory effect on the secretion of ecdysteroids (Nijhout 1998). Interference with moulting as observed in the present study has also been observed by an insect ecdysis inhibitor from the medicinal plant, *Plumbago capensis* when administered to the lepidopteran, *Bombyx mori* (Kubo *et al.* 1983).

The formation of larval-pupal, pupal-adult and larval-pupal-adult mosaics/chimeras as a consequence of treatment of the last instar larvae of *P. ricini* is similar to that produced as a result of treatment of larval instars of lepidopteran insects with IGRs such as lufenuron on *H. armigera* (Butter *et al.* 2003) or juvenile hormone analogues ZR-619-ethyl 11-methoxy-3,7,11-trimethyl (2E, 4E-dodecadienethiolate) on *Epiphyas postvittana* (Singh and Dugdale 1979), pyriproxyfen on *Papilio demoleus* (Singh and Kumar 2011b) and pyriproxyfen and diofenolan on *S. litura* (Singh and Kumar 2015). Production of such intermediate forms or chimeras have also been observed in larval instars of lepidopteran insects treated with plant extracts (Nathala and Dhingra 2006; Nathan and Sehoon 2006; Baskar *et al.* 2012). It has been suggested that this could be due to interference with the titre and activity of JH (Nathala and Dhingra 2006). Infact, administration of fruit extracts of the *M. azedarach* plant significantly increased the juvenile hormone titre as compared to that of control in lepidopterans, *S. littoralis* and *Agrotis ipsilon* resulting in the prolongation of larval life and larval-pupal intermediates. It has been concluded that *M. azedarach* fruit extract exerts a neuroendocrine control (Schmidt *et al.* 1998). Thus, the presence of a high titre of JH at an inappropriate time when it is not desired in the insect system may switch off the larval-pupal programme of differentiation (Riddiford *et al.* 2003; Wilson 2004) leading to the formation of nonviable intermediate forms (Bowers 1971; Dhadialla *et al.* 2005). This inhibitory effect on the larval-pupal development due to high JH titre has been attributed to an inhibitory effect on PTTH which in turn has an indirect inhibitory effect on the secretion of ecdysteroids (Nijhout 1998). The formation of larval-pupal-adult chimeras resulting from treatment with root extracts of *W. somnifera* has also been observed in treatment with JHA, diofenolan

(Singh and Kumar 2011a). It has been suggested that plant extracts are juvenile hormone mimics (Munoz *et al.* 2013) and are capable of arresting the larval-pupal or pupal-adult transformation (Sahayaraj and Sathyamoorthi 2010).

The seed extract of medicinal plant, Ashwagandha, *W. somnifera* is more effective than the root extract since it completely suppresses pupation and adult emergence whereas treatment with the latter caused a significant decline in pupation and adult emergence. Similar results have also been observed when larvae of *S. littoralis* are treated with extracts of *Commiphora molmol* (Shonouda *et al.* 2000) or *S. frugiperda* larvae are treated with *Calceolaria talcana* extracts (Munoz *et al.* 2013). It appears that the plant extracts are mimicking the action of JH which suppresses pupation (Eto 1990) and may also block the synthesis/release of eclosion hormone since phytochemicals are known to suppress the synthesis of ecdysteroids or eclosion hormone (Rembold *et al.* 1982).

Malformed pupae and adultoids, like those found in the present study, have also been reported in cases of treatment with other plant extracts (Jeyasankar and Chinnamani 2014; Paulraj *et al.* 2014; Soonwera and Phasomkusolsil 2016). The results are similar to those obtained with exogenous administration of JHA, pyriproxyfen (Ghasemi *et al.* 2010). This clearly demonstrates the insect growth regulatory properties of these plant extracts in a manner similar to those found as a result of administration of juvenoids. Insect metamorphosis is controlled and regulated by several hormonal factors such as neuropeptides, ecdysteroids and juvenile hormone (Hoffman and Lorenz 1998; Goodman and Granger 2005). Any disruption in the developmental process as a result of hormonal fluctuations may prove detrimental and lead to the formation of malformed pupae, adultoids or chimeras.

Thus, it is clear, that both seed and root extracts of Ashwagandha, *W. somnifera,* were highly effective against the polyphagous pest, *P. ricini* causing disruption of moulting and metamorphosis. The plant extracts from *W. somnifera* may be safely and judiciously used under field conditions along with other biorational control measures in insect pest management (IPM).

References

Ahmed S., Riaz M.A., Malik A.H., Shahid M. 2007. Effect of seed extracts of *Withania somnifera, Croton tiglium* and *Hygrophila auriculata* on behavior and physiology of *Odontotermes obesus* (Isoptera, Termitidae). Biologia, Bratislava 62 (6): 770–773. DOI: 10.2478/s11756-007-0134-0

Arora M., Sharma J., Singh A., Negi R.S. 2011. Larvicidal property of aqueous extracts of *Withania somnifera* on *Tribolium castaneum*. Indian Journal of Fundamental and Applied Life Sciences 1 (2): 32–36.

Ascher K.R.S., Schmutterer H., Glotter E., Kirson I. 1981. Withanolides and related ergostane-type steroids as antifeedants for larvae of *Epilachna varivestis* (Coleoptera: Chrysomelidae). Phytoparasitica 9 (3): 197–205.

Bansal S.K., Singh K.V., Sharma S., Sherwani M.R.K. 2011. Comparative larvicidal potential of different plant parts of *Withania somnifera* against vector mosquitoes in the semi-arid region of Rajasthan. Journal of Environmental Biology 32 (1): 71–75.

Baskar K., Maheswaran R., Ignacimuthu S. 2012. Bioefficacy of *Ceasalpinea bonduc* (L.) Roxb. Against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Archives of Phytopathology and Plant Protection 45(10): 1127–1137. DOI: http://dx.doi.org/10.1080/03235408.2012.657931

Bowers W.S. 1971. Insect hormones and their derivatives as insecticides. Bulletin of the World Health Organization 44 (1–3): 379–389.

Brittain C.A., Vighi M., Bommarco R., Settele J., Potts S.G. 2010. Impacts of a pesticide on pollinator species richness at different spatial scales. Basic and Applied Ecology 11 (2): 106–115. DOI: https://doi.org/10.1016/j.baae.2009.11.007

Butter N.S., Singh G., Dhawan A.K. 2003. Laboratory evaluation of the insect growth regulator lufenuron against *Helicoverpa armigera* on cotton. Phytoparasitica 31 (2): 200–203.

Chockalingam S., Manoharan T., Kumar U.S. 1992. Ovicidal, larvicidal and pupicidal activities of an indigenous plant extract against *Pericallia ricini* (Arctiidae: Lepidoptera). Journal of Environmental Biology 13 (3): 197–199.

Ciemior K.E., Sehnal F., Schneiderman H.A. 1979. Moulting, growth and survival of *Galleria mellonella* L. (Lep., Pyralidae) treated with juvenoids. Journals of Appplied Entomology 88 (1–5): 414–425. DOI: 10.1111/j.1439-0418.1979.tb02518.x

Damalas C.A., Eleftherohorinos I.G. 2011. Pesticide exposure, safety issues, and risk assessment indicators. International Journal of Environment Research and Public Health 8 (5): 1402–1419. DOI: 10.3390/ijerph8051402

Dar N.J., Hamid A., Ahmad M. 2015. Pharmacologic overview of *Withania somnifera*, the Indian Ginseng. Cellular and Molecular Life Sciences 72 (23): 4445–4460. DOI: 10.1007/s00018-015-2012-1

David B.V., Ananthakrishnan T.N. 2004. General and Applied Entomology. Tata McGraw-Hill Publishing Company Limited, New Delhi, India, 543 pp.

Dhadialla T.S., Retnakaran A., Smagghe G. 2005. Insect growth and development-disrupting insecticides. p. 55–116. In: "Comprehensive Insect Molecular Science" (L.I. Gilbert, I. Kostas, S.K. Gill, eds.). Vol. 6, Pregamon Press, New York, NY.

El-Wakeil N.E. 2013. Botanical pesticides and their mode of action. Gesunde Pflanzen 65 (4): 125–149.

Eto M. 1990. Biochemical mechanism of insecticidal activities. p. 65–107. In: "Controlled Release, Biochemical Effects of Pesticides, Inhibition of Plant Pathogenic Fungi. Chemistry of Plant Protection". Vol. 6. Springer, Berlin, Heidelberg. DOI: https://doi.org/10.1007/978-3-642-46674-8_2.

Gaur R., Kumar K. 2010. Insect growth-regulating effects of *Withania somnifera* in a polyphagous pest, *Spodoptera litura*. Phytoparasitica 38: 237–241. DOI: 10.1007/s12600-010-0092-x

Ghasemi A., Sendi J.J., Ghadamyari M. 2010. Physiological and biochemical effect of pyriproxyfen on Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Journal of Plant Protection Research 50 (4): 416–422. DOI: https://doi.org/10.2478/v10045-010-0070-9

Gilbert S.F. 2006. Hormonal control of insect metamorphosis. p. 1–4. In: "Developmental Biology" (S.F. Gilbert, ed.). 8th ed. Sinauer Associates, Inc., 751 pp.

Gill H.K., Garg H. 2014. Pesticides: Environmental Impacts and Management Stategies. p. 187–230. In: "Pesticides – Toxic Aspects" (M.L. Larramendy, S. Solaneski, eds.). In Tech, 238 pp.

Gnanamani R., Dhanasekaran S. 2013. Growth inhibitory effects of azadirachtin against *Pericallia ricini* (Lepidoptera:

Arctiidae). World Journal of Zoology 8 (2): 185–191. DOI: 10.5829/idosi.wjz.2013.8.2.746

Goodman W.G., Granger N.A. 2005. The juvenile hormones. p. 319–408. In: "Comprehensive Insect Molecular Science" (L.I. Gilbert, K. Latrou, S.S. Gill, eds.). Vol. 3, Oxford: Elsevier Ltd., 896 pp.

Govindachari T.R., Narasimhan N.S., Suresh G., Partho P.D., Gopalakrishnan G. 1996. Insect antifeedant and growth-regulating activities of salannin and other c-seco limonoids from neem oil in relation to azadirachtin. Journal of Chemical Ecology 22 (8): 1453–1461. DOI: 10.1007/BF02027724.

Gupta L., Srivastava M. 2008. Effect of *Withania somnifera* extracts on the mortality of *Callosobruchus chinensis* L. Journal of Biopesticides 1 (2): 190–192.

Hangartner W., Masner P. 1973. Juvenile hormone: inhibition of ecdysis in larvae of the German cockroach *Blatella germania*. Experientia 29: 1358–1359.

Hoffman K.H., Lorenz M.W. 1998. Recent advences in hormones in insect pest control. Phytoparasitica 26 (4): 223–230.

Huang S.H., Xian J.D., Kong S.Z., Li Y.C., Xie J.H., Lin J., Chen J.N., Wang H.F., Su Z.R. 2013. Insecticidal activity of pogostone against *Spodoptera litura* and *Spodoptera exigua* (Lepidoptera: Noctuidae). Pest Management Science 70: 510–516. DOI: 10.1002/ps.3635

Ibrahim Y.A. 2016. Health and environmental impacts of pesticides: A responsibility principle and two novel systems for hazard classification and external cost determination. Journal of Toxicology and Health 3 (1): 1–9. DOI: 10.7243/2056--3779-3-1

Isman M. 2002. Insect antifeedants. Pestic Outlook 13: 152–157. DOI: 10.1039/b206507j

Jain P.C., Verma J.P., Mathur Y.K. 1972. Occurrence of *Pericallia ricini* Fabr. (Lepidoptera: Arctiidae), on zinnia (*Zinnia grandiflora*) and balsam (*Impatiens balsamina*) and notes on its biology. Indian Journal of Entomology 33 (2): 214.

Jeyasankar A., Chinnamani T. 2014. Bioactivity of *Pseudocalymma alliaceum* (Lam.) Sandwith (Bignoniaceae) against *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Journal of Coastal Life Medicine 2 (4): 302–307. DOI: 10.12980/JCLM.2.2014JCLM-2014-0009

Jeyasankar A., Chennaiyan V., Chinnamani T., Ramar G. 2014. Feeding and growth inhibition activities of *Tragiain volucrata* Linn (Euphorbiaceae) on *Achaea janata* (Linn.) (Noctuidae: Lepidoptera) and *Pericallia ricini* (Fab.) (Lepidoptera: Arctidae). Open Access Library Journal 1: 1–7.

Joseph B., Sujatha S., Jeevitha M.V. 2010. Screening of pesticidal activities of some marine sponge extracts against chosen pests. Journal of Biopesticides 3 (2): 495–498.

Karmegam N., Sakthivadivel M., Anuradha V., Daniel T. 1997. Indigenous-plant extracts as larvicidal agents against *Culex quinquefasciatus* say. Bioresource Technology 59 (2–3): 137–140. DOI: https://doi.org/10.1016/S0960-8524(96)00157-5

Khan M.M., Srivastava B.B.L. 1992. Penfluron induced sterility in adults of black hairy caterpillar *Pericallia ricini* and hairy caterpillar *Euproctis icilia*. Indian Journal of Agricultural Sciences 62 (6): 414–417.

Krishnan M., Chockalingam S. 1989. Effect of diflubenzuron on the bioenergetics of a hairy caterpillar, *Pericallia ricini*. Journal of Environmental Biology 10 (4): 383–392.

Kubo I., Uchida M., Klocke J.A. 1983. An insect ecdysis inhibitor from the African medicinal plant, *Plumbago capensis* (Plumbaginaceae); a naturally occurring chitin synthetase inhibitor. Agricultural and Biological Chemistry 47 (4): 911–913. DOI: http://dx.doi.org/10.1080/00021369.1983.10865746

Lapcharoen P., Apiwathnasorn C., Komalamisra N., Dekumyoy P., Palakul K., Rongsriyam Y. 2005. Three indigenous Thai medicinal plants for control of *Aedes aegypti* and *Culex quinquefasciatus*. The Southeast Asian Journal of Tropical Medicine and Public Health 36 (Suppl): 167–175.

Madkour M.H., Zaitoun A.A., Singer F.A. 2013. Repellent and toxicity of crude plant extracts on saw-toothed grain beetle (*Oryzaephilus surinamensis* L.). Journal of Food, Agriculture and Environment 11 (2): 381–384.

Mahmood I., Imadi S., Shazadi K., Gul A., Hakeem K.R. 2016. Effect of pesticides on environment. p. 253–269. In: "Plant, Soil and Microbes" Vol. 1. Implications in Crop Science (K.R. Hakeem, M.S. Akhtar, S.N.A. Abdullah, eds.). Springer, Cham, Switzerland, 2016. DOI https://doi.org/10.1007/978-3-319-27455-3

Mayuravalli V.V.L., Reddy G.P., Murthy M.Mk. 1989. Effect of diflubenzuron on larval and post-larval stages of garden hairy caterpillar *Pericallia ricini* (Lepidoptera: Arctiidae). Indian Journal of Agricultural Sciences 59 (3): 193–194.

Mead H.M., El-Shafiey S.N., Sabry H.M. 2016. Chemical constituents and ovicidal effects of mahlab, *Prunus mahaleb* L. kernels oil on cotton leafworm, *Spodoptera littoralis* (Boisd.) eggs. Journal of Plant Protection Research 56 (3): 279–290. DOI: https://doi.org/10.1515/jppr-2016-0044

Mehrotra S., Kumar S., Zahid M., Garg M. 2016. Biopesticides. p. 273–292. In: "Principles and Applications of Environmental Biotechnology for a Sustainable Future" (R.L. Singh, ed.). Springer, Singapore, 487 pp.

Munoz E., Lamilla C., Marin J.C., Alarcon J., Cespedes C.L. 2013. Antifeedant, insect growth regulatory and insecticidal effects of *Calceolaria talcana* (Calceolariaceae) on the *Drosophila melanogaster* and *Spodoptera frugiperda*. Industrial Crops and Products 42: 137–144. DOI: https://doi.org/10.1016/j.indcrop.2012.05.014

Nathala E., Dhingra S. 2006. Biological effects of *Caesalpinia crista* seed extracts on *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its predator, *Coccinella septempunctata* (Coleoptera: Coccinellidae). Journal of Asia-Pacific Entomology 9 (2): 159–164. DOI: 10.1016/S1226-8615(08)60287-3

Nathan L.S., Nathan R.J. 2011. Effect of chitin biosynthesis inhibitor-diflubenzuron on the fifth instar larvae of *P. ricini* Fabr. The Asian Journal of Animal Science 6 (1): 66–70.

Nathan S.S., Sehoon K. 2006. Effects of *Melia azedarach* L. extract on the teak defoliator *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). Crop Protection 25: 287–291. DOI: https://doi.org/10.1016/j.cropro.2005.03.023

Nijhout H.F. 1998. Insect Hormone. 4th ed. Princeton University Press, Princeton, NJ, 267 pp.

Pant M., Dubey S., Patanjali P.K. 2016. Recent advancements in bio-botanical pesticide formulation technology development. p. 117–126. In: "Herbal Insecticides, Repellents and Biomedicines: Effectiveness and Commercialization" (V. Veer, R. Gopalakrishnan, eds.). Springer, New Delhi, India, 258 pp.

Paulraj M.G., Shanmugam N., Ignacimuthu S. 2014. Antifeedant activity and toxicity of two alkaloids from *Adhatoda vasica* Neem leaves against diamondback moth *Plutella xylostella* (Linn.) (Lepidoptera: Plutellidae) larvae. Archives of Phytopathology and Plant Protection 47 (15): 1832–1840. DOI: http://dx.doi.org/10.1080/03235408.2013.859402

Pratibha C., Madhumati B., Akarsh P. 2013. Therapeutic properties and significance of different parts of Ashwagandha – a medicinal plant. International Journal of Pure and Applied Bioscience 1 (6): 94–101.

Rajapakse R.H.S., Ratnasekera D., Abeysinghe S. 2016. Biopesticides research: current status and future trends in Sri Lanka. p. 219–234. In: "Agriculturally Important Microorganisms". Springer, Singapore, 2016. DOI: https://doi.org/10.1007/978-981-10-2576-1

Ramakanth G.S.H., Kumar C.U., Kishan P.V., Usharani P. 2016. A randomized, double blind placebo controlled study of efficacy and tolerability of *Withania somnifera* extracts in knee joint pain. Journal of Ayurveda and Integrative Medicine 7 (3): 151–157. DOI: 10.1016/j.jaim.2016.05.003

Razavi N., Ahmadi K. 2016. Compatibility between four ethanolic plant extracts with a bug predator *Orius horvathi* (Reuter) (Heteroptera: Anthocoridae) used for controlling the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Journal of Plant Protection Re-

search 56 (1): 89–94. DOI: https://doi.org/10.1515/jppr-2016-0015

Rembold H., Sharma G.K., Czoppelt Ch., Schmutterer H. 1982. Azadirachtin: a potent insect growth regulator of plant origin. Journal of Applied Entomology 93 (1–5): 12–17. DOI: 10.1111/j.1439-0418.1982.tb03564.x

Riddiford L.M., Hiruma K., Zhou X., Nelson C.A. 2003. Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manduca sexta* and *Drosophila melanogaster*. Insect Biochemistry and Molecular Biology 33 (12): 1327–1338. DOI: https://doi.org/10.1016/j.ibmb.2003.06.001

Rountree D.B., Bollenbacher W.E. 1986. The release of the prothoracicotropic hormone in the tobacco hornworm, *Menduca sexta*, is controlled intrinsically by juvenile hormone. Journal of Experimantal Biology 120: 41–58.

Sahayaraj K., Borgio J.F. 2012. Screening of some mycoinsecticides for the managing hairy caterpillar, *Pericallia ricini* Fab. (Lepidoptera: Arctiidae) in Castor. Journal of Entomology 9 (2): 89–97.

Sahayaraj K., Sathyamoorthi P. 2010. The toxicity and biological effect of *Pedalium murex* L. extracts on the tobacco cutworm, *Spodoptera litura* (Fabr.) larvae. Archives of Phytopathology and Plant Protection 43 (18): 1768–1780. DOI: http://dx.doi.org/10.1080/03235400902753626

Sakthivadivel M., Daniel T. 2008. Evaluation of certain insecticidal plants the control of vector mosquitoes viz. *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. Applied Entomology and Zoology 43 (1): 57–63. DOI: http://doi.org/10.1303/aez.2008.57

Schmidt G.H., Rembold H., Ahmed A.A.I., Breuer M. 1998. Effect of *Melia azedarach* fruit extract on juvenile hormone titre and protein content in the hemolymph of two species of noctuid lepidopteran larvae (Insecta: Lepidoptera: Noctuidae). Phytoparasitica 26 (4): 283–291.

Shonouda M.L., Farrag R.M., Salama O.M. 2000. Efficacy of the botanical extract (myrrh), chemical insecticides and their combinations on the cotton leafworm, *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae). Journal of Environmental Science and Health 35 (3): 347–356. DOI: http://dx.doi.org/10.1080/03601230009373275

Shophiya N., Sahayaraj K. 2014. Biocontrol potential of entomophagous predator *Eocanthecona furcellata* (Wolff) against *Pericallia ricini* (Fab.) larvae. International Journal of Current Research 6 (10): 9052–9056.

Shophiya N., Sahayaraj K., Kalaiarasi J.M.V., Shirlin J.M. 2014. Biocontrol potential of entomopathogenic fungus *Beauveria bassiana* (Balsamo) against *Pericallia ricini* (Fab.) (Lepidoptera: Arctiidae) larvae. Biolife 2 (3): 813–824.

Singh P., Dugdale J.S. 1979. Morphogenetic and physiological effects of a juvenile hormone analogue on the light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae). New Zeeland Journal of Zoology 6 (2): 381–387. DOI: http://dx.doi.org/10.1080/03014223.1979.10428377

Singh A., Gandhi S. 2012. Agricultural insect pest: occurrence and infestation level in agricultural fields of Vadodara, Gujarat. International Journal of Scientific and Research Publications 2 (4): 1–5.

Singh N.K., Jyoti Vemu B., Nandi A., Singh H., Kumar R., Dumka V.K. 2014a. Laboratory assessment of acaricidal activity of *Cymbopogon winterianus, Vitex negundo*, and *Withania somnifera* extracts against deltamethrin resistant *Hyalomma anatolicum*. Experimental and Applied Acarology 63 (3): 423–430.

Singh N.K., Jyoti Vemu B., Nandi A., Singh H., Kumar R., Dumka V.K. 2014b. Acaricidal activity of *Cymbopogon winterianus, Vitex negundo*, and *Withania somnifera* against synthetic pyrethroid resistant *Rhipicephalus* (*Boophilus*) *microplus*. Parasitology Results 113 (1): 341–350. DOI: 10.1007/s00436-013-3660-4

Singh S., Kumar K. 2011a. Diofenolan: a novel insect growth regulator in common citrus butterfly, *Papilio demoleus*. Phytoparasitica 39: 205–213. DOI: 10.1007/s12600-011--0154-8

Singh S., Kumar K. 2011b. Effect of the juvenile hormone agonist pyriproxyfen on larval and pupal development of the citrus swallowtail *Papilio demoleus* (Lepidoptera: Papilionidae). International Journal of Tropical Insect Science 31 (3): 192–198. DOI: 10.1017/S1742758411000269

Singh S., Kumar K. 2015. Comparative efficacy of phonoxy derivative JHAs pyriproxyfen and diofenolan against polyphagous pest *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera). Phytoparasitica 43: 553–563. DOI: 10.1007/s12600-015-0473-2

Soonwera M., Phasomkusolsil S. 2016. Effect of *Cymbopogon citratus* (lemongrass) and *Syzygium aromaticum* (clove) oils on the morphology and mortality of *Aedes aegypti* and *Anopheles dirus* larvae. Parasitology Results 115 (4): 1691–1703. DOI: 10.1007/s00436-016-4910-z

Suvanthini S., Mikunthan G., Thurairatnam S., Pakeerathan K. 2012. Evaluation of *Withania somnifera* on a storage pest Rice weevil, *Sitophilus oryzae*. Annals of Biological Research 3 (11): 5050–5053.

Thyagaraja B.S., Kelly T.J., Masler E.P., Gelman D.B., Bell R.A., Imberski R.B. 1992. Kopec revisited – Neuroendocrine regulation of metamorphosis in the gypsy moth (*Lymantria dispar*): Development of a larval *in vivo* assay for prothoracicotropic hormone. Journal of Insect Physiology 38 (11): 925–938. DOI: https://doi.org/10.1016/0022-1910-(92)90104-L

Tunaz H., Uygun N. 2004. Insect growth regulators for insect pest control. Turk. J. Agric. For. 28: 377–387.

Vanitha K., Karuppuchamy P., Sivasubramanian P. 2011. Pests of vanilla (*Vanilla planifolia* Andrews) and their natural enemies in Tamil Nadu, India. International Journal of Biodiversity and Conservation 3 (4): 116–120.

Wilson T.G. 2004. The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: how these compounds kill insects. Journal of Insect Physiology 50 (2–3): 111–121. DOI: https://doi.org/10.1016/j.jinsphys.2003.12.004

Yankanchi S.R., Gadache A.H. 2010. Grain protectant efficacy of certain against rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Journal of Biopesticides 3 (2): 511–513.

Zacharia J.T. 2011. Ecological effects of pesticides. p. 129–142. In: "Pesticides in the Modern World – Risk and Benefits". (M. Stycheva, ed.). INTech. DOI: 10.5772/949