

Disturbed Survival, Growth and Development of the Desert Locust *Schistocerca gregaria* by Different Extracts of *Azadirachta indica* (Meliaceae) and *Nigella sativa* (Ranunculaceae).

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ABSTRACT

The desert locust *Schistocerca gregaria* is an economically dangerous pest invading several countries in North Africa and West Asia. The present work aims at assessing the effects of *Nigella sativa* extracts, compared to *Azadirachta indica* extract Neemazal, on survival and development of this pest. Treatment of the newly moulted penultimate instar or the newly moulted last instar nymphs with Neemazal leads to the decrease in gained somatic weight. It, also, exhibited an inhibitory effect on the development of penultimate instar nymphs and intervenes with the metamorphosis program because that some nymphal-adult intermediates were formed. After treatment of the newly moulted penultimate instar nymphs with *N. sativa* extracts, the lethal effects of both the methanolic and petroleum ether extracts were exerted early in the same treated nymphs but no mortality rats were detected among the treated last instar nymphs by methanolic extract. Treatments of the same nymphs with *N. sativa* extracts led to the decrease in the body weight gain. The methanolic or petroleum ether extract significantly inhibited the nymphal development. Treatment with n-butanolic extract resulted in disrupted metamorphosis because some nymphal-adult intermediates appeared proportionally to the concentration level. Treatments of the newly moulted last instar nymphs with *N. sativa* extracts deprived the nymphs to obtain normal somatic weight. Methanolic extract caused a slightly suppressed developmental rate. The n-butanolic extract exhibited a reverse action because significantly accelerated developmental rates, were recorded.

Keywords: *Schistocerca gregaria*, Neemazal, *Nigella sativa*, mortality, growth, development.

INTRODUCTION

Damage caused by the desert locust *Schistocerca gregaria* is a consequence of its polyphagous behaviour, high density of the population, and the nature to aggregate and swarm. Each individual gregarious locust is able to consume roughly its own weight (about 2 grams, or 0.7 ounces) in foliage daily (Lindsey, 2002). This partly explains how dense swarms of adults, or marching bands of hoppers, can inflict considerable economic harms during only a short time.

The use of chemical pesticides has been the main insect controlling approach during recent decades, but the widespread use of such chemicals has significant drawbacks, such as the development of strain resistance to insecticides (Garriga and Caballero, 2011), increased costs, handling hazards, concerns about insecticide

residues, and great threats to both human and environmental health. Many international institutions have intensified their research efforts in the search for integrated locust control methods. Much attention has been devoted to locust pathogens (Prior and Greathead, 1989) and the use of plant constituents that have an insecticidal effects "biocides" (Rembold and Mwangi, 1989; Schmutterer, 1990 a, b).

The botanical control agents are generally pest-specific and relatively harmless to non-target organisms including man. They are biodegradable and harmless to the environment (Rembold, 1994). Furthermore, unlike conventional synthetic insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus, the chances of pests developing resistance to such substances are less likely (Saxena, 1987). The neem tree (*Azadirachta indica* A. Juss), as for example, is known to has strong insecticidal properties (Schmutterer and Ascher, 1984; Jacobson, 1989). The most important active ingredient, azadirachtin (a tetranorterpene, $C_{35} H_{14} O_{16}$) is mostly concentrated in the seed kernels (Stoll, 1986). More than 200 insect species are reported to be controlled by the pesticides derived from the neem tree (Hamilton, 1992).

The present study was planned and conducted for investigating the possible effects of the *Nigella sativa* extracts on different survival, developmental and morphogenic of the dangerous orthopteran *S. gregaria* in comparison with the results obtained by the *Azadirachta indica* extract, Nemazal.

MATERIALS AND METHODS

Experimental Insect:

The desert locust *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae) was used as an experimental insect in the present study. The present culture was originated by a lot of gregarious nymphs obtained from Locust Research Division, Plant Protection Research Institute, Ministry of Agriculture, Doqqi, Giza.

Insects were reared in wooden cages furnished with a sandy layer of 20 cm depth and with 10-15% humidity to be suitable for egg laying. An electric bulb (100 watt) was adjusted to maintain a continuous photoperiod of 12 L: 12 D in each cage as well as in order to maintain an ambient temperature of $32\pm 2^{\circ}C$. The insects were reared and handled under the crowded conditions outlined by Hunter-Jones (1961). Fresh clean leaves of the berseem *Medicago sativa*, in winter, and the leaves of leguminous plant *Sesbania aegyptiaca*, in summer, were used as a food for insects. On the other hand, the berseem leaves only were offered as food for insects during the experimental work.

Plant Extracts:

Nemazal is a 20% azadirachtin extract from *Az. indica* A. Juss (Meliaceae). It was used in the present study as a comparable extract for different extracts from *N. sativa* (Ranunculaceae). Samples of *N. sativa* seeds were purchased from an Egyptian market. The samples were air-dried, powdered and kept in tightly closed amber coloured glass containers for protecting from light, at low temperature. Dried and pulverized powder of *N. sativa* (2 kg) was exhaustively separately extracted with methanol (1.7 Lx3). The combined alcohol extracts were concentrated to 400 ml, diluted with 400 ml of water and the next successively extracted with petroleum ether (5x400 ml) was concentrated to dryness under reduced pressure giving (11 and 90 g). The n-butanol (5x400 ml) extracts were concentrated to dryness under reduced pressure giving (75 and 55 g).

Nymphal treatments:

A series of Neemazal concentration levels (7.5, 3.7, 1.8, 0.9, 0.4 & 0.2%) was prepared. Different solvents were used for extracting *N. sativa*. A series of concentration levels from each of Methanolic extract, Petroleum ether extract and n-Butanol extract (30, 15, 7.5, 3.7 & 1.8%) was prepared.

The 0-day old 4th (newly moulted penultimate), 0-day old 5th (newly moulted last) instar nymphs of *S. gregaria* were fed on fresh leaves of *M. sativa* after dipping in different concentration levels of each extract. A day after treatment, all nymphs (treated and control) were provided with untreated food plant. Ten replicates (one individual of nymph per each) were used for each concentration. Each individual nymph was isolated in a glass vial provided with a thin layer of sterilized sand as a floor. All vials were kept in a large cage having a suitable electric bulb. After feeding for 24 hrs on the treated leaves, all physiological criteria were studied.

Criteria studied:

Survival potential of the treated nymphs, or their control congeners, is a reverse ratio of the mortality % in the meaning of more mortalities lesser survival potential. The insect growth is usually predicted by the weight gain. It was calculated as follows:

initial weight (before the beginning of experiment) - final weight (at the end of experiment).

The developmental rate of the nymphs can be expressed by the developmental duration. Developmental duration was calculated by the Dempster's equation (1957) and the developmental rate was calculated by Richard's equation (1957).

Nymphal-adult intermediates were observed and calculated in % as disturbed morphogenesis of the desert locust.

Statistical Analysis of Data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

RESULTS**A) Mortal Potency of the Plant Extracts Against Nymphs of *S. gregaria*.**

Five concentration levels of methanolic, petroleum ether or n-butanolic extract of each of the cultivated plant *Nigella sativa* (Ranunculaceae) as well as six concentration levels of the neem extract, Neemazal (*Azadirachta indica*, Meliaceae) were applied against the early penultimate (4th) or last (5th) instar gregarious nymphs of *Schistocerca gregaria*. After 24 h of treatment, all mortalities were observed for nymphs. Also, the nymphal mortality was recorded along each of the nymphal instars.

After treatment of penultimate instar nymphs with the highest concentration level, all insects died while 60.0% mortality was recorded at conc. level 3.7% of Neemazal. However, other mortalities were recorded in the resulted 5th instar nymphs (40.0 and 20.0 % mortalities at conc. levels 3.7 and 1.8 %) (Table 1). After treatment of last instar nymphs, all insects died at the highest conc. level while 70.0% mortalities was recorded at conc. levels 3.7 and 1.8% (Table 2).

Data of the mortal potency of the *N. sativa* extracts are arranged in Table (3). After treatment of penultimate instar nymphs, the lethal effects of both the methanolic and petroleum ether extracts were exerted early in the same treated nymphs but the lethal effects of n-butanolic extract lately appeared during the next nymphal instar.

Some different results of toxicity were obtained for the *N. sativa* extracts after treatment of the last instar nymphs (see Table 4). No mortality %s were observed among the treated last instar nymphs by the methanolic extract. According to the data, the lethal action of petroleum ether extract increased as the concentration level was increased (20 and 30 mortality %s at concentration levels 15 and 30 %). A similar dose-dependent mortality could not be observed after treatment of the last instar nymphs with n-butanolic extract which caused the maximal % (30%) at concentration level 7.5%.

B) Influenced Growth and Development of *S. gregaria* by the Plant Extracts:

1- Effects of Neemazal:

As summarized in Table (1), penultimate instar nymphs gained somatic weight less than that of control congeners (as for examples: 245.3±50.0 mg at concentration level 3.7% vs. 358.5±69.4 mg of control congeners). Also, the successfully moulted last instar nymphs were prohibited to gain normal somatic growth, i.e. the weight gain decreased, albeit insignificantly, by the action of Neemazal. Such decreasing weight gain was in a dose-dependent course.

Table 1: Survival, growth and developmental effects of the neem extract, Neemazal, on the desert locust *Schistocerca gregaria* after treatment of the early penultimate instar nymphs.

Conc. (%)	Nymphal instars								
	4 th instar nymphs				5 th instar nymphs				
	Mortality %	weight gain (Mean mg ± SD)	Duration (Mean days ± SD)	Develop. rate	Mortality %	weight gain (Mean mg ± SD)	Duration (Mean days ± SD)	Develop. rate	Nymphal-Adult Inter. (%)
7.5	100.0	-	-	-	---	-	-	-	-
3.7	60.0	245.3 ± 50.0 c	19.0 ± 2.8 d	05.3	40.0	---	---m	---	---
1.8	0.0	315.0 ± 64.4 a	17.7 ± 3.2 d	05.6	20.0	639.3 ± 112.5 a	15.8 ± 4.0 c	6.3	30.0
0.9	0.0	309.4 ± 70.1 a	13.2 ± 2.8 b	07.6	10.0	650.9 ± 110.1 a	15.9 ± 3.9 c	6.3	50.0
0.4	0.0	307.8 ± 53.7 a	15.5 ± 3.9 c	06.5	10.0	677.7 ± 140.2 a	12.7 ± 3.2 a	7.9	40.0
0.2	0.0	353.9 ± 51.2 a	11.9 ± 3.6 a	08.4	10.0	677.7 ± 133.1 a	14.1 ± 3.0 a	7.1	30.0
Controls	0.0	358.5 ± 69.4	09.6 ± 2.6	10.4	10.0	680.5 ± 135.8	12.3 ± 1.2	8.1	20.0

Conc.: See footnote of Table (1). Develop. rate: Developmental rate ; Inter.: intermediates ; Mean ± SD followed with the same letter (a): is not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001). m: all penultimate instar nymphs died.

Depending on the data of the same table, Neemazal exhibited an inhibitory effect on the development of both penultimate and last instar nymphs since the nymphal duration in penultimate instar was highly significantly prolonged (especially at the concentration level: 3.7, 1.8, 0.9 and 0.4%: 19.0±2.8, 17.7±3.2, 13.2±2.8 and 15.5±3.9 vs. 09.6±2.6 days days of control nymphs). A similar inhibitory effect of this neem extract on the development of last instar nymphs was calculated (especially at the concentration levels 1.8 and 0.9%: 15.8±4.0 and 15.9±3.9 days vs. 12.3±1.2 days of control nymphs). Another calculated parameter may substantiate this finding where the developmental rate of penultimate instar nymphs was conspicuously regressed by the action of Neemazal parallelly to the concentration level. On the other hand, less suppressing action of the extract could be detected in the last instar nymphs.

Also, the program of development and metamorphosis was disrupted because some nymphal-adult intermediate creatures appeared (Plate 1). However, no certain trend of such disrupted program was recorded.

Data arranged in Table (2), show a slight depressing effect of Neemazal on the weight gain. Also, Neemazal exhibited pronouncedly inhibitory effect on the developmental duration of nymphs nearly in a dose-dependent manner. The most detrimental effect (18.1 ± 2.1 vs. 12.9 ± 2.9 days of control congeners) was recorded at the concentration level 0.9%. Subsequently, the developmental rate decreased as the concentration level was increased.

Table 2: Survival, growth and developmental effects of the neem extract, Neemazal on the desert locust *Schistocerca gregaria* after treatment of early last instar nymphs.

Conc. (%)	5 th instar nymphs				
	Mortality %	weight gain (Mean mg \pm SD)	Duration (Mean days \pm SD)	Develop. rate	Nymphal-Adult Inter. (%)
7.5	100.0	-	-	-	-
3.7	70.0	649.5 ± 114.0 a	18.0 ± 2.0 d	5.6	20.0
1.8	70.0	600.0 ± 135.0 a	18.0 ± 2.2 d	5.6	10.0
0.9	10.0	644.1 ± 107.1 a	18.1 ± 2.1 d	5.5	10.0
0.4	10.0	634.1 ± 110.0 a	13.8 ± 2.7 a	7.2	00.0
0.2	10.0	662.2 ± 114.5 a	13.3 ± 2.2 a	7.5	00.0
Controls	10.0	674.0 ± 165.4	12.9 ± 2.9	7.8	00.0

Conc., Develop. rate, Inter., a, d: See the footnote of Table (1).

Neemazal, at these three concentration levels, intervened with the metamorphosis program since some nymphal-adult intermediates were formed (20, 10 and 10% at concentration levels 3.7, 1.8 and 0.9%, respectively) (Plate 1).

1) Effects of the *N. sativa* extracts:

Table (3) contains data of the most important growth and development parameters of *S. gregaria* after treatment of penultimate instar nymphs with the *N. sativa* extracts. With regard to the somatic growth of penultimate instar nymphs, depressed weight gain was generally recorded as an effect of all *N. sativa* extracts. The most dramatically depressed weight gain was observed at the highest concentration level of methanolic extract and n-butanolic extract (197.6 ± 76.3 vs. 417.6 ± 47.0 mg of control nymphs and 269.7 ± 50.2 vs. 361.3 ± 61.9 mg of control nymphs, respectively) but at concentration level 7.5% of petroleum ether extract (337.3 ± 51.6 vs. 417.6 ± 47.0 mg of control nymphs).

The data in the same table show a significant inhibitory effect on the developmental duration of penultimate instar nymphs, unexceptionally, by the methanolic extract (10.61.4, 10.7 ± 1.5 , 10.1 ± 1.3 and 10.1 ± 1.2 days at concentration levels 15.0, 7.5, 3.7 and 1.8 % of methanolic extract, compared with 8.3 ± 2.1 days of control nymphs) and petroleum ether extract (10.9 ± 1.9 , 10.9 ± 2.1 , 11.0 ± 1.5 and 10.9 ± 1.9 days at concentration levels 15.0, 7.5, 3.7 and 1.8 %, compared with 8.3 ± 2.1 days of control nymphs). The n-butanolic extract exhibited remarkably inhibitory effect or slightly enhancing one (11.1 ± 1.0 at concentration level 30.0 % vs. 9.2 ± 1.9 days of control nymphs) on such duration. An odd datum of the developmental duration after treatment with methanolic extract was 3.6 ± 1.2 days (in comparison with 8.3 ± 2.1 days of control nymphs) i.e., such extract enhanced the development at the highest concentration level. However, no certain trend could be observed for the enhancing effect of the methanolic extract and petroleum ether extract. Furthermore, the developmental duration was prolonged or shortened in no certain trend after treatment with n-butanolic extract.

Also, the growth and developmental criteria of the next instar nymphs were affected by these *N. sativa* extracts as clearly shown in the same table. Depending on the available data, the successfully moulted last instar nymphs appeared with depressed weight gain, irrespective of the *N. sativa* extract or its concentration level. However, the most drastic depressing effect was observed for only the two higher concentration levels of methanolic extract (518.1 ± 109.3 and 550.0 ± 117.5 mg at concentration levels 30.0 and 15.0%, respectively, vs. 688.9 ± 110.3 mg of control nymphs).

Table 3: Survival, growth and developmental effects of *Nigella sativa* extracts on the desert locust *Schistocerca gregaria* after treatment of the early penultimate instar nymphs.

Solvent	Conc. (%)	Nymphal instars								
		4 th instar nymphs				5 th instar nymphs				
		Mortality %	weight gain (Mean mg \pm SD)	Duration (Mean days \pm SD)	Develop. rate	Mortality %	weight gain (Mean mg \pm SD)	Duration (Mean days \pm SD)	Develop. rate	Nymphal-Adult Inter. (%)
Methanol	30.0	20.0	197.6 \pm 76.3 d	0 3.6 \pm 1.2 d	27.8	37.5	518.1 \pm 109.3 b	09.6 \pm 1.5 b	10.4	00.0
	15.0	30.0	303.6 \pm 43.7 d	10.6 \pm 1.4 b	09.4	14.3	550.0 \pm 117.5 b	10.1 \pm 1.3 a	09.9	00.0
	7.5	10.0	291.0 \pm 65.8 d	10.7 \pm 1.5 b	09.3	11.1	650.3 \pm 142.3 a	10.4 \pm 1.0 a	09.6	00.0
	3.7	20.0	340.3 \pm 59.1 c	10.1 \pm 1.3 b	09.9	12.5	678.6 \pm 130.9 a	11.5 \pm 0.9 a	08.7	00.0
	1.8	10.0	369.3 \pm 44.4 b	10.1 \pm 1.2 b	09.9	11.1	680.1 \pm 120.3 a	11.6 \pm 1.4 a	08.6	00.0
	Controls	10.0	417.6 \pm 47.0	08.3 \pm 2.1	12.0	11.1	688.9 \pm 110.3	11.9 \pm 1.8	08.4	00.0
Petroleum ether	30.0	80.0	414.0	12.0*	08.3	0.0	630.0	13.5	07.4	00.0
	15.0	30.0	358.7 \pm 53.8 b	10.9 \pm 1.9 b	09.2	14.2	673.2 \pm 112.5 a	11.0 \pm 0.6 a	09.1	00.0
	7.5	20.0	337.3 \pm 51.6 c	10.9 \pm 2.1 b	09.2	12.5	670.0 \pm 132.3 a	10.8 \pm 1.0 a	09.3	00.0
	3.7	20.0	417.4 \pm 42.5 a	11.0 \pm 1.5 c	09.1	12.5	678.6 \pm 127.2 a	11.0 \pm 1.0 a	09.1	00.0
	1.8	10.0	415.2 \pm 50.0 a	10.9 \pm 1.9 b	09.2	11.1	680.8 \pm 120.7 a	11.0 \pm 0.9 a	09.1	00.0
	Controls	10.0	417.6 \pm 47.0	8.3 \pm 2.1	12.0	11.1	688.9 \pm 110.3	11.9 \pm 1.8	08.4	00.0
n-butanol	30.0	50.0	269.7 \pm 50.2 b	11.1 \pm 1.0 b	09.0	40.0	609.9 \pm 123.2 a	0 7.3 \pm 1.5 d	13.7	60.0
	15.0	0.0	288.1 \pm 45.7 b	09.1 \pm 1.7 a	11.0	10.0	623.0 \pm 119.7 a	09.9 \pm 2.2 b	10.1	40.0
	7.5	0.0	283.4 \pm 53.4 b	09.2 \pm 0.4 a	10.9	20.0	644.2 \pm 110.5 a	09.6 \pm 3.1 b	10.4	40.0
	3.7	0.0	288.1 \pm 65.1 b	09.3 \pm 1.5 a	10.8	30.0	666.3 \pm 130.4 a	09.7 \pm 2.2 b	10.3	20.0
	1.8	0.0	311.0 \pm 72.7 a	09.0 \pm 1.8 a	11.1	20.0	685.4 \pm 114.2 a	09.3 \pm 2.8 b	10.8	10.0
	Controls	0.0	361.3 \pm 61.9	09.2 \pm 1.9	10.9	10.0	690.1 \pm 122.3	12.6 \pm 2.6	07.9	10.0

Conc., Develop. rate, Inter., a, b, c, d: See the footnote of Table (1). *: One nymph only could survive.

It is important to mention that the treatment with only n-butanolic extract resulted in disrupted metamorphosis because some nymphal-adult intermediates appeared proportionally to the concentration level (Plate 1).

Just a look at the data of Table (4) reveals some insignificant effects of *N. sativa* extracts on growth and development of *S. gregaria* after treatment of last instar nymphs. Also, these last instar nymphs were deprived to obtain normal somatic weight but their calculated weight gain slightly decreased, irrespective of the extract. For some details, treatment with methanolic extract resulted in a slight prolongation of the developmental duration and subsequently slightly depressed developmental rate. The n-butanolic extract exhibited a reverse action because insignificantly shortened durations, and subsequently slightly fastened developmental rates, were recorded.

In addition, no effect was observed on the metamorphosis program by methanolic or n-butanolic extract. Only the petroleum ether extract exerted a drastic effect on such program because some intermediates appeared (40% of nymphal-adult intermediates at 15.0%).

Table 4: Survival, growth and developmental effects of *Nigella sativa* extracts on the desert locust *Schistocerca gregaria* after treatment of the early last instar nymphs.

Solvent	Conc. (%)	5th instar nymphs				
		Mortality %	weight gain (mean mg \pm SD)	Duration (mean days \pm SD)	Develop. rate	Nymphal-Adult Inter. (%)
Methanol	30.0	0.0	699.6 \pm 110.0 a	11.6 \pm 1.6 a	8.6	00.0
	15.0	0.0	667.1 \pm 191.1 a	11.4 \pm 2.1 a	8.8	00.0
	07.5	0.0	707.3 \pm 142.6 a	11.5 \pm 1.9 a	8.7	00.0
	03.7	0.0	706.2 \pm 142.5 a	11.1 \pm 1.4 a	9.0	00.0
	01.8	0.0	712.0 \pm 137.5 a	11.0 \pm 1.1 a	9.1	00.0
	Controls	0.0	744.4 \pm 108.1	10.6 \pm 1.4	9.4	00.0
Petroleum ether	30.0	30.0	840.0 \pm 131.1 a	10.7 \pm 1.5 a	10.0	10.0
	15.0	20.0	848.0 \pm 096.8 a	11.1 \pm 1.0 a	09.0	40.0
	07.5	0.0	851.6 \pm 104.3 a	11.1 \pm 1.4 a	09.0	10.0
	03.7	0.0	853.2 \pm 093.3 a	11.0 \pm 0.7 a	09.1	10.0
	01.8	0.0	866.0 \pm 110.0 a	11.0 \pm 1.0 a	09.1	10.0
	Controls	0.0	924.5 \pm 097.7	11.1 \pm 1.3	09.0	00.0
n-butanol	30.0	10.0	685.4 \pm 193.4 a	7.5 \pm 1.5 c	13.3	0.0
	15.0	10.0	679.9 \pm 151.0 a	6.7 \pm 2.0 c	14.9	0.0
	07.5	30.0	664.5 \pm 131.6 a	6.2 \pm 1.4 c	16.1	0.0
	03.7	10.0	696.3 \pm 123.2 a	8.2 \pm 2.6 a	12.2	0.0
	01.8	10.0	690.3 \pm 153.1 a	8.9 \pm 2.1 a	11.2	0.0
	Controls	10.0	706.3 \pm 126.5	9.6 \pm 1.5	10.4	0.0

Conc., Develop. rate, Inter., a,c: See the footnote of Table (1).

DISCUSSION

1) Insecticidal Efficiency of the Plant Extracts against *S. gregaria*:

So many institutions have been engaged with the search for some environmentally safe control agents in order to avoid the disadvantages and hazards of the synthetic insecticides. A great part of efforts have been achieved for the investigation and re-examination of plant sources to obtain natural compounds which may have toxic, repellent, anti-feedant or anti-hormonal characteristics (Thomas and Callaghan, 1999). The most famous plant species for several decades ago is the neem tree *Azadirachta indica* from which hundreds of products and preparations were extracted and assessed against different insect pests for protecting the health and agronomical systems. As a contribution in this context, in the present study, *Nigella sativa* (Ranunculaceae) was extracted by methanol, petroleum ether and n-butanol for investigating its potential effects against the economically destructive desert locust *S. gregaria*. Also, Neemazal (with 20% azadirachtin content) was assessed as a comparative parameter for these extracts.

Despite of the plenty of literature about the bioactivity of the azadirachtin (Azt.) and other neem products and preparations against several insect species, it is sufficient

to look on the following few examples. High mortality rates of the brown plant hopper *Nilaparvata lugens* (Homoptera) were caused by higher concentrations of Azt. (Saxena and Khan, 1985). Some products of *Az. indica* exhibited a larvicidal activity in the horn fly *Haematobia irritans*, stable fly *Stomoxys calcitrans* and house fly *Musca domestica* (Miller and Chamberlain, 1989). The oils pressed from seeds of *Az. indica* achieved mortality rates of 65-100 % in *S. gregaria* (Schmutterer and Freres, 1990; Nicol and Schmutterer, 1991). On the other hand, no acute toxic effect of azadirachtin applied against the 5th instar larvae of *Spodoptera mauritia*, was observed (Jagannadh and Nair, 1992). Osman (1993) observed some different mortalities of *Pieris brassicae* after treatment of 1-day old 5th instar larvae with 5.0 and 2.5 % azadirachtin. Also, high mortality of *S. gregaria*, red locust *Nomadacris septemfasciata* and variegated grasshopper *Zonocerus variegates* was caused by the neem oil (Schmutterer *et al.*, 1993). The neem seed and kernel powder applied directly, or as aqueous extract, killed *Clavigralla tomentosicollis* (Mitchell *et al.*, 2004). Azadirachtin, also caused complete mortality of *N. lugens* nymphs at 1.0 ppm azt. (Senthil Nathan *et al.*, 2007). Mortality of *N. lugens* was associated mainly with failure to moult and the nymphs died after several days (Senthil Nathan *et al.*, 2007). Azadirachtin caused increasing mortality of *Spodoptera exigua* (Yoshida and Toscano, 1994), *Trialeurodes vaporariorum* (von Elling *et al.*, 2002) and *Cnaphalocrocis medinalis* (Senthil Nathan *et al.*, 2006 a). Recently, Azadirachtin exerted a lethal action increasingly with the age of pupae after treatment of the prepupae of *Rhynchophorus ferrugineus* (Abdel-Ghaffar *et al.*, 2008). Also, a significant reduction of the larval and pupal survival of the blowfly *Chrysoma megacephala* and *M. domestica* after mixing the fresh beef (as a food) with a neem product (0.24 % Azt. content) (Siriwattananarungsee *et al.*, 2008).

Some other neem seed extracts and preparations were assessed against several insect pests, such as Margosan-O (0.2 % Azt. content) and Neemazal (20 % Azt. content). After treatment of larvae with Margosan-O, various degrees of mortality of *S. littoralis* (Meisner and Nemny, 1992), of *Earias insulana* (Meisner *et al.*, 1981), of *Ostrinia nubilalis* (Meisner *et al.*, 1991), of the European leafroller *Archips rosanus* (AliNiazee *et al.*, 1997), of the false stable fly *Muscina stabulans* was caused after continuous feeding of larvae on Margosan-O treated diet (Ghoneim and Al-Dali, 2002), of *M. domestica* (Amer *et al.*, 2004) were recorded. Referring to another famous neem seed extract, Neemazal, the lethal effects were reported for several insect pests, such as *S. littoralis* (Ghoneim *et al.*, 2000), *M. domestica* (Mohamed *et al.*, 2000), *Tribolium castaneum* (Athassiou *et al.*, 2005).

Outside the neem extracts, strong larvicidal activity was exhibited by the extracts from *Dysoxylum malabaricum* (Meliaceae) against *Anopheles stephensi* (Senthil Nathan *et al.*, 2006 b), by the Jojoba oil against *Rhynchophorus ferrugineus* (Abdel-Ghaffar *et al.*, 2008), by the extracts from *Centaurium erythraeae*, *Peganum harmala*, *Ajuga iva*, *Aristolochia baetica*, *Pteridium aquilinum* and *Raphanus raphanistrum* against *T. castaneum* (Jbilou *et al.*, 2008).

In the light of all these aforementioned results for the neem products against the survival of different insect pests, and as a contribution for searching alternative control agents to the desert locust *S. gregaria*, the lethal action of Neemazal was investigated in the present study. The newly moulted nymphs of the later instars (4th and 5th) of *S. gregaria* were treated (through the fresh food plant) with Neemazal (20 % Azt. content) for assessing its mortal potency because the toxicity of plant extracts depends on the physiological age of larvae (Adeyeye and Blum, 1989). After treatment of penultimate or last instar nymphs with the highest concentration level, all

insects died. However, the present results of Neemazal toxicity on *S. gregaria* agree, to a great extent, with other results on different insect species. The partial or complete mortality of *S. gregaria*, in the present study, may be attributed to the feeding inhibition which usually leads to continuous starvation and subsequently death (Ghoneim *et al.*, 2000). Specifically, the deaths of botanical-treated penultimate instar nymphs of *S. gregaria* in the present study may be due to the inability of the moulting bodies to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis (Mordue and Evans, 1987; Linton *et al.*, 1997). In addition, the deaths of last instar nymphs of *S. gregaria* may be due to a metamorphosis inhibiting effect of the plant extract, which is possibly based on the disturbance of the hormonal regulation (Al-Sharook *et al.*, 1991) because the prevention of the metamorphosing ecdysis, and subsequently death, could be attributed to the reduction in ecdysteroid peak or interference with the release of eclosion hormone (Sieber and Rembold, 1983).

The present results of toxicity caused by *N. sativa* come in an agreement with those results reported by several authorities using different plant species against various insect pests. Just a look on the following examples may be sufficient, Chiu *et al.* (1985) reported that at 400 ppm toosendanin (extract from *Melia toosendan*) caused 80 % mortality to 3rd instar and 580 ppm caused 75 % mortality to 5th instar *Pieris rapae*. Water based extracts from the fruits and leaves of *Melia azadirach* yielded mortality rates between 93 and 100 % in nymphs of *Locusta migratoria migratorioides* (Wen and Schmutterer, 1991). The aqueous extract of *Koelreuteria paniculata* (Laxm.) (0.15%) causing a significant increase in the larval mortality (68.3%) of *Anticarsia gemmatilis* (Huebner) (Martins *et al.*, 2012). Application of the palm oil on *S. gregaria* induced mortality rate that increased with dosage (Wilps *et al.*, 1993). Against the same acridid species, topical application of *Melia volkensii* extracts caused 32.5 % mortality in the laboratory-reared nymphs and 27.8 % mortality in the field-collected nymphs (Nasseh *et al.*, 1993). Feng *et al.* (1995) observed no mortality of *Spodoptera litura* larvae even at 600 or 3000 ppm toosendanin. Methanolic extracts from the leaves of *Chukrasia tabularis* var *velutina* and *Swietenia macrophylla* caused high mortality among the Chinese rice grasshopper *Oxya chinensis* (Xiao Dong *et al.*, 1997). Pascual-Villalobos and Robledo (1998) reported higher mortalities in larvae of *T. castaneum* by the extract from *Ajuga iva*. Topical application of leaf and whole plant extracts of *Ageratum conyzoides* (Asteraceae) to the penultimate instar nymphs of *S. gregaria* resulted in nymphal mortality (Sharda *et al.*, 2000). Crude ethanolic extracts of *A. iva* or *Ajuga pseudoiva* have insecticide activity against some Lepidoptera (Simmonds and Blaney, 1992 ; Benjannet *et al.*, 2001). Topical application of ethanolic extracts from *Cyprus rotendus* (Cyperaceae) to penultimate or last instar nymphs of *S. gregaria* caused increasing nymphal mortality parallelly to the dose level (El-Sokkary, 2003). However, the present plant extracts may contain certain ingredients affecting the homeostasis leading to increased body water loss and subsequently the death (Amer *et al.*, 2004). Because the plant species, *Peganum harmala*, as for example, is a rich source of β -carboline alkaloids which, and other secondary metabolites, may explain the toxic effects on *S. gregaria* (Kartal *et al.*, 2003). Further investigation should be conducted in future for exploring the secondary metabolites, alkaloids or other active components, in the extracts of the present tested plant *N. sativa* which cause the disturbance or imbalance of the enzymatic pattern or hormonal hierarchy responsible for the maintenance of life and regulation of different physiological processes (Dorn *et al.*, 1986).

1) Inhibited Growth, Retarded Development and Disturbed Metamorphosis of *S. gregaria* by the Plant Extracts:

a) *Inhibited Growth of S. gregaria:*

Because the body weight, and hence the weight gain, is one of the valuable indicators for evaluating growth (Armbruster and Hutchinson, 2002), the weight gain of nymphs was determined in the present study. After treatment of the penultimate instar nymphs of *S. gregaria* with Neemazal, the somatic weight gain was less than of control nymphs. Also, the treatment of the last instar nymphs with Neemazal resulted in a slight decreased body weight gain. These results are, however, in accordance with several results using the azadirachtin or other neem preparations. Growth inhibition was recorded for the migratory locust *L. migratoria* after treatment with a compound from the neem tree *Az. indica* (Sieber and Rembold, 1983), the lepidopteran *Spodoptera mauritia* larvae after treatment with azadirachtin (Jagannadh and Nair, 1992), the stable fly *S. calcitrans* and house fly *M. domestica* by azadirachtin (Miller and Chamberlain, 1989), the desert locust *S. gregaria* by azadirachtin (Annadurai and Rembold, 1993), the cotton leaf worm *S. litoralis* after treatment of larvae with the neem preparation, Neemazal (Ghoneim *et al.*, 2000) or Margosan-O (Mohamed *et al.*, 2000), the false stable fly *Muscina stabulans* after treatment of larvae with Margosan-O (Al-Dali *et al.*, 2003), the house fly *M. domestica* after larval treatment with Margosan-O (Amer *et al.*, 2004), the brown plant hopper *Nilaparvata lugens* after treatment the nymphs with different neem extracts (Senthil Nathan *et al.*, 2007), the blowfly *Chrysoma megacephalala* and house fly *M. domestica* after mixing a neem product (0.24 % Azt. content) with beef (as a food) (Siriwattananarungsee *et al.*, 2008), and the red palm weevil *Rh. ferrugineus* after treatment of prepupae with azadirachtin (Abdel-Ghaffar *et al.*, 2008). In contrast, no significant effect of a neem extract on the body weight gain of *Pieris brassicae* larvae was observed (Osman, 1993).

In addition to the neem seed extract, Neemazal, different extracts from *N. sativa* were assayed in the present study to investigate possible effects on growth of *S. gregaria* in the present study. Concerning the growth inhibition by the *N. sativa* extracts, treatment of penultimate instar nymphs resulted in suppressed body weight gain. However, the most drastic suppressing effect on the weight gain was exhibited at the highest concentration level (30%) of the methanolic and n-butanolic extracts. After treatment of the last instar nymphs, the normal somatic growth was prohibited when compared with that of the control congeners.

As clearly seen in the present study, methanolic, petroleum ether and n-butanolic extracts from *N. sativa* prohibited the growth of *S. gregaria* nymphs. These results agree with several reported results for other insect species using other plant extracts. Essential oils of garlic significantly suppressed the growth rate of the coleopterans *Sitophilus zeamais* and *T. castaneum* (Huang *et al.*, 2000). Acetonic and ethanolic extracts from tubercula and various compounds of *Aristolochia pubescens* inhibited the larval growth of *T. castaneum* (Nascimento *et al.*, 2004). *Melia volkensii* (Meliaceae) extract exhibited a potent growth inhibitor effect on the cabbage looper *Trichoplusia ni* (Lepidoptera) (Akhtar and Isman, 2004). A methanolic extract from the roots and aerial parts of *Myrtillocactus geometrizans* (Cactaceae) disturbed the growth of *Spodoptera frugiperda* and *Tenebrio molitor* (Cespedes *et al.*, 2005). Similar growth inhibition was reported for *Trichilia americana* extract in *S. littoralis* larvae and for *Melia. azedarach* extract in the rice leaf folder *Cnaphalocrocis medinalis* (Lepidoptera) (Senthil Nathan, 2006). Jojoba oil prohibited the pupal growth of the red palm weevil *Rh. ferrugineus* (Abdel-Ghaffar *et al.*, 2008) but did not affect the growth of larval growth of *M. domestica* (Amer *et al.*, 2004), etc.

The growth inhibition in *S. gregaria*, by the action of azadirachtin or other plant extracts in the present study, may however be caused as a result from the blocked release of morphogenic peptides, causing alteration in ecdysteroid and juvenoid titers as suggested by Sieber and Rembold, 1983; Barnby and Klocke, 1990 and Linton *et al.*, 1997. Also, some possible direct effects of azadirachtin (as represented by Neemazal, in the present study), *N. sativa* extracts may affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994).

a) Retarded development of *S. gregaria*:

In the present study on *S. gregaria*, the treatment of penultimate instar nymphs with Neemazal, resulted in retarded development since the developmental duration was prolonged and developmental rate was regressed parallelly to the concentration level. Also, a similar inhibitory effect of Neemazal was exerted on the development after treatment of the last instar nymphs nearly in a dose-dependent fashion... More or less, prolonged developmental duration was a good indicator to the inhibited development of the migratory locust *L. migratoria* after treatment with azadirachtin (Urishalom *et al.*, 1988), of the desert locust *S. gregaria* after treatment with the seed oil from *Az. indica* (Nicol and Schmutterer, 1991), of *S. mauritia* after treatment with azadirachtin (Jagannadh and Nair, 1992), of the lepidopteran *S. exigua* after treatment with a neem extracts (Yoshida and Toscano, 1994), of the hemipteran *Spilostethus ponchrus* after treatment with azadirachtin (El-Sherief, 1998), of the orthopteran *Euprepocnemis plorans* after treatment with the neem preparation, Margosan-O (Mohamed, 1998), of the dipteran *M. domestica* after treatment with the neem preparation, Neemazal (Mohamed *et al.*, 2000), of the lepidopteran *S. littoralis* after treatment with the neem preparation, Neemazal (Ghoneim *et al.*, 2000), of the homopteran *Trialeurodes vaporariorum* after treatment with Azt. (von Elling *et al.*, 2002), of the dipteran *M. stabulans* after treatment with the neem preparation, Margosan-O (Al-Dali *et al.*, 2003), of the coleopteran *Rh. ferrugineus* by azadirachtin (Abdel-Ghaffar *et al.*, 2008), etc... On the contrary, shortened developmental duration indicating an induced development had not observed for *S. gregaria* after treatment the nymphs with Neemazal, but reported for other insect species such as: *M. domestica* (Amer *et al.*, 2004)

In the present study, treatment of the penultimate instar nymphs with *N. sativa* extracts resulted in changed development of treated nymphs by exhibited a remarkable inhibitory effect (significantly prolonged developmental duration). Also, slightly retarded development by methanolic extract but slightly enhanced development by n-butanolic extract was recorded after treatment of the last instar nymphs. Shortly, *N. sativa* caused a significant retardation or enhancement of the development of *S. gregaria* depending on the extracted ingredients because the butanolic extract (after treatment of the penultimate instar nymphs) and methanolic extract (after treatment of the last instar nymphs) caused a retarded development while other extracts promoted this serious physiological process. However, the retarded development of *S. gregaria* by some extracts of the present two plant species (neem and *N. sativa*) agree with some results obtained for several insect species by the action of extracts from different plant species as reported herein.

Extracts from *M. volkensii* (Meliaceae) caused a conspicuous prolongation of larval development in the orthopteran *S. gregaria* and the mosquito *Aedes aegypti* (Mwangi and Rembold, 1988; Wilps *et al.*, 1993). Also, Amr *et al.* (1995) observed a significant prolongation in the larval duration of *S. littoralis* by the ethanolic extract from *Nerium oleander* which, also, caused similar prolongation in the developmental duration of *M. stabulans* (El-Shazly *et al.*, 1996). Similar effects were observed for

Eichlornia crassipes (Ponteriaceae) on the coleopteran *T. castaneum* (Rani and Jamil, 1989), for *A. conyzoides* on the orthopteran *S. gregaria* (Sharda *et al.*, 2000), for *Rhododendron molle* (Ericaceae) on the lepidopteran *Pteris rapae* (Zhong *et al.*, 2001), for *Pelargonium citrosa* (Graminaceae) on the mosquito *Anopheles stephensi* (Jeyabalan *et al.*, 2003), for *M. geometrizzans* on the lepidopteran *S. frugiperda* and coleopteran *T. molitor* (Cespedes *et al.*, 2005), for *Raphanus raphanistrum* (Brassicaceae) and *Peganum harmala* (Zygophyllaceae) on the coleopteran *T. castaneum* (Jbilou *et al.*, 2008).

On the other hand, the enhanced development (as indicated by the remarkably shortened developmental duration) in the desert locust *S. gregaria* by various extracts from *N. sativa*, in the present study, is in agreement with similar enhancing effects of different plant species like *Annona squamosa* (Annonaceae) against the mosquito *An. stephensi* (Saxena *et al.*, 1993), *Ajuga reptans reptans* (Lamiaceae) against the sarcophagid *Neobellieria bullata* (Darvas *et al.*, 1996), *Launaea arborescens* (Asteraceae), *Pteridium aquilinum* (Polypodiaceae) against the coleopteran *T. castaneum* (Jbilou *et al.*, 2008), and Jojoba oil against the coleopteran *Rh. ferrugineus* (Abdel-Ghaffar *et al.*, 2008). Moreover, no effect on the developmental duration was reported for some plant species such as *M. volkensii* (Meliaceae) on the mosquito *Culex pipiens* (Al-Sharook *et al.*, 1991) and the Jojoba oil on the muscid *M. domestica* (Amer *et al.*, 2004).

The prolongation of the developmental periods and subsequently the retarded development of *S. gregaria*, in the present study, can be explicated by the delaying effects of the plant extracts on the ecdysis and transformation of insects (Quadri and Narsalah, 1978; Gaaboub and Hayes, 1984; Linton *et al.*, 1997). On the other hand, the shortened developmental durations, and hence the enhanced development, as caused by the present plant extracts, may be due to a specific physiological elasticity in the insect body for overcoming the adverse conditions (like the penetrating extracts) by shortening the time interval during which the insect would be more tolerant.

b) Disrupted metamorphosis of *S. gregaria*:

Some authorities documented an inhibitory action of Azt., Azt. preparations, or extracts from other plants on the immature-adult transformation program while others reported no effects or even contradictory effects, depending on the activity of the plant species and the susceptibility of the insect species (Hashem and Youssef, 1991; Jagannadh and Nair, 1992; Khalaf and Hussein, 1997; Shaurub *et al.*, 1998; Ghoneim *et al.*, 2000; Hassan, 2002; Al-Dali *et al.*, 2003). Also, some nymphal-adult intermediates were observed in the orthopteran *S. gregaria* by an essential oil of *A. conyzoides* (Pari *et al.*, 2000) and by *C. rotendus* (El-Sokkary, 2003).

Disturbed metamorphosis of *S. gregaria* was caused by the neem extract, Neemazal, or extracts from *N. sativa*, in the present study. Neemazal intervened with the metamorphosis program because some nymphal-adult intermediates were produced, irrespective of the time of treatment or the concentration level. Only after treatment of the penultimate instar nymphs with n-butanolic extract from *N. sativa*, disturbed metamorphosis was caused because various nymphal-adult intermediates appeared proportionally to the concentration level. The formation of nymphal-adult intermediates of *S. gregaria*, in the present study, possibly indicated the disturbance of the normal ecdysone or ecdysteroid titer which is usually needed for the perfect metamorphosis. The inhibition of ecdysteroid titers by Azt., as for example, had been reported (Truman, 1981; Sieber and Rembold, 1983; Subrahmanyam *et al.*, 1989). In addition, Azt. treatment leads to the inhibition of neurosecretion (prothoracicotropic

hormone) causing the inhibition or delay of a number of physiological processes, such as metamorphosis (Josephraj Kumar *et al.*, 1999). Extracts of different species of Rutaceae interfered with neuronal as well as neuroendocrine and endocrine components of moult regulation (*cf.* Richter *et al.*, 1997). Hence, the neem extract Neemazal, *N. sativa* extracts affected the hormonal events essential to the nymphal-adult transformation in *S. gregaria*, in the present study (moulting, juvenile and eclosion hormone, in particular). Also, the suggestion of Senthil Nathan *et al.*, (2007) may be appreciated because the feeding of *N. lugens* nymphs on neem-treated plants for some days resulted in damage to physiological processes essential to the development. However, further investigation should be conducted in the future to disclose this questionable issue to point out the mode of action of the active components contained in the present plant extracts.

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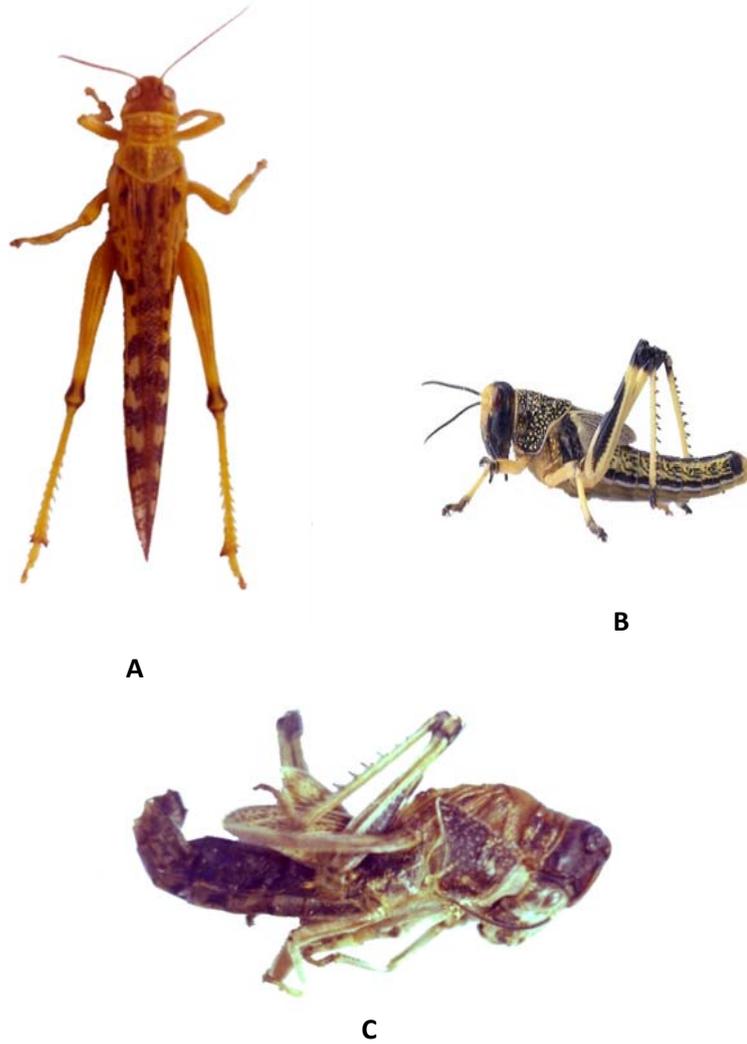


Plate 1: Nymphal-adult intermediates of *Schistocerca gregaria* as a disturbed metamorphosis program after the nymphal treatments with the present plant extracts. (A) Normal adult. (B) Normal last instar nymph. (C) a nymphal-adult intermediate.

ARABIC SUMMERY

اختلال الكفاءة المعيشية والنمو والإنماء في الجراد الصحراوي شبيستوسيركا جريجاريا بمستخلصات مختلفة من النيم (أزاديراختا إندিকা) وحبّة البركة (نيجيلا ساتيفا)

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من المعروف أن الجراد الصحراوي شبيستوسيركا جريجاريا آفة إقتصادية خطيرة تغزو دول عديدة في شمال إفريقيا وغرب آسيا، وتدمر العديد من المحاصيل الزراعية التي تهبط فوقها الأسراب. وفي مجال مكافحة هذه الآفة، فإن الدراسة الحالية تهدف إلى تحديد تأثيرات مستخلصات مختلفة من بذور حبّة البركة "ليجالا ساتيفا" مقابلّة بتأثيرات مستخلص النيم "أزاديراختا إندিকা" (ممثلاً في نيمزال)، وذلك في الكفاءة المعيشية والنمو والإنماء. أدبت معاملة حوريات كل من الدور الأخير والدور قبل الأخير بالنيمزال إلى حدوث نقص في الوزن المكتسب بالجسم. كما أبدى النيمزال تأثيراً تثبيطياً في إنماء الدور الحوري قبل الأخير وأفسد برنامج التحول إذ شوهدت أشكال وسيطة بين الحورية واليافاعات. وأما التأثيرات المسجلة لمستخلصات نيجالا ساتيفا، فإنه بعد معاملة الحوريات حديثة الانسلاخ من الدور قبل الأخير بمستخلصات الميثانول والإيثر البترولي قد وقعت وفيات مبكرة فيما بين الحوريات المعاملة وإن لم تسجل وفيات فيما بين حوريات الدور الأخير المعاملة بمستخلص الميثانول. كما أدت معاملة نفس الحوريات بمستخلصات نيجالا ساتيفا إلى حدوث نقص في الوزن المكتسب بالجسم. وقد لوحظ أن المعاملة بمستخلص كل من الميثانول والإيثر البترولي أدت إلى تثبيط النمو في اليرقات، وأدت المعاملة بمستخلص ن-بيوتانول إلى حدوث اضطراب في التحول، وذلك لظهور أشكال وسيطة بين الحوريات واليافاعات. ومما يذكر، أيضاً، أن معاملة الحوريات حديثة الانسلاخ من الدور الأخير بمستخلصات نيجالا ساتيفا، قد أدت إلى حرمان الحوريات من اكتسابها لوزن الجسم المعتاد، بينما تسببت المعاملة بالمستخلص الميثانولي إلى حدوث نقص طفيف في معدل الإنماء. ولكن المستخلص ن-بيوتانول أبدى تأثيراً عكسياً ملحوظاً حيث أدت معاملة الحوريات به إلى ارتفاع معدل الإنماء.