

Juvenillizing action of the desert locust *Schistocerca gregaria* egg pod extract

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ABSTRACT

The froth of *Schistocerca gregaria* egg pods was extracted in hexane, and tested against one day old 4th instar nymphs of the desert locust to study the juvenilizing action of the extract. To confirm the obtained results of egg pod extract, another group of 4th instar nymphs of *S. gregaria* were treated with different doses of the juvenile hormone analogue, fenoxycarb (2.5, 5.0 and 7.5µg / nymph). The two applications induced an increase in insect body weight, a prolongation in of the nymphal durations and high percentages of the adultoied insects from the emerged 5th instar nymphs. The most important juvenillizing feature is the adultoied insects which are characterized with adult wings, the pronotom and legs of the 5th instar nymphs. This feature is considered as an indicator of juvenilizing like effect of the egg pod extract.

Keywords: Juvenilizing action, egg pod extract, locust, *Schistocerca gregaria*, fenoxycarb

INTRODUCTION

Extracts and volatiles collected from the froth of egg pods of *Schistocerca gregaria* were attractive to its gravid adult females. Results with froth extracts obtained by sequential extraction with non-solvents of increasing polarity suggest that both non-polar and polar compounds are involved in the attraction of these females (Saini *et al.*, 1995). These compounds are found to influence the development of the embryos within eggs exposed to them soon after laying (McCaffery *et al.*, 1998). Moreover, El-gammal *et al.*, (2002) found that ethanol extracts of *S. gregaria* egg pod significantly reduced the numbers of produced egg pods, laid eggs, hatching percent and the reproductive potential of the treated solitary or gregary females of *S. gregaria*. Some trials were made to identify these effective compounds using GC- Ms analysis. The obtained results indicated the

presence of several volatile compounds such as eugonal, isoeugonal, benzaldehyde and phenol derivative (El-gammal *et al.*, 2003).

The juvenile hormone analogue, fenoxycarb was tested against 5th instar nymphs of *S. gregaria*. The dose of 15µg/g. body wt. produced green solitary colour, meanwhile, 8 µg / g body wt. induced 98 % adultiform (El-gammal *et al.*, 1989). Also, El-gammal *et al.*, (2004) suggested the dual effects of working as an RNA and protein synthesis inhibitor, cycloheximide. They stated that, the lowest doses of cycloheximide (10 and 20 µg / insect) produced solitary green colour in the resulting 5th instar nymphs, which is considered as an indicator for the high level of juvenile hormone in their haemolymph.

The present study is an attempt to detect a juvenile like effect of *S. gregaria* egg pod froth extract on their own 4th instar nymphs, compared to the juvenillizing action of the JH-analogue, fenoxycarb, as a new contribution to using a natural extract from some tissues of the same insect.

MATERIALS AND METHODS

Insects: The first instar nymphs of *Schistocerca gregaria* (Forsk.) were collected from the gregarious stock colony which had been maintained under the crowded conditions (Hunter-Jones, 1961) in Locust and Grasshoppers Department, Plant Protection Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt. These hoppers were Kept in wooden cages (30 x 30 x 30 cm) at 32 ± 2 °C and 65 ± 5 % R. H. The clover, *Sativium alexandrinum* leaves were daily provided as a feeding material.

Froth (Foam) Extraction: Froth from the gregarious egg masses was extracted in hexane by single-solvent extraction (El-gammal *et al.*, 2003). Froth was allowed to dry at ambient temperature (25 – 27 °C) for 6hrs. Six ml of hexane were added, and kept for 48 hrs before filtration.

Treatments: Five µl / nymph of the hexane froth extract, were topically applied to the neck inter-membrane of the one day old 4th instar nymphs. Three doses (2.5, 5.0 and 7.5 µg / nymph) of fenoxycarb [ethyl-2 (phenoxy) ethyl carbamate] (Ro13-5223), were topically applied to one day old 4th instar nymphs of the desert locust. These doses were dissolved in acetone. The applications of froth extract and fenoxycarb as a positive control were done using Hamilton microsyring (type 701-Nch). The control insects of

froth extract treatment received 5 µl hexane / nymph, while the control ones of fenoxycarb were treated with acetone.

Parameters studied: The treated and untreated control nymphs were kept under the crowded conditions in wooden cages which were incubated at 32±2 °c and 65 % R.H. The treated nymphs were daily weighed and examined for changes in nymphal colour according to the phase theory of Locusts (Uvarov, 1928). The durations of these nymphs were estimated by Dembester equation (1957). Meanwhile, the percentage of mortality, failure in ecdysis and changing to adultoids form were calculated.

RESULTS AND DISCUSSION

To justify the juvenillizing effects of hexane extract of *Schistocerca gregaria* egg pod froth, it was necessary to evaluate this action compared to fenoxycarb, a chemical with Juvenile hormone (JH) activity (Reeds *et al.*, 1985).

Table (1) shows that, the application of the three doses, 2.5, 5.0 and 7.5 µg / 4th instar nymph, induced considered percentages of mortality during the 4th instar. These percentages were 16.7, 33.3 and 25.0 for each dose, respectively. High percentages of 5th instar green colour nymphs were produced being 83.3, 66.7 and 75.0 % for each dose, respectively. This feature suggests the juvenillizing action of fenoxycarb in *S. gregaria* inducing the green colour of solitary phase (Fig.1)

Table (1): Morphogenetic effects of Juvenile hormone analogue, fenoxycarb on the last nymphal instars of *Schistocerca gregaria*

Doses µg / nymph	4 th nymphal instar		5 th nymphal instar	
	% Mortality	% perfect green 5 th instar	% Failur in last ecdysis	% adultoids * *
2.8 (24)	16.7 (4)	83.3 (20)	20.0 (4)	80.0 (16)
5.0 (24)	33.3 (8)	66.7 (16)	25.0 (4)	75.0 (12)
7.5 (24)	25.0 (6)	75.0 (18)	11.1 (2)	88.9 (16)
Control (24)	0.0	0.0	0.0	0.0

*The figures in paranthesis represent the numbers of insects for each indicated group.

** The adultiform (adultoids) characterized with adult wings and the pronotum and legs of 5th instar, these adultoids died 3 days after emmergence.



Fig. (1): The development of solitary green colour in the resulting 5th instar (left) from the treated 4th instar nymph (right) with fenoxycarb

Cassier *et al.*, (1976) stated that the presence of ecdysone and juvenile hormones together during the first two days of the 5th instar nymphs of *S. gregaria* favoured solitarious development.

Also, Table (1) indicates that, the produced green colour 5th instar nymphs failed to reach the adult stage. Some of them could not moult to the adult (Fig. 2-A), and the other moulted to adultiform insects (Fig. 2-B). The percentages of failure of the last ecdysis were 20.0, 25.0 and 11.1 % for 2.5, 5.0 and 7.5 μg / nymph, respectively. The produced adultiform in the

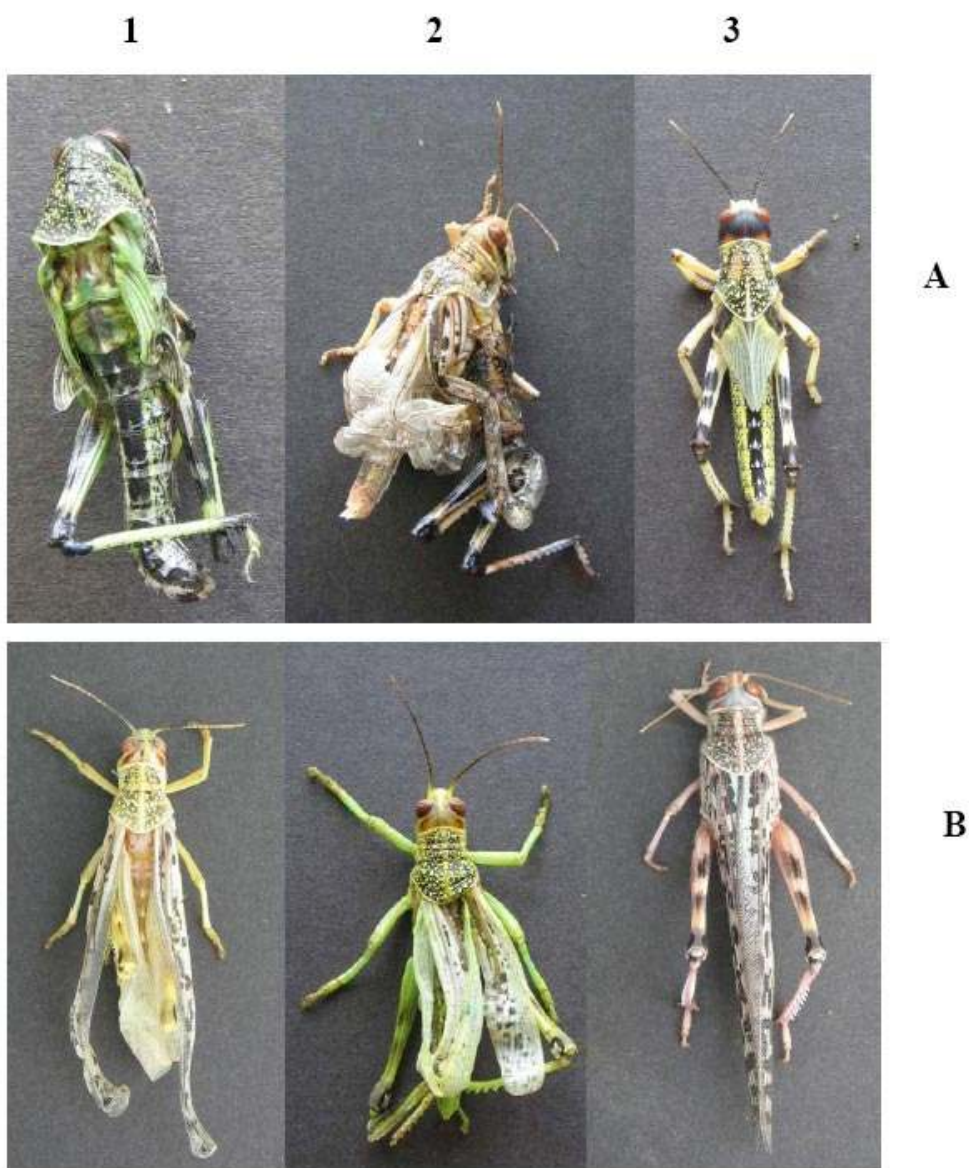


Fig. (2): A illustrates, the failure in ecdysis to the adult stage (A-1) after treatment with fenoxycarb, while (A-2) indicates the failure in ecdysis to the adult stage after treatment with hexane froth extract. B shows the produced adultoides (B-1) after treatment with fenoxycarb and the other adultoid (B-2) after treatment with hexane froth extract compared to the normal adult (B-3)

present study may be due to the presence of JH in the haemolymph of the treated insect. It is in agreement with Gilbert and King (1973) who stated that JH is a morphogenetic hormone in insects and the continuity of its presence assures that the developing insects maintain immature characteristics. That may explain why the adultiforms were produced with the adult wings and the pronotum and the legs of the 5th instar nymphs (Fig. 2-B).

Accordingly, the froth extract of *S. gregaria* egg pods induced juvenillizing effect in the treated 4th and 5th instar nymphs, especially producing adultiform with the same feature of those which produced after treatment with fenoxycarb (Fig. 2-B). So, Table (2) shows that the durations of the treated 4th instar nymphs with froth extract slightly higher than the control. The mortality percent was 33.3 % during the 4th instar. The duration of 5th nymphal instar was also prolonged, it was about 11.3 day compared to 8.3 for the untreated control. Twenty percent of the produced 5th instar failed in ecdysis to the adult stage. The other 5th instar nymphs moulted to adultiforms with the same features produced after fenoxycarb application (Fig. 2-B).

Table (2): Morphogenetic effect of *Schistocerca gregaria* froth extract on the last nymphal instars of this insect pest.

Treatment*	4 th nymphal instar			5 th nymphal instar			
	Duration (days)	Mortality %	Perfect 5 th % instar	Duration (days)	% Failure in ecdysis	% Adultoid	% Perfect adult
Froth extract (30)	8.3	33.9 (10)	66.7 (20)	11.3	20.0 (4)	80.0 (16)	0.00
Control (30)	7.6	0.0 (0.0)	100 (30)	8.3	0.0	0.0	100 (30)

* The figures in paranthesis represent the number of each indicated group.

The growth rates of the treated 4th instar nymphs and the produced 5th instar were evaluated. Fig. 2 illustrates that fenoxycarb increased the growth rate of both instars. The application of froth extract almost induced the same trend but less than fenoxycarb.

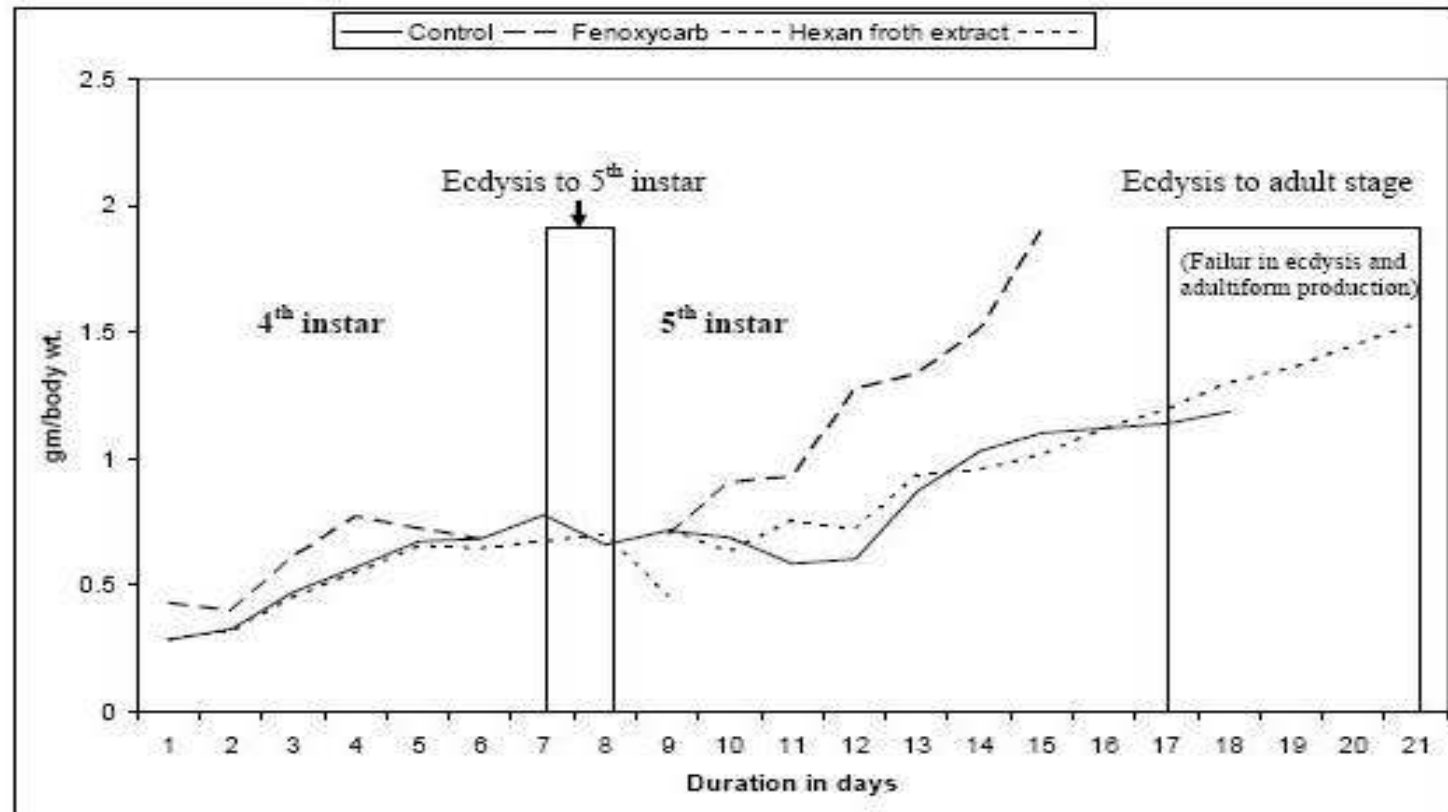


Fig. (3): Growth rate of the treated 4th and 5th instar female nymphs with hexane froth extract, fenoxycarb and the untreated control

Generally, the production of the adultiforms by fenoxycarb and froth extract suggested that froth extract has juvenile hormone like action on nymphal instars of 4th and 5th instar of *S. gregaria*. In this respect, El-gammal *et al.*, (2002) stated that the application of egg pod ethanol extract to the ovipositing sites of gravid adults of *S. gregaria* resulted in a disturbance in their harmolymph main metabolites associated with the reduction in their reproductive potential. While in the present study hexane froth extract of *S. gregaria* egg pod previously exhibited JH like action on the 5th instar nymphs, of *S. gregaria*. The mode of action of this natural extract from *S. gregaria* egg pods may be fenoxycarb like action as suggested by Ajami (1975) who found that carbamate derivative (fenoxycarb) inhibited hydrolytic decomposition of JH *Manduca sexta* pupae causing a high titre of this hormone at the end of the larval development. So, the failure in abolysis and the production of the adultiforms (Adultoids) by the application of hexane froth extract in this study can be explored by the findings of Cassier *et al.*, (1976) who stated that the necessary hormonal conditions for the differentiation of gland cells are established at the end of the 4th instar and during the first two days of the 5th instar of *S. gregaria*, at which time, the hypodermis is exclusively subjected to actions of ecdysis. This phenomena (Adultiforms) could be also, considered as an indicator of JH high level in the treated 4th and 5th instar nymphs with froth extract, as discribed by Gilbert and King (1973) who mentioned that JH is a morphogenetic hormone in insects and the continuinty of its presence assure that the developing insects maintain immature characteristics.

Thus, this study could be considered as new contribution to a novel research approach for controlling insects by natural extracts from their own.

REFERENCES

- Ajami, A. M. (1975). Inhibitors of ester hydrolysis as synergists for biological activity of *Cecropia* juvenile hormone. J. Insect Physiol., 21:1017-1025.
- Cassier, P. and C. Delmore-Joulie (1976). The imaginal differentiation of the integument in *Schistocerca gregaria* .2-Development during the imaginal moult and its determination in gregarious males. Ann. Sci. Nat. (Zool. Biol. Anim.), 18: 295-309.

- Dembester, J. P. (1957). The population dynamics of moroccan locusts *Dociostarus marcocanus* in cyprus. *Anti-Locust Bull.*
- El-gammal, A. M.; M. S. Zohny; G. Z. Taha and M. I. Abdel-Hamid (1989). The metabolic effect of the insect growth regulator, Fenoxycarbe on *Schistocerca gregaria* last nymphal instar. *Agric. Res. Review*: 67: 125-132.
- El-gammal, A. M.; M. E. Osman, M. T. Mohamed and H. A. El-Gawhary (2002). Experimental evidence for the suppressive effect of ethanol egg pod extract of *Schistocerca gregaria* (Forsk.) on their reproductive potential. 2nd International Conference, Plant Protection Res. Institute, Cairo, Egypt, 21-24, December, 2002.
- El-gammal, A. M.; M. T. Mohamed; M. E. Osman and H. A. El-Gawhary (2003). Effects of *Schistocerca gregaria* (Forsk.) egg pod extract in hexane on its reproductive process with special trials to identify the active compounds in this extract. *Egypt. J. Appl. Sci.*, 18 (3B) 631-641.
- El-gammal, A. M.; T. A. Abdel-Fattah; M. T. Mohamed and H. A. El-Gawhary (2004). The anti-juvenile hormone action of cycloheximide (RNA and protein synthesis inhibitor) in some nymphal instars of *Schistocerca gregaria* (Forsk.). *Egypt J. Agric. Res.*, 82 (4): 1561-1571.
- Gilbert, L. I. and D. S. King (1973). Physiology of growth and development. PP. 249-370. In: M. Pockstein (ed.) *The physiology of insects*. 1st, 2nd eds. Academic Press. London.
- Hunter-Jones, P. (1961). Rearing and breeding locusts in the laboratory. *Bull. Anti-locust Res. Centre, London*, 12 pp.
- Mc Caffery, A. R.; S. J. Simpson; S. Islam and M. S. Roessirgh (1998). Agregarizing factor present in the egg pod foam of the desert locust, *Schistocerca gregaria*. *J. Experimental Biol.*, 21: 347-363.
- Reed, R. H.; P. Alkima and L. H. M. Blommers (1985). The use of the IGR, fenoxycarb and epofenonane against leafrollers in integrated pest manement in apple archards. *Entomol. Exp. Appl.*, 39: 265-272.

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Saini, R. K.; M. M. Rai; A. Hassanali and J. Wawiye (1995).
Semiochemicals from froth of egg pods attract ovipositing female
Schistocerca gregaria. . Insect Physiol., 41: 711-716.

Uvarov, B. P. (1928). Locusts and grasshoppers, a handbook for their study
and control. Imp. Bur. Entomol., London, 352pp.