Indian Journal of Entomology 86(1): 81-86 (2024) DoI. No.: 10.55446/IJE.2023.872

JUVENILE HORMONE-INDUCED ABNORMALITY OF *PERIPLANETA AMERICANA* (L.)

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ABSTRACT

Application of juvenile hormone III (JH III) and a JH analogue, methoprene to last instars of *Periplaneta americana* suppressed adult emergence and caused morphological abnormalities such as twisted wings and asymmetry of appendages resulting in formation of either nymphoids or giant supernumerary nymphs and adultoids. The effects of JH III and methoprene were concentration dependent in inducing aberrations in morphogenetic moult as well as in significantly lowering the body mass (BM) and lipid reserves (LR) in *P. americana*.

Key words: *Periplaneta americana* growth hormone, JH analogue, methoprene, morphology, appendages, body, lipid content, nymphoids, giant nymphs, adultoids

The American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae**)** is the largest of the common peri-domestic cockroaches, adults measuring 39.43 to 43.30 mm in length (Borah and Hazarika, 2019a). The species is ubiquitous (Schal et al., 1984; Mudoi et al., 2019) and adults live and actively feed for over 300 days. As a domestic pest, the *P. americana* causes economic losses to stored products and food and poses public health concerns in houses, hotels and public transports (Bell and Adiyodi, 1982; Bell et al., 2007). To manage this pest, synthetic insecticides ("blatticides," Wilson, 2007) are extensively applied as spray, dusts or baits, which may be an economic burden and cause environmental contamination (Schal and Hamilton, 1990; Mudoi et al., 2019). Therefore, there is a dire need to investigate alternatives and insect hormones and their analogues could be promising candidates (Alamer and Hoffman, 2013). Hormones that regulate physiological, morphogenetic, developmental and reproductive processes in insects are of particular interest. This paper reports aberrations in morphogenetic moults caused by Juvenile Hormone III (JH III) and methoprene, a synthetic JH alongue that serves as an insect growth regulator.

One of the target sites of JH is the fat body, an organ analogous to vertebrate liver and adipose tissues; fat body constitutes 50% of the fresh BM, and is the major metabolic centre (Gilbert and Chino, 2001; Arrese and Soulages, 2010) integrating hormonal and nutritional signals centrally to regulate reproduction (Arrese and Soulages, 2010), growth, development and metamorphosis, body size, circadian rhythms, diapauses, longevity and feeding behaviour, partially under the neuroendocrine direction of the brain (Hazarika and Gupta, 1987; Colombani et al., 2005; Adamo, 2012; Li et al., 2019). Lipids accumulated in the fat body during active feeding stages, and LR accumulate and are used in an age-specific manner in insects (Anand and Lorenz, 2008). The process of mobilization of fat body energy reserves is essentially regulated by the action of hormones like adipokinetic hormones, JH, ecdysteroids and various neurohormones (for recent review see Li et al., 2019). We are also interested to understand how exogenous application of hormones brings about changes in the LR and consequently upon the BM of the *P. americana*.

MATERIALS AND METHODS

The experiment was conducted from 2013-2017 at Physiology Laboratory, Department of Entomology, Assam Agricultural University, Jorhat, Assam, India The cockroaches used in these experiments were raised in the Physiology Laboratory, Department of Entomology at 28±3°C, 70 to 85% relative humidity and 12:12 L: D cycle (light was switched on at 0500 h and switched off at 1700 h) in wooden rearing cages (90× 65× 60 cm) fixed with nylon netting on four sides and the top. Rearing procedures were described by Borah and Hazarika (2019a) and Mudoi et al. (2019). Laboratory cockroaches were age-synchronized by

moving freshly emerged adult males and females within one hour after the final moult (designated as 1 day old) into new glass jars, each measuring 32 cm tall and 22 cm diameter (Borosil Glass Works Ltd., Mumbai, India) with the open top covered with a piece of muslin cloth held in place with a rubber band.

To study the effect of hormones on adult BM and LR, 30 adult cockroaches of a specific age were selected randomly, and then subjected to lipid estimation by the method described by Folch et al. (1957). After weighing the sample, an extract was made by homogenizing the insect in chloroform-methanol (2:1 V/V) using a grinder. The homogenate was filtered through a sintered glass filter to a separating funnel. The residue was washed with 20 ml of chloroform-methanol. The residue collected from the filter paper was oven dried at 80°C to a constant mass for determining the lean dry sample mass. The pooled chloroform-methanol sample filtrate was purified by washing with 20 ml of 3% NaCl solution and the chloroform layer containing the lipids was withdrawn. The remaining aqueous layer was washed two times with 10 ml chloroform and put in a pre-weighed beaker. Anhydrous sodium sulphate was added to the combined layers to remove water. The chloroform was allowed to dry in an oven and finally the total mass of the lipids was taken as (weight of beaker + lipid) – weight of beaker.

To study the effect of JH III and methoprene, experiments were conducted using a Completely Randomized Design (CRD) with five replications and four treatments (three1-day-old 6^{th} instars per treatment) including the untreated control. Three concentrations (1, 3 and 5 ppm) of JH-III (Sigma-Aldrich) and methoprene (Sigma-Aldrich) were prepared in physiological saline (using double-distilled water) using ethanol as solvent. JH III was less soluble in water. JH III is the most common compound of the JH family in insects. JH III or methoprene were either topically applied or injected. Topical application was performed onto the dorsal surface of the abdomen of the insect during day time (Alamer, 2013). Injection of the hormone was administered into the cervix region of the test insect individually with the help of a Hamilton Microsyringe (25µl capacity) that delivered 1 µl to each insect. Control insects were treated with physiological saline alone. Mortality and deformities of appendages, effects of hormones on BM and LR and reproduction in the control and treated insects were recorded at 7, 15 and 30 days after treatment (DAT). All data were subjected to CRD analysis of variance (ANOVA) (Panse and Sukhatme, 1985). The difference in hormone-induced deformities of the body parts between untreated and treated insects were separated using Student's T test (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

We observed morphological deformities (demorphogenesis) in the form of intermediate forms, i.e. nymphoids (Fig. 1a), giant nymphs (Fig. 1b) and adultoids (Fig. 1c) in a dose-dependent manner as a result of exogenous application of JH III and methoprene. These results were similar to those reported by other workers (Das and Gupta, 1974; Kawada et al., 1989; Fathpour et al., 2007) on the German cockroach. As stated above, amongst the four doses of JH III and methoprene tested, 5 ppm was the most effective. Comparison of morphometrics, such as body length, head width, and pronotum width of the control and the treated individuals (giant nymph) is presented in Table 1, which revealed that all the body parameters were significantly different between the treated and the control insects. Borah and Hazarika (2019) reported morphometrics of the *P. americana* in untreated individuals. Exogenous application of JH and JHA during the sensitive period (King and Bennet, 1991) would lead to production of another larval stage by altering the gene expression and deactivating the PTG for ecdysteroids synthesis (Lanzrein et al., 1985; Hazarika and Gupta, 1986; Chapman, 2004). Kawada et al. (1989) also found that exposure of nymphs to pyriproxyfen (a JHA) suppressed adult emergence and caused morphological abnormalities in emerged adults.

The effect of various concentrations of JH III and methoprene on the appendages of male and female *P. americana* are represented in Fig. 1d with 1i. Besides demorphogenesis of the body, appendages were also affected. The morphological abnormalities we recorded on nymphoids, giant nymphs and adultoids were asymmetry of antennae, wings and cercus (Fig. 1d with 1g) as well as "amputation" of antennae, legs and cerci (Fig. 1h and 1i). Twisted wings were observed as was earlier reported by Lim and Yap (1996). Since exogenous application of JH and JHA might have caused reduction of ecdysteroid titre in the haemolymph of the *P. americana*, which might have caused amputation of the appendages as was earlier reported in *Rhodnius prolixus* Stal (Hemiptera: Reduvidae) due to temporary reduction of ecdysteroid level in the hemolymph (Hackney and Cherbas, 2014). Nevertheless, few works have been reported abnormal

effects of JHAs on appendages (Hazarika and Baishya, 1997; Wheeler and Gupta, 1988; Fathpour et al., 2007).

We also observed that females treated with methoprene and JH III were unable to produce oothecae (NB, personal observation). Previously, Alamer (2013) reported that methoprene treatment of females resulted in prevention of ootheca formation or complete resorption of the ootheca, depending upon the time of treatment. Sterility might be related to effect of these hormones on the process of development of internal reproductive organs, sex pheromone production or on mating. These compounds might have affected chemoreceptors on the antennae as a result of which the male might have failed to detect pheromones needed

Fig. 1a-i. Effect of Methoprene and JH III on *P. americana* a. resultant nymphoid (dorsal and ventral view); b. giant nymph; c. adultoid showing distinct twisted tagmina; nymphoid of *P. americana* due to application of methoprene showing asymmetrical development of d. antennae; e. wings; nymphoid of *P. americana* due to application of JH III showing f-g. breakage of hind legs and asymmetrical growth of circus (dorsal and ventral view); h. "amputation" of antennae and legs; i. "amputation" of legs and cercus.

Table 1. Comparison of morphometrics (Mean± SEM) between normal and giant nymphs of *P. americana*

Nymph	Body length (mm)	Head width (mm)	Pronotum length (mm)	Pronotum width (mm)	Body mass (g)
Normal	39.03± 1.78	4.05± 0.17	7.96± 0.06	9.00± 0.17	1.21± 0.09
Giant	43.00± 6.48*	5.02± 0.15*	9.45± 0.57*	10.21± 1.06*	1.39± 0.08*

Sample size = 30; *Significant at p=0.05

for mating. This needs to be researched in more detail in the future.

The effect of topical application and injection of various concentrations of JH III and methoprene on BM and LR of *P. americana* are presented in Tables 2, 3, 4 and 5. The BM and LR of the treated individuals were significantly lower than those of the controls and the effect was age- as well as dose-dependent, because both parameters varied with age or days after treatment (DAT). Method of application also influenced the results, as the lowest BM was recorded with 5 ppm methoprene treated insects (0.31 g and 0.29 g of topical and injection, respectively on 30 DAT) and 5 ppm JH III treated insects (0.35 g and 0.26 g of topical and injection, respectively on 30 DAT). Irrespective of doses, it was found that injection of JH III and methoprene suppressed the BM and the LR more compared with those individuals that were topically applied. Similarly, both hormones were also found to be equally effective in lowering the LR of the treated insects (Table 4 and 5). Methoprene reduced LR in *Dicladispa armigera*

Table 2. Effect of JH III on body mass (g) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	1.01	1.14	1.23	1.27	0.97	1.09	1.12	1.14
1	1.19	1.03	0.84	0.75	1.05	0.94	0.84	0.64
3	1.10	0.86	0.81	0.63	1.05	0.68	0.45	0.41
5	1.01	0.39	0.38	0.35	1.03	0.34	0.29	0.26
S.Ed. (±)		0.12	0.10	0.11		0.11	0.11	0.12
$CD_{0.05}$	NS	0.28	0.22	0.25	NS	0.25	0.25	0.27
CV (%)		16.92	13.02	14.97		14.87	15.24	17.12

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

Table 3. Effect of methoprene on body mass (g) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	1.06	1.11	1.20	1.22	1.04	1.16	1.23	1.27
1	0.96	0.93	0.61	0.59	0.97	0.82	0.71	0.62
3	1.10	0.87	0.50	0.49	1.06	0.52	0.50	0.47
5	1.07	0.53	0.33	0.31	0.95	0.32	0.30	0.29
S.Ed. (±)		0.09	0.14	0.13		0.12	0.12	0.11
$CD_{0.05}$	NS	0.20	0.31	0.29	NS	0.27	0.26	0.26
CV (%)		11.50	19.08	18.28		16.75	16.26	16.11

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

Table 4. Effect of JH III on lipid reserves (mg) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	326.16	328.21	331.41	332.10	323.88	324.50	325.55	330.98
1	324.44	323.98	321.10	318.76	323.87	311.22	304.08	294.42
3	327.98	325.02	322.22	317.22	325.27	294.92	284.12	271.73
5	330.80	312.78	308.30	306.20	324.72	283.30	263.40	247.78
S.Ed. (±)		5.05	7.96	7.81		7.52	17.88	12.99
$CD_{0.05}$	NS	11.01	17.35	17.02	NS	16.40	38.97	28.32
CV (%)		2.21	3.51	3.46		3.50	8.59	6.42

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

Table 5. Effect of methoprene on lipid reserves (mg) of *P. americana*

Dose (ppm)	Topical application				Injection			
	Pre treatment	Post treatment			Pre treatment	Post treatment		
		7 DAT	15 DAT	30 DAT		7 DAT	15 DAT	30 DAT
0	324.68	329.50	330.70	332.30	328.28	329.10	332.55	336.18
1	324.57	319.43	314.63	308.89	329.77	319.80	309.64	287.02
3	325.53	310.39	305.87	300.57	328.53	315.60	294.12	265.52
5	326.28	307.00	302.42	300.12	330.28	308.94	278.82	240.98
S.Ed. (±)		6.94	5.98	5.14		7.03	18.63	17.31
$CD_{0.05}$	NS	15.12	13.05	11.21	NS	15.33	40.61	37.73
CV (%)		3.10	2.70	2.34		3.12	8.67	8.67

DAT = Days after treatment; NS = Non significant; Data based on mean of 5 replication (3 insects per treatment)

(Olivier) (Coleoptera: Chrysomelidae) (Baishya and Hazarika, 1996), *Gryllus firmus* Scudder (Orthoptera: Gryllidae) (Zera and Zhao, 2004), mosquito pupae (Downer et al., 2012) by substantially decreasing *in vivo* biosynthesis of total lipid and triglycerides.

We conclude that different concentrations of a juvenile hormone and its analogue (JHA) caused morphological and reproductive disorders and lowered the BM and LR of the insect. Thus insect growth regulators can be successfully utilized for its management. Moreover, JH or JHA act on the ultimate instar during the critical period only when endogenous JH is absent in the haemolymph, resulting in an additional nymph or nymphoid and adultoid development. Since application of JH and JHA was done on the ultimate instar i.e. 6th instar, only those insects which were at the critical period were affected, as a result 20-30% tested individuals developed abnormalities. However, sterility was evident in the remaining 70-80% due to occurrence of abnormalities that they failed to produce oothecae.

ACKNOWLEDGEMENTS

The authors thank the Department of Science and Technology- INSPIRE for providing financial assistance and also grateful to Dr. Coby Schal, Blanton J. Whitmire Distinguished Professor, Department of Entomology & Plant Pathology, 3107 Gardner Hall Campus Box 7613 (Courier: 100 Derieux Pl.), North Carolina State University Raleigh, NC 27695-7613 for critically reviewing, reading and correcting the manuscript.

FINANCIAL SUPPORT

The authors thank the Department of Science and Technology, Government of India for providing INSPIRE Fellowship and to the Assam Agricultural University, Jorhat, Assam's authority for proving financial support.

AUTHOR CONTRIBUTION STATEMENT

LKH conceived and designed the experiment. NB conducted experiments, analysed data and wrote manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Adamo S A. 2012. The importance of physiology for ecoimmunology: Lessons from the insects. pp. 413-439.

Alamer A H. 2013. Endocrine control of fat body composition and effects of the growth regulators methoprene and pyriproxyfen on the development and reproduction of Argentinian cockroach, *Blaptica dubia* Serville (Blattaria: Blaberidae). Doctoral Thesis, Bayreuth, Germany.

Alamer A H, Hoffman K H. 2013. Endocrine aspects of ovary growth and gestation in the Argentinian cockroach, *Blaptica dubia* (Dictyoptera: Blaberidae). Invertebrate Reproduction and Development 58 (1): 34-41.

Anand A N, Lorenz M W. 2008. Age-dependent changes of fat body stores and the regulation of lipid synthesis and metabolism by adipokinetic hormone in the last larval instar of the cricket, *Gryllus bimaculatus*. Journal of Insect Physiology 54: 1404-1412.

Arrese E L, Soulages G L. 2010. Insect fat body: energy, metabolism, and regulation. Annual Review of Entomology 55: 207-225.

Baishya R L, Hazarika L K. 1996. Effect of methoprene and diflubenzuron on water, lipid, protein and chitin content of *Dicladispa armigera* (Coleoptera: Chrysomelidae). Entomon 21: 7-11.

Bell W J, Adiyodi K G. 1982. The American Cockroach. Chapman and Hall, London 1-273.

Bell W J, Roth L M, Nalepa C A. 2007. Cockroaches: Ecology, Behavior, and Natural History. The Johns Hopkins University Press, Baltimore, pp. 18-247.

Borah N, Hazarika L K. 2019a. Biology and morphometrics of

Periplaneta americana. Journal of Entomology and Zoology Studies 7(1): 1206-1210.

Borah N, Hazarika L K. 2019b. Water requirement and effect of deprivation on ootheca formation by *Periplaneta americana* (Blattodea: Blattellidae). Journal of Entomology Zoology Studies 7(4): 1396-1399.

Chapman R F. 2004. The Insect Structure and Function. Cambridge University Press. pp 674-696.

Colombani J, Bianchini L, Layalle S, Pondeville E, Dauphin V C, Antoniewski C, Noselli S, Leopold P. 2005. Antagonistic action of ecdysone and insulins determine final size in *Drosophila*. Science 310: 667-670.

Das Y T, Gupta A P. 1997. Effects of three juvenile hormone analogues on the female German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Experientia 30: 1093-1095.

Downer R G H, Spring J H, Smith M. 2012. Effect of an insect growth regulator on lipid and carbohydrate reserves of mosquito pupae (Diptera: Culicidae). Canadian Entomology 108: 627-630.

Fathpour H, Noori A, Zeinali B. 2007. Effects of a juvenoid pyriproxyfen on reproductive organ development and reproduction in German cockroach (Dictyoptera: Blattellidae). Iranian Journal of Science and Technology 31: 89-98.

Folch J, Lees M, Sloane-Stanley G W. 1957. A simple method for isolation and purification of total lipids from animal tissues. Journal of Biology and Chemistry 226: 497-509.

Gilbert L I, Chino H. 1974. Transport of lipids in insects. Journal of Lipid Research 15(5): 439-456.

Hackney J F, Cherbas P. 2014. Injury response and developmental timing in insects. Fly (Austin) 8: 226-231.

Hazarika L K, Baishya R L. 1997. Effects of methoprene on the developmental stages of rice hispa. Pesticide Research Journal 9(1): 109-112.

Hazarika L K, Gupta A P. 1986. Structure, innervations, persistence and effects of juvenile hormone on the prothoracic glands in adult *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Zoological Science 3: 145-150.

Hazarika L K, Gupta A P. 1987. Anatomy of the retrocerebral complex of *Blattella germanica* (L.) Dyctyoptera: Blattellidae. Zoologica Anzeiger 219 (5-6): 257-264.

Kawada H, Kojima I, Shinjo G. 1989. Laboratory evaluation of a new insect growth regulator pyriproxyfen, as a cockroach control agent. Japanese Journal of Sanitary Zoology 40: 195-201.

King J E, Bennett G W. 1991. Sensitive developmental period of last-instar German cockroaches (Dictyoptera: Blattellidae) to fenoxycarb and hydroprene. Journal of Medical Entomology 28 (4): 514-517.

Lanzrein B, Gentinetta V, Abbegglen H, Baker F C, Miller C A, Schooley D A. 1985. Titers of ecdysone, 20- hydroxyecdysone and juvenile hormone III throughout the life cycle of a hemimetabolous insect, the ovoviviparous cockroach, *Nauphoeta cinerea.* Experimentia 41: 913-917.

Li N, Zeng M, Xiao H, Lin S, Yang S, Huang H, Zhu S, Zhao Z, Ren C, Li S. 2019. Alteration of insulin and nutrition signal gene expression or depletion of Met reduce both lifespan and reproduction in the German cockroach. Journal of Insect Physiology 118: 103934.

Lim J L, Yap H H. 1996. Induction of wing twisting abnormalities and sterility on German cockroach (Dictyoptera: Blattellidae) by a juvenoid pyriproxyfen. Journal of Economic Entomology 89(5): 1161-1165.

Mudoi A, Das P, Hazarika L K, Das K, Roy S. 2019. Variation in haemocyte profile induced by *Beauveria bassiana* (Bals.) Vuill. in *Periplaneta americana* (L.) (Blattodea: Ectobiidae). International Journal of Tropical Insect Science 40: 81-91.

Panse V G, Sukhatme P V. 1985. Statistical Methods for Agricultural Workers. ICAR, New Delhi.

Schal C, Gautier J Y, Bell W J. 1984. Behavioural ecology of cockroaches. Biological Reviews 59: 209-254.

Schal C, Hamilton R L. 1990. Integrated suppression of synanthropic cockroaches. Annual Review of Entomology 35: 521-551.

Wheeler M C, Gupta A P. 1988. Effect of two juvenile hormone analogues (R-20458, RO 203600) and three juvenile hormone (JH 1, 2 and 3) on the number and distribution of sensilla on the seventh abdominal tergite in the male German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). JEZ-A Ecological and Integrative Physiology 248(2): 243-246.

Wilson E O. 2007. Foreword, in: Bell W J, Roth L M, Nalepa C A. 2007. Cockroaches: Ecology, Behavior, and Natural History. The Johns Hopkins University Press, Baltimore, pp. 1-2.

Zera A J, Zhao Z. 2004. Effect of a juvenile hormone analogue on lipid metabolism in a wing polymorphic cricket: Implications for the endocrine-biochemical bases of life-history trade-offs. Physiological and Biochemical Zoology 77(2): 255-266.

(Manuscript Received: November, 2022; Revised: May, 2023;
Accepted: May, 2023; Online Published: May, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23872