

*Full Length Research Paper*

# **Evaluation of the systemic action of neem (*Azadirachta indica* A. juss) seed products against the desert locust immature *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae)**

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Received 20 May, 2019; Accepted 21 June, 2019

The current study was done to investigate the potential of the systemic growth regulatory effects of various neem seeds products against immature stages of desert locust infesting potted millet plants in Sudan. The tests also covered the stability of the systemic action of neem seed powder (NSP) under conditions of water stress. Some (33-80%) of the exposed nymphs developed to the third stage without further moulting. Medium lethal time for neem seed water extracts (NSWE), neem seed organic extract (NSOE) and NSP ranged from 166 to 248 hours. All neem seed product induced significant systemic antifeedant activity, ranging from 52 to 99% against the immatures. Based on these findings, NSP, the simplest form, was found to possess systemic activity comparable to complicated forms of neem products. NSP was stable under conditions of delayed watering up to 10 days and the latter had effects on development, mortality and feeding comparable to immediate watering. All aspects studied indicated the superior systemic activity of various neem seed products. The fact that they were able to delay development, prevent further moulting of instars, stabilize under conditions of delayed watering, enabled them to confine the desert locust to their breeding sites as immatures without threat of swarm formation and limited damage to local growers.

**Key words:** Desert locust, neem seed products, pearl millet, Sudan.

## **INTRODUCTION**

Man suffers tremendous losses from feeding and other activities of insects. Many insects feed on the plants man cultivate, some feed on valuable stored materials, clothing, or wood, while others feed on man and other animals directly (Borror et al., 1981). Among these insects are locusts, the most historic world pest. Locusts

are extraordinary insect whose name is synonymous with famine among one-eighth of the world population (Baron, 1972; Edward, 2017). About 200 species of grasshoppers and locusts with different food preferences and geographical distribution are known to be agricultural pests, in Africa (Office of Technology Assessment, 1990).

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The most important locust species in Africa are the desert locusts, *Schistocerca gregaria gregaria* Forskal in east Africa and *S. gregaria flaviventris* Burmeister in South West Africa; the brown locust, *Locurta pardalina* Walker; the African migratory locust, *Locusta migratoria migratorioides*; the red locust, *Nomadacris septemfasciata* Serville and the tree locust, *Anacrieedium melanorhodon* Walker. Among these, the desert locust *S. gregaria* Forskal is the most important because of its serious damage to crops, and its vast invasion area, which covers 29,000,000 km<sup>2</sup> equivalent to 20% of the world land surface (Meinzingen, 1993; Showler, 2013). Over the years, man had faced desert locust plagues with the most futile attempts to control it. However, since early forties, synthetic insecticides have become the remedy; particularly, the long lasting chlorinated hydrocarbons, such as dieldrin, appear to be the ideal solution (Krall and Wilps, 1994). The extensive use of these substances has led to serious negative effects on the environment such as long environmental persistence, accumulation in food chain and body fats of higher animals as well as many cases of insect resistance. The universal banning of these compounds started in 1973 in USA placed a significant pressure on necessity of alternative control measures (Krall, 1994) with short persistence pesticides. These latter pesticides (of short environmental persistence) necessitate repeated blanket spray with negative impact on the environment and enormous increase in the cost of control, in addition to the enhanced risk to humans and other non-target organisms (Fadl-Elmawla 2004; Ilboudo et al., 2014).

Considering the ongoing threat of desert locust, hazards associated with conventional synthetic pesticide to the environment and the progressive increase in the cost of chemical control, the current study was initiated to throw light on possible alternative control measures that are effective, environmentally safe, and cheap. The use of microbial control agents as well as pheromone in combinations with fractional pesticide against locust were reported (Hosny, 2012; Bashir et al., 2016). Botanicals are groups of biodegradable pesticides with greater selectivity and low mammalian toxicity. They have received worldwide interest as one of the safe substitutes of conventional pesticides (Mamadou and Sarr, 2009; Hosny, 2012). The neem tree, *Azadirachta indica* A. Juss, out of 2000 plant species has been reported as one of the most promising source of natural pesticide (Maute et al., 2016). Neem tree is an abundant evergreen plant in various parts of Sudan. It was reported as an excellent source of botanical pesticides with multiple actions against various types of insects, virus, mite, nematode, fungal and bacterial management (Schmutterer, 2002; Krall and Wilps, 1994). Many workers (Elamin, 2002; Abd El Rheem, 2005; Hummel et al., 2012) confirmed its activity as contact and/or systemic insecticide, especially against immature stages. The systemic growth regulatory activity action of neem product, its availability and

cheapness under local condition makes it a suitable alternative of great potential for use in confinement of locust swarms in their breeding sites as immature. This initiated our interest for thorough investigation of neem products as systemic pesticides against immature stages of desert locust. Therefore, this study aim to evaluate the systemic activity of different neem seeds products on development, antifeedant activity and water-stress of the immature stages of desert locust.

## MATERIALS AND METHODS

### Rearing of the desert locust nymphs:

Egg laying tubes of 3.5 cm diameter and 10.5 cm deep, containing egg pods of desert locust *S. gregaria* were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Port Sudan, Sudan. The tubes were placed under rearing cages (54 cm x 42.5 cm x 42.5 cm) in a room measuring 2 x 5 m at room temperature of 26 ± 2°C during the day (11 hours), and 20 ± 2°C during the night (13 h). The relative humidity was 60-70%. Extra heat was provided by electric bulb (60 watt) placed at the top of the cage to enhance eggs hatching. Hatching instars were fed pear millet seedlings grown in pots of 7 cm diameter and 10 cm deep, plus wheat bran. The cages were cleaned daily.

### Experimental cages

The cages used are of the standard type described by Harvey (1990), with some modifications. They measured 54 x 42.5 x 42.5 cm, with-stands of 15 cm. The front side was covered with plywood, with a 13.7 cm diameter opening fitted with cloth sleeves to facilitate feeding, cleaning and handling of insects. The other sides were fitted with wire mesh.

### Preparation of natural products

#### *Preparation of neem seeds powder (NSP)*

Mature seeds of neem (*A. indica*) were collected in July from trees grown for shade at Shambat area. The seeds were left to dry under shade for 10 days. The dried seeds were crushed with sticks to remove the shell, while keeping the seed intact. The seed were then ground by pestle and mortar into fine powder, stored in tightly closed glass jar, wrapped with aluminum foil, and kept at room temperature until required for extraction and/or bioassay.

#### *Preparation of neem seeds aqueous extracts (NSAE)*

Neem seeds aqueous extracts were prepared following the method of Siddig (1991). Ten grams seed powder were soaked in 1000 ml distilled water, left for 24 h at room temperature, while thoroughly stirred by a piece of wood every eight hours for 5 min. The mixture (1% w/v) was then filtered through light cloth and the filtrate was used on the same day. Other concentrations (5, 10 and 20% w/v) were prepared following the same method.

#### *Preparation of neem seeds organic extract (NSOE)*

Prepared neem seeds powder (300 g) was placed in a Soxhlet apparatus. The powder was extracted for 12 h with 1000 ml of hexane (boiling point 67-79°C). The added hexane was removed

using rotary evaporator at 45°C. The obtained oil was kept in a flask, tightly closed, wrapped with aluminum foil and stored in the refrigerator at 5°C till needed for bioassay. Various dilutions needed (10, 15, and 20% v/v) were done by serial dilution.

### **Production of millet seedlings**

Pearl millet seeds (local variety), were purchased from local market, grown in pots of 7 cm diameter and 10 cm deep with 200 g of alluvial soil mixed with sand (5:2 parts respectively) to improve soil texture, then two grams of seeds were sown per plot. Irrigation water (30 ml/pot) was given every 3 days.

### **The systemic action of neem seeds products on the development of the desert locust immatures**

The following neem seeds products used were:

- (i). Neem seeds organic extract (NSOE), at concentrations (1, 5, 10 and 20% w/v).
- (ii). Neem seeds water extracts (NSWE), at concentrations (1, 5, 10 and 20% w/v).
- (iii). Neem seed powder (NSP), at concentrations (1, 5, 10 and 20% w/v).
- (iv). Azadirachtin (as azal) at the recommended dose (2 L/ha).
- (v). Carbofuran (as furadan) as standard systemic insecticide at the recommended dose (4 kg/ha).
- (vi). Control (potted pearl millet seedlings) without any treatment.

Chemical treatments were directly applied to the soil of 3 days old seedlings, and left for an extra 72 hours to allow the uptake of active ingredient (a-i) of the products by the seedlings. Five desert locust nymphs (2nd instar) were introduced into each cage, containing ten treated seedlings, and daily observed until the control treatments reached the adult stage (fledglings). Each treatment was replicated three times and units were assigned in randomized complete block design (RCBD). The daily observations included mortalities, deformation, developmental period and weight gain. Data were recorded every 24 h, through the entire developmental period. Necessary corrections were done according to Abbott's formula (1925).

$$P_1 = \frac{P_T - P_c}{100 - P_c} \times 100$$

Where;

$P_1$ : Corrected mortality%;  $P_T$ : Treated mortality and  $P_c$ : control mortality %.

Corrected mortality was subject to probit analysis.

### **Effects of water stress on the efficacy of the systemic action of neem seeds powder**

Different concentrations (1, 5, 10 and 20% w/v) of neem seeds powder were applied to the soil prior sowing. Irrigation water (30 ml/pot) was given either immediately (NSP1) or after 7 days (NSP7) or 10 days (NSP10). Potted seedlings were maintained in cages placed in the Wire house, Faculty of Agriculture, University of Khartoum. Plants were left for 72 hours after irrigation to ensure the uptake of the active ingredient by the germinated seedlings. Five individuals of the desert locust nymphs (2<sup>nd</sup> instar), were introduced

separately into each cage. Units were assigned in randomized complete block design (RCBD), with three replicates. The development of the caged desert locust nymphs was observed until the control treatments reached the adult stage (fledglings). Daily observation includes mortalities, deformation and developmental period. The 24 hours mortality data was subject to necessary corrections according to Abbott's formula and the data was subject to probit analysis.

### **Systemic antifeedant action of different neem seeds products**

The systemic antifeedant effect of neem seeds products on different nymphal instar (2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>) of desert locust was studied. Soils of potted pearl millet seedlings were treated with different concentrations (1, 5, 10 and 20%) of various neem products (NSWE, NSOE, NSP, Azadirachtin 1% and carbofuran (as Furdan) 1%). Treated seedlings were left for 72 hours to allow uptake of the active ingredient. Patches of desert locust nymphs (5 each) were weighed and separately introduced into different cages. Treated seedlings were then chopped at soil level and provided as food to test insects. Experimental insects were starved for 24 hours prior to testing. Treated nymphs were daily provided with sufficient amount of seedlings.

In parallel, the similar amount of untreated seedlings were chopped and placed in a cage under the same condition to estimate natural water loss. Treatments were assigned in randomized complete block design (RCBD) with three replicates. The amount of food consumed by the different desert locust nymphs in various treatments was recorded after 24 hours by reweighing the remaining amount. Care was taken to correct the evaporation losses.

### **Statistical analysis**

The results were statistically analyzed using F-test and means separated with the least significant difference test (LSD) at  $p = 0.05\%$ .

## **RESULTS**

### **The effect of systemic action of neem seed products on development of desert locust nymphs**

#### **Nymphal duration**

The effect of various neem seed products on developmental period was summarized in Table 1. All treatments were significantly different from the control. The results indicated that the nymphal duration progressively increased with the increase in concentration of neem seed products and the effects were dose-related.

The duration of nymphal instar (2<sup>nd</sup>) reared on pearl millet seedlings treated with different concentrations of soil applied neem seed water extracts (NSWE) were summarized in Table 1a. The increase in nymphal duration of the second nymphal instars were 24, 33, 43 and 24% for the concentrations 20, 10, 5 and 1%, respectively.

Only 40, 40, 53 and 80% of the test instars succeeded

**Table 1.** The systemic action of various neem seed products on the development of desert locust nymphs.

Conc. (%)	Duration				
	2 <sup>nd</sup> instar duration (days)	% moulted to 3 <sup>rd</sup> instar	3 <sup>rd</sup> instar duration (days)	Total duration (days)	%mortality 3 <sup>rd</sup> instar
<b>(a) NSWE</b>					
20	9.50 <sup>c</sup> (24)	40	12.00 <sup>b</sup> (41)	21.5(33)	100
10	10.16 <sup>bc</sup> (33)	40	11.80 <sup>bc</sup> (39)	21.96(36)	100
5	11.00 <sup>b</sup> (43)	53	11.00 <sup>bc</sup> (29)	22(36)	100
1	9.50 <sup>c</sup> (24)	80	10.33 <sup>bc</sup> (18)	19.5(21)	100
Azal 1%	12.30 <sup>a</sup> (64)	73	28.667 <sup>a</sup> (237)	41(126)	100
0.0	7.667 <sup>d</sup> (0.0)	100	8.50 <sup>c</sup> (0.0)	16.16	6.6
Lsd	1.226		3.009		
<b>(b) NSOE</b>					
20	15.00 <sup>a</sup> (100)	47	11.00 <sup>bc</sup> (29)	26(62.5)	100
10	14.00 <sup>a</sup> (86.6)	33.3	12.00 <sup>b</sup> (41)	26(62.5)	100
5	9.50 <sup>c</sup> (27)	73	11.50 <sup>b</sup> (35)	21(31)	100
1	9.66 <sup>c</sup> (29)	53	10.50 <sup>bc</sup> (24)	20.16(26)	100
Azal 1%	12.33 <sup>b</sup> (64)	41	28.66 <sup>a</sup> (237)	41(126)	100
0.0	7.50 <sup>d</sup> (0.0)	100	8.5 <sup>c</sup> (0.0)	16	6.6
Lsd	1.116		2.905		
<b>(c) NSP</b>					
20	10.00 <sup>b</sup> (42)	60	12.00 <sup>b</sup> (41)	22(42)	100
10	8.25 <sup>c</sup> (17)	66	11.00 <sup>bc</sup> (29)	19.5(24)	100
5	10.00 <sup>b</sup> (42)	53	10.00 <sup>bc</sup> (18)	20(29)	100
1	12.00 <sup>a</sup> (70)	66	10.00 <sup>bc</sup> (18)	22(42)	100
Azal 1%	12.30 <sup>a</sup> (69)	73	28.66 <sup>a</sup> (237)	41(126)	100
0.0	7.50 <sup>b</sup>	100	8.50 <sup>c</sup>	16	6.6
Lsd	1.749		2.867		

Mean values having different letters in each column differ significantly ( $P \leq 0.05$ ) according to LSD test.

Values between brackets represent the percentage increase in nymphal duration.

NSWE: Neem seed water extracts

NSOE: Neem seed organic extracts.

to moult to the third instar for the respective concentration of NSWE 20, 10, 5 and 1%. The respective increase in the nymphal duration of the third instars was 41, 30, 29 and 18% for the concentrations 20, 10, 5 and 1%, respectively. No further moulting occurred and nymphs died as overaged instar. The increase in the total developmental period for the two stages ranges from 21-36%. All treatments were significantly different from the control. However, differences between the various concentrations were sometimes non-significant at  $P = 0.05$ .

The duration of the second nymphal instars reared on pearl millet seedlings treated with different concentrations of soil applied NSOE were summarized in Table 1b. The respective increases in nymphal duration were 100, 86, 27 and 29% for the 20, 10, 5 and 1% concentrations, respectively. Percentage instar succeeded to moult in the third stage, ranging from 33 to 73%. Those moulted instars experienced longer duration during the third instar

period of 29, 41, 35 and 24% for the 20, 10, 5 and 1% concentrations, respectively. Again, no further moulting occurred and nymphs died as overaged instars. The percentage increase in the total developmental period for the two stages (2<sup>nd</sup> and 3<sup>rd</sup>) were 62.5, 26, 31 and 26% for the 20, 10, 5 and 1% concentrations, respectively. All treatments were significantly different from the control ( $P = 0.05$ ) (Table 1b). Table 1c shows the effect of neem seed powder (NSP) on the duration of the second nymphal instars reared on pearl millet seedlings treated with soil applied NSP. The respective increases in the duration of the second instar were 42, 17, 42 and 17% for the 20, 10, 5 and 1% concentrations, respectively. Lower concentration gave longer duration.

Individuals succeeded to the next moult, ranging from 53 to 66%. The percent increase in duration of the third nymphal instar were 41, 29, 18 and 18% for the 20, 10, 5 and 1% concentrations, respectively. No further moult occurred and all individuals died as overaged nymphs.

The increase in the total developmental period (2<sup>nd</sup> and 3<sup>rd</sup>) ranges from 29 to 42%. All treatments were significantly different from the control. Statistical differences between various levels were sometimes noticed at  $P = 0.05$  (Table 1c).

The soil applied Azadirachtin (Azal 1%) gave 64 and 237 % increase in the duration of the second and third nymphal instars, respectively. About 73% second instars moulted to third instar with no further moult and individual died as overaged nymphs. The total developmental period of the two stages were increased by 126 % as compared to the control. All nymphs exposed to pearl millet seedlings treated with soil applied Carbofuran died within 6 days following the application.

### ***Mortality cases resulting from the systemic action***

The mortality cases among various nymphal instars of desert locust fed on pearl millet seedlings treated with soil-applied neem seed products were given in Table 2. Table 2 showed the mortality cases noticed among various nymphal instar fed on pearl millet seedlings treated with soil applied neem seed water extract (NSWE). The different concentrations brought about significant deaths among test insects compared to the control. Mortality in most cases occurred before moulting. Mortality rate is dose-related with higher dosage generally resulting in higher and faster mortality rate compared to lower dosage. Complete mortality of test insects occurred between 15 and 24 days, depending on the concentration; while insects tested with Furadan died within 6 days. Those treated with Azal 1% reached 100% mortality after 33 days.

The mortality cases occurred among desert locust nymphal instars fed on pearl millet seedlings and treated with soil-applied neem seed organic extract (NSOE) are given in Table 2. All tested concentrations caused significant mortality compared to the control and response is dose-related; higher dosage gave quick and fast mortality. Complete mortality of treated insects was noticed between 18 and 24 days (Table 2) depending on the concentration. Table 2 showed the mortality of the desert locust nymphs treated with soil-applied neem seed powder (NSP). All treatments significantly differ from the control. The mortality rate is dose-related with higher doses resulting in higher and faster mortality rate compared to lower dosage. Complete mortality was noticed between 15-18 days, depending on the concentration.

### ***Morphological deformations on the insect***

The number of insects that showed damaged parts (antennae, hind legs, fore legs and wings) were ranged from 6.6 to 13%.

## **PROBIT ANALYSIS OF RELATIVE TOXICITIES OF DIFFERENT NEEM SEEDS PRODUCTS**

### **Time response at 5%**

Table 3a and Figure 1 showed the comparative time related toxicities data of the 5% concentration of NSWE, NSOE, NSP<sub>1</sub>, NSP<sub>7</sub> and NSP<sub>10</sub>. The corresponding percentage mortality responses are shown in Table 2. The results indicated that, the slope of the mortality regression lines (LT-P lines) were steep and positive, indicating homogenous population. The homogeneity is also evident from the narrow  $LT_{90}/LT_{50}$  values and fiducial limits. Chi-square values (2.6, 9.5, 6.5, 3.3 and 2.6 for NSWE, NSOE, NSP<sub>1</sub>, NSP<sub>7</sub> and NSP<sub>10</sub> respectively) were small, indicating a good line fit and good execution. Responses appear more variable at  $LT_{10}$ , while they become close at the middle ( $LT_{50}$ - $LT_{90}$ ). The results indicated the following overall effectiveness NSP<sub>1</sub> > NSP<sub>7</sub> > NSP<sub>10</sub> > NSWE. NSOE, with relative potencies (relative to  $LT_{50}$  of NSOE) 1.5, 1.3, 1.05, 1.01 and 1.0, respectively.

### **Time response at 10%**

The time related toxicities data for the 10% concentration are given in Table 3b, and Figure 2. The test products showed a relative increase in efficiency with dose as evident from the decrease in  $LT_{50}$ ,  $LT_{90}$  values compared to that of 5% concentration. Similar to the previous observation, test population was quite homogenous in its response as indicated by the steep line slopes, narrow  $LT_{90}/LT_{50}$  ratios and fiducial limits. Chi-square values (7.7, 12.5, 4.4, 5.2 and 3.8 for NSWE, NSOE, NSP<sub>1</sub>, NSP<sub>7</sub> and NSP<sub>10</sub>) were low, indicating good execution and line fit. The test neem seed products showed an overall order of effectiveness as follows NSP<sub>7</sub> > NSP<sub>1</sub> > NSOE. NSP<sub>10</sub> > NSWE (based on  $LT_{50}$  of NSWE).

The responses to test compounds appeared relatively closer at  $LT_{50}$  compared to  $LT_{10}$  and  $LT_{90}$  as indicated by the respective differences in values of  $LT_{10}$  and  $LT_{90}$  as well as line slope (Figure 2).

### **Time response at 20%**

Table 3c and Figure 3 showed the comparative time related toxicities of the five-neem seed products to the test nymphs. The test compounds showed a relative increase in efficacy as evident from the lower values of  $LT_{50}$ ,  $LT_{90}$  compared to those of 5 and 10% concentrations. The homogeneity of test population was evident by the narrow values of  $LT_{90}/LT_{50}$  ratios. The  $LT_{50}$  values ranges between 119 and 174 hours for various products. The results indicate that LT-P lines are almost

**Table 2.** Mortalities among desert locust nymphs (2nd instar) fed on pearl millet seedlings treated with soil applied by NSWE, NSOE and NSP.

Conc. (%)	Mortality through time (days)											
	3	6	9	12	15	18	21	24	27	30	33	
NSWE	20	0.33 <sup>a</sup> (6.6)	2.33 <sup>a</sup> (46.6)	2.667 <sup>a</sup> (53.3)	4.33 <sup>a</sup> (68.6)	5.00 <sup>a</sup> (100)						
	10	0.33 <sup>a</sup> (6.6)	1.33 <sup>ab</sup> (26.6)	2.33 <sup>ab</sup> (46.6)	4.33 <sup>a</sup> (68.6)	5.00 <sup>a</sup> (100)						
	5	0.33 <sup>a</sup> (6.6)	1.00 <sup>ab</sup> (20)	2.33 <sup>ab</sup> (46.6)	3.33 <sup>ab</sup> (66.6)	4.33 <sup>ab</sup> (86.6)	5.00 <sup>a</sup> (100)					
	1	0.33 <sup>a</sup> (6.6)	1.33 <sup>ab</sup> (26.6)	2.33 <sup>ab</sup> (46.6)	3.00 <sup>b</sup> (60)	4.00 <sup>a</sup> (80)	4.33 <sup>a</sup> (86.6)	4.66 <sup>a</sup> (93.3)	5.00 <sup>a</sup> (100)			
	Azal 1%	0.00 <sup>(-)</sup>	0.00 <sup>b</sup> (-)	0.33 <sup>b</sup> (6.6)	1.00 <sup>c</sup> (20)	1.66 <sup>b</sup> (33)	2.66 <sup>b</sup> (53)	3.33 <sup>b</sup> (66.6)	3.33 <sup>b</sup> (66.6)	3.66 <sup>a</sup> (73)	4.00 <sup>a</sup> (80)	5.00 <sup>a</sup> (100)
	0.0	0.00 <sup>(-)</sup>	0.00 <sup>a</sup> (-)	0.33 <sup>a</sup> (6.6)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)
	LSD	0.838	1.779	2.045	1.110	1.949	1.258	0.727	0.592	1.869	1.608	0.856
NSOE	20	1.667 <sup>a</sup> (33)	3.00 <sup>a</sup> (60)	4.66 <sup>a</sup> (93)	4.66 <sup>a</sup> (93)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)					
	10	1.00 <sup>ab</sup> (20)	2.00 <sup>ab</sup> (40)	2.66 <sup>b</sup> (53)	2.66 <sup>b</sup> (53)	3.33 <sup>ab</sup> (66.6)	3.66 <sup>ab</sup> (73)	5.00 <sup>a</sup> (100)				
	5	0.00 <sup>b</sup> (-)	2.00 <sup>ab</sup> (40)	2.33 <sup>ab</sup> (46.6)	2.33 <sup>b</sup> (46.6)	3.00 <sup>bc</sup> (60)	3.33 <sup>a</sup> (66.6)	5.00 <sup>a</sup> (100)				
	1	0.00 <sup>b</sup> (-)	0.33 <sup>ab</sup> (6.6)	1.00 <sup>bc</sup> (20)	2.00 <sup>bc</sup> (40)	2.66 <sup>bc</sup> (53)	2.66 <sup>a</sup> (53)	3.66 <sup>b</sup> (73)	5.00 <sup>a</sup> (100)			
	Azal %	0.00 <sup>(-)</sup>	0.00 <sup>c</sup> (-)	0.33 <sup>c</sup> (6.6)	1.00 <sup>cd</sup> (20)	1.66 <sup>cd</sup> (33)	2.66 <sup>a</sup> (53)	3.33 <sup>b</sup> (66.6)	3.33 <sup>b</sup> (66.6)	3.66 <sup>a</sup> (73)	4.00 <sup>a</sup> (80)	5.00 <sup>a</sup> (100)
	0.0	0.00 <sup>(-)</sup>	0.00 <sup>c</sup> (-)	0.33 <sup>c</sup> (6.6)	0.66 <sup>b</sup> (13)	0.66 <sup>d</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)
	LSD	1.110	1.677	1.569	1.110	1.728	0.727	0.592	1.869	1.608	1.608	0.856
NSP	20	0.33 <sup>bc</sup> (6.6)	2.66 <sup>a</sup> (53)	3.33 <sup>a</sup> (4.6)	4.00 <sup>a</sup> (80)	5.00 <sup>a</sup> (100)						
	10	0.33 <sup>bc</sup> (6.6)	2.33 <sup>ab</sup> (46.6)	3.33 <sup>a</sup> (66.6)	3.66 <sup>a</sup> (73)	5.00 <sup>a</sup> (100)						
	5	1.33 <sup>a</sup> (27)	2.00 <sup>ab</sup> (40)	3.00 <sup>a</sup> (60)	4.00 <sup>a</sup> (80)	5.00 <sup>a</sup> (100)						
	1	1.00 <sup>ab</sup> (20)	1.66 <sup>b</sup> (33)	3.00 <sup>a</sup> (60)	3.00 <sup>a</sup> (60)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)					
	Azal %	0.00 <sup>(-)</sup>	0.00 <sup>(-)</sup>	0.33 <sup>b</sup> (6.6)	1.00 <sup>b</sup> (20)	1.66 <sup>b</sup> (33)	2.66 <sup>b</sup> (53)	3.33 <sup>a</sup> (66.6)	3.33 <sup>a</sup> (66.6)	3.66 <sup>a</sup> (73)	4.00 <sup>a</sup> (80)	5.00 <sup>a</sup> (100)
	0.0	0.00 <sup>(-)</sup>	0.00 <sup>(-)</sup>	0.33 <sup>b</sup> (6.6)	0.66 <sup>b</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)
	LSD	0.727	0.727	1.391	1.185	0.727	1.185	0.592	1.050	1.869	1.6082	0.856

-Mean values having different letters in each column differ significantly ( $P \leq 0.05$ ) according to LSD test.

-Values between brackets represent the percentage mortality.

-The std systemic insecticide (Furdan) curve 100% mortality within 6 days.

-NSWE: Neem seed water extracts

-NSOE: Neem seed organic extracts

-NSP: Neem seed powder.

parallel with the following overall order of effectiveness; NSOE>NSP<sub>7</sub>>NSP<sub>1</sub>>NSWE.NSP<sup>10</sup> with relative potencies of 1.46, 1.35, 1.02, 1.02 and 1.0, respectively based on LT<sub>50</sub> of NSP<sub>10</sub>.

### Systemic effects of neem seed products on food intake

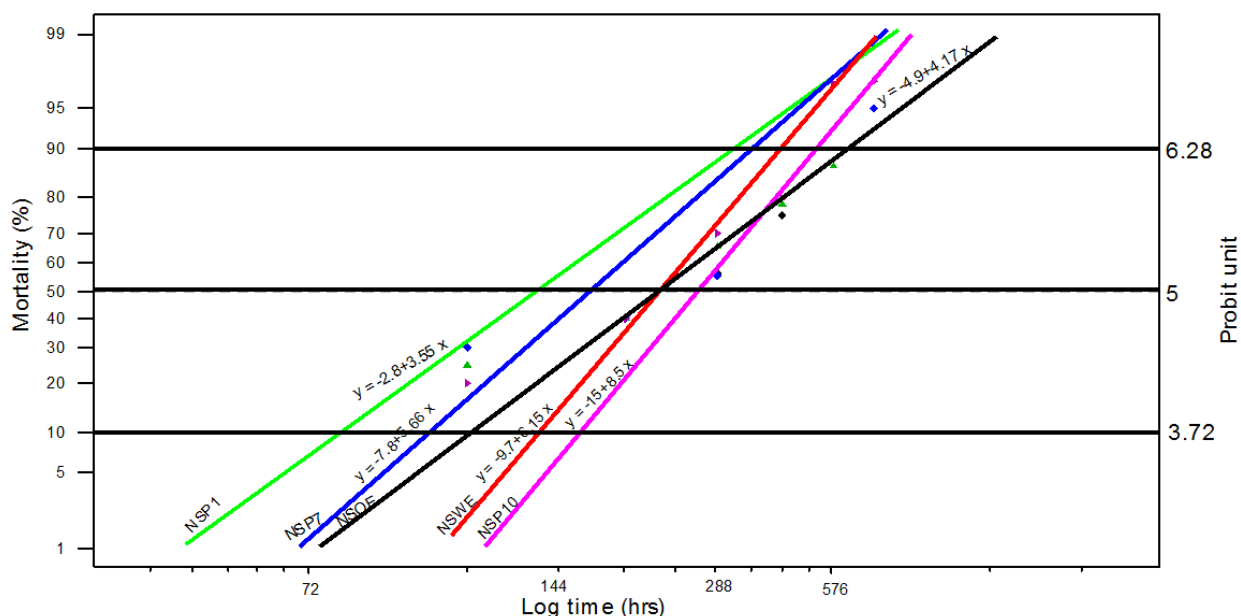
Results summarized in Table 4 showed the

amount of food ingested by different nymphal instars of desert locust (2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>) fed on pearl millet seedlings treated with various types of soil applied neem seed products viz., NSWE,

**Table 3.** Time response (mortality) data of the nymphal instar of desert locust reared on pearl millet seedling treated with soil applied neem seed products (at concentrations 5, 10 and 20%).

Insecticide	LT <sub>50</sub>	LT <sub>90</sub>	LT <sub>90</sub> /LT <sub>50</sub> ratio	Slope	Fiducial limits	Chi-square	D.F	Relative potency
<b>based on NSP<sub>10</sub></b>					<b>(a) Conc. 5%</b>			
NSWE	245	396	1.6	6.15	1.966	2.6	10	1.01
NSOE	248	504	2.0	4.17	1.969	9.5	10	1.00
NSP <sub>1</sub>	166	380	2.3	3.55	1.970	6.5	10	1.50
NSP <sub>7</sub>	186	313	1.7	5.66	1.973	3.3	10	1.3
NSP <sub>10</sub>	234	330	1.4	8.58	1.966	2.6	10	1.05
<b>based on NSOE</b>					<b>(b) Conc. 10%</b>			
NSWE	198	312	1.6	6.60	1.965	7.7	10	1.00
NSOE	192	611	3.2	2.53	1.975	12.5	10	1.03
NSP <sub>1</sub>	188	374	1.9	4.29	1.969	4.4	10	1.05
NSP <sub>7</sub>	167	271	1.66	6.11	1.966	5.2	10	1.2
NSP <sub>10</sub>	197	276	1.4	8.85	1.965	3.8	10	1.005
<b>based on NSWE</b>					<b>(c) Conc. 20%</b>			
NSWE	170	301	1.8	5.15	1.967	7.2	10	1.02
NSOE	119	272	2.3	3.6	1.971	12.2	10	1.46
NSP <sub>1</sub>	170	334	1.9	4.42	1.968	6.8	10	1.02
NSP <sub>7</sub>	128	226	1.8	5.09	1.967	3.3	10	1.35
NSP <sub>10</sub>	174	273	1.6	6.56	1.965	4.0	10	1.00

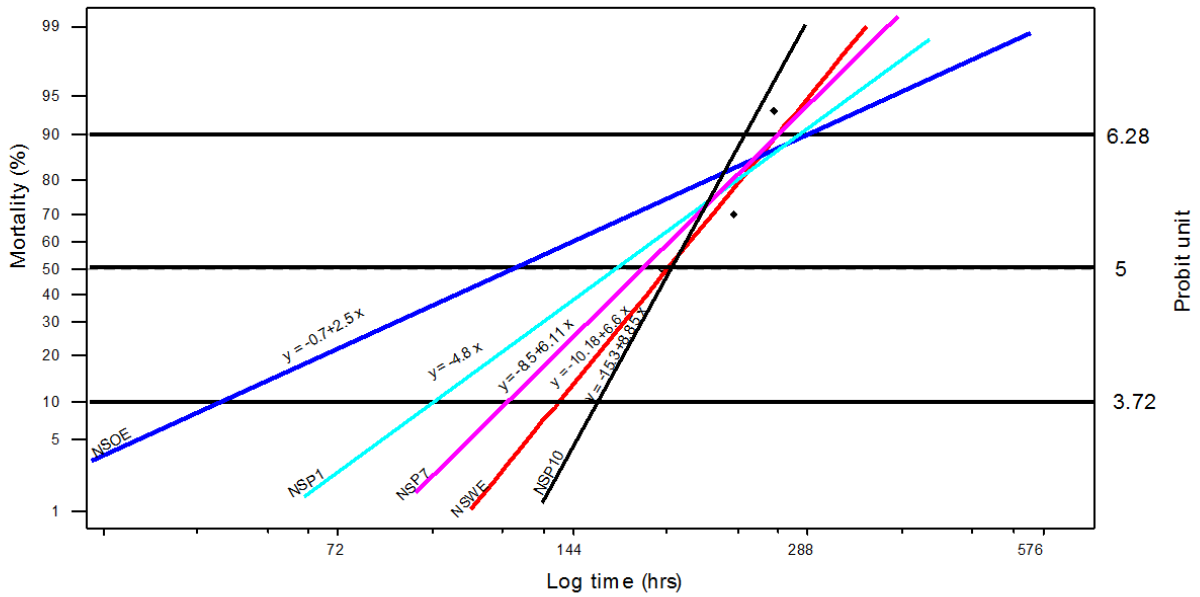
NSWE: Neem seed water extracts  
 NSOE: Neem seed organic extracts  
 NSP1: Effect of one day delayed watering of treated seedlings  
 NSP7: Effect of 7 day delayed watering of treated seedlings  
 NSP10: Effect of 10 days delayed watering of treated seedlings.



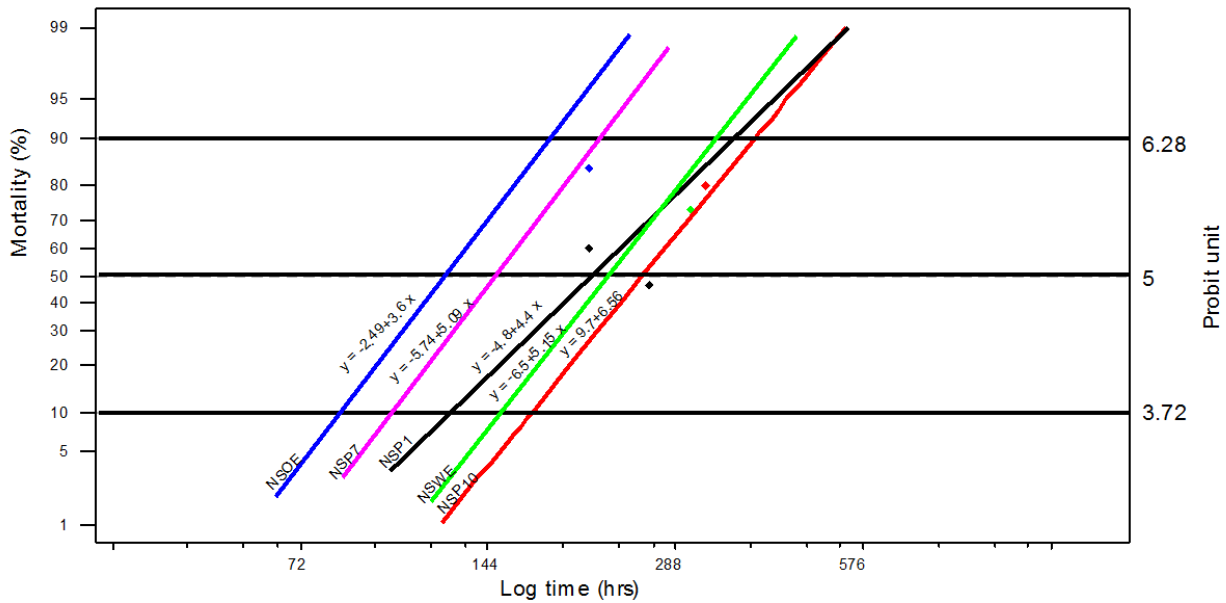
**Figure 1.** Logtime-Probit-line of the 2nd nymphal instar of Desert locust fed on pearl millet seedling treated with soil applied Neem seed products at concentration 5%.

NSOE and NSP at various concentrations (20, 10, 5 and 1%) and Azal 1% (as standard antifeedant).

The results clearly indicated that the amount of ingested food is inversely related to the dose. Increasing



**Figure 2.** Logtime-Probit-line of the 2nd nymphal instar of Desert locust fed on pearl millet seedling treated with soil applied Neem seed products at concentration 10%.



**Figure 3.** Logtime-Probit-line of the 2<sup>nd</sup> nymphal instar of Desert locust fed on pearl millet seedling treated with soil applied Neem seed products at 20% concentration.

the concentration of neem products results in progressive decrease for food ingested. The extent of reduction in food intake for the different neem treatments is extremely high, exceeding 52% at the lowest concentrations tested in various treatments. Neem seed water extracts causes reduction in food intake, ranging from 72-90.5, 86-93, 96-

99 and 97-99% for the second, third, fourth and fifth nymphal instars, respectively. The overall percentage reduction in food intake by all nymphal instars ranges from 86.75-95.4% for the various concentrations of neem seed water extracts. All concentrations significantly suppressed the feeding rate of test insects compared to



**Table 4.** Amount of ingested food and %reduction in food intake of various desert locust instars (2<sup>nd</sup>-5<sup>th</sup>) fed on pearl millet seedlings treated with soil applied neem seed products.

Conc. (%)	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar
<b>(a) NSWE</b>				
20	0.267 <sup>d</sup> (90.5)	0.507 <sup>c</sup> (93)	0.333 <sup>b</sup> (99)	0.400 <sup>b</sup> (99)
10	0.33 <sup>d</sup> (89)	0.667 <sup>bc</sup> (91)	0.733 <sup>b</sup> (98)	0.900 <sup>b</sup> (98)
5	0.50 <sup>d</sup> (83)	0.920 <sup>bc</sup> (88)	0.900 <sup>b</sup> (97)	1.400 <sup>b</sup> (97.7)
1	0.80 <sup>c</sup> (72)	1.040 <sup>bc</sup> (86)	1.233 <sup>b</sup> (96)	1.600 <sup>b</sup> (97)
Azal 1%	1.33 <sup>b</sup> (50)	1.400 <sup>b</sup> (77)	2.467 <sup>b</sup> (90)	2.400 <sup>b</sup> (96)
0.0	2.867 <sup>a</sup> (-)	7.667 <sup>a</sup> (-)	31.4 <sup>a</sup> (-)	62.461 <sup>a</sup> (-)
Lsd	0.2813	0.7795	3.168	4.045
<b>(b) NSOE</b>				
20	0.267 <sup>c</sup> (90.5)	0.533 <sup>e</sup> (93.7)	1.333 <sup>b</sup> (96)	1.333 <sup>c</sup> (98)
10	0.467 <sup>c</sup> (83)	2.333 <sup>d</sup> (73)	3.533 <sup>b</sup> (89)	3.667 <sup>c</sup> (94)
5	0.933 <sup>b</sup> (66.7)	3.767 <sup>c</sup> (56)	5.067 <sup>b</sup> (84)	7.600 <sup>b</sup> (88)
1	1.333 <sup>b</sup> (52.5)	5.000 <sup>b</sup> (59)	6.067 <sup>b</sup> (81)	9.533 <sup>b</sup> (85)
Azal 1%	1.333 <sup>b</sup> (50)	1.400 <sup>de</sup> (77)	2.467 <sup>b</sup> (90)	2.400 <sup>c</sup> (96)
0.0	2.800 <sup>a</sup> (-)	8.533 <sup>a</sup> (-)	31.133 <sup>a</sup> (-)	62.677 <sup>a</sup> (-)
Lsd	0.4248	1.218	5.739	4.738
<b>(c) NSP</b>				
20	0.400 <sup>d</sup> (86)	0.600 <sup>c</sup> (92)	0.867 <sup>b</sup> (97)	0.733 <sup>d</sup> (99)
10	0.600 <sup>cd</sup> (79)	1.400 <sup>b</sup> (82)	1.033 <sup>b</sup> (96.7)	4.00 <sup>cd</sup> (94)
5	0.933 <sup>bc</sup> (67)	1.500 <sup>b</sup> (80)	1.267 <sup>b</sup> (95)	7.067 <sup>bc</sup> (87)
1	1.200 <sup>b</sup> (58)	1.800 <sup>b</sup> (76)	1.600 <sup>b</sup> (94.9)	8.733 <sup>b</sup> (86)
Azal 1%	1.333 <sup>b</sup> (50)	1.400 <sup>b</sup> (77)	2.467 <sup>b</sup> (90)	2.400 <sup>d</sup> (96)
0.0	2.867 <sup>a</sup> (-)	7.667 <sup>a</sup> (-)	31.600 <sup>a</sup> (-)	62.267 <sup>a</sup> (-)
Lsd	0.527	0.7209	2.957	3.667

Mean values having different letters in each column differ significantly ( $P \leq 0.05$ ) according to Lsd test.

Values between brackets represent the percentage reduction in food intake.

NSWE: Neem seed water extracts

NSOE: Neem seed organic extracts

NSP1: Neem seed powder.

the control. The reduction in food intake caused by NSWE is superior to that caused by Azal 1% (Table 4a).

Neem seed organic extracts induce reduction in feeding rate, ranging from 52.5-90.5, 59-93.7, 81-96 and 85-98% for the second, third, fourth and fifth instars, respectively. The overall percentage reduction in food intake by all nymphal instars ranges from 69.4-94.5% for the various concentrations of neem seed organic extracts. All concentrations of NSOE significantly suppressed the feeding rate of test insects compared to the control (Table 4b). The reduction in food intake caused by NSOE is superior to that caused by the standard (recommended) dose of Azal 1%.

Neem seed powder (Table 4c) cause's reduction in feeding rate, ranging from 58-85, 76-92, 94-97 and 86-99% for the second, third, fourth and fifth instars, respectively. The overall percentage reduction in food intake ranges from 78.7-93.5% for the various neem seed powder treatments. All neem seed powder (NSP) concentrations significantly suppressed the feeding rate

of test insect compared to the control. The reduction in feeding rate exceeds that caused by the standard dose of Azal 1%. Azadirachtin as Azal 1% at the recommended dose reduced the feeding rate by 50, 77, 90 and 90% for the second, third, fourth and fifth nymphal instars, respectively. It also showed a significant suppression in food intake rate of test insects compared to the control.

#### Effect of water stress on the efficacy of systemic action of NSP in nymphal duration increase

Table 5 showed the effect of water stress on the efficacy of the systemic action of neem seed powder. Watering was performed either immediately following sawing (NSP<sub>1</sub>), after 7 days (NSP<sub>7</sub>) or after 10 days (NSP<sub>10</sub>). NSP<sub>1</sub> resulted in increased duration of the second nymphal instar by 47, 17, 42 and 70 hours for the concentrations of 20, 10, 5 and 1%, respectively (Table 5-a) as explained before; while NSP<sub>7</sub> resulted in

**Table 5.** The effect of various water regimes on the efficacy of NSP as related to development of 2<sup>nd</sup> instar

Conc. (%)	Duration				
	2 <sup>nd</sup> instar duration (days)	%moulted to 3 <sup>rd</sup> instar	3 <sup>rd</sup> instar duration (days)	Total duration (2 <sup>nd</sup> and 3 <sup>rd</sup> ) (days)	%mortality 3 <sup>rd</sup> instar
<b>(a) NSP1</b>					
20	10.00 <sup>ab</sup> (47)	60	12.00 <sup>a</sup> (41)	22(42)	100
10	8.25 <sup>bc</sup> (17)	66	11.00 <sup>b</sup> (29)	19.5(24)	100
5	10.00 <sup>ab</sup> (42)	53	10.00 <sup>c</sup> (18)	20(29)	100
1	12.00 <sup>a</sup> (70)	66	10.00 <sup>c</sup> (18)	22(42)	100
0.0	7.06 <sup>b</sup> (-)	100	8.50 <sup>d</sup> (-)	15.5	6.6
Lsd	2.152		0.8420		
<b>(b) NSP7</b>					
20	10.00 <sup>a</sup> (33.3)	13.3	12.00 <sup>a</sup> (41)	22(37.5)	100
10	12.00 <sup>b</sup> (60)	13.3	11.00 <sup>a</sup> (29)	23(44)	100
5	11.00 <sup>b</sup> (47)	20	10.00 <sup>a</sup> (18)	21(31)	100
1	18.00 <sup>ab</sup> (140)	20	15.00 <sup>a</sup> (76)	33(106)	100
0.0	7.50 <sup>c</sup> (-)	100	8.50 <sup>a</sup> (-)	16	6.6
Lsd	1.684		5.119		
<b>(c) NSP<sub>10</sub></b>					
20	11.00 <sup>b</sup> (47)	13.3	12.00 <sup>a</sup> (41)	23(44)	100
10	8.00 <sup>b</sup> (7)	13.3	12.00 <sup>a</sup> (41)	20(25)	100
5	14.00 <sup>a</sup> (87)	20	11.00 <sup>b</sup> (29)	25(56)	100
1	17.33 <sup>a</sup> (131)	26.6	11.00 <sup>b</sup> (29)	28(75)	100
0.0	7.50 <sup>b</sup> (-)	100	8.50 <sup>c</sup> (-)	16(-)	6.6
Lsd	5.514		0.421		

Mean values having different letters in each column differ significantly ( $P \leq 0.05$ ) according to Lsd test.

Values between brackets represent the percentage increase in nymphal duration.

NSP1: Effect of one day delayed watering of treated seedlings

NSP7: Effect of 7 day delayed watering of treated seedlings

NSP10: Effect of 10 days delayed watering of treated seedlings.

prolongation of the second instar duration by 33.3, 60, 47 and 140% for the 20, 10, 5 and 1% concentrations, respectively. Generally, effects were inversely related to dose. The individual succeeded to moult to the third instar, ranging from 7 to 13.3% (Table 5b). The increase in the duration of the third nymphal instars were 41, 29, 18 and 78% for the concentrations 20, 10, 5 and 1%, respectively. No further moult was observed. The increase in the total developmental period (2<sup>nd</sup> + 3<sup>rd</sup>) was 37.5, 44, 31 and 105% for the concentrations 20, 10, 5 and 1%, respectively. All concentrations were significantly different from the control.

The percentage increase in nymphal duration of the second nymphal instar exposed to NSP<sub>10</sub> treated seedlings was 47, 7, 87 and 131% for the concentrations 20, 10, 5 and 1%, respectively (Table 5-c). Prolongation was inversely related to dose. Percentage of the second instar moulted to the third stage, ranging from 7-13%. No further moult was recorded. On the other hand, the increase in the duration of the third nymphal instar

ranged from 29-51%, while the increase in the total period (2<sup>nd</sup> and 3<sup>rd</sup>) ranged from 25-75%. All treatments were significantly different from the control and effects were almost dose related.

### Mortality cases

Mortality of desert locust nymphs fed on pearl millet seedlings treated with soil applied NSP watered after 7 days were given in Table 6-b. All concentrations gave significant deaths (at  $P = 0.05$ ) among test insects compared to the control. The mortality is dose related; higher concentration resulted in higher mortality and at faster rate compared to lower dosage. In most cases, mortality occurred before moulting. Lower dosage cause death after a long period and insects died as overaged nymphs. Complete mortality of test insects occurred between 12-21 days depending on dosage. On the other hand, NSP<sub>10</sub> (Table 6c) induced significant mortality

**Table 6.** Mortality cases among desert locust nymph (2<sup>nd</sup> and 3<sup>rd</sup>) fed on pearl millet seedlings treated with soil applied NSP.

Conc. (%)	Mortality through time (days)							
	3	6	9	12	15	18	21	24
<b>(a) NSP<sub>1</sub></b>								
20	0.33 <sup>bc</sup> (6.6)	2.66 <sup>a</sup> (53)	3.33 <sup>a</sup> (66.6)	4.00 <sup>a</sup> (80)	4.00 <sup>a</sup> (80)			
10	0.33 <sup>b</sup> (6.6)	2.33 <sup>ab</sup> (46.6)	3.33 <sup>a</sup> (66.6)	3.66 <sup>a</sup> (73)	3.66 <sup>a</sup> (73)			
5	1.33 <sup>a</sup> (27)	2.00 <sup>ab</sup> (40)	3.00 <sup>a</sup> (60)	4.00 <sup>a</sup> (80)	4.00 <sup>a</sup> (80)			
1	1.00 <sup>a</sup> (20)	1.66 <sup>ab</sup> (33)	3.00 <sup>a</sup> (60)	3.00 <sup>a</sup> (60)	3.00 <sup>a</sup> (60)	5.00 <sup>a</sup> (100)		
0.0	0.00 <sup>b</sup> (-)	0.00 <sup>b</sup> (-)	0.33 <sup>b</sup> (6.6)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (26)	0.66 <sup>b</sup> (13)		
Lsd	0.8136	0.8136	1.409	1.3280	0.6635	0.8564		
<b>(b) NSP<sub>7</sub></b>								
20	1.33 <sup>a</sup> (26)	2.66 <sup>a</sup> (53)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)	-			
10	0.60 <sup>ab</sup> (13)	0.60 <sup>b</sup> (13)	4.33 <sup>ab</sup> (87)	4.66 <sup>ab</sup> (93)	5.00 <sup>a</sup> (100)			
5	0.60 <sup>ab</sup> (13)	1.66 <sup>ab</sup> (32)	4.33 <sup>ab</sup> (87)	4.33 <sup>ab</sup> (87)	5.00 <sup>a</sup> (100)			
1	0.30 <sup>b</sup> (6.6)	1.33 <sup>b</sup> (26)	3.33 <sup>b</sup> (67)	3.33 <sup>b</sup> (67)	3.33 <sup>b</sup> (67)	3.66 <sup>a</sup> (73)	5.00 <sup>a</sup> (100)	
0.0	0.00 <sup>b</sup> (-)	0.00 <sup>c</sup> (-)	0.33 <sup>c</sup> (6.6)	0.66 <sup>c</sup> (13)	0.66 <sup>c</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	
Lsd	0.8136	0.9337	1.5062	0.4923	0.3828	0.5010	0.8564	
<b>(c) NSP<sub>10</sub></b>								
20	1.00 <sup>a</sup> (20)	1.67 <sup>a</sup> (33.4)	4.66 <sup>a</sup> (93)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)			
10	0.66 <sup>ab</sup> (13)	1.00 <sup>ab</sup> (20)	4.66 <sup>a</sup> (93)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)			
5	0.66 <sup>ab</sup> (13)	0.66 <sup>bc</sup> (13)	3.66 <sup>a</sup> (73)	4.00 <sup>a</sup> (80)	4.66 <sup>a</sup> (93)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)	
1	0.33 <sup>ab</sup> (6.6)	0.66 <sup>bc</sup> (13)	3.00 <sup>a</sup> (60)	3.33 <sup>a</sup> (66.6)	4.33 <sup>a</sup> (87)	4.33 <sup>a</sup> (87)	4.66 <sup>a</sup> (93)	5.00 <sup>a</sup> (100)
0.0	0.00 <sup>b</sup> (-)	0.00 <sup>b</sup> (-)	0.33 <sup>b</sup> (6)	0.66 <sup>b</sup> (66)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)	0.66 <sup>b</sup> (13)
Lsd	0.8136	0.8136	2.048	1.243	1.050	1.632	0.9413	0.8564

Mean values having different letters in each column differ significantly ( $P \leq 0.05$ ) according to Lsd test.

Values between brackets represent the percentage mortality.

NSP<sub>1</sub>: Effect of one day delayed watering of treated seedlings

NSP<sub>7</sub>: Effect of 7 day delayed watering of treated seedlings

NSP<sub>10</sub>: Effect of 10 days delayed watering of treated seedlings.

among desert locust nymphs with all treatments significantly different from the control. The mortality rate is dosage related and increases progressively with the increase in the dosage. Most of the mortality cases occurred before moulting. Lower doses resulted in late mortality and insects died as overaged nymphs. Probit analysis of mortality as related to dose or time was explained earlier.

### Effects on food intake

Results summarized in Table 7 showed the amount of food ingested by different nymphal instar of desert locust. It is clear from the table that, the amount of food ingested is negatively related to the dose. Increasing the concentration of neem seed products resulted in progressive suppression in the amount of food ingested. This observation is true for all tested instars (2<sup>nd</sup>-5<sup>th</sup> nymphal instars of desert locust). The extent of suppression in food intake for NSP<sub>7</sub> and NSP<sub>10</sub> treatments is very high that is, more than 56% suppression was induced by the lower concentration

(1%). All treatments significantly suppressed the feeding rate of test insects compared to the control and gave similar results compared to NSP<sub>7</sub> treatments. For NSP<sub>7</sub>, the reduction ranges from 57-85%, 76-91%, 95-97% and 85.1-98.5% for the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> nymphal instars, respectively. While for the NSP<sub>10</sub>, the respective reduction ranges from 56.5-84%, 76-92%, 95-97.1% and 84.1-98%.

### Morphological deformations on the insect

Deformation cases were noticed on desert locust nymphs fed on pearl millet seedlings treated with soil applied NSP subjected to various types of water regimes (7 and 10 days). The percentages of damaged insects noticed in various treatments ranged from 6.6 to 13%. No deformation was noticed in the control counterparts.

### DISCUSSION

An important tactical component in the preventive control strategy of desert locust is to locate hoppers during

**Table 7.** Amount of ingested food (mg)/insect and percentage reduction in food intake of various locust instars (2<sup>nd</sup>-5<sup>th</sup>) fed on pearl millet seedlings treated with soil applied NSP.

Conc. (%)	Instars			
	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
<b>(a) NSP</b>				
20	0.400 <sup>d</sup> (86)	0.600 <sup>c</sup> (92)	0.867 <sup>b</sup> (97)	0.733 <sup>d</sup> (99)
10	0.600 <sup>cd</sup> (79)	1.400 <sup>b</sup> (82)	1.033 <sup>b</sup> (96.7)	4.00 <sup>cd</sup> (94)
5	0.933 <sup>bc</sup> (67)	1.500 <sup>b</sup> (80)	1.267 <sup>b</sup> (95)	7.067 <sup>bc</sup> (87)
1	1.200 <sup>b</sup> (58)	1.800 <sup>b</sup> (76)	1.600 <sup>b</sup> (94.9)	8.733 <sup>b</sup> (86)
Azal 1%	1.333 <sup>b</sup> (50)	1.400 <sup>b</sup> (77)	2.467 <sup>b</sup> (90)	2.400 <sup>d</sup> (96)
0.0	2.867 <sup>a</sup> (-)	7.667 <sup>a</sup> (-)	31.600 <sup>a</sup> (-)	62.267 <sup>a</sup> (-)
Lsd	0.527	0.7209	2.957	3.667
<b>(b) NSP<sub>7</sub></b>				
20	0.443 <sup>d</sup> (85)	0.700 <sup>c</sup> (91)	0.867 <sup>b</sup> (97)	0.933 <sup>d</sup> (98.5)
10	0.640 <sup>cd</sup> (79)	1.600 <sup>b</sup> (79)	0.873 <sup>b</sup> (97)	5.333 <sup>c</sup> (91.5)
5	1.166 <sup>bc</sup> (61)	1.677 <sup>b</sup> (78.5)	1.267 <sup>b</sup> (96)	7.233 <sup>b</sup> (88)
1	1.300 <sup>b</sup> (57)	1.900 <sup>b</sup> (76)	1.600 <sup>b</sup> (95)	9.500 <sup>b</sup> (85)
0.0	3.000 <sup>a</sup> (-)	7.800 <sup>a</sup> (-)	31.600 <sup>a</sup> (-)	62.267 <sup>a</sup> (-)
Lsd	0.5489	0.6216	3.164	3.317
<b>(c) NSP<sub>10</sub></b>				
20	0.483 <sup>c</sup> (84)	0.657 <sup>c</sup> (92)	0.890 <sup>b</sup> (97)	1.067 <sup>d</sup> (98)
10	0.657 <sup>c</sup> (78.5)	1.650 <sup>b</sup> (80)	1.100 <sup>b</sup> (96.5)	5.967 <sup>c</sup> (90.5)
5	1.267 <sup>b</sup> (59)	1.700 <sup>b</sup> (79)	1.400 <sup>b</sup> (95.5)	7.667 <sup>b</sup> (88)
1	1.333 <sup>b</sup> (56.5)	1.967 <sup>b</sup> (76)	1.667 <sup>b</sup> (94.7)	10.067 <sup>b</sup> (84)
0.0	3.067 <sup>a</sup> (-)	8.167 <sup>a</sup> (-)	31.633 <sup>a</sup> (-)	63.200 <sup>a</sup> (-)
Lsd	0.5457	0.5585	3.091	2.934

Mean values having different letters in each column differ significantly ( $P \leq 0.05$ ) according to Lsd test.

Values between brackets represent the percentage increase in nymphal duration.

NSP1: Effect of one day delayed watering of treated seedlings

NSP7: Effect of 7 day delayed watering of treated seedlings

NSP10: Effect of 10 days delayed watering of treated seedlings.

upsurges and mount control operations with pesticides to halt their exponential growth into large swarms (Magor et al., 2008; Leach et al., 2009; Lecoq, 2010). The strategy is built on improved knowledge of locust ecology, more efficient monitoring of pest levels in various areas, use of more effective pesticides, and improved application methods. The botanical compounds, although they are slow in action, are easily degradable under field conditions; thus requiring repeated spraying on crops.

One of the promising sources of natural products is neem tree. Neem seed products possess systemic activity, which was first proved by Gill and Lewis (1971), who reported a systemic antifeedant action of three neem products viz.; azadirachtin (tetracyclic triterpenoid), ethanolic extract from neem seeds, and aqueous suspension of ground seed kernel, as against the desert locust, *S. gregaria*.

The study of Gill and Lewis (1971) indicated that the desert locust caused slight damage to bean plants grown

in treated soil. Bean seedlings grown from seed soaked in a solution of 0.01% azadirachtin, 0.1% alcohol extract, or 0.1% aqueous kernel extract gave protection against *S. gregaria* adults for one week after germination. Ruscoe (1972) who found that azadirachtin is an ecdysteroids in habit, acts as ecdysone analogue, and ecdysone is a hydrophilic in nature also reported the systemic activity of neem products. Attri (1975) reported that the active principle of neem is hydrophilic rather than lipophilic in nature. Saxena (1984) found that, after oil expression from neem seeds, the seed residue (seedcake) gave good protection to crops when applied to the soil.

The fact that the extract can be taken by plants and confer protection from within is one of the neem's interesting and potential use. The various merits of the systemic actions of neem products such as enhanced margin of safety to human and environment, protection from photo degradation, the longer period of activity, the

reduction in number of sprays (thus cost) as well as the possible systemic growth regulatory and/or antifeedant activity initiated the authors interest to investigate the efficacy of its systemic action in the control of desert locust at immature stages. The idea of using systemic antifeedant/growth regulatory agent could prevent locust immatures from reaching adults stages, and this may result in confining the locust as immatures in their breeding sites, with little threat of swarm formation and limited damage to local growers. The preliminary work from this group on the antifeedant and growth regulatory effect and the work of previous authors (Govindachari et al. 2000; Elamin 2002; Schmutterer 2002; Abd El Rheem 2005; Mohamed 1999) on the subject further strengthen these goals.

In the current study, various aspects of systemic activity of different neem seed products (NSWE, NSOE, NSP and azadirachtin as azal 1%) were tested against various instars of desert locust. Aspects covered include evaluation of effects on development of immature, deformation, mortality and food intake. Effects of various water regimes on stability of powder products under pots conditions were also investigated.

Results indicated that, various neem seed products are capable of delaying the developmental period of all tested instars. The delay in development, which is dose-related in most cases, is very prominent in both instars tested (2<sup>nd</sup> and 3<sup>rd</sup>). The majority of tested 2<sup>nd</sup> instar individuals either never moult to the third stage, died as overaged 2<sup>nd</sup> instars, or moult to the third instar with no further moulting and died as overaged 3<sup>rd</sup> instar nymphs. Death may occur before or during moulting. Prolongation (delay) of development by neem seed products is well documented and current results agree with the findings of previous authors (Elamin, 2002; Abd El Rheem, 2005).

Complete mortalities of test nymphs subject to various treatments occurred within 2-3 weeks. Many of the test insects were deformed prior to death. Although most test insects died in stage treated, yet many of the nymphs, who succeeded to proceed to the next instars were unable to complete shedding their nymphal exuviae or moult to further instar, and others had some morphological abnormalities. All these observations agree with previous reports of Ruscoe (1972) and Nasseh et al. (1993).

Neem seed powder and neem water extract (the simplest forms of neem) gave comparable or sometimes superior results compared to NSOE and azal. Thus, deserve further investigation under field conditions. The facts that they are less expensive, simple to prepare and with comparable superior systemic action, which prevent further moulting give strong support to this argument.

Field application of these products especially in locust breeding areas could prevent swarm formation by keeping the locust in the early stages of development until they die as overaged immatures. The finding of the limited water stress experiments carried indicated the

stability of the powder for up to 10 days if the area receives no rains as would be explained later.

The time related insecticidal activities of neem seed products, were subject to probit analysis, the data indicated that all neem seed products have similar and relatively slow action. This is clear from the time response mortality data, which shows an increase in efficacy with the increase in exposure time of the nymph to treated pearl millet seedlings, evident from the decrease in the values of  $LT_{50}$  and  $LT_{90}$ . The line slopes for the concentrations 5, 10 and 20% were steep and positive, indicating a homogenous test population. The homogeneity of responses is also evident from the narrow  $LT_{90}/LT_{50}$  ratios and narrow fiducial limits. Suitable lines fit and good execution is also evident from the low chi-square values. The time response of late watering treatments (7 and 10 days) of NSP gave results comparable to those of immediate watering.

Active principle of neem could mostly kill test insects through feeding suppressing and/or growth regulatory effects on immatures stages. As reported by Ruscoe (1972) and Ruskin (1991) azadirachtin, the active principle of neem, is structurally similar to ecdysone, the ecdysteroid hormones controlling metamorphosis in insects. Azadirachtin affect the corpus allatum, which secrete vital hormones in insects. Azadirachtin blocks the secretion and release of ecdysone and this may delay the moulting process and therefore prolong the duration of the immature stages and causes the associate deformations and mortality before or during moulting process (Rembold et al. 1982).

Reduction in food intake might also lead to the prolongation of the nymphal period and deformation of test immatures, and they must reach a critical body weight for moulting (Elamin 2002). Azadirachtin seem to prolong the time needed by the immatures to gain the critical body weight through its suppression of feeding rate (Mohamed, 1999). The fact that test nymphs either died within the 2<sup>nd</sup> nymphal stage or succeeded to moult to the third stage without further moulting, could indicate also the role of amount of food consumed (as reflected by the weight gain) in triggering the moulting process. Individuals who are able to consume larger amount of food were able to moult, since they reached the critical body weight for moulting faster. Once the nymphs enter the 3<sup>rd</sup> instar and are continuously exposed to neem seed products through food ingestion, their feeding rate decrease and therefore, remain as immatures without further moulting. This argument needs further investigation for clarification. The results of the current study showed a dose dependent antifeedant effect of various neem seed products as indicated by the significant reduction in food intake. All forms of neem seeds products gave superior suppression of food intake compared to Azal 1%.

Pradhan et al. (1962); Gill and Lewis (1971) and Ridha et al. (2018) reported the antifeedant effect of neem. The

active antifeedant principle in neem includes Salanin, Salannol acetate, 3-deacetyl salannin, 4-epoxy azadirachtin, gedunin, nimbenin and deacetyl nimbinen (Schwinger et al., 1984). Neem exerts its antifeedant action through gustation at physiological site associated with chemoreceptors (Pradhan et al., 1962; Fagoonee, 1981; Gill and Lewis, 1971; Ridha et al., 2018). Beside the regulatory growth and the suppression in food intake, mortality might result from the direct toxic action, which was also reported as mode of action of neem (Fagoonee and Lauge 1981; Saxena and Khan, 1985).

The current study investigates the effect of various water regimes on stability of neem seeds products. The results indicated that applying neem seed powder to the soil and irrigation after a period of water stress up to 7 or 10 days do not adversely affect the efficacy of neem seed powder on the prolongation of the nymphal duration, mortality or deformation. This superior stability, though not tested more than 10 days, indicated the stability of neem seed powder for field application in locust breeding areas, where watering depends on sporadic irregular rainfall. Results reported here were very encouraging as it demonstrates the possibility to confine the locust in the breeding area as tiny immatures, which pose little hazards to crops in the breeding site and/or invasion zone (as no swarms will be formed).

## Conclusion

The current study has shed light on the efficacy of systemic action of various neem seed products against immature stages of desert locust. Superior suppression of moulting with subsequent delay in development, deformation, mortality, suppression of feeding rate and stability of products under conditions of delayed rainfall were noticed in the current study. Results reported here indicate the suitability of soil applied (systemic) neem seed products in management of desert locust in their breeding site. Efficacy of seed treatment with the products was not tested for some logistic reasons, but it deserves investigation in future work. Other aspects related to the validation and use of these products under field conditions needs further investigation.

## CONFLICT OF INTERESTS

The authors declare no competing financial interest.

## ACKNOWLEDGEMENT

The authors appreciate the University of Khartoum, Sudan for the financial support.

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