Life Table Data Analysis of *Locusta migratoria migratorioides* (R,F) and Effect of Pheromones Phenylacetonitrile (PAN) on Feeding, mortality and Survival of the Nymphs

By

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DEDICATION

To my beloved mother

To the Soul of my Father

To my brothers and sisters

To my dear wife

To my children

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In the beginning I render my acknowledgement to the Almighty Allah. I am indebted to and express my deepest thanks, sincere gratitude and appreciation to my supervisor Professor Magzoub Omer Bashir for his continuous guidance, my grateful thank are also extended to Team who collected with me the locust in the field, my grateful thank are also extended to Dr Abdu Elwhab Hassan Abdalla for his help in doing the data analysis. Finally, I wish to thank anyone who supported and encouraged me throughout my study .

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ABSTRACT

The laboratory experiments were conducted at the Unit of Biological Control, of the Department of Crop Protection, Faculty of Agriculture University of Khartoum, Shambat, during the period September 2014, to Marsh 2015. The experiments were carried out under laboratory conditions, normal day light and under semi-field conditions (cage-experiment). The study was conducted to determine life history parameters of *Locusta migratoria migratorioides* collected from North Elgedaref coordinate N 14 31 144 E 035 12 450.Based on laboratory studies, duration of egg, nymphal, and adult were 14 days, 29 days, 67 days respectively.The sex ratio of emerging adults was 1:1.Fecundity mean was 55 egg/ female and the mean number of pods was 3 / female.Maximum values of the fecundity rates were reached after 16 weeks.The values of the net reproductive rate (RO), weighed generation time (T), Doubling time (DT), innate capacity of numerical increase (rm), and the finite rate of increases(ʎ) were 738.71, 98.8, 10.36, 0.067 and 1.166 respectively.The second part of the studywas conducted to determine the effect of Phenylacetonitrile (PAN)pheromone of the desert locust *Schistocerca gregaria* (Forskal) gregarious adult on mortality and feeding rate on the third instar of *Locusta migratoria migratorioides* (R,F).The tested concentrations were 5ml, 10ml, 15ml and 20ml,diluted in diesel to the dose application rate was 2 literper hectare and Malathion 96% was used as areference. The mortality effect of these concentration appeared after four days except the concentration 15 ml where the effect appeared after 24 hour. The effect of PAN concentrations 5 , 10 , 15 and 20ml,Control, Diesel , and Malathion 96% was 0%, 5% , 16.66% , 23.33% , 26.66% , 8.33% , 100% respectively. The feeding rate (g/day) of nymphs treated with Phenylacetonitrile (PAN)

concentrations of 5ml , 10ml,15mland 20ml,untreated control , diesel treated and Malathion 96% were 5.69 ± 0.35, 4.9 ± 0.48, 2.87 ± 0.11, 1.3 ± 0.19 , 1.31 ± 0.04, 1.41 ± 0.15 , 0.32 ±0.021 gram, respectively.The research recommendsthe use of the pheromone Phenylacetonitrile (PAN) as alternative of chemical pesticides against the African migratory locust *Locusta migratoria migratorioides* control management campaigns. It is also recommended that tests be conducted on its use against other locust species.

مستخلص الأطروحة

:-اجريت التجارب المعمليه بوحدة المكافحة الاحيائية التابعة لقسم وقاية المحاصيل بكلية الزراعة جامعة الخرطوم شمبات في الفترة من سبتمبر 2014 حتى مارس 2015.اجريت التجارب تحت الظروف المعمليه وضؤ النهار العادي وتحت ظروف مشابهه للحقل (تجربة الاقفلص).اجريت الدراسة لتحديد دورة الجراد الافريقي الرحال الذي جمع من شمال ولاية القضارف (إحداثيات: شمال 14°31 144" وشرق 035° 12 450°).فترة نمو البيض ,الحورية ,والطور الكامل الاناث والذكور كانت 14 ,29 , 67, و 49 يوم اعلي التوالي. نسبة الجنس بعد بذوغ الطور الكامل كانت 1:1.بلغ متوسط معدل الخصوبة 55 بيضه ومتوسط اكياس البيض 3 للانثي , اعلي معدل الخصوبة للمجموعة كان في الاسبوع السادس عشربعد الفقس.صافي معدل التكاثر (RO) , زنة عمر الجيل (T),الوقت الذي يستغرقه الجيل(DT) ,ال قدره الفطرية للتزايد(rm) وحد معدل التضاعف(٨)كانت 738.71 , 98.8 ,, 10.36 , 0.067 و1.166 علي التوالي .الهدف الثاني من التجربة كان تحديد اثر فيرمون التجمعي للجراد الصحراوي البالغ علي الموت و معدل التغذية للطور الثالث للجراد الافريقي الرحال .كانت التراكيز المستخدمة من الفيرمون 5 , 10 , 15 و 20 مل . مخففه بواسطة الديزيل لحجم المعاملة بمقدار 2لتر في الهكتار وأيضا استخدم الملاثيون 96 % كشاهد. إتضح تأثير الفيرمون لكل التراكيز بعد مرور اربعة ايام ما عدا التركيز 15 مل اتضح اثره بعد مرور 24 ساعة من التطبيق .وكان تاثيرتركيز الفرمون5 , 10 , 15 و 20 مل ,الشاعد,الديزيل والملاثيون 96%كالاتي:-0%, 5% , 16.66% ,23.33% ,26.66% ,8.33 % , 100% علي التوالي .تغذي الحوريات (جرام في اليوم) المعاملة بالفيرمون تركيز 5 , 10 , 15 و 20 مل , الشاهد, الديزيل والملاثيون 96% كالاتي:-

1.41 , 0.03± 1.31 ,0.19±1.3 ,0.11±2.87 , 0.48±4.9 , 0.345±5.69

±0.15 و 0.32 ±0.02 علي التوالي. البحث يوصي بإستخدام الفيرمون كبديل للمبيدات الكيميائية في حملات مكافحة الجراد الافريقي الرحال. كما يوصي بإجراء الإختبارات على إستخدامه ضدالأنواع الأخرى من الجراد.

CHAPTER ONE
INTRODUCTION

Locusts are potentially the most destructive insect pests in the world. They often form swarms and migrate over long distances. Locust swarms may consist of billions of individuals and cause serious damage to agricultural crops (Chapman, 1976).

Locusts are short horned grasshoppers, a large group in the order Orthoptera. There are six major locust species in Africa:

- the desert locust, *Schistocerca gregaria gregaria*(Forscal),
- the African migratory locust, *Locusta migratoria migratorioides* (R & F),
- the Red locust, *Nomadacris septemfasciata* (Serville),
- the Brown locust, *Locustana pardalina* (Walker),
- the Moroccan locust, *Dociostaurus maroccanus* (Thur.berg! , and
- the Tree locust, *Anacridium melanorhodon melanorhodon*(Walker).

The first four species are major agricultural pests in Africa, but the desert locust is regarded as the single most serious pest due to its polyphagous feeding behaviour, and its migratory habits (Popov *et al*. 1991;Steedman, 1988; Meinzingen, 1993).

While the desert locust is widely distributed in Africa, the Middle-East, and parts of Southwest Asia, the African migratory locust is restricted to Africa where it breeds in three ecological zones including the Sahelian, Sudanese and Guinean zones (Zolotarevsky,1938;Showler 1995).

The Migratory Locust , the African migratory locust, *Locusta migratoria migratorioides* (Reiche and Fairmaire), is an order Orthoptera family Acrididae, is one of 20,000 grasshopper species described to date.

The gregarious migratory locust is polyphagous, but to a lesser extent compared to the desert locust, and is restricted to graminaceous wild and cultivated plants

such as millet, sorghum, maize, rice, sugar cane, and bamboo (Meinzingen, 1993). No yield loss assessment exists for this species .

The major control strategy is based on the use of insecticides. All the insecticides sprayed had some potentially negative environmental effects. Rembold (1994) adverted to the rapidly increasing insect tolerance against any type of neurotoxic insecticide, and all insecticides given their wide spectrum of action undoubtedly had substantial side-effects on the non-target fauna (Müller, 1988). Thus, alternatives to these harmful pesticides must be found. Peveling, *et al*. (1994) showed that, there was no evidence of serious side-effects of the alternative control agents tested so far on epigeal arthropods when compared with conventional insecticides. One of these promising alternatives is the gregarious adult desert locust pheromone Phenylacetonitrile (PAN) 98% 10ml/ha (recommended dose). Diesel was used as a dilutant in all formulations. To study effect on *Locusta migratoria migratorioides* .

The objectives of the study include the following :

1) To gather information about fecundity , egg hatchability , survival rate and duration of the various instar reared in cage under laboratory conditions .
2) To study the effect of Phenylacetonitrile (PAN) on Nymphal of *Locusta migratoria migratorioides* on:

    I) Feeding

    II) Mal-moulting

    III) Deformation and malformation

    2) Mortality.

CHAPTER TWO

2.LITERATURE REVIEW

2.1. Classification

Kingdom : Animalia

Phylum : Arthropoda

Class : Insecta

Order : Orthoptera

Family : Acrididae

Genus : Locusta

Species : migratoria

Scientific Name:

Locusta migratoria migratorioides (R,F)

2.2. Distribution of Migratory Locust

Its range extends through the Atlantic Islands, Africa, Asia and parts of Australia. The Migratory Locust is widespread in grassland areas south of the Sahara and has its main outbreak area in the middle Niger flood plain in Mali where the last plague (1928–41) originated (Steedman, 1990). Figure (1) .

2.2.1 Distribution in Sudan

Migratory locust spread in Sudan in different States: Gedaref, Kassala, Gezira, and Sennar (from PPD2010) .

2.2.2 Distribution in Gedaref

Migratory locust spread in Gedaref, Elgadamblia, Elshwak, Samsam, om senat, Elhwata, Elmganis and Kasamour (From PPD2010) .

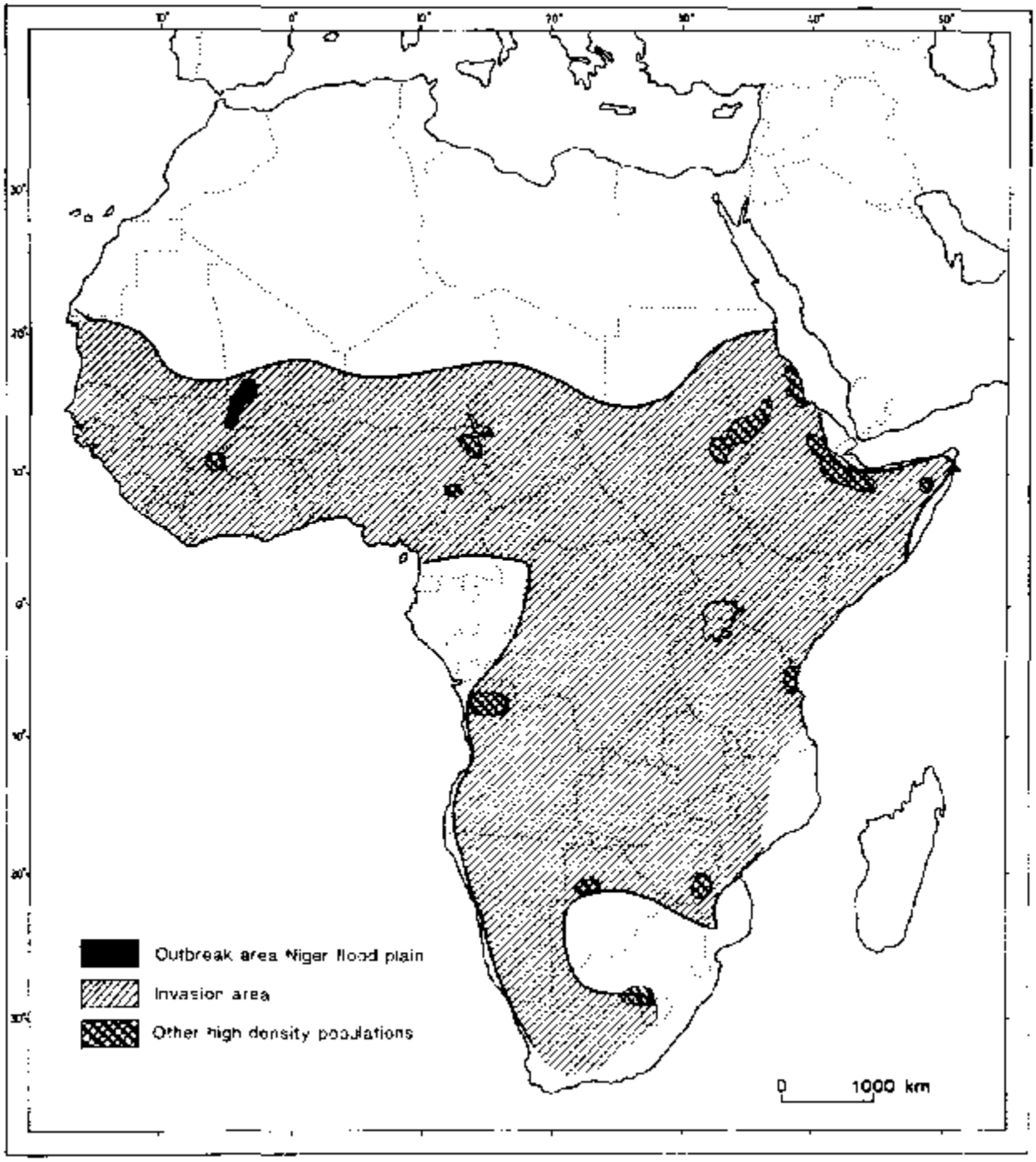

Figure (1)Distribution of *Locusta migratoria migratorioides* in Africa

Steedman (1988) Locust hand book .

2.3 Outbreak causes in Gedaref

According to PPD (2005) the beginning of proliferation in Gedaref Infected covered 4152 hectares which were 4152 hectares were treated with pesticide using 4055 liters ulv and 265 kg powder, Table (1) .

In 2007 *Locusta migratoria migratorioides* spread in Elgadamalia, Elshwak, Samsam, infected area was 62140 hectares and the control area was 56995 hectares and the insecticide used were 27485 liters ulv Table (2). (PPD 2007)

In 2010, there was a large spread in all regions of *Locusta migratoria migratorioides*. This included Gedaref, Elgadambalia, Elshwak, Eldarout Samsam , Elhawata , Om belial Dowka , Elmganis ,and Kasamor. Infected area was 60777. Table (3). And in (2010) spread in all regions of *Locusta migratoria migratorioides* in Sudan. Table (4)(PPD 2010)

Table (1) Outbreak of *Locusta migratoria migratorioides* in Gedaref area season 2005

State	infested Are hactar	Control Are hactar	ulv litter	EC litter	Powder Kg
Gedaref	4152	4152	4055	0	265

PPD (2005)

Table (2) Outbreak of *Locusta migratoria migratorioides* in Gedaref area season 2007

State	infested Area (hactar)	Control Area (hactar)	ULV (Litter)	EC (Litter)	Powder (Kg)
Gedaref	62140	56995	27485	0	0

PPD(2007)

Table (3) Outbreak of *Locusta migratoria migratorioides* in Gedaref area season2010

State	Infested Area(hactar)	Control Area (hactar)	ULV (Litter)	EC (Litter)	Powder Kg
Gedaref	66777	55490	33665	6820	13540

PPD(2010)

Table (4) Outbreak in the infestation area in Sudan 2010

State	Infested Area(hactar)	Control Area (hactar)	ULV (Litter)	EC (Litter)	Powder (Kg)
Sennar	6835	6435	2200	0	3270
Gedaref	66777	55490	33665	6820	13450
Elgzera	3950	3250	1265	150	500
Kassala	10667	8428	2893	3243	4004
Total	88229	73603	40023	10213	21224

Annual Report of plant protection directory(2010)

.

Table (5) Rain(ml) in Gedaref area season 2005

April	May	June	July	Ogast	Septembe r	October	Total
4.2	42.4	48	158.7	205.5	36.4	4.1	499.3

Annual Report of Plant Protection Directory PPD(2005)

Table (6) Rain(ml) in Gedaref area season 2007

April	May	June	July	August	Septembe r	October	Total
7.8	1.3	77.3	200.5	259.3	53.5	5.3	552.7

Annual Report of Plant Protection Directory PPD(2007)

Table (7) Rain(ml) in Gedaref area season 2010

April	May	June	July	August	September	October	Total
3.4	14.9	76.1	216.2	208.8	94.6	35.2	647.2

Annual Report of Plant Protection Directory PPD(2010)

2.4 Life cycle:

The locust is hemimetabolous with a lifecycle comprised of three stages: egg, nymph and adault. Developmental time depends on a variety of factors including temperature, humidity, soil conditions, and food availability (Rottey *et al*, 2003).

The outstanding characteristic of locusts is their ability to show density-dependent phase polymorphism, involving graded changes in morphological, physiological and behavioral traits. At low density, nymphs of the migratory locust, *Locusta migratoria*., assume various body colors including green ,yellow, brown, reddish and black depending on the habitat background color and humidity. Adults are either greenish or brownish in color. Both nymphs and adults show little tendency to aggregate and they are sedentary. At high density, all nymphs look similar with black and orange body coloration. They show a strong tendency to aggregate and move in bands. Adults are dark brown and often undergo long-distant migration in swarms. Because of the distinct differences in appearance and behavior, the individuals at low density were once designated as *L. danica* and those at high density as *L. migratoria* until Uvarov (1921) formulated the phase polymorphism theory. He described that these two species constitute a single species that changes from one phase to another in response to population density and suggested this species be called *L. migratoria*. This theory was extended to include the desert locust, *Schistocerca gregaria* Foskål (Uvarov, 1923, 1966).

One of the most important characteristics of phase polymorphism is the presence of intermediate forms called transient phase in addition to the two extreme phases, solitarious and gregarious phases occurring at low and high density, respectively. A shift from solitarious to gregarious, or

vice versa, does not take place in one generation, but takes several generations (Uvarov, 1966).

Locusts during the transient period or at intermediate densities show intermediate characteristics. Thus, the variation is continuous, the situation being different from other types of polymorphism or polyphenism in which morphologically distinct morphs such as long-winged and short-winged or apterous morphs occur without forming intermediate morphs (Harrison, 1980; Pener, 1985; Dingle, 1996).

2.5 Developmental life cycle

The developmental cycle of both *L. migratoria* and S.*gregaria* compri three stages: egg, nymph, and adult(Steedman, 1988; Meinzingen, 1993) .

2.5.1 Egg development

Eggs are laid in the soil where, under favorable conditions of soil humidity and temperature, they take, on the average, 15 days to incubate. Their development is affected by environmental conditions. For example,Ackowor and Vajime (1995) found that in the lake Chad breeding area of *L. migratoria migratorioides,* egg development is closely related to season, soil structure, and natural enemy complex in the area. The fastest egg development was recorded during the main rainy season in sandy soil at temperatures of around 29°C with an incubation period of 13.6 days while the slowest was recorded during the Harmattan (dry) season temperatures ranging from 19°C to 26°C.incubation time was 24 days in clayey soils.

No distinct diapauses has been observed in the egg development in both *S. gregaria* and *L. m. migratorioides,*even though Meinzingen (1993), and Ackowor and Vajime (1995) showed that in the field, some slowing

or temporary arrest of the egg development is possible in conditions of low soil humidity and temperature conditions. In *L. migratoria*, the incubation period ranges between 20 - 40 days in cold conditions, but only 10 - 20 days in warm conditions (Price and Brown, 1990; Meinzingen, 1993) .

2.5.2 Nymphal development :

Through five to six instars depending on their phase and species before fledging into adults (Uvarov, 1966; Steedman, 1988; Meinzingen, 1993).

L. migratoria, nymph development goes through five instars regardless of the phase, over aperiod of 24 to 35 days. However, under adverse conditions, solitarious individuals may go through six or seven instars and development could last as long as 60 days (Meinzingen, 1993) .

2.5.3 First instar hopper :

The first is whitish when newly hatched but turns mainly black during (1-2hours) .Average length is approximately 7mm and average weight is about , 30-40 mg(Steedman, 1990) .

2.5.4 Second instar hopper:

It is difficult to distinguish the second instar from the first, but with experience one can see that the pale colour pattern is more obvious and the head is much larger , the average length is about approximately 15mm and the average weight 50-80 mg (Steedman,1990).

2.5.5 Third instar hopper:

This instar is easily recognized by the two pairs of wing bud which can be seen projecting from underneath, the pronotum on each side of the thorax . The average length is approximately 20mm and the average weight is 120-200mg (Steedman,1990**).**

2.5.6 Fourth instar hopper:

There can then be a very wide range of colours including green, grey, buff, brown, red and black, often resembling the background colour of their habitat , The average length is approximately 26_27 mm and the average weight is 430_500mg (Steedman,1990).

2.5.7 Fifth instar hopper :

There can then be a very wide range of colours including green, grey, buff, brown, red and black, often resembling the background colour of their habitat , The average length is approximately 34_37 mm and the average weight is 1000_1220 mg (Steedman,1990).

2.5.8Adult sexual maturation:

L. migratoria, this number varies from 55-140 with an average of 67 eggs/pod (Steedman, 1988; Meinzingen , 1993) in the solitaries phase, and an average of 39.4 eggs/pod in the gregarious phase (Meinzingen, 1993).

L. migratoria can produce up to five generations per year in the main outbreak area, but only two elsewhere in Africa (Meinzingen, 1993).

Adult sexual maturation is affected by such factors as rainfall, humidity, temperature (Norris, 1954; 1957; Steedman, 1988), chemical stimuli (Norris, 1954, 1968; Loher, 1960; Mahamat *et al*., 1993; Assad, 1995),host and non host plants (Jackson, *et al*., 1978; Assad, 1995).

2.6. Phenylacetonitrile (PAN)

Locusts are the swarming phase of short-horned grasshopper of the family Acrididae. These are species that can breed rabidly under suitable condition and subsequently become gregarious and migratory . They form bands as nymphs and swarms as adults _both of which can travel great distances , rapidly stripping fields and greatly damaging crops .

In the desert locust , the mating males emit a courtship-inhibiting pheromone to repel other rival males. the pheromone was identified as Phenylacetonitrile (PAN) (Obeng- ofori *et al* , 1993)

Phenylacetonitrile (PAN) solitarising agregarious population , it has some effects on the nymphs , immune factors .Gregarious nymphs showed asignificant decreases in total haemocyte count (THC) reflected in the decreases in the all three types of haemocytes , granulocytes in CO (Kane ,2004)

The optimum dosage of the pheromone is 10ml ha .The application of the pheromone on gregarious nymphs causes increased cannibalistic tendency lowered feeding rate and causes shifts in the circadian rhythm . Nymphs were hyperactive and tend to continuously move rather than feed or roost (Hassnali and Bashir , 2010).

2.7 Control

2.7.1. Control Measures:

The existence of known major and minor outbreak areas makes it feasible to prevent the development of further plague by controlling the locust in the outbreak areas, so preventing formation of swarms. Field investigations were carried out immediately after the beginning of the last plague in 1928 and this led to the formation in 1948 of an internationally supported control organization, followed, in 1952, by the signing of a convention formally establishing Open Innovation Center Advanced Materials (OICMA). This is now supported by 18

Africa countries representing most of those liable to invasion by this Locust in times of plague (COPR, 1982).

2.7.2 Chemical control

The aerial surveys and control measures in the officially designated outbreak areas are now necessary, in the majority of years. In 1951-52 over 17,000 hopper bands were destroyed in north of the equator, in Sudan and Ethiopia. Due to the activities of OICMA, no plague of this Locust has developed since the last plague ended in 1941. Control by individual governments after the escape of swarms would obviously be more difficult and expensive and, in fact, no such improvement of these survey and control operations (COPR,1982). In recent years spray able concentrations of this locust have infested many areas in central and southern Africa. It appears that the African Migratory Locust finds favorable breeding conditions in irrigated lands such as those found in sugarcane estates. Heavy and widespread infestations were recorded north of the equator, in Sudan and Ethiopia, in 1974, 1978, 1982 and 1987 (Meinzingen, 1993).

Chemical Pesticides are applied against Locust and grasshopper in one of three forms baits , dusts and sprays . bait are usually prepared by mixing the insecticides with acarrier that is eaten by the Locust . Carbamates such as H-C-H and dieldrin , were used in baiting form against locust and grasshopper (A.O.A.D ,1987 and Steedman 1987) .

2.7.3. Natural control

Destruction of the eggs by insect parasites and predators is considerable, and eradicates about 30% of the eggs. The most important parasites and predators are the wasp *Sceliosuda nensis* (Ferriere), Carabid beetle larvae *Chlaeniusquadrinotatus* (Dej) and the predator *Homalolachnussexmaculatus* (Dej). Also the dipteran *Sarcophagamezzadrii* (Seguy), nematodes worms and ants *A Coccobacillus* have been found attacking young hoppers and infecting the adults . Many birds prey on hoppers and adult . The most important

may be Storks carmine the bee-eater *Meropsnubicus* (Gmel) , Abyssinian roller *Coraciasabyssinica* (Herm) , black kite *Milvus migrans* (boddaert), Little egret Egretta *garzetta*, guinea fowl *Numidia meleagrismirata* (pall), turtle doves and francolins. Also the March harrier *Circus aeruginisus* and marabou stork *leptoptiloscrumeniferus* (lesson). In one such case a population reduction of 75% was estimated in the Sahel and Sudan. These natural enemies are credited with the destruction of three eggs, hopper bands and adults (COPR, 1982).

CHAPTER THREE

MATERIALS AND METHODS

This study was carried out under laboratory conditions , at the Insectary of the Department of Crop Protection , Faculty of Agriculture University of Khartoum ,Shambat , during the period from September 2014 to Marsh 2015.

3.1Rearing of *locusta migratoria*:

The initial material adult and nymph , were received from Elgedaref State , Elmganis North Elgedaref coordinate(N 14 31 144 E 035 12 450) . The third generation were reared up to the third instar were then used as the experimental insects .

3.1.1 Hopper rearing cage :

The hopper rearing cage measured (80cm/60cm/50cm) , was made of wood and wired sides which were covered by alight cloth on one side of the cage , fastener, in from of the strip was fitted . This zipper was made to facilitate caring out of the activities of feeding and cleaning by hand inside the cage ,wit out the insects being able to escape away from the rearing cage Plate(1.a).

3.1.2 Food materials :

Sorghum plant *Sorghum bicolor* were grown in the field and , and Millet plant grown in cups , and used for the daily feeding of the nymph and adult plate(1.b) , in addition to wheat bran which was provided for additional supplementary feeding plate(1.c) . The cage were checked daily for cleaning the hopper faeces and changing the food . Adult locusta (fledgling) that emerged from the nymphs were transferred to the egg laying cage .

Plate (1) Rearing cage and food material of Locusta migratoria migratorioides (1a -1c) :

1a. Rearing cage

1b. Food Sorghum

1c. Food Wheat bran

3.1.3 Egg laying cage :

Each egg-laying cage measured (100cm/75cm/55cm) , with five faces , four of them covered with wire mesh , the other one faces were covered by wood and cloth to facilitate the easy handling of insect , cleaning and feeding . On the bottom surface of the cage , about eight holes were made for fixing plastic cups filled with moist sandy soil , these cups were used to provide site for egg laying . Mature adult were put in the egg laying cage Plate(2.c) . After the egg had been laid , the cups which contained sufficient egg pods were removed and replaced by new ones . The egg pods were covered with light cloth and moisture with little water until they hatch .

3.1.4 Experimental cage :

The cage used in experimental were made of wood and wire mash . Each cage measured (30/30/30) cm Plate(2.b) .

The same experimental cage were repeated three times .

3.2 Biological Studies :

These included the following :

3.2.1 Fecundity

Five pairs were collected randomly from the rearing cage each pairs was kept in separate cage (40 cm, 40 cm, 40 cm) made of wood , plywood and mosquito wire net . At the floor of cage one hole each (12cm) indiameter , to fit oviposition Plate(2.a) .

3.2.2 Egg incubation period :

In this test the egg- pods in cups were incubated in outdoor cage . Just before hatching the cups covered with muslin cloth secured by a rubber band until hatching . This facilitated the counting of the hatching and incubation period Plate(2.d) .

Plate (2) Oviposition, Experimental cage, Egg laying and Egg incubation cups (2a-2d) :

2a.Oviposition cage

2b. Experimental cage

2c. Egg laying activity

2d.Egg Incubation cubs

3.2.3 Nymph Development :

To study the development period of *Locusta migratoria migratorioides* isolated and crowded nymph instar , a number of experiments were carried out .

3.2.3.1 Isolated rearing :

Twenty , 2-days old first instar nymph were collected randomly from the stock culture , and reared separately under laboratory condition .

3.2.4 Life tables studies :

Cups containing egg pods were observe until hatching. The hatched nymphs were collected. The remains of the egg pods were inspected to determine hatching percent and fertility. The collected nymphs were placed each 20 in a wire mesh cage (40X40X40 cm). These were observed until fledging. The duration and mortality rate in each stage was recorded. The emerging adults were placed each pair in the above mentioned cages and data on pre-oviposition, oviposition rate and mortality collected from these data the life tables data and parameters were analyzed and Charted:

Net Reproductive Rate (Ro) = $\sum$ Lx*Mx.

Weighed Generation Time (T) =$\sum$(X*Lx*Mx)/Ro.

Doubling Time DT = Natural Anti log 2/rm.

RO = Female eggs per female per generation.

T = Period from egg to adult mortality.

DT = The time required by the species to double its population size.

3.3 Preparation of the test product:

3.3.1 Preparation of phynilacetonitiril(PAN) :

Four dose of Phynilacetonitiril (PAN) (5% ,10% ,15% ,20%) were tested. These were diluted in diesel to bring the volume to 2 liter .

3.3.2 Diesel

Diesel was often used as acarrier when spraying *Locusta migratoria* with control agent. Here all treatments in this investigation were prepared with diesel as acarrier. Consequently , a diesel treatment was included in the tested as control.

3.4 The application techniques :

3.4.1 Application of the Phynilacetonitiril (PAN).

The aggregation Pheromone Phenylacetonitrile (PAN) formulation was manufactured by Sigma–Aldrich Chemie Gmb H, Steinheim, Germany,

and obtained from International Center of Insect Physiology and Ecology (ICIPE) stock at station in Port-Sudan. The formulation was used at a rate 5, 10, 15and 20 ml, of the active ingredient of the (PAN) pheromone as the four tested concentrations and diluted to make 2000 ml by the appropriate amount of diesel fuel .

An appreciable amount of diluted concentrations of the aggregation pheromone was applied on a piece of cotton swap, and then hanged with a string from the top center of the cage. This step is to ensure a cloud of the pheromone vapour inside the cage.

3.4.2 Application of Malathion 96% :

Applications were carried out by a hand-held ULVA + sprayer which were powered by D- cell / R20 batteries. An electric motor spins the atomizer disc to produce uniform spray droplet size.

3.5 The experiment :

The third nymphal instar were expose to PAN . Each cage contained twenty nymphs and was considered as an experimental unit plate. The experiment was arranged in a completely randomized design and replaced three time .

3.6. Statistical analysis

A Completely Randomized Design (CRD) was used for setup of the experiments. The obtained data were analyzed according to SAS programmer version3, SAS, 1997 and using SPSS 16 version for windows. Using analysis of variance (ANOVA). The accepted level of significance as ≤ 0.05 and means were separated using the least significant difference (LSD) according to Gomez and Gomez (1984).

CHAPTER FOUR

RESULT

4.1 Biological Studies

4.1.1 Egg stage :

4.1.2 Copulation period :

The result in table (8) showed that , the period after fledging to copulation ranges between (11,14) days with the means (12.5) days .

4.1.3 Fecundity :

The result in table (8) showed that , the mean number of egg- pods per female ranges between (2,3) and (4) egg pods , with a mean of (2.2) egg-pods per female and egg per pods ranges between (42,68) with Means (55) egg per pods .

4.1.4 Egg incubation period :

As shown in table (8) Plate, the incubation period of egg pods range between(11,12,13) and (14) days with the mean (12.5) days.

4.2 Nymphal Development :

4.2.1 Nymphal stage :

Table (9) , showed the percent survival and developmental period of the different instars , the total developmental period from first instars to adult emergence varied between(25_29)days with the means of 27 days Plate(3f) .Result showed the range of duration of the different instars. First instar 4-5 days with means 4.5days , Plate (3.a). Second instar 4-5 days with means 4.5 days, Plate (3.b). Third instar 4.5 days with means 4.5 days, Plate (3.c). Four instar (5-7) days with means 6 days, Plate (3.d). Five instar 6-7 days with means 6.5 days, Plate (3.e).

Plate (3) Development Stages of *Locusta migratoria migratorioides*(3a-3f)

3a :First instar

3b: Second instar

3c: Third instar

3d: Fourth instar

3e: Fifth instar

3f: Adult *Locusta migratoria*

Table (8) Inset of copulation, number of pods , number of eggs per pod and incubation period of *Locusta migratoria migratorioides*:

Cage	Copulation after fledging	Numbers of Pods	Numbers of egg	Incubation Period
Pair 1	**11 Days**	**2 Pods**	**111 eggs**	**11 Days**
Pair 2	**13 Days**	**4 Pods**	**238 eggs**	**14 Days**
Pair 3	**14 Days**	**3 Pods**	**154 eggs**	**12 Days**
Pair 4	**11 Days**	**2 Pods**	**112 eggs**	**13 Days**
Pair 5	**0**	**0**	**0**	**0**

Table (9) Development period in days from First instar to Fledging of *Locusta migratoria migratorioides* in three experimental cages:

Days	**Number/cage**						**Fledging %**
	Cage 1		**Cage2**		**Cage 3**		
25	**1**		**0**		**0**		**1.67 %**
26	**5**		**3**		**2**		**15 %**
27	**11**		**4**		**0**		**11.67 %**
28	**18**		**10**		**12**		**11.67 %**
29	**20**		**18**		**17**		**38.33 %**
Total	**20**		**18**		**17**		**91.67 %**
	m	**f**	**m**	**f**	**m**	**f**	
	8	**12**	**10**	**8**	**9**	**8**	**1: 1=**

*****m= male

*****f= female

4.2.2 Life Table data :

Life table data are present in figures (2) and (3) the data was analyzed according to the formulae in material and methods :

The life table parameter of *Locusta migratoria migratorioides* the survival rate from first instar to die last one of adult , the net reproductive rate(RO) was (738.71) , the weight generation time (T) was (98.8), while the doubling time (DT) was (10.36) , innate capacity of numerical increase (rm) was (0.067) and the finite rate of increases(ʌ) was (1.166).

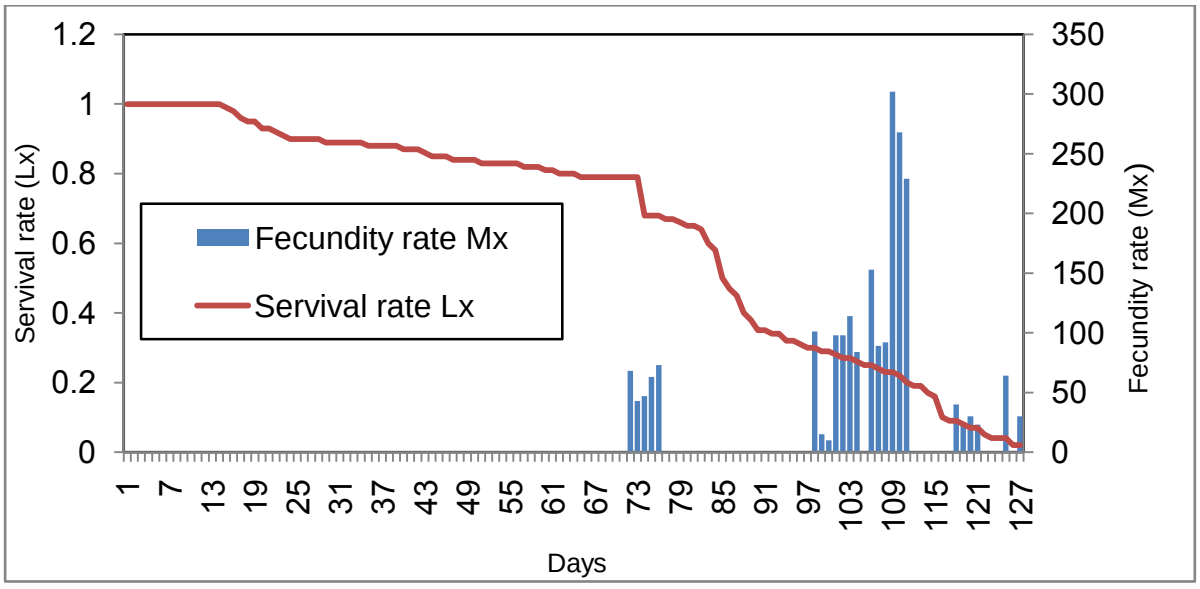

Figure (2) Life tables data chart of *Locusta migratoria migratorioides* reared on Sorghum

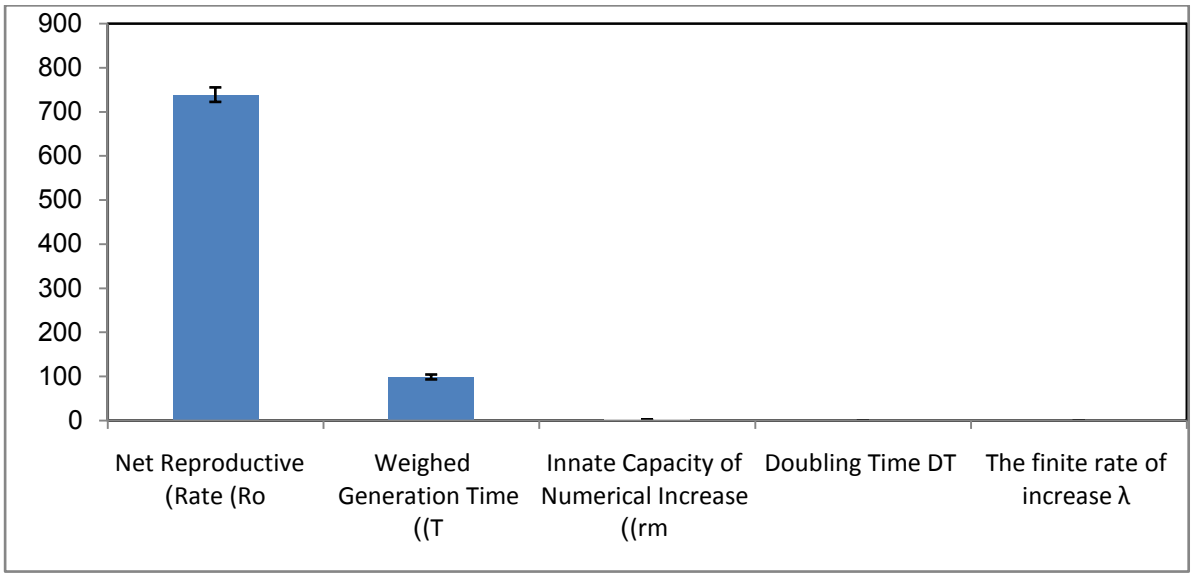

Figure (3) life table data parameter of *Locusta migratoria migratorioides* reared on Sorghum

4.3 The effect of Phenylacetonitrile(PAN)on survival rate of *Locusta migratoria migratorioides*:

The result of data present in table (10) and figure (4) , showed the significant effect of different concentrations of the gregarious adult pheromone of desert locust Phenylacetonitrile (PAN) when used alone .

All treated nymphal of *Locusta migratoria migratorioides* did not die instantly but only after four days except the concentration 15% PAN they died after 24 hour. The result show the effect of PAN on nymphal *Locusta migratoria migratorioides* was increasing according to the less concentration . The effect of PAN Control, Diesel ,5% PAN , 10% PAN , 15% PAN ,20% PAN , and Malathion 96% were 5.43±2.17, 38.76±00, 70.09±8.57, 86.33±3.839, 92.02±8.241 ,49.8±3.187, 270±00 respectively .

4.4 The effect of Phenylacetonitrile (PAN) on the morphology of *Locusta migratoria migratorioides:*

The effect in deformation and failure of molting (Plate 4.a ,4.b), seen the colouration is dark brown (Plate 4.c) .

Table (10) Mortality of *Locusta migratoria migratorioides* after treatment with PAN and Malathion 96% fed on Sorghum:

Treatments	Days after treatment application				
	3 days	6 days	9 days	12 days	15 days
Control	5.43±2.17	5.43±2.17	5.43±2.17	5.43±2.17	5.43±2.17
Diesel	5.43±2.17	16.54±6.414	27.65±6.414	38.76±00	38.76±00
5% PAN	5.43±2.17	44.28±3.187	54.15±4.947	66.88±6.674	70.09±8.527
10%PAN	5.43±2.17	16.54±6.414	52.4±7.875	74.44±7.590	86.33±3.839
15%PAN	37.52±10.496	54.93±14.289	81.65±7.768	89.06±6.745	92.02±8.241
20%PAN	5.43±2.17	27.65±6.414	27.65±6.414	38.76±00	49.8±3.187
Malathion96%	270±00	270±00	270±00	270±00	270±00

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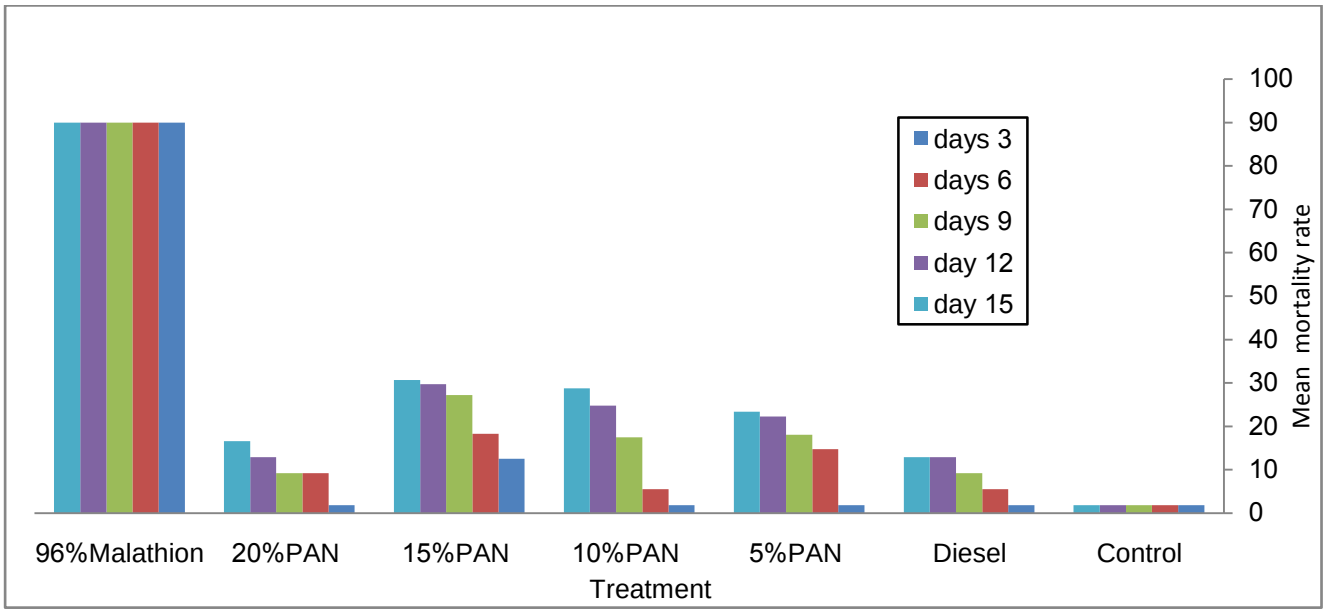

Figure (4) Mortality of *Locusta migratoria migratorioides* after treatment with PAN and Malathion 96% fed on Sorghum.

4a. Deformation due to PAN exposure

4b:Failure Molting due to PAN exposure

4c: Change in colour due to PAN exposure.

Plate (4): Deformation ,moulting failure and changing in colour exposed to PAN (4a-4c)

4.5: The effect of Phenylacetonitrile (PAN)on feeding rate per gram of *Locusta migratoria migratorioides* :

The effect of Phenylacetonitrile in the concentrate 10% and 15% is highly significantly compared with the control and the other concentration .

The result showing in table (15) figure (5) the concentration control , Diesel , 5% , 10% ,15% ,20% and malathion 96% were 5.69±0.345, 4.9±0.484 , 2.87±0.105, 1.3±0.191, 1.31±0.035, 1.41±0.151, 0.32±0.021 respectively .

Table (11) Effect of PAN and Malathion 96% on feeding rates of *Locusta migratoria migratorioides* fed on Sorghum.

Concentration	Consumed Food per Grams				Percentage of consumed food
	Cage 1	Cage 2	Cage 3	Means	
Control	2.06	1.5	2.13	1.9	32.1 %
Diesel	1.56	1.19	2.15	1.63	27.53 %
5% PAN	0.85	0.96	1.06	0.96	16.22 %
10 % PAN	0.49	0.59	0.22	0.53	9 %
15 % PAN	0.44	0.4	0.47	0.44	7.43 %
20 % PAN	0.3	0.59	0.52	0.47	7.94 %
Malathion 96%	0.1	0.09	0.13	0.11	1.9%

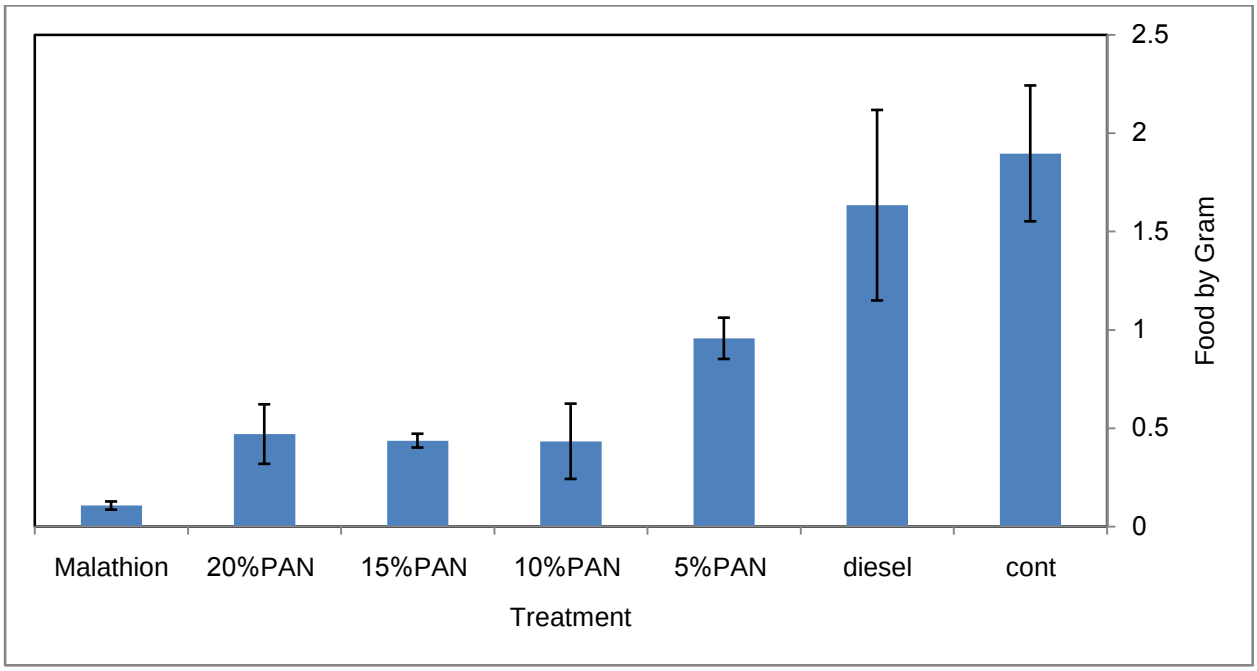

Figure (5) Effect of PAN and Malathion 96% on feeding rates of *Locusta migratoria migratorioides* fed on Sorghum.

Chapter five

DISCUSSION

Fecundity as in table (8), the egg-pods per female range between (2-4) egg-pods and egg per pods range between (42-68). This result is similar to that reported by Steedman (1988).The number of pods laid by a single female varies considerably as does the interval between successive laying . During the winter months in the outbreak area locusts have been found to lay (1-3) pods each at intervals of about 20 days, while in the summer they may lay(2-4) egg pods each at intervals of only (3-4) days. Solitary locusts lay about 65 eggs (range 55-110 per pod which falls to 39 eggs per pod for gregarious females) .

Nymphal development in table (9) plate (1.b), the total period from first instar to adult emergence varied between (25-29) days. This result is similar to that reported by Steedman (1988). The duration of each instar is, as in the Desert Locust, dependents on environmental factors of which the most important is temperature. In the laboratory hoppers developed faster in humid conditions (38 days) than in dry conditions (57 days). Records from the last plague in both East and West Africa show that the whole hopper period lasts (30-60) days for the five instars. In the Middle Niger area, hopper development lasts only (24-35) days.

The incubation period in table (8) plate (2.a) range between (11- 14) days this result similar to that reported by Steedman (1988). For successful development *Locusta* eggs must absorb water from the soil in the early stages. Provided that there is adequate water in the soil the incubation period is dependent upon temperature. In the outbreak area of the Niger flood plains the incubation period is generally(10-20) days in the summer and (20-40) days

in the winter. Eggs laid in dry soil, or in soil that dried out rapidly after laying, may take over (100) days to hatch .

Phenylacetonitrile (PAN) is the major component of gregarious adult pheromone . Recently Phenylacetonitrile (PAN) combined with the fungus *Metarhizium acridum* was classified as an efficient alternative for chemical pesticides (Hassanali and Bashir 1999, Hassanali and Bashir 2010 , and Ferenz *et al*. 1993).

The present study also included investigation and evaluation of the insecticidal effect of the aggregation pheromone (PAN) on the growth, development and mortality rates of *Locusta migratoria migratorioides* nymphs .

According to the result (Table 10, Figure 4), the effect of Phenylacetonitrile (PAN) on the instars treated with 5 ml, 10ml , 15ml , 20ml) showed that they are more sensitive to PAN concentrations at both 10 ml, 15 ml. These results were similar to that obtained by Ferenz *et al* (1993); Hassanali and Bashir (2010) who showed that PAN combined with the fungus *Metarhizium acridum* is more efficient and can be an alternative for chemical insecticides against the Desert Locust.

Also the result showed that the treated insects with different PAN concentrations do not die instantly but only after the four days after application expect 15 ml the mortality was after 24 hour. This finding is in agreement with Seyam (2012) who stated that PAN alone did not cause satisfactory mortality to the desert locust at initial time of application.

Also the result of this study is in agreement with Ould Ely *et al* (2006) who stated that studies of PAN combined with Green Muscle gave good results in the management of desert locust. This point strengthened that PAN alone has low effect but, when combines with fractional doses of insecticide has high effect.

The result of the effect of PAN concentration 10 ml , 15 ml 20 ml (table 15 Figure 5) revealed good anti feeding to Locusta migratory and there was no significant difference between the three concentrations. This is in agreement with results

stated by Kooyman (2003) who showed that the application of PAN at concentration 0.5% on desert locust nymphal instars affected the feeding behaviour one hour after treatment. This confirms that the PAN has the potential to influence the feeding behaviour as antifeedant.

Results of PAN concentrations in (Table10 and Plate 4.a ,4.b ,4.c) revealed different other lethal effects such as deformation and moulting failure in nymphs. This finding is in line with Kane (2004) who showed that PAN caused significant decrease in total haemocytes count which has direct effect on moulting processes. Also the result is in agreement with Mohamed *et al* (2000) who stated that PAN in general enhanced mortality rate and induced disruptive effects characterized by delay moulting and malformations which mostly affected wings, legs and antennae. Also Kane (2004) stated that PAN showed deleterious effects on conspecific nymphs and whenever combined with Dursban insecticide at low dose, PAN caused significant mortality of hoppers within 24 hours more than PAN alone or *Metarhizium* alone. Also the result in this study is in line with findings by Lecoq (2010) who reported that low concentration of PAN application has resulted in good effect in gregarization behaviour and enhanced the efficacy of Fenitrothion insecticide on late instar nymphs and enhanced mortality rate.

CONCLUSION and RECOMMENDATIONS .

-The results showed that the Phenylacetonitrile (PAN) pheromone possesses a potential role in the future of management programmed of *Locusta migratoria migratorioides* like the Desert locust *Schistocerca gregaria* .

-Significant differences were found among the treatments or concentrations of (PAN) pheromone such as in food intake, and mal-moulting, deformation and malformation, and mortality of nymphs.

- Further testing of different doses of Phenylacetonitrile (PAN) on different nymphal instars of *Locusta migratoria migratorioides* is needed.

- Further research in need to investigate the economical cost of using Phenylacetonitrile (PAN).

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Appendixes

Appendix 1 .Analysis of Variance of Table(10) 3Days After Treated with PAN :

ANOVA

Source	df	ss	ms	F ratio
Treatment	6	19484.87	3247.478	206.3421**
Error	14	220.3365	15.73832	
Total	20	19705.2		

High significances at the LSD>5%

Appendix 2 .Analysis of Variance of Table(10) 6Days After Treated with PAN :

ANOVA

Source	df	ss	ms	F ratio
Treatment	6	17380.34	2896.724	60.03103**
Error	14	675.5528	48.25377	
Total	20	18055.89		

High significances at the LSD>5%

Appendix 3 .Analysis of Variance of Table(10) 9Days After Treated with PAN：

ANOVA

Source	df	ss	ms	F ratio
Treatment	6	16111.16	2685.193	82.03666**
Error	14	458.2427	32.73162	
Total	20	16569.4		

High significances at the LSD>5%

Appendix 4 .Analysis of Variance of Table(10) 12Days After Treated with PAN :

ANOVA

Source	df	ss	ms	F ratio
Treatment	6	15089.85	2514.974	119.2299**
Error	14	295.3088	21.09349	
Total	20	15385.15		

High significances at the LSD>5%

Appendix 5 .Analysis of Variance of Table(10) 15Days After Treated with PAN :

ANOVA

Source	df	ss	ms	F ratio
Treatment	6	14721.17	2453.529	103.7598**
Error	14	331.0474	23.64624	
Total	20	15052.22		

High significances at the LSD>5%

Appendix 5 .Analysis of Variance of Table(15)

ANOVA

Source	df	ss	ms	F ratio
treatment	6	8.285581	1.38093016	22.69667**
Error	14	0.8518	0.06084286	
Total	20	9.137381		

High significances at the LSD>5%