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Influence of neem kernel extract on morphogenesis and vitellogenic oocyte development in *Trogoderma granarium* everts

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Abstract. Neem seed kernel extracts adversely affect the growth and morphogenesis of insects. In *Trogoderma granarium*, neem seed kernel extract was found to inhibit normal pupal-adult development. The malformed adults comprised a heterogenous group of transitory forms between the pupal and adult types. Older pupae were less sensitive to the compound. In the seemingly normal surviving adults of *Trogoderma granarium* a reduction in the number of vitellogenic oocytes was found. The active compound(s) in the neem seed kernel extracts is proposed to produce the observed effects by regulating morphogenetic hormone titres, though the precise mode of action cannot be safely defined.

Keywords. *Trogoderma granarium*; neem seed extract; morphogenesis; vitellogenic oocytes; juvenile hormone.

1. Introduction

The active principles in neem have been identified to be tetranortriterpenoids (Krause and Adami 1984). These adversely affect the growth and morphogenesis of insects (McMillian *et al* 1969; Steets 1975; Meisner *et al* 1976, 1978; Saxena *et al* 1981; Schmutterer 1985). In neem seeds, azadirachtin has been found in the highest concentration and was therefore considered to serve as a substance representative of the biological activity (Schneider and Ermel 1987). However, Schmutterer and Zebitz (1984) indicated that other compounds also exert synergistic or antagonistic effects on insects.

Comparatively little information is available on the influence of neem seed kernel extracts (NKE) on the development of vitellogenic oocytes in insects. Schulz and Schluter (1984) revealed the histopathological effects of NKE on egg maturation in *Epilachna varivestis*. They had reported degenerative changes in the oocytes and disturbances in yolk deposition during vitellogenesis. Related to these degenerative changes, significant disruptive changes in the corpus allatum and alterations in the corpus cardiacum and neurosecretory cells in the pars intercerebralis are established.

The growth disruptive action of methanolic NKE was studied in a stored grain pest, *Trogoderma granarium*. The influence of NKE on the development of vitellogenic oocytes was also examined. In *T. granarium*, the development of the oocytes occurs during the late pupal period and the adults emerge with full complement of eggs. The adults are short lived and do not feed. *T. granarium* is therefore an ideal insect to study the influence of NKE on oocyte development. Oocyte development of seemingly normal adults produced after NKE treatment is reported here.

2. Materials and methods

T. granarium (Coleoptera; Dermestidae) was maintained on crushed wheat at

35 ± 1°C. Fifth instar larvae were collected and maintained separately for pupation. Pre-pupae roll slightly and lie on their back, motionless for a few hours. Afterwards their body becomes stretched and a split of the larval cuticle appears on the dorsal side. This marked the initiation of pupation and such pupae were taken as 0 h old.

Neem kernel was extracted with methanol repeatedly and the solvent evaporated off. Known quantity of the residue was dissolved in methanol and topically applied (1 μl/pupa) to the pupae (10/test/concentration/replicates) with a Hamilton microsyringe. Four concentrations—10, 20, 50 and 100 μg per μl per pupa were applied on 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90 and 96 h old pupae. Methanol treated (1 μl/pupa) pupae served as controls. After treatment, pupae were maintained for adult emergence. At the end of the normal pupal period, the individuals were examined for any abnormalities and a record of normal and abnormal adults was kept.

In order to study the vitellogenic oocyte development, ovaries were dissected from seemingly normal adults within 24 h of emergence and examined under the microscope. The number of vitellogenic oocytes in each female was determined. As small adults produce lesser number of eggs (Karnavar 1972), pupae of the same size and weight served as experimental and control (20 insects/batch; 17 batches were used). The transformed (square root) data of the vitellogenic oocyte number were analyzed using an analysis of variance (ANOVA), followed by multiple comparison procedures using the least significant difference (LSD) procedure (Ott 1984). The data were transformed in order to fulfill the assumption of normally distributed observations for statistical tests.

3. Results

3.1 *Influence on morphogenesis*

The per cent of abnormal adults obtained is presented in figure 1. The malformed adults comprised a heterogenous group of transitory forms between the pupal and adult types. Based on the extent of malformations, the individuals were differentiated into 6 grades (Karnavar 1973a). In grade-I forms the morphogenetic aberrations were observed to be maximum. Grade-II individuals showed morphogenetic development slightly advanced compared to the grade-I forms. The fore and hind

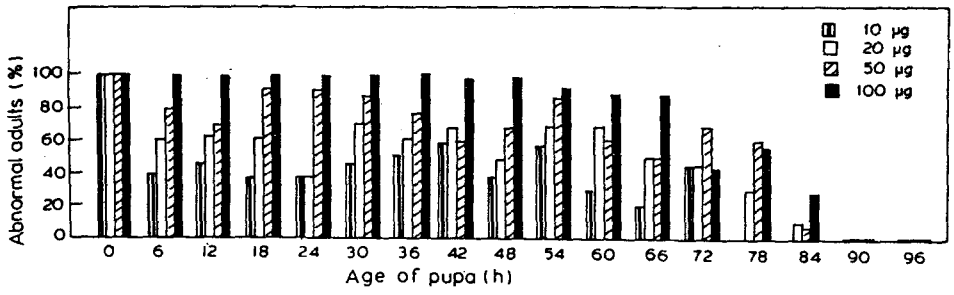

Figure 1. Per cent of abnormal adults derived from whole neem extract treated *T. granarium* pupae.

wings were slightly curled away from the body. The hind wing buds were observed to be swollen and filled with a fluid. In grade-III forms, tanning was observed on the entire anterior ventral and on the head region. The development of the wings and the legs was not complete. Grade-IV individuals showed morphogenetic development very much advanced. The wings were not fully formed. The legs were well developed. The adults were capable of independent movement. In grade-V individuals, adult characteristics were predominant. Grade-VI individuals were normal-looking adults with wings imperfectly developed. The adult-like forms were able to move about and mate. Pupae in the later stages of development i.e. 84, 90 and 96 h old are relatively insensitive to the NKE treatment. In all 4 dosages, 70–100% morphologically normal adults were obtained from 84 h pupa, whereas in 90 and 96 h pupa, adult development was 100%. All the control pupae developed normally.

3.2 *Influence on vitellogenic oocyte development*

Table 1 shows the number of vitellogenic oocytes in treated and control insects.

The ANOVA of the vitellogenic oocyte number (table 2) show that statistically significant ($\alpha \leqslant 0{\cdot}05$) differences exist among the factors age (17 levels; 0 through 96 h) and treatment (2 levels; control and experimental). The effect due to the different concentrations (10, 20, 50 and 100 μg) was not statistically significant indicating that the concentration does not affect the growth of eggs. All the two-way interactions between the factors (age × treatment, age × concentration, and treatment × concentration) were significant. However, the 3-way interaction was not significant.

4. Discussion

In *T. granarium*, NKE was found to inhibit normal pupal-adult development. The resulting adultoids were of several types, showing preservation of pupal features to different extent. Older pupae were found to be less responsive. Distinct morphogenetic disorders, following treatment with neem seed extracts have also been reported in *Manduca sexta* (Haasler 1984) and *Antestiopsis orbitalis bechuana* (Leuschner 1972). The insect growth regulating effects of neem compounds have been explained to result from disturbances in the endocrine system. In the present studies with NKE, the characteristics of pupal-adult intermediates obtained resembled those obtained from *T. granarium* following application of juvenile hormone analogues (Karnavar 1973a). Maintenance of a high juvenile hormone titer during metamorphosis was interpreted to cause the disruption of development in neem seed extract-treated *M. sexta* fifth instar larva (Haasler 1984). Juvenilizing effect of neem kernel suspension was also reported in *Chilo partellus* and *Heliothis armigera* larvae (Jotwani and Srivastava 1984). In *Aedes aegypti* also the impairment of pupal and adult development was related to changes induced by synthetic juvenoids such as altosid in the insect (Zebitz 1984). In *T. granarium* also, a disturbance in the hormone titers could be generalized to result from NKE application.

In the seemingly normal surviving adults of *T. granarium*, a reduction in the number of vitellogenic oocytes was observed. A dose-related effect was not found,

Table 1. The effect of whole neem extract on the vitellogenic oocyte number in Trogoderma.

Age of pupa (h)	Dose of whole neem extract (μg/pupa)							
	10 μg		20 μg		50 μg		100 μg	
	Number of vitellogenic oocytes per female (mean ± SD)							
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
0	60·0 ± 12·0	61·0 ± 11·0	64·0 ± 16·18	68·5 ± 16·17	54·85 ± 12·60	68·57 ± 5·42	49·0 ± 13·70	63·75 ± 6·04
6	68·75 ± 13·98	75·5 ± 5·07	63·77 ± 11·33	71·11 ± 4·63	57·77 ± 21·74	74·29 ± 4·54	42·57 ± 16·48	73·71 ± 4·59
12	62·5 ± 11·52	68·4 ± 8·71	65·33 ± 12·86	71·56 ± 11·49	67·5 ± 15·63	72 ± 11·56	49·56 ± 15·96	64·44 ± 7·60
18	58·44 ± 12·99	68·44 ± 9·18	45·25 ± 11·70	70·0 ± 8·72	45·33 ± 14·94	70·67 ± 7·83	53·0 ± 23·17	75·0 ± 5·19
24	57·11 ± 13·37	65·78 ± 9·21	60·8 ± 11·39	66·2 ± 5·83	63·0 ± 5·20	67·5 ± 3·27	76·0 ± 4·0	78·0 ± 6·0
30	64·5 ± 12·68	72·0 ± 84·9	57·14 ± 14·61	67·14 ± 5·64	61·25 ± 12·37	63·75 ± 10·93	47·6 ± 15·81	74·4 ± 4·8
36	52·67 ± 16·22	64·44 ± 8·93	57·25 ± 13·15	66·0 ± 6·08	66·0 ± 6·0	67·0 ± 5·0	53·2 ± 18·14	65·6 ± 9·24
42	44·0 ± 5·29	63·0 ± 7·21	42·67 ± 8·84	59·67 ± 7·52	50·0 ± 6·32	61·5 ± 6·38	38·0 ± 7·48	64·67 ± 5·24
48	63·8 ± 9·31	70·67 ± 7·94	67·2 ± 10·09	72·2 ± 6·66	51·56 ± 18·11	66·67 ± 7·72	57·5 ± 5·55	66·0 ± 9·27
54	55·56 ± 14·04	68·44 ± 9·46	48·22 ± 9·82	64·89 ± 6·40	60·0 ± 14·80	75·0 ± 5·20	44·29 ± 11·58	72·57 ± 6·57
60	69·0 ± 5·19	73·25 ± 2·63	67·33 ± 6·79	70·22 ± 4·93	55·71 ± 16·68	69·43 ± 6·21	45·71 ± 15·06	63·71 ± 7·89
66	56·67 ± 12·36	65·11 ± 5·74	53·0 ± 12·57	64·0 ± 5·48	54·0 ± 12·42	68·29 ± 8·31	62·0 ± 10	67·25 ± 7·28
72	61·42 ± 13·29	68·86 ± 7·77	57·11 ± 10·71	65·78 ± 5·37	58·0 ± 14·42	64·89 ± 11·97	71·25 ± 3·42	72·0 ± 3·16
78	63·2 ± 10·13	69·6 ± 6·92	58·4 ± 13·99	67·6 ± 8·85	50·25 ± 14·05	64·75 ± 7·87	63·6 ± 15·54	72·4 ± 9·24
84	69·0 ± 14·06	75·0 ± 6·71	66·2 ± 12·88	69·2 ± 9·6	64·6 ± 12·13	68·4 ± 5·49	64·4 ± 12·83	72·8 ± 5·88
90	59·0 ± 10·96	71·8 ± 11·58	55·4 ± 13·8	67·8 ± 5·25	63·2 ± 9·09	72·6 ± 12·71	67·2 ± 14·15	78·4 ± 7·63
96	62·4 ± 11·93	73·6 ± 7·79	63·0 ± 16·86	72·2 ± 7·29	58·0 ± 11·06	72·6 ± 5·89	63·2 ± 15·29	70·8 ± 8·4

Table 2. The three-factor ANOVA of the transformed oocyte number in *T. granarium* when crude neem kernel extract was used.

Source of variation	*df*	*ss*	*F*	*PR* > *F*
Age	16	52·6154	5·40	0·0001
Treatment	1	139·5381	243·26	0·0001
Concentration	3	3·1567	1·83	0·1392
Age × treatment	16	20·8765	2·27	0·0029
Age × concentration	48	60·0180	2·18	0·0001
Treatment × concentration	3	26·7013	0·97	0·5330
Age × treatment × concentration	48	26·7013	0·97	0·5330
Error	931	534·0363	—	—
Total	1067	841·6999	—	—

df, Degrees of freedom; *ss*, sum of squares; *F*, table value; *PR*, Probability.

showing that the concentration of neem extract does not influence the development of vitellogenic oocytes in this insect. However, significant differences exist among the factors, age of pupa and neem extract treatment (control and experimental). Comparatively few reports are available on the specific effects of NKE during the course of reproductive development. When fifth instar nymphs of *Dysdercus fasciatus* were treated with neem extracts, fecundity of the adults as well as the hatchability of the eggs were lowered, while topical treatment of third instar nymphs was shown to have no effect on the fecundity of resulting females (cf. Schmutterer 1984). Larvae of *C. partellus* that survived the neem kernel suspension treatment were reported to from abnormal adults, incapable of producing another generation (Jotwani and Srivastava 1984). Influence of various neem extracts on the development of the Egyptian cotton leaf worm *Spodoptera littoralis* larvae was studied by Ascher *et al* (1984) and strong fecundity-reducing effects in the females that survived the larval treatment was observed. In *Tetranychus cinnabarinus*, repellency and reduction of fecundity and also mortality of adults was observed when extracts were sprayed directly on adult female mites on bean leaf discs. Severe damage was noticed in the germarium and vitellarium zones of the ovarioles of *E. varivestis* females fed on NKE, in addition to effects on developing oocytes (Schulz and Schluter 1984). The effects were not found to depend on the dose of neem and a correlation between the time of treatment on one side and the physiological state of the individuals on the other was indicated. These reports show that the influence of NKE on the development of vitellogenic oocytes in *T. granarium* is not unusual. Schulz and Schluter (1984) investigated the inhibitory effects of neem on oocyte development in *E. varivestis* in some detail. They had found a decrease of protein concentration in the haemolymph and oocytes in the neem treated beetles. They also had described changes in the corpus allatum of 3 and 6 day old treated beetles which appeared to indicate lytic processes and alternations in the corpora cardiaca and the neurosecretory cells. In *T. granarium*, differences in oocyte and fat body protein concentration in females obtained from NKE treated pupa and controls was observed (S Chellayan and G K Karnavar, unpublished results). Oocyte protein composition was observed to be low in association with neem treatment while the fat body protein showed a higher level when compared to the controls. In most insects, precursors of the major yolk proteins are synthesized in the fat body, released into the haemolymph and

sequestered by the ovaries (Keeley 1978; Hagedorn and Kunkel 1979). Control of egg development is accomplished by hormones, primarily juvenile hormone and in certain species like *A. aegypti*, *Drosophila melanogaster*, ecdysteroids or both (Engelmann 1986). Concerning the results of the present experiments it may be assumed that the active compound(s) in the NKE produce the observed effects by regulating the titres of the morphogenetic hormones, though the precise action cannot be safely defined.

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