

Faculty of Science Dept. of Entomology

Laboratory studies of the effect of some juvenoids on the two-spotted spider mite *Tetranychus urticae* and the associated predaceous mite *Amblyseius swirskii*

A thesis

Submitted in Partial fulfillment of the requirement for the degree of the Master of Science (In Entomology)

 $\mathcal{B}y$

Hala Mohamed Sayed Metwally

Supervised by

Prof. Dr. Mohamed Talaat El-Ibrashy

Prof. Dr. Jehane Abdel Aleem Hafez

Prof. Dr. Maher Abdel Kader El-Borolossy

Ain Shams University

Faculty of Science

Name: Hala Mohamed Sayed Metwally

Title: Laboratory studies of the effect of some juvenoids on the two-spotted spider mite Tetranychus urticae and the associated predaceous mite Amblyseius swirskii.

Degree: Master degree of science.

Supervisors:

1- Dr. Mohamed Talaat El-Ibrashy

Professor Emeritus of Insect Physiology National Research Centre

2- Dr. Jehane Abdel Aleem Hafez

Professor of Entomology
Faculty of Science
Ain Shams University

3- Dr. Maher Abdel Kader El-Borolossy

Professor of Acarology National Research Centre



سمش زبيد قعمام خابة العادم هسة قسم علم المشراب

دراسات معملية على تأثير بعض متشابهات هرمون الشباب الحشرى على الحلم العنكبوتى الأحمر ذو البقعتين تتيرانيكس يورتيكا والآكاروس المفترس المصاحب له امبليسيس سويرسكياى

رسالة مقدمة كجزء من متطلبات الحصول على درجة الماجستير في العلوم (علم الحشرات)

مقدمة من

هاله محمد سید متولی (بکالوریوس علوم)

تحت إشراف

أ.د. محمد طلعت الإبراشي أ.د. جيهان عبد العليم حافظ أ.د. ماهر عبد القادر البرلسي

> جامعة عين شمس كلية العلوم

اسم الطالبة : هاله محمد سيد متولى

عنوان الرسالة : دراسات معملية على تأثير بعض متشابهات هرمون الشباب الحشرى على الحلم العنكبوتي الأحمر ذو البقعتين تتيرانيكس يورتيكا وعلى الآكاروس المفترس المصاحب له أمبليسيس سويرسكياي

الدرجة : الماجستير في علم الحشرات

لجنة الإشراف

1- أ.د/ محمد طلعت الإبراشي أستاذ متفرغ فسيولوجيا الحشرات المركز القومي للبحوث

2- أ.د/ جيهان عبد العليم حافظ أستاذ علم الحشرات بكلية العلوم جامعة عين شمس

3- أ.د/ ماهر عبد القادر البرلسى أستاذ علم الآكاروس المركز القومي للبحوث

ACKNOWLEDGEMENTS

With a warm feeling of entire complacence and a sigh of repose, I find myself at the moment in a situation to thank all those who have contributed in one way or another to make this study possible.

Gratefully, the present research was undertaken at the Department of Plant Protection, National Research Centre of Egypt (NRC), under the inspirational and pertinacious supervision of Prof. Dr. Mohd. Talaat El-Ibrashy, Professor Emeritus of Insect Physiology of NRC, who suggested the study, planning out the whole experimentation with its systematic profile down to the last detail. Veraciously, I should admit that he patiently guided me past many pitfalls throughout the whole work, otherwise, I would have floundered. I am further, deeply indebted to him for his constructive discussion, stimulating criticism, critical reading, correction of the manuscript, and for fitting me tenaciously with outpouring of literature supply. His keen interest and the ample time he freely devoted to the various aspects of the study should be greatly acknowledged and never be forgotten. Special appreciation I should like to express for developing some statistical formulations quite appropriate for the comparative resolution and data processing, the implementation of which was also exerted by himself personally.

My adequate thanks are impossible to **Prof. Dr. Jehane Abdel-Aleem Hafez**, Department of Entomology, Ain-Shams University, for her overwhelming enthusiasm, credible comments and helpful suggestions and to whom I owe profound gratitude for official sponsorship and kind consolidation of the study.

Hearty thanks are due to **Prof. Dr. Maher A. El-Borolossy**, Professor of Acarology (NRC), for unfailing help and sagacious advice he essentially gave throughout the predatory mite experimentation.

Illimitable assistance and good working relationships which my authorities and colleagues at NRC have gracefully instituted towards me will remain evocative, and I whish to thank, in particular, **Drs. Wafaa L.** Abdou, El ham Abdel-Hakim and M. A. Gisraha.

Capable technical assistance and vigilant care given to mite laboratory cultures by the late Mr. Sayed Hussam El-Din should deserve a token of my respect and admiration.

LIST OF ABBREVIATIONS

AST	allatostatin
ATT	allatotropin
CA	corpus allatum gland/corpora allata glands
CC	corpus cardiacum gland/corpora cardiaca glands
DN	deutonymph
DNq	deutochrysalis
20E	20-hydroxyecdysone
FA	farnesoic acid
IGR	insect growth regulator
IPM	integrated pest management
JH	juvenile hormone
JHA	juvenile hormone analogue
JHB ₃	bis-epoxide form of JH III
MF	methyl farnesoate
MO	crustacean mandibular organ
PG	prothoracic gland
PIM	post-imaginal moult
PN	protonymph
PNq	protochrysalis
PTTH	prothoracicotropic hormone
RCO	retrocerebral organ complex
XO/SG	crustacean x-organ/sinus gland complex

Abstract

In the present study – based on immature feeding of *T. urticae* on a total of 12 potent juvenoids having different functional moieties – a detailed account is given for the first time, suggesting the putative occurrence of endogenous juvenile-and moulting-like substances in this mite species and perhaps in acarines as a whole. In the light of this well-documented account, a hypothesised model is advanced and discussed in which all three mite pre-imaginal developmental stages might rather assume various endocrinological events equivalent to those occurring all along the metamorphic last stadium of an Insect. Virtually identical to insects, the treated mites exhibited several detrimental consequences, the most common of which was the juvenoid induction of lethal disturbances in metamorphosis as signalled by morphogenetic aberrations of mouthparts (chelicerae and pedipalpi) and /or legs, leading seriously to ecdysial failures and eventual death. Sporadically, some immature intermediate creatures were formed early at the larval/protonymphal moult. In addition, several other developmental and biological phenomena were recorded in corroboration of the possible occurrence in mites of the putative JH-like substance to be functioning – analogous to the insect JH in Insecta – as the pleiotropic master regulator. Most prominent and amazing among these phenomena was the induction in some determined juvenoid treatments of: a drastic prolongation of post-embryonic development duration, culminating in unconscionable deutochrysalis phase (a diapause-like state, effectuated by a "fail-safe mechanism"); a great deal of sterile and ephemeral "female superimagoes" (a "status quo" action); a precocious metamorphosis, resulting in eclosion of a number of impotent and short-lived males (cases of "progeria"); all eclosing males being sterile either physiologically or physically, thus resulting only in male progenies instituted by the arrhenotokous parthenogenetic mode of mite reproduction; eclosing males, showing vigorous sexual excitability even in complete absence of eclosing female congeners (cases of a "male pheromonal implication"); normally looking females, depositing even though unhatched or partially hatched eggs (cases of a "latent anti-gonadotropic effect"); a sex-reversal phenomenon in both sexes, provided that the treated mites should a priori be monoecious by nature or disposed by accident. In view of the findings obtained, together with the harmless nature of all the juvenoids assayed versus biological qualities and predation efficiency of the predaceous mite, A. swirskii, it will become attractive to apply them as highly selective and compatible compounds with the foreseeable "holistic" IPM programmes.

Keywords: *Tetranychus urticae*, *Amblyseius swirskii*, juvenoids, morphogenetic aberrations, fail-safe mechanism, superimago, parthenogenesis, *progeria*, sex-reversal, antigonadotropic action, *status quo* action.

CONTENTS

	Page
Acknowledgements	
Abstract	
1. INTRODUCTION	
2. REVIEW OF LITERATURE	
2.1.Neuroendocrine regulation of post- embryonic development and reproduction in Arthropoda: historic perspective and general consideration	6
2.2.Neuroendocrine regulation of moulting and metamorphosis in Mandibulata–Insecta and Crustacea: a comparison	15
2.3.Neuroendocrine regulation of reproduction in Mandibulata–Insecta and Crustacea: a comparison	26
2.4.Neuroendocrine regulation of moulting, metamorphosis, and reproduction in Chelicerata as represented by Acarina (ticks and mites)	31
2.5.Economic and medical importances of Acarina– ticks and mites	37
2.6.Potential use of insect growth regulators as viable alternatives to neurotoxic pesticides in integrated pest management (IPM) programmes	40

3. MATERIALS AND METHODS

3.1.Rearing colonies and caring of test organisms	4
3.1.1.The Two-spotted spider mite, <i>Tetranychus</i>	
urticae Koch (Acarina: Tetranychidae)	4
3.1.2. Amblyseius swirskii Athias-Henriot (Acarina: Phytosiidae)	4
3.2.Test juvenoids	
3.3.Experimental setting and juvenoid assessment: general test procedure	4
3.4.Relative susceptibility to juvenoid treatments of mite pre–imaginal mobile developmental stages: parameters and evaluations	4
3.5. Data processing and statistical analyses	
4. EXPERIMENTAL RESULTS	
4.1.Effects of immature feeding on various juvenoids on post-embryonic developmental rate and reproduction in T. urticae adults with particular reference to females	
4.1.1.Effects due to juvenoid feeding to larvae	
4.1.2.Effects due to juvenoid feeding to protonymphs	
4.1.3.Effects due to juvenoid feeding to deutonymphs	

4.3.Biological qualities and predation efficiency of A. swirskii in response to juvenoid dipping of post-embryonic immatures into an aqueous acetone solution of CGA-28772	113
5. GENERAL DISCUSSION	
5.1.Post-embryonic development of <i>T. urticae</i> as influenced by juvenoid feeding to immatures	120
5.2.Reproductive biology of <i>T. urticae</i> as influenced by juvenoid feeding to immatures	139
5.3. Putative hormones involved in the endocrine regulation of post-embryonic development and reproduction in <i>T. urticae</i> : a tentative profile	148
5.4.Prospective use in cropping systems of juvenoids as a potential chemical tool in soft/holistic IPM strategy: feasibilities for	1.50
T. urticae and an overview	152
6. SUMMARY AND CONCLUSIONS	
7. REFERENCES	168
ARABIC SUMMARY	

1. INTRODUCTION

In arthropod endocrinology, research has long been concentrated on endocrine regulation of development and reproduction mainly in the two classes of the Insecta and Crustacea (for comparative reviews and contained references, consult e.g. Downer and Laufer, 1983,1988; Watson et al., 1989; Gupta, 1990; Koolman, 1990; Smith and Sedlmeier, 1990; Cusson et al., 1991a; Chang, 1993; Roe et al., 1993; Gilbert et al., 2004). On the other hand, in specifically class Arachnida and the in acarine endocrinology, despite occurrence of an ample literature pertaining mainly to the sub-order Ixodida (= ticks) there is of knowledge intermingled with insubstantial data remaining to be worked out (see e.g. reviews by Obenchain and Galun, 1982; Griffiths and Bowman, 1984; Sauer and Hair, 1986; Gupta, 1987; Engelmann, 1990; Sonenshine, 1991; Oliver and Dotson, 1993; Adiyodi and Adiyodi, 1997; Lomas et al. 1997; Connat, 2000; Neese et al., 2000; Chang and Kaufman, 2004). In the sub-order Actinedida, almost nothing of considerable importance has so far been materialised with regard to the mite endocrinology (for reviews and contained references, see e.g. Rodriguez, 1979; Evans, 1992; Chambers et al., 1996). But, from an evolutionary comparative standpoint, ectodermal glands that might secrete (by analogy with insects) an endogenous juvenile and moulting hormone-like substances might well be suggested to generally exist within the arachnid prosoma, and the acarines are not exceptions (Jenkin, 1970; Manton and Anderson, 1979; Rodriguez, 1979; Weygoldt, 1986).

Most members of actinedid mites are of agricultural and/or medical concern (see reviews by Yunker, 1973; Jeppson et al., 1975; Helle and Sabelis, 1985; Lane and Crosskey, 1993) of which the spider mites constitute a large

family (Tetranychidae) many species of which are of paramount economic importance. Being of a phytophagous habit, they are generally found throughout those parts of the world where higher plants flourish and occur, in effect, on virtually every major food crop and ornamental plants, thus causing serious injury and in some cases the entire death of the host (reviews, op. cit.).

The two-spotted spider mite, Tetranychus urticae Koch (Acarina: Tetranychidae), is a cosmopolitan pest attacking and causing severe damages of economic importance not only to a great variety of field and orchard crops but to greenhouse ornamentals and vegetables as well (Albajes et al., 1999; Lentern, 2000). In Egypt, as in several other cotton-exporting countries, T. urticae forms in addition one of the major components of any cotton arthropod pest complex, whereby creating with other accompanying pest components an established niche in any cotton biocenosis (Pimentel. 1981: Perkins. Matthews and Tunstall, 1994; Metcalf and Luckmann, 1994; Dent, 1995). This awkward situation is not at all surprising if one knows that this mite pest, like many other species of the spider mites – due to their very short life cycles and their inherent ability to rapidly build up an awful number of generations of high-density populations in one season – has already gained a cross-resistance to a wide range of conventional pesticides which, in fact, are still in current use today (see e.g. Otto and Weber, 1992; Ishaaya, 2001). The over-use of such non-selective pesticides has brought about an enormous destruction of many beneficials including natural enemies such as the predaceous mites and insects as well as resulted in many other undesirable sideeffects, the most prominent amongst which are the deterioration of the environment and the biosphere in the greatest part along with multiple hazards to human health and wealth.

All these drawbacks have imposed a global bounden duty to overmaster, or at least to mitigate, such an awkward situation. As a result, the "integrated pest management" (IPM) concept has been thought to be worked out and implemented with no delay, instead of the unilateral use of any means of pest combat and thus more research into non-chemical methods of crop and food production could play the leading rôle. Indeed, biological measures have been looked ahead as the most attractive to be justified as potential forms of pest control that could have only few environmental disadvantages (see e.g. Waage and Greathead, 1986; Lentern, 1990; Mackauer et al., 1990; Hedin, 1991; Jervis and Kidd, 1994; Driesche and Bellows, 1996; Gurr and Wratten, 2000).

While many components of IPM programmes have been implemented for various crops in a considerable number of highly industrialised nations, the main intervention for the management of key pests continues, unfortunately, to be of a classical pesticide nature, though (Boller et al., 1998; Jegger, 2000; Urech, 2000; Schneider et al., 2004).

Rather earlier, El-Ibrashy (1987,1988a,b), in his comprehensive reviews of this subject-matter, has discussed the philosophy and paragmatism of what he termed the "holistic" IPM strategy, emphasising meanwhile the importance of including chemical pesticides as essential and even key components if successful programmes are to be pragmatically devised in the context of sustainable agriculture, provided that these chemicals should prove "selective" enough to impose no or negligible negative impacts on non-target organisms. In 1990, El-Ibrashy extended his arguments to propose the use of what are currently known as the insect growth regulators (IGRs) (see e.g. Retnakaran et al., 1985; Bowers, 1990; Ishaaya and Degheele, 1998), being preferred as the most appropriate

candidates and the soft alternatives to the harmful "neurotoxic" (conventional) pesticides in the prospective IPM programmes.

In fact, El-Ibrashy and his co-workers (El-Ibrashy and Mansour, 1970; Farrag, 1983; El-Ibrashy and Aref, 1985; El-Ibrashy et al., 1986; Abdou, 1992; Abdel-Hakim, 1996; El-Ibrashy et al., 1996; El-Ibrashy, 1997; El-Ibrashy al.,2001) have perseveringly undertaken a wide laboratory screening programme on the structure-activity relationship of the IGRs particularly those with juvenile hormone (JH) activity. Initially in 1974 these IGRs were termed "juvenoids" by Slàma et al. and since then have been known also as JH analogues or JH mimics (for reviews and contained references see e.g. Staal, 1975; EL-Ibrashy, 1982, 1984; Sehnal, 1983; Retnakaran et al., 1985; Ishaaya and Degheele, 1998). In this context, it should be further stressed that the "aromatic" juvenoids – particularly those containing a phenoxy or pyridyloxy functionality in place of the methyl ester moiety of the JH molecules as well as those in which phenyl rings interbridged with oxygen atoms substitute for two or all the three isoprene units – have proved comparatively to be the highly efficacious forms to come to the fore (El-Ibrashy and Aref, 1985; El-Ibrashy, 1987). These aromatic juvenoids, apart from having intrinsically a potent JH activity against several pest species, are technically readily accessible for chemical production and reasonably stable under a wide range of practical field situations (op.cit.)

The aim of the present study

In the present study, a detailed account is given of an investigation in which a whole selected dozen of such aromatic juvenoids (inclusive, only one of a cyclic chemical nature) were tested against a laboratory strain of *T. urticae*, having been raised for several years on mulberry leaves

maintained under constant conditions of 25±2°C, 65±5% RH and continuous fluorescent illumination.

As evident, this mite species was chosen specifically from both basic and applied perspectives, aiming at eliciting primarily whether there exists an organ equivalent to the corpus allatum gland (CA) of insects able to secrete a JHlike secretion that may determine the time of occurrence of metamorphosis in mites generally and perhaps in the class Arachnida as a whole. To substantiate the feasibility of developing a paragmatic IPM plan essentially for *T. urticae* in which a test aromatic juvenoid will prove higly rewarding so as to be considered the key chemical element, an attempt was consequently made to assay the selectivity of such a rewarding juvenoid versus the predaceous mite, Amblyseius swirskii Athias-Henriot (Acarina: Phytoseiidae), a common predator associating the spider mite, T. urticae, populations whenever occurred both in orchards and agroecosystems and in greenhouses alike.