

# The toxic effects of *Nerium oleander* on larvae of the desert locust *Schistocerca gregaria* (Forskål, 1775) (Orthoptera, Acrididae)

## Efectos tóxicos de *Nerium oleander* en larvas de la langosta del desierto *Schistocerca gregaria* (Forskål, 1775) (Orthoptera, Acrididae)

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**Palabras clave:** *Schistocerca gregaria*, *Nerium oleander*, comportamiento alimenticio, toxicidad, mortalidad.

### ABSTRACT

This study assesses the toxic effects of *Nerium oleander* leaves (Apocynaceae) used as staple food on the development, food intake and mortality of fourth instar larvae of the desert locust *Schistocerca gregaria* under laboratory conditions. The results revealed that larvae fed with *Nerium oleander* could not moult and food intake was lower compared to control locusts (fed with *Brassica oleracea*), which had completed their moult. Larval development was affected, reaching a cumulative mortality rate over 50% from the 4<sup>th</sup> day and total mortality at the 12<sup>th</sup> day. The digestive coefficient (D.C.) and efficiency of digestive conversion (E.D.C.) were lower in *Nerium oleander* fed larvae than in control larvae. The consumption of *Nerium oleander* leaves by 4<sup>th</sup> instar female larvae was assessed by measuring the weight of ingested food, weight of excreted faeces, and weight of the insects. The results showed that though *Nerium oleander* was consumed in small quantities by desert locust larvae, it was not assimilated. The toxic effect of *Nerium oleander* on desert locust larvae could be due to the toxic secondary compounds likely contained in the leaves.

## RESUMEN

Este estudio aborda los efectos tóxicos de las hojas de *Nerium oleander* (Apocynaceae) como principal fuente alimento sobre el desarrollo, consumo de alimento y mortalidad de larvas de 4º instar de la langosta del desierto *Schistocerca gregaria* en condiciones de laboratorio. Los resultados revelaron que las larvas alimentadas con *Nerium oleander* no pudieron mudar y que el consumo de alimento fue menor en comparación con las langostas control (alimentadas con *Brassica oleracea*), que habían completado su muda. El desarrollo larvario se vio afectado, alcanzando una tasa de mortalidad acumulada superior al 50% desde el cuarto día y una mortalidad total al duodécimo día. El coeficiente de utilización digestiva (D.C.) y la eficiencia de conversión digestiva (E.D.C.) fueron menores en las larvas alimentadas con *Nerium oleander* que en las larvas control. El consumo de hojas de *Nerium oleander* por hembras de 4º instar se abordó midiendo el peso del alimento ingerido, peso de heces eliminadas y peso de los insectos. Los resultados mostraron que aunque las larvas de la langosta del desierto consumieron pequeñas cantidades de *Nerium oleander*, no la asimilaron. Los efectos tóxicos de *Nerium oleander* sobre las larvas de la langosta del desierto podrían deberse a los compuestos secundarios tóxicos que probablemente contienen las hojas.

## INTRODUCTION

The desert locust is a fearsome pest with a high reproductive potential. Its migration ability and its polymorphism enable it to adapt to different ecological situations. This pest can damage crops and pastures when it is in hopper bands and swarms in the invasion area (Popov, 1958; Uvarov, 1977; Lecoq, 2005). It is a polyphagous insect that feeds on a wide range of plant species on which no aboveground part escapes its voracity by devouring stems, bark, flowers, fruits and seeds. However, a complete or partial refusal to eat some plants species by this locust was observed (Rao & Mehrotra, 1976; Latchininsky & Launois-Luong, 1997). Phytophagous insect species evolved in parallel with plants throughout millennia (Blum, 1983; Rembold, 1994). Phytophagous insects have developed the capacity to recognize plants that have toxic substances allowing them to defend themselves (Frankel, 1959; Lebreton, 1982; Rembold, 1994). In nature, plants preferred by the desert locust are generally those that provide necessary nutrients for its development and reproduction. The quest for food depends on the capacity of locusts for moving, the characteristics of plants and the environmental conditions (Duranton *et al.*, 1982); also, the choice of their food source is made according to visual, olfactory or gustatory criteria (Le Gall, 1989).

Previous research on the feeding behavior of *Schistocerca gregaria* in its habitat has shown that the presence of this locust is linked to plants

consumed by this insect such as *Tribulus terrestris* L. and *Schouwia thebaica* Webb (Popov, 1965; Roffey *et al.*, 1968; Ghaout, 1990), while other species such as *Calotropis procera* (Abbassi *et al.*, 2004) and *C. gigantea* (Rao & Mehrotra, 1976; Pari *et al.*, 1998), *Peganum harmala* (Abbassi *et al.*, 2003a; Idrissi Hassani, 2000), *Azadirichta indica*, *Melia volkensii* (Schmutterer, 1990; Rembold, 1997; Ould El Hadj *et al.*, 2006), and *Cestrum parqui* (Barbouche *et al.*, 2001), studied in the searching for new insecticidal substances, were classified as plants with a repellent or deterrent effect on locusts. A large number of insects avoid consuming some plant species thanks to the presence of toxic and/or repellent secondary compounds contained in these plants (Bruneton, 1996).

Several plant species affect differentially the fertility, development, behaviour, and mortality of the desert locust (Ghaout, 1990; Despland & Simpson, 1999; Idrissi Hassani, 2000; Abbassi *et al.*, 2003b; Abbassi *et al.*, 2004). Using plants as a means to control locust populations could be an alternative to reduce/avoid the harmful effects caused by intensive spraying of pesticides. Indeed, prior results on the effects of plant extracts on the desert locust were encouraging for implementing an alternative method to chemical control (Nasseh *et al.*, 1993; Idrissi Hassani, 2000; Abbassi *et al.*, 2003b; Wilps & Diop, 1997). *Nerium oleander* (Apocynaceae) is among the unpalatable plants to locusts. All the parts of this plant are poisonous to man, animals and certain insects (Langford & Boor, 1996; Adom *et al.*, 2003; Barbosa, *et al.*, 2008). In this study, we compare the effects of *Brassica oleracea* (a plant readily consumed by this insect) and *Nerium oleander* leaves on the development, food intake and survival of fourth instar larvae of *Schistocerca gregaria* under laboratory conditions

## MATERIALS AND METHODS

### 1. Insect culture

Locusts used in the experiments were obtained from the mass rearing facility of the National Center of Locust Control (Ait-Melloul, Agadir). Locusts were reared in aluminum cages of 37 × 37 × 50 cm and 100 × 50 × 54 cm size. The front top of the cages is equipped with an opening clamshell to allow the introduction of food, aeration, cleaning and handling of individuals. Plexiglas boxes of 23.5 × 17.5 × 9.5 cm size were also used for larvae tested individually. A perch was placed vertically inside each box to allow the larvae to moult. The cages were maintained in a room at 32 ± 2°C temperature. The lighting of cages was provided by incandescent bulbs of 40 watts.

## 2. Plant material

During reproduction, the locusts were reared with a diet of alfalfa (*Medicago sativa*), foraged cabbage (*Brassica oleracea*) and other seasonal plants such as *Diploptaxis harræ* and wheat bran.

During the experiments, fresh leaves of *Nerium oleander* were provided as the staple food to 4<sup>th</sup> instar desert locust larvae. The leaves used to feed locusts were collected from early flowering plants in the Ait-Melloul area. *Nerium oleander* leaves provided as food for the larvae during the experiment were only fresh apical leaves, to avoid the limited edibility of old leaves caused by the hardness of their epidermis.

## 3. Experimental design

Third instar larvae were selected for synchronization of moulting. Next, fourth instar female larvae were placed individually in plexiglass boxes. A perch was placed inside each box to help the moult of larvae. Two groups of up to 30 larvae each were used for the experiment: Three replicates of 10 larvae each were performed for each diet. The first one was fed with *Nerium oleander* leaves, and the second one, used as a control treatment, with *Brassica oleracea*. The boxes were placed in a room at a  $32 \pm 2^\circ\text{C}$  temperature. The lighting of boxes was provided by incandescent bulbs of 40 watts, linked to a timer to control for a 12/12 hours photoperiod. Evapotranspiration of *Nerium oleander* and *Brassica oleracea* leaves was taken into account to adjust the amount of plant material consumed by the larvae. Evapotranspiration was measured using four boxes without larvae, containing only leaves of each plant species which were weighed and then reweighed after a 24 hours period.

The mortality rate of larvae fed on *Nerium oleander* compared with that of *Brassica oleracea* was followed during the 4<sup>th</sup> larval stage during 12 days. After fasting for 12 hours, 14 larvae were sacrificed and transferred to an oven at  $65^\circ\text{C}$  temperature for 24 hours to get initial dry weight, then experimental larvae were weighed when they die during the experiment. Leaves used to feed the larvae were weighed and renewed daily in the morning. The ingested amount and assimilated portion were evaluated. Plant material not consumed by the larvae and produced faeces were collected and weighed daily to determine the digestive utilization coefficient (D.C) and the efficiency in which digested food is converted into body substances (E.C.D.) according to Waldbauer (1968) equations:

$$D.C. = (\text{Ingested amount} - \text{weight of faeces}) \times 100 / \text{Ingested amount}$$

$$E.C.D. = \text{Dry weight gain} \times 100 / (\text{Dry weight of ingested plant} - \text{Dry weight of faeces})$$

#### 4. Statistical analysis

The analysis of food intake data was carried out by means of an analysis of variance (ANOVA) test since data were normally distributed. The significance level was set at 5%, using the SPSS statistical software.

### RESULTS

#### 1. Effect on the development and mortality

Larvae fed on *N. oleander* leaves were weak and showed a reduced locomotory activity starting from the second day of the experiment. Morphological examination of larvae showed no distinct deformations on the insects. The mortality rate differed between the groups of 4<sup>th</sup> instar larvae fed on *Nerium oleander* and *Brassica oleracea* (Fig. 1). In *Nerium oleander* fed larvae, cumulative mortality rate of over 50% was obtained from the fourth day and total mortality was recorded at the 12<sup>th</sup> day. However in control larvae fed on *Brassica oleracea*, the mortality rate in these dates is 6.7% and 13.34% respectively (Fig. 1). Larval development was also affected by the diet of *Nerium oleander* leaves. No larvae fed with *Nerium oleander* molted during the experimental period, while control larvae, fed on *Brassica oleracea*, carried out their normal development to reach the 5<sup>th</sup> larval stage.

#### 2. Effect on food intake

Food intake by the larvae fed on *Nerium oleander* was very low compared to the amount observed in *Brassica oleracea* (Fig. 2). When the *Nerium oleander* leaves were introduced to feed the larvae, they rejected it in the beginning, but after some hesitations that lasted for hours, they started to approach them. Then larvae bit at the base of leaves near the petiole and on the midvein. Faeces of the larvae fed on leaves of *N. oleander* were very small compared to control ones.

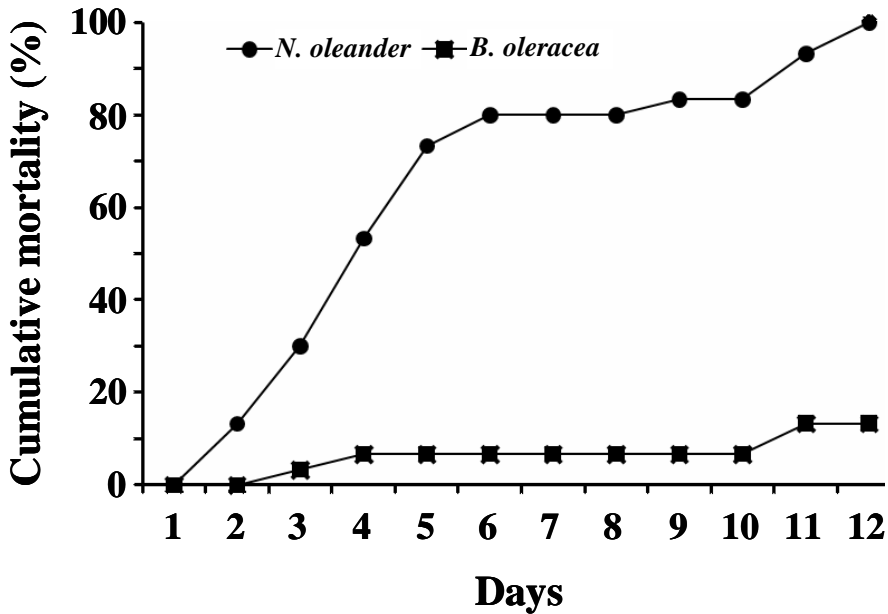


Fig. 1.—Cumulative mortality rate of 4<sup>th</sup> instar desert locust larvae fed on *Nerium oleander* and *Brassica oleracea*.

Fig. 1.—Tasa acumulativa de mortalidad de larvas del 4<sup>o</sup> instar de la langosta del desierto alimentadas con *Nerium oleander* y *Brassica oleracea*.

The results obtained on the consumption by 4<sup>th</sup> instar larvae of the tested plant *N. oleander* compared to the control plant *B. oleracea*, showed that the average amount ingested was  $0.032 \pm 0.012$  g/day for the tested plant and  $0.606 \pm 0.054$  g/day for the control plant (Fig. 2). The difference in food intake on these two plants was significant ( $F = 129$ ,  $p < 0,001$ , d.f. = 1, 385).

Parameters related to the amount of ingested food, the weight gain/loss of insects and the dry weight of faeces ( $0,016 \pm 0,002$  and  $0,066 \pm 0,006$  for *N. oleander* and *B. oleracea*, respectively) were used to determine the digestive coefficient (D.C) and the efficiency of digestive conversion (E.C. D). The estimated values of these two indices of consumption and the mean weight gain/loss of larvae fed on *Nerium oleander* and *Brassica oleracea* are presented in Table I. These results show that *Nerium oleander* was not only scarcely consumed, but also that there was no conversion of this ingested amount into body substances in desert locust larvae, with a consequent slight decrease in larval weight (Table I). For control larvae

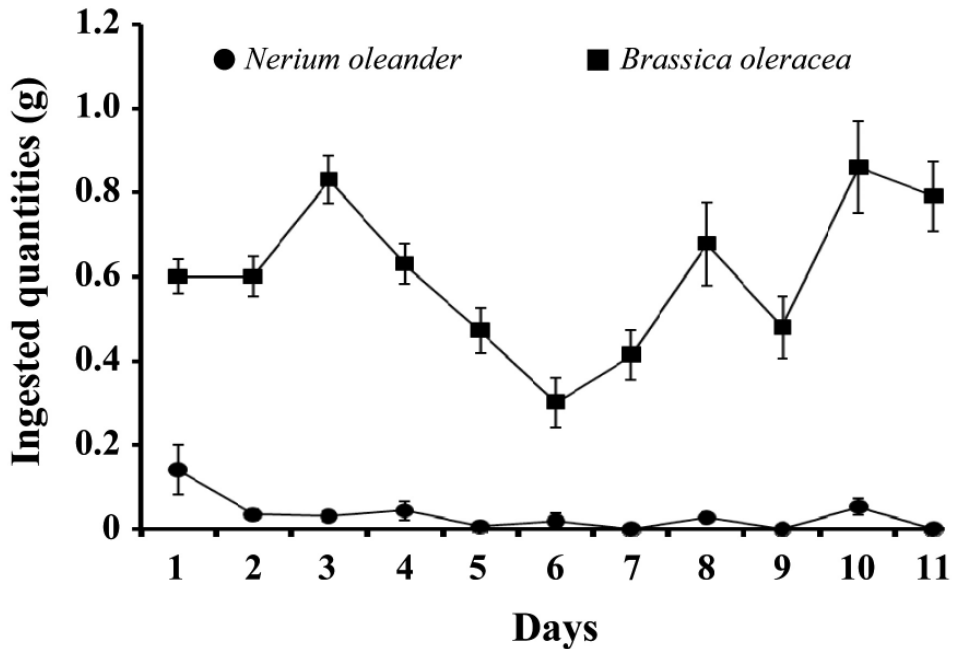


Fig. 2.—Daily consumption of *Nerium oleander* and *Brassica oleracea* by 4<sup>th</sup> instar desert locust larvae.

Fig. 2.—Consumo diario de *Nerium oleander* y *Brassica oleracea* por larvas de 4<sup>o</sup> instar de la langosta del desierto.

Table I.—Weight gain/loss, digestive utilization coefficient (D.C.) and assimilation (E.C.D.) of *Nerium oleander* and *Brassica oleracea* by desert locust larvae.

Tabla I.—Ganancia/pérdida de peso, coeficiente de utilización digestiva (D.C.) y asimilación (E.C.D.) de *Nerium oleander* y *Brassica oleracea* por larvas de la langosta del desierto.

<i>Tested plants</i>	<i>Weight gain/loss</i> (g)	<i>Mean D.C.</i> (%)	<i>Mean E.C.D.</i> (%)
<i>N. oleander</i> (n = 30)	-0.007 ± 0.001	30.20 ± 5.04	0
<i>B. oleracea</i> (n = 30)	0.201 ± 0.015	40.00 ± 1.68	66.6 ± 2.89

fed with *Brassica oleracea*, the digestibility of the plant was higher and the assimilation of ingested vegetation in bodily substances was relatively high resulting in a normal development with a net weight gain at the end of the experiment (Table I).

## DISCUSSION

The results of this study showed that the consumption of *Nerium oleander* was very low compared to the control plant due to the unpalatability and deterrent effect of this plant on locusts. The toxic effect of this plant caused a slowdown of locomotory activities on locust larvae. It has been reported by several authors that *Nerium oleander* is toxic to humans, birds and insects (Alfonso *et al.*, 1994). Food intake by *Schistocerca gregaria* and *Locusta migratoria migratorioides* depends on the nutritional value of the food (see Sinoir, 1968) and may also be influenced by the temperature, physical and ethological (solitary vs. gregary) state of the insect. The study on the toxic effect of plants and their extracts on food intake of the desert locust showed that *N. oleander* is a deterrent while *Inula viscosa* is unpalatable (Tail, 1998). The use of *N. oleander* cuttings to control the white grub larvae of Rhizotrogini beetles has a deterrent effect on these insects and limited the damage to cereals crops (Madaci *et al.*, 2008). *Nerium oleander* extracts tested on *Lymantria dispar* larvae, showed insecticidal activity (Kerris *et al.*, 2008). According to Lokesh *et al.* (2010), *N. oleander* extracts contain active compounds that are able to eliminate mosquito effectively by affecting their reproduction. The decomposition of *N. oleander* leaves leads to an important deoxygenation of aquatic receptors having a toxic effect on the freshwater snails *Melanopsis preamorsa* (Haddy, 2006).

The direct use of several plants or their extracts has given encouraging results to control the development and reproduction of desert locust. While in some plants the different parts can be toxic, in other cases toxicity is only caused by specific organs. The study of the effect of leaves of *Calotropis procera* on desert locust fifth instars showed a mortality rate of 100% after 15 days (Abbassi *et al.*, 2004). Plants such as *Melia volkensii* may play an important role in protecting crops against locust damages in the context of integrated pest management. The use of neem powder seeds for maize protection against *Nomadacris septenfasciata* damage gave interesting results (Langwald *et al.*, 1995), and thus many researchers gave importance to pursuing research in this domain. Indeed, the use of plants or their extracts have shown promising results, but in practice, such use is still limited in protecting the crops against many insect pests.

In conclusion, the diet based on *Nerium oleander* leaves has halted the development of the desert locust *Schistocerca gregaria* larvae. Weight loss of treated individuals is due to the low food intake closely related to the repellent and anti-palatable effect of this plant. Indeed, using this plant in preventive control may play an important role in the close protection of crops against locusts, reducing the intensive use of pesticides and minimizing the



consequences of the ecological imbalance. The repellent effect and reduced assimilation of *Nerium oleander* could be due to secondary compounds contained in the leaves which play an important role in affecting the development of desert locust.

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