Sudan University of Science and Technology

College of Graduate Studies

Biology, Ecology and Biocontrol Trials of Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in Sudan

بيولوجية و بيئة وتجارب مكافحة بيولوجية للبق الدقيقى فى السودان

A Thesis submitted in Fulfillment of the Requirements for the Degree of Ph.D in Plant Protection

By:

Nawal Ahmed Mohamed Abdel Rahman

B.Sc. (Plant Protection) University of Khartoum, 1985.

M.Sc. (Environmental Science), University of Khartoum, 2000.

Supervisor: Prof. Dr. Awad Khalafalla Taha

Co Supervisor: Dr. Saif Eldin Mohamed Khair

College of Agricultural Studies - Shambat

Department of Plant Protection

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بسم الله الرحمن الرحيم

Dedication

I dedicate this thesis to the soul of my lovely mother (Zeinab) and father (Ahmed), God bless them.

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I am very indebted to many people who helped me from the begining of this research to finishing this thesis; therefore, it pleases me to express my deep gratitude to them.

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ABSTRACT

During 2012/ 2013 high infestation with mealybugs was recorded from different States of Sudan, on vegetables, fruits, ornamental plants and weeds; therefore, the objectives of this study were to investigate some aspects of this invasive pest in order to come out with effective strategies for its management.

Surveys were conducted during the period 2015- 2019 to collect samples of mealybugs species in Khartoum State, and some other States in Sudan (Kassala, Gazira and Northern States) for identification and afterward, studying the biology, host preference, seasonality and incidence of infestation, in addition to management by different control measures.

All samples of mealybug collected were identified as Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). It was observed after the rainy season on Okra and on weeds, and migrates and become a source of infestation to the winter crops. From the results of the surveys, the population of mealybugs was high in March 2016 on tomato and egg plant (season 2015/ 2016) and low in Season 2016/ 2017 due to high rain fall.

The biological parameters of *P solenopsis* were evaluated under laboratory conditions on four important crops: cotton (*Gossypium* sp), Okra *(Abelmoschus esculentus*), Tomato (*Lycopersicon esculentus*) and Sweet peper (*Capsicum annuum*), during the period January to April 2016.

The longest period of development for the female and male was observed on nymphs reared on cotton.the longest oviposition period and the average total fecundity were recorded on cotton and okra.

For food preference of *P. solenopsis* , maximum population were recorded on plants of family Malvaceae at interval of 24 hours, particularly, on Okra, followed by cotton and hambouk as compared with the control (*Hibiscus rosa-sinensis*). While, the proportions were very low on unpalatable plants; raba and eggplant.

In Sudan, a diversity of indigenous parasitoids and predators has been reported associated with mealybugs. Two of which were chosen: the Predator, *Exochomus nigromaculatus* (Goeze) (Coleoptera: Coccinellidae) and the parasitoid, *Aenasius arizonensis* (Girault) (Hymenoptera,Chalcidoidea).Their biological chararcteristics were studied to evaluate their effectiveness as a successful biocontrol agents of *P. solenopsis* .

From the results, Predator, *Exochomus nigromaculatus* consumed and preferred the 3rd instar nymphs of *P. solenopsis* (when fed on a mixed diet of the 3rd and adult females). The coccinellid females consumed more prey than males and the 4th instar grub.

From the results, the parasitoid, *Aenasius arizonensis* was available most of the year. It was highly effective on adults and the 3rd instar nymph of *P. solenopsis,* with a total percent parasitism of 31.26%

in the field, and 20 to 24 in terms of number of Parasitized host female / day under laboratory conditions.

The botanical insecticides Kafur, *Eucalyptus camaldulensis*,was evaluated against *P. solenopsis* using four concentrations (2.5%, 5%, 7.5% and 10%) . The highest percent mortality of Eucalyptus essential oil extracts was obtained by the topical method; where Ethanol extracts gave 100% mortality, followed by petroleum ether extracts, and lowest mortalities recorded from the aqueous extracts for both topical and dipping methods.

The total life cycle of Cotton mealybug ranged from 21 to 39 days for female and 16 to18days for male, according to the host plants. Family Malvaceae was the most preferred and play an important role towards pest maturity and oviposition.

The present study confirmed that, the natural enemies studied, have a potential future in pest control if subjected to further studies. In addition, more research is needed to identify the main active ingredients in the different parts of Eucalyptus tree.

ملخص البحث

فى الاونة الأخيرة , خلال الاعوام 2012- 2013 سجلت اصابات عالية بالبق الدقيقى على الخضروات ، الفاكهة ، نباتات الزينة و الحشائش فى ولايات السودان المختلفة ، لذا هدفت هذه الدراسة لبحث بعض المفاهيم حول هذه الآفة الغازية للخروج باستراتيجية فعالة لإدارتها.

أجريت مسوحات حقلية في الفترة من 2015 – 2019 لجمع عينات البق الدقيقي من ولاية الخرطوم وبعض الولايات الاخرى من السودان (كسلا ، الجزيرة و الولاية الشمالية) لتصنيف الآفة ودراسة بيولوجيتها ، عوائلها المفضلة و موسميتها بالاضافة الى إدارتها بطرق مكافحة مختلفة.

اكد التصنيف ان كل العينات التى جمعت هى بق القطن الدقيقى (أو البق الدقيقى) المعروف باسم :

Cotton mealybug, *Phenacoccus solenopsis* **Tinsley**

(Hemiptera: Pseudococcidae)

والذى لوحظ بعد فصل الخريف على محصول البامية الخريفية و بعض الحشائش , و كانت مصدراً لنقل الإصابة للمحاصيل الشتوية . نتائج المسوحات أثبتت كذلك أن كثافة الآفة كانت عالية فى مارس 2016 على محصولى الطماطم و الباذنجان (موسم 2015/ 2016) و بسيطة موسم 2017/2016 لكل محاصيل الدراسة.

تم تقيم البيولجية لآفة البق الدقيقى تحت ظروف المعمل على اربعة محاصيل : القطن (*Gossypium* sp) ، البامية (*Abelmoschus esculentus*) ،الطماطم (*Lycopersicon esculentus*) و الفلفلية (*Capsicum* annuum) فى الفترة من يناير الى ابريل 2016. سجلت أطول فترة نمو للإناث و الذكور على القطن، بينما سجلت اطول فترة وضع البيض و أعلى متوسط للخصوبة (متوسط عدد الحوريات / انثى) على القطن و البامية.

سجلت دراسة سلوك و افضلية الغذاء للبق الدقيقى *P.solenopsis* و كانت أعلى كثافة للآفة (بعد 24 ساعة)على العائلة الخبازية (Malvaceae) بالاخص على البامية , يليها القطن ثم الهمبوك (*Abutilon pannosum*) مقارنة بالشاهد (الهبسكس *Hibiscus rosa senensis*) , وكانت ادنى كثافة للآفة على الباذنجان(*Solanum melongena* L.) و الربعة (*Zaleya pentandra*) مما يؤكد عدم افضليتها و ملاءمتها لتغذية الآفة.

رصدت أنواع من الاعداء الحيوية فى البيئة السودانية تتغذى على البق الدقيقى ، تم اختيار اثنين منها لدراسة فعاليتها فى مكافحة الآفة ,هما :

1. The Predator, *Exochomus nigromaculatus* (Goeze)
2. The Parasitoid, *Aenasius arizonensis* (Girault)

اثبتت الدراسات البيولوجية أن المفترس *Exochomus nigromaculatus* يفضل الطور الثالث على الطور الكامل (الاناث) من البق الدقيقى (عندما أعطى خليط من الطورين) . سجلت الاناث اعلى نسبة افتراس للآفة مقارنة بالذكور و الطور الرابع .

الطفيل *A. arizonensis* متواجد طول السنة متطفلاً على البق الدقيقى على الطور

الثالث و الطور الكامل (الاناث) , وسجلت النسبة المئوية للتطفل فى الحقل ب 31.26 % و فى المعمل قدر من حيث عدد الاناث التى تم التطفل عليها ب 20-24 فى اليوم.

تم تقييم فعالية مستخلص نبات الكافور *Eucalyptus camaldulensis* بالتركيزات

(2.5%، 5%، 7.5%، 10%) على الطور الكامل اناث و سجلت أعلى نسبة موت باستخدام الطريقة الموضعية حيث اعطى مستخلص الايثانول نسبة موت 100% , يليه مستخلص بتروليم ايثر. المستخلص المائى سجل اقل نسب موت بالطريقة الموضعية وطريقة الملامسة.

من هذا البحث نجد ان دورة حياة البق الدقيقى تتراوح بين 21 الى 39 يوم للانثى و

16 الى 18 يوم للذكر على كل النباتات تحت الدراسة، و ان العائلة الخبازية لها الافضلية لبلوغ الآفة مرحلة النضج و التبويض.

اثبتت هذه الدراسة أن الاعداء الحيوية التى تمت دراستها لها مستقبل واعد فى المكافحة و تقليل الاصابة و ذلك من خلال مزيد من الدراسات , بالاضافة الى تكثيف البحث لتعريف اكثر للمواد الفعالة للاجزاء المختلفة لشجرة الكافور.

CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

Khartoum State ranks top among other States in Sudan regarding the production and consumption of vegetables that relates to the higher increase in population growth, income level and nutritional awareness (Imam, 2010).

Many types and cultivars of fruits and vegetables can be produced almost all the year round due to the climatic variation plus available land and water (Elbashir and Imam, 2010). The vegetable crops are subject to pest damages on seeds, fruits, leaves, stems and roots which ranged from reduced plant vigor to plant death and crop loss (Rondon *et al.*, 2008).

Polyphagous insects such as whitefly, aphids and African boll worm are considered as major pests, inflicting economic damage on the crops which are mainly produced during winter season (Elshafie and Abdel-Raheem, 2012). Hence, very devastating species of alien pests were reported inflicting serious economic damage on various field and horticultural crops and store products. Most of these pests, including species of fruit flies, bollworms and the green pit scale insect of date palm, still causing economic impact and financial burden to the country (Satti, 2011).

A number of mealybug species have been reported attacking vegetables, fruit trees and field crops all over the world. More than 160 mealybug species have been identified as pests worldwide and most of them are invasive species (Shah *et al.*, 2015).

Recently, *phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has acquired the status of major insect pest in most growing areas of the world, and there are difficulties facing entomologists in management of *P. solenopsis* because it is polyphagous in nature. It infests more than 154 plant species in 53 families and level of infestation varied among different plant species (Vennila *et al.*, 2010).

The mealybug, *phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has a wide geographical distribution, with its origin in Central America followed by reports of the Caribbean and Ecuador, Chile, Argentina and Brazil. *Phenacoccus solenopsis* has been described as a serious and invasive pest of cotton in Pakistan and India, and on *Hibiscus rosa-sinensis* in Nigeria. Latest reports on the invasiveness of *P. solenopsis* have been reported from the eastern region of Seri lanka on ornamentals, vegetable crops and weed, and from China on cotton (vennila *et al.*, 2010).

In Sudan, Schumutterer (1969) recorded three species of mealybug, *Ferrisia virgata*, guava mealybug, *Phenacoccus hirsutus*, hibiscus mealybug and *Pseudococcus citri*, the citrus mealybug. Guava mealybug, *Ferrisia virgata* has been found in the Northern Province, Khartoum Province , Blue Nile Province, Kordofan Province, Darfur Province and Kassala Province. The species is most probably present in the whole country wherever its host plants occur. While hibiscus mealybug, *Phenacoccus hirsutus* is common in irrigated areas of Khartoum, Blue Nile and Kassala Provinces.

The citrus mealybug, *Pseudococuus citri* is a common insect in Equatoria Province where it occurs mainly in coffee plantations and fruit gardens. In addition, Satti and Abdalla (2014) studied the biology, ecology, damage, natural enemies and host range of the mealy bug, *Planococcus citri* (Risso) of citrus, during 2004-2005 in Gazira State.

During 2012/ 2013 infestation with mealybugs was recorded from cultivated and wild plant species in different States of the country, so a survey was conducted in Gazira and Khartoum States during 2015, where samples of mealy bugs from Cotton, Okra and *Abutilon* spp. were sent to Dr. Gillian W.Watson (Department of Food and Agriculture, Plant Pest Diagnostic Centre, Sacramento, CA, U.S.A.) Who confirmed the specimens as *Phenacoccus solenopsis* Tinsley. The survey showed that, at least 26 host plant species that belong to 16 plant families are host plants (Mohamed *et al.*, 2015).

Mealybugs are small sap sucking insects that cause severe economic damage to cotton and a wide range of vegetables, horticultural and other field crops. Plant infested by mealy bugs during vegetative phase exhibit symptoms of distorted, bushy shoots, crinkled and stunted plants that dry completely in severe cases (Nagrare *et al.*, 2011). The principal damage associated with mealy bugs arises also from their secretion of honeydew, which encourages the growth of sooty moulds such as *Aspergillus* spp. Heavy infestations of mealy bugs can result in premature leaf fall, which may affect the ability of a canopy to mature a crop or storage of carbohydrates. Mealy bugs have also been identified as vectors of grapevine leaf roll-associated viruses. Some of these viruses can reduce vegetative growth, yield and fruit quality (Braybrook, 2012).

The mealybug has ability to build populations within shoots and apexes and become difficult to control with foliar application of pesticides because of the waxy secretions on their body surface (Shah *et al.*, 2015).

Justification of the study:

Mealy bugs are spreading all over Khartoum State damaging vegetables, fruits and ornamental plants; no information is currently available in Sudan on its biology, ecology and management. Therefore, this study was conducted to investigate some aspects of this invasive pest in order to come out with effective strategies for its management.

Objectives of the study:

1. 1dentify mealy bugs species found on vegetable, field crops and ornamental plants in Khartoum State and some other areas in the Sudan.

2. Study the biology and ecology of the most important species of mealy bugs.

3. Investigate the potential of using Eucalyptus leaf extracts against mealy bugs as alternative to chemical pesticides.

4. Identify the active constituents of the most effective plant extracts.

5. Study the biology and investigate the predatory potential of ladybeetle, *Exochomus nigromaculatus* (**Goeze**) as one of the natural enemies of the pest.

6. Study the biology and parasitism rate of *Aenasius arizonensis* (Girault) on cotton mealybug '*Phenacoccus solenopsis*'.

CHAPTER TWO

LITERATURE REVIEW

2. LITERAURE REVIEW

2.1. Background to Scale insects and Mealybugs:

The Scale insects and mealy bugs fall under the super family Coccoidea, order Hemiptera. The name refers to their secretion of waxy scales that serves as a protective covering. They range in size from 1.5 mm to 25 mm in length. They are economically important insects, since many species attack different parts of plant and feed by sucking the plant sap. On the other hand, a few species of scale insects are beneficial to mankind, e.g. the cochineal insect (*Dactylopius indicus*) yields a carmine dye and the lac insect (*Kerria lacca*) is the source of shellac (Ben-Dov *et al*., 1997).

The soft scale insects (Insecta: Hemiptera: Coccoidea: Coccidae) constitute a family among the 21 families of scale insects, Coccoidea. The soft scales are plant – feeding insects which develop mainly on perennial, but occasionally on annual plants. Member of this family, about 1100 described species, are distributed in all zoogeographical regions extending to the north and south latitudes 60-65◦. The family, like other families is characterized by a very distinct sexual dimorphism. The adult female is always wingless

with complete of the head, thorax and abdomen into a flattened or globular sac- like body, while the male is usually winged insects with a clear division of the body into head, thorax and abdomen (Ben-Dov *et al*., 1997). Eggs or first instars are normally laid in an ovisac that can enclose all or part of the body of the female (Miller, 2005).

A bout 7,500 species of Scale insects and mealy bugs are known world wide, and inhibits almost all parts of the globe, in all floral habitats, from the tundra to the tropics (Varshney *et al*, 2002).

Mealybugs can be thought of as a kind of soft scale that does not form the protective cover that most scales produce for protection. The eggs hatch into mobile nymphs, adult of most species are also active , unlike scales where the crawler find a suitable site for feeding and then remain fixed, mealybugs will move about to find feeding sites (Johnson,2009).

Mealybugs occur in all zoogeographic regions of the world and are abundant in most ecosystems. There are 1,989 species and 271 genera worldwide and in the United States there are 351 species in 48 genera; and in the Southern region of the United States there are 155 species and 37 genera (Miller, 2005).

Mealy bugs (Hemiptera: Pseudococcidae) are small, soft bodied plant sap-sucking insects that constitute the second largest family of scale insects (Hemiptera: Coccoidea). During the last few years mealy bugs, which were considered to be minor pests in many crops, have acquired the status of major pests especially in cotton, vegetables and fruits (Banu and Devaraj, 2013).

There are numerous species of mealy bugs (Pseudococcidae) recorded from cotton, many of them having a world wide distribution. The mealy bugs are relatively mobile, soft- bodied, oval insects, few millimeters long, of which the body segments are well – defined, covered in powdery wax, and have usually a fringe of waxy filaments.

The nymphs and adult females resemble each other in form, the male is rarely seen and is minute delicate- winged insect quite unlike the female (Pearson, 1958).

2.2. Origin and Distribution of Cotton mealybugs:

Species are considered invasive if they are transported outside their native range and become established, spread, and adversely impact the environment by threatening the ecosystems. Effort has been devoted to determine shared characteristics of invasive species in order to evaluate the risk of invasion by new species and to identify approaches for management of those already established (Helms and Vinson, 2002).

Phenacoccus solenopsis was originally discovered in U.S.A. by Tinsley (1898) on cotton and was considered as a minor pest of cotton until 1990. It attained invasive status when reported from other countries. In Chile, from 1995 to 1997, it was seen as a pest of Papino (*Solanum mericatum* Aiton, Solanaceae), in Brazil (2005) as a pest of tomato (*Lycopersicon esculentum* Miller, Solanaceae), and in Pakistan, it was reported in 2005 as a pest of cotton. Then in India (2008) cotton mealybug was reported from all the cotton growing centers (Shahid *et al.,* 2012). In Egypt, the occurrence of *Phenacoccus solenopsis* infestation was recorded on weed plants by Abd-rabou *et al.,* (2010) and later recorded on tomato plants by Ibrahim *et al.,* (2015).

The genus *phenacoccus* currently contain about 180 species and is one of the largest genera in the pseudococcidae (Ben-Dov, 1994).

2.2.1. Taxonomy and Nomenclature of *P.solenopsis*:

Mealybug species was first described by Tinsley (1898) and it was named as *Phenacoccus solenopsis* by Ferris (1950). Further, the taxonomic description of this mealybug based on the morphology of adult female has been provided by many workers from different parts of the world. The present taxonomic position of *P. solenopsis* is as shown in the taxonomic tree below (Fand and Suoshe, 2015).

Phylum: Arthropoda

Subphylum: Uniramia

Class: Insecta

Order: Hemiptera

Sub order: Sternorrhyncha

Super family: Coccoidea

Family: Pseudococcidae

Genus: *Phenacoccus*

Species: *Phenacoccus solenopsis* Tinsley

2.2.2. Species Description:

Adult females of *P. solenopsis* measure about 2- 5mm in length, and 2-4 mm in width. They are covered with a powdery, waxy secretion with six pairs of transverse, dark band that located across the thoracic segments. A series of waxy filaments extend from around the margin of the body with the pair of longest terminal filaments. Females possess nine segmented antennae and five segmented legs with translucent pores on femur and tibia of hind legs. Ventrally female is clearly distinguished by the absence of quinqueloclar pores. Dorsum bears 18 pairs of cerarii (Miller, 2005).

2.3. Feeding and Damage Symptoms of Mealybugs:

Large populations of mealybugs cause general weakening, defoliation, and death of susceptible plants. Indirectly, it may also damage plants by serving as vectors of plant diseases. Moreover, the honeydew excreted by the mealybugs causes growth of sooty moulds and other secondary infections that decreases photosynthesis and reduces the marketability of the plant products (Ibrahim *et al.*, 2015).

Scales and mealybugs prefer nitrogen rich succulent tissues to feed on, more nitrogen contents in host plants increased the survival, longevity, fecundity, and hatchability of sucking insect pests, as well as hatching capacity of their eggs (Dogar *et al.*, 2018). Over fertilization and watering, can lead to problems with these pests (Goble *et al.*, 2012).

They are found on nearly all parts of the host plant including the leaves, branches, trunks, fruits, and roots. They sometimes occur under bark and may cause various kinds of plant deformities including chlorotic spots, pits, and galls. They cause severe damage to economically important plants like sugarcane, tea, coffee, cotton, sorghum, vegetables, greenhouse plants and almost all kinds of fruit trees (Varshney *et al*, 2002).Once the species has established on a host plant, it has the capabitity of rapid growth resulting in significant damage to the crop. Sharma (2007) documented a seasonal outbreak of *P. solenopsis* on Okra (*Abelmoschus esulentus*) in 2007, which developed into a heavy infestation on the crop by the end of the season and resulted in a 90% loss of seeds.

2.4. Life Cycle of Mealybugs:

The life cycle of the females contain two or three nymphal instars and the adult females. The male develops through two nymphal instars plus a pre-pupa and a pupa before emerging as the winged adult male. The dorsum of all stages is always covered by a soft, waxy covering which varies considerably in both texture and structure between the various sub- families (Ben-Dov and Hodgson 1997).

Mealybugs have a three stage of life history egg, nymph or crawler and adult. The egg hatch into mobile crawlers which are the most active stage that can move between plants and develop through several growth periods before becoming adults. Adults of most species are also active move about to find feeding sites. Male mealybugs do little feeding only in their youngest crawler stages. Mature males are small (1.5-2.5 mm) winged, whose primary function is to mate and then die. In temperate region, mealybugs usually have only one or two generations per season. In a warm green house or in doors there may be upwards of 8 over lapping generations per year (Johnson, 2009). In Central Europe, native species generally complete one to three

generations per year, while other species with an agricultural impact complete a higher number of over lapping generations, two to seven per year, in the Mediterranean Basin (Ivars, 2014). The pest is reported to complete 10 over lapping generations under laboratory conditions (26±2◦c) (Arif *et al.*, 2012), and a maximum of 12-15 generations in field conditions (Tanwar *et al.*, 2007).

The developmental period from immature crawler to adult stage was greater for males compared to females, probably due to the additional molt to the pupal stage in males. Survival of the second instars was lower (45.5%) than the first and third instars (71.4%).

Parthenogenesis with ovoviviparity (96.5%) was dominant over the oviparous (3.5%) mode of reproduction with a very short egg incubation period ranging from few minutes to a maximum of 2 hr. More than 10 crawlers per day were produced by a female with a mean reproductive period of 17.2±4.3 days, during which 97.3% of crawlers were produced (Vennila *et al.*, 2010).

Eggs are normally laid in a white waxy ovisac. The fecundity of *P. solenposis* is highly variable and temperature dependant, showing response with maximum at temperature between 25 and 32◦c (Fand and Suroshe, 2015).

2.5. Favourable Conditions:

P. solenopsis is widely spread in tropics and subtropics and is likely to invade many other countries due to predicted climate change.

The pest invasion, establishment, survival and multiplication in various geographical regions based on temperature conditions (Fand *et al.*, 2014).

Sharma (2007) reported that *P. solenopsis* has been capable of surviving temperatures ranging from 0- 45ºC, throughout the year; whereas Braybrook (2012) reported mealybugs prefer mild warm conditions with temperatures around 25◦C and high humidity.

2.6. Host Range of Cotton mealybug, *P. solenopsis:*

Mealybugs are polyphagous and multiply on different hosts, producing as many as 15 generations per year. Cotton mealybug , *Phenacoccus solenopsis* has a wide range of variation in morphological character , biological adaptation and ecological adjustability, it has been recorded from 154 plant species including field crops, vegetables, ornamentals, weeds, bushes and trees belonging to the families Malvaceae, Solanaceae, Asteraceae, Euphorbiaceae, Amaranthaceae and Cucurbitaceae,. However, the economical damage has been observed on Cotton, Brinjal, Okra, Tomato, Sesame, Sunflower and China rose with plant death in severe conditions (Arif *et al.,* 2009; lysandrou *et al.,* 2012).

Different workers recorded alternative host plants of *P. solenopsis*, 183 plant species from 52 families (Ben-Dov *et al.,* 2008), 154 plant species from 53 families (Vennila *et al.,* 2011) in the globe.

2.7. Host Preferences:

The major hindrance in the management of cotton mealybug is its polyphagus nature, the pest inflicted significant damage to cotton followed by okra, tomato, eggplant, sesame and sunflower. Ornamental shrub, China rose was found severely infested, which also support the carryover of the pest, even during the off season when cotton crop is not available in the fields (Fand and Suroshe, 2015).

Mealybugs of genus *solenopsis* attack a variety of crops, fruits, vegetables, ornamental and weeds but cotton is the prime target. In the initial stage, it breeds on all types of weeds throughout the year and then migrates to cotton crop. It establishes and spreads more easily than many other insect pests because of high reproductive rate, ability to spread through natural carriers (wind, water, bird and human beings) and the waxy coating that protects it from insecticides and natural mortality factors (Rashid *et al.,* 2012).

2.8. Association with ants:

A number of ant species are commonly found in association with mealybug infestation. The ants feeding on their secretions of honey dew and, in return, protecting them from predators (Braybrook, 2012).

The ant has also been observed carrying about young mealybug crawlers, aiding in their dispersal and has long been known to aggravate mealybug populations and other honey dew producing

insect species by disrupting the natural biological controls on these species (Phillips and Sherk, 1991). Ants build earthen shelters protecting mealybugs from adverse weather, feeding on honey dew, thereby preventing accumulation of honey dew and attacking of fungi, presumably, immature mealybugs get stuck in honey dew and die (Jahn *et al.*, 2003).

2.9. Management of Cotton mealybugs *P. solenopsis*:

Integrated pest management of mealybugs could be the safer and cheapest method of pest control. Nicotine based insecticides, non insecticidal chemical, include use of petroleum spray, oils and soap spray have a control measure in developed countries. Biological control has proved very effective, keeping in view the hazardous nature of insecticides. So, management approaches by integrating insecticides, plant extracts and biological control evolved effective and efficient strategies of cotton mealybug suppression (Ahmed *et al.*, 2011).

2.9.1 Chemical Control:

Chemical control of mealybugs with conventional insecticides are not giving satisfactory control because of its high reproductive potential and strong biophysical resistance due to egg- sac and thick waxy covering of the body (Joshi *et al.*, 2010; Arif *et al.*, 2011) and the tendency to hide in protected locations and form dense colonies. Contact insecticides, such as insect growth regulators, insecticidal soap and horticultural oil will kill the young nymphs (Pundt, 2013).

Systemic insecticides are not effective in stages were the insect do not feed such as eggs, adult males, and some gravid females. Over use of some active ingredients has led to resistance in some species of mealybugs and harmful to natural enemies and pollinators (Ivars, 2014). However, pesticides are inevitable for management of mealybug outbreaks, several insecticides belonging to different groups have been documented as effective against cotton mealybug, profenofos 50 EC and chlorpyrifos 50EC, sulfoxaflor, other organophosphate insecticides has been documented as an effective control measure against many mealybug species (Ahmed *et al.,* 2011; Lysandrou *et al.,* 2012).

2.9.2. Biological control of *phenacoccus solenopsis*:

For the sustainable management of *P. solenopsis*, biological control represents the most important method of controlling mealybugs, and under pesticide free conditions several species of predators, parasites and pathogens attack *P. solenopsis* and can effectively regulate mealybug populations (Solangi *et al., 2012)*. The chemical control of Mealybugs is not only expensive, but it also disturbs the habitats of natural enemies and has negative impacts on different ecosystems and human health (Abbas *et al.*, 2010, Abdin *et al.*, 2012).

To avoid such problems, several parasitoids and predators were used to control the spread and damage that can be caused by Mealybugs and keep the pest populations below the economic injury level (Nagrare *et al.*, 2011) .Tanwar *et al*. (2007) reported coccinellid beetles such as *Cheilomenes sexmaculatus, Rodolia fumida, Scymnus coccivora* and *Nephus regularis* as important predators of mealybug nymphs.

In Egypt, studies were carried by Shanab *et al*. (2010) on the life and fertility tables of the predatory cocciellid, *Exochomus nigromaculatus* (Coleoptera: coccinellidae) as an important indigenous predator of mealybugs on *Maconellicoccus hirsutus*, the results exhibited different biological parameters when reared on various host plants.

The use of biorational approach is necessary for better environmental protection. Aromatic plants, and their essential oils, are among the most efficient botanicals. The plants showing fumigant and topical toxicity extracts as well as anti-feedant or repellent effects. Botanical products like tobacco extract, neem oil and extracts, which can be easily and cheaply collected, have been found promising and useful for pest control, therefore, the plant products can be utilized for preparing biochemical products which are a good alternative to conventional synthetic insecticides as they are safe, economical and available in many areas of the world (Singh *et al.,* 2012).

2.9.2.1. The Predator, *Exochomus nigromaculatus :*

The coccinellidae (Coleoptera) is the largest family, which include almost 6000 species all over the world, including the most important predatory groups in biological control of pests in general, and specialiezes in feeding upon scale insects, mealybugs, aphids and white flies (Kaydan *et al.,* 2012). They are commonly called lady beetles, lady bugs, and coccinellids. Most lady birds species are carnivores; both adults and larvae are primarily predators of aphids and other pest species (Nazari, 2008), they play an important role in regulating the population of mealybugs (Chrysantus, 2012). There have been many successful classical biological and integrated pest management examples using coccinellids to control member of the Coccoidea, such as *Cryptolaemus montrouzieri* Mulsant on *Planococcus citri* (Risso) (Pseudococcidae), *Rodolia cardinalis* Mlsant on *Icerya purchase* Maskell (Monophlebidae) (Kaydan et al., 2012).

In Egypt, *Exochomus nigromaculatus* (Coleoptera: Coccinellidae), is an important predator of mealybugs (Shanab *et al*., 2010).

2.9.2.2. The Parasitoid, *Aenasius arizonensis:*

The most effective natural enemy of cotton mealybug, was first reported from Tandojam (Sindh), Pakistan in 2009, and identified as *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). It has an enormous impact on the development, fecundity and population growth of the host (Shahzad *et al.,* 2016), has high searching capacity and attacked cotton mealybug in colonies and scattered individual on plants.host specific parasitoid, completed development in *Phenacoccus solenopsis* only (Solangi and Mahmood, 2011).

Mealybug- parasitizing encrtids primary endoparasitoid; most of them undergo solitary development. Parasitoids are better fitted to survive at low mealybug densities and have the ability to disperse over a rather large area in their search for mealybug colonies (Chrysantus, 2012).

The family Encyrtidae (Hymenoptera, Chalcidoidea) is an important entomophagous group of insects that are parasitoids of a wide range of insects and other Arthropods. Several species of the family have been successfully used as biological control agents in some parts of the world (Fallahzadeh *et al.*, 2014).

The parasitoid, *Aenasius arizonensis* (Girault), Family Encyrtidae (Hymenoptera, Chalcidoidea) was described from the USA as *Chalcaspis arizonensis* (Girault, 1915), and later transferred to *Aenasius* (Noyes, *et al.*, 1994). It is a solitary aggressive endoparasitoid of *P. solenopsis* under natural conditions, and a most successful example of many biological control agents (Ahmed *et al.*, 2015; Shahzad *et al.*, 2016), this is due to the fact that, this parasitoid has some characteristics of adaptation to different environmental conditions, multiply faster than the host, short life Cycle (17-20 days), high host searching capacity and synchronise life cycle with host, and parasitism ranging from 5-100% (Nagrare, *et al.*, 2011) .

The parasitoid was recorded from USA, India, Pakistan, China, Iran, Egypt, Turkey and Iraq (Girault, 1915; Abdul- rassoul, 2018) .

Solangi and Mahmood (2011) studied the host specificity of the parasitoid, *Aenasius bambawalei* (syn. of *A. Arizonensis*) to six species of Mealybugs and their suitability for development of the parasitoid. Only mealybug, *Phenacoccus solenopsis* was found suitable for development and none of the other species of Mealybugs were parasitized, which means that, host specificity is confined to *P. solenopsis*.

In Sudan, the first record of the parasitoid, *A. arizonensis* was by Mohamed, *et al.* (2017) during monthly surveys in the Gezira Research Station. They observed the presence of Mealybug mummies containing parasitoid or its emergence holes on Hambouk (*Abutilon* spp), Datura (*Datura stramonium*) and Okra (*Hibiscus esculentus*).

2.9.2.3. Eucalyptus Extracts:

Eucalyptus species belong to family Myrtaceae, are native to Australia, New Guinea, and Indonesia. Its leaves contain volatile compounds such as α-and β-Pinene, globulol, terpineol, and 1, 8-cineole, the last one is the predominant monoterpene in the most Eucalyptus species. These oils have antimicrobial, antifungal, antiviral,

and insecticidal activities (Russo *et al.*, 2015). Eucalyptus oils are characterized as fumigants and contact toxicants with variations in chemical ingredients according to tree age, species, chemotypes, geographical origin and extraction procedure. Eucalyptus leaf oils exhibit the inhibition of acetylcholine esterase activity and showed the common symptoms of a neurotoxic mode of action (Siramon *et al.*, 2008).

The genus Eucalyptus contains about 600 species, fast growing tree in forest plantation (Takahashi *et al*., 2004). The family Eucalyptus (Myrtaceous) plants are source of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol derivatives and tannins. More than 300 species of genus Eucalyptus contain volatile oils in their leaves and contain 37 compounds (Getahun and Welderufael, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Identification of Mealybugs Species:

3.1.1 Mealybug Specimen's Collection:

A total of 49 specimens of mealybugs were collected from infested plants on different locations (Khartoum, Kassala, Gazira and Northern Sudan). The infested parts of the plant were collected and placed in labeled paper pags. In the laboratory, a very fine paint brush wetted with alcohol was used to pick the mobile females and nymphs and transfer them to alcohol.

3.1.2. Preparation of slide mounts:

For quick identification of samples, a simplified preparation method described by Sirisena *et al.*, (2013) (Figure 1), was applied for preparation of both temporary and permanent slide mounts of Coccoidea.

The specimens and the prepared slides of mealybugs were sent to Taxonomy Department, Agricultural Research Corporation, wad Medani, Sudan, for proper identification. The results of the species identification were confirmed by Mohamed *et al*., (2015) as *Phenacoccus solenopsis.*

3.2. Seasonal Population of Cotton mealybug:

3.2.1. The study sites:

This study was carried out during the seasons: 2015/ 2016-2016/2017, in four main Agricultural locations in Khartoum State: Soba Scheme (Khartoum), Algammuia Scheme (Omdurman), Silait Scheme (East Nile) and Khartoum North area (**Figure 2**).

3.2.2. Infestation and Seasonality of Cotton mealybug:

To study the seasonal population, plant susceptibility, distribution, degree of infestation and carryover of cotton mealybug *Phenacoccus solenopsis*, three of the main host plants were chosen, i.e., okra ,tomato and eggplant. From each location, one progressive vegetable grower's farm was selected. Each selected farm was divided into four sites, and in each site 20 plants were randomly selected for observations. Observations on the occurrence and infestation of mealybug nymphs and adult females were recorded at fortnight intervals on 30 cm apical shoot (twig) and 5 leaves taken randomly (i.e., a total of 80 specimens per site). In order to study the influence of abiotic factors on mealybug population, the meteorological data on maximum and minimum temperature, relative humidity and rainfall were bought from the Mererlogical authority, Ministry of Water Resources, Irrigation and Electricity, Khartoum.

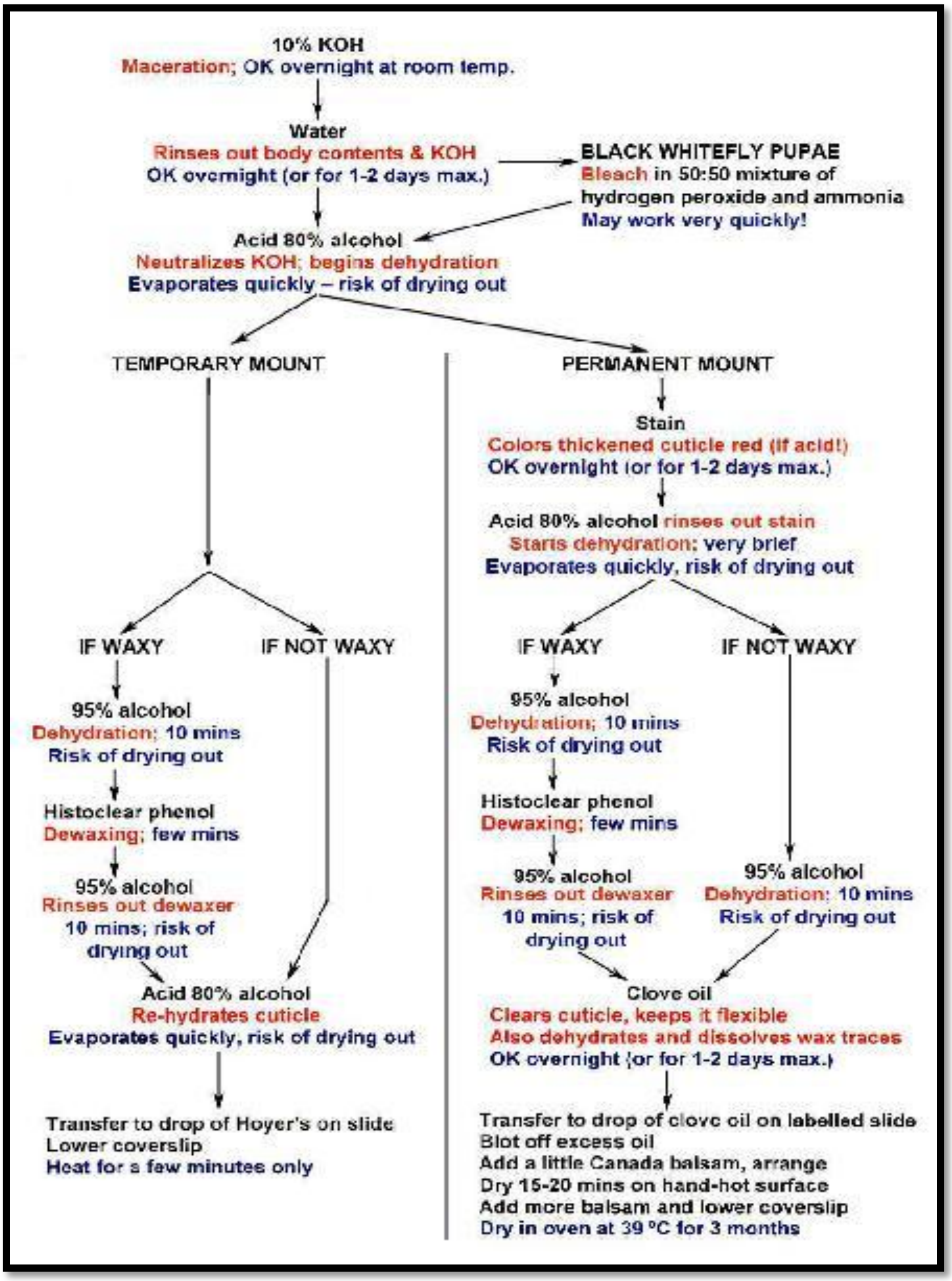

Figure 1. A flow diagram of the technique for preparing temporary and permanent slide mounts of soft-bodied Sternorrhyncha (Sirisena *et al.*,2013).

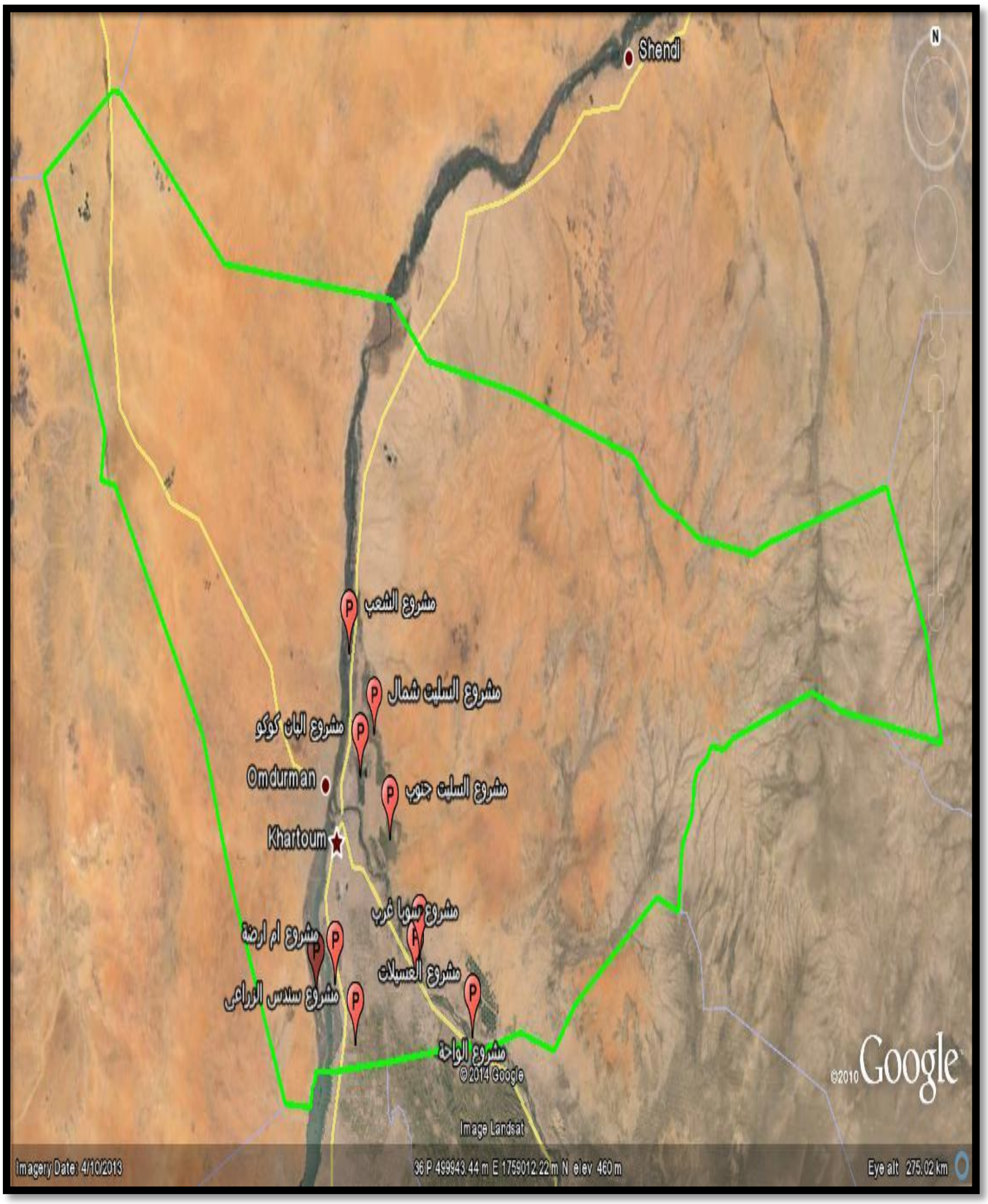

Figure 2. The main study sites of mealybugs in Khartoum State (Ministry of Agriculture, Animal wealth and Irrigation, Khartoum).

3.3. Biology of Cotton Mealybug '*Phenacoccus solenopsis*':

A series of experiments were conducted to study the biology of *P. solenopsis* on four main host plants: Cotton (*Gossypium sp.*), okra *(Abelmoschus esculentus*), tomato (*lycopersicon esculentum*) and sweet pepper (*Capsicum annuum*) under laboratory conditions.

3.3.1 Sample Collection and Rearing of '*P. solenopsis'*:

The biology of *P. solenopsis* was studied using samples collected from unsprayed, infested vegetables and ornamental plants, during the period from January up to April 2016 at the Integrated Pest Management Laboratory, Ministry of Agriculture, Khartoum State.

Twigs of plants infested with reproducing females of *P. solenopsis* were brought to the laboratory, and individual females were separated. Leaves of the study plants, having the same size were collected from the field (free from mealybugs) ,washed under tap water and shade dry from water; these leaves were used as food for this sap sucking insect (**Plate 1**). The leaf petioles of the selected host plants were wrapped with cotton wool dipped in water to keep the leaves turgid and fresh (**Plate 2**). Each leaf was infested with an adult female and put in a separate Petri- dish (9x2 cm) (**Plate 3**).

The maximum and minimum temperature and mean relative humidity of the laboratory were ranging between 27°c - 32 $^{\mathbf{o}}$c and 24-27 % RH, respectively.

Plate 1. Cotton leaves washed and shade dry from water

Plate 2. Cotton leaves wrapped with cotton wool dipped in water, to be kept turgid and fresh.

Plate 3. Release of mealybug adult females on leaves in Petri- dish

The females in the Petri -dish plates were examined daily. After the formation of the ovisac at the posterior part of the abdomen, the first nymphal instars emerged from the sacs, and were accumulated on the leaves seeking for food. Then, by a soft brush, the newly emerged nymphs were taken to another Petri-dish plate and examined daily for moulting, which was confirmed by the presence of exuviae on the leaves.

Range and mean values for the developmental period of each instar to females and males, pre oviposition and oviposition periods, fecundity and longevity were calculated. The numbers of male and female nymphs, out of the total population that survived to adult stage, were also calculated.

3.3.2. Mode of Reproduction of *Phenacoccus solenopsis'*:

For studying mode of reproduction, the first instars were gently taken from crawler sacs of the female and transferred to a Petri- dish containing cotton leaves for rearing. Forty female nymphs were selected for further rearing. Twenty female nymphs of which were transferred to another Petri- dish plate and provided with male cocoons to ensure they could have an opportunity to mate with the emerging males. The other twenty female nymphs were kept in isolation (without males) until they died. The number of offspring produced by each female was recorded.

3. 4. Host Preference of "*Phenacoccus solenopsis*":

3. 4.1. Population Culture of *P. solenopsis*:

The adults of the cotton mealybug were collected from infested vegetables, ornamental shrubs and weeds, and were reared in the laboratory at a temperature of 25-28°c and a relative humidity of 25-28%.

3. 4. 2. Host Plants:

A total of 8 host plant species commonly available in Khartoum State were used in these studies. These included three vegetables, two ornamentals, two weeds and one field crop; viz., okra (*Abelmoschus esculentus)*, Egg plant (*Solanum melongena*), Tomato (*Lycopersicon esculentum*), Lantana (*Lantana camara*), Chinese

rose (*Hibiscus rosa- sinensis*), Raba (*Trianthema pentandra*), Hambouk (*Abutilon sp.*) and Cotton (*Gosspium sp*.) The host plant species were evaluated against 1st , 2nd and 3rd nymphal instars and the adult females of cotton mealybug following multi- choice experiments under laboratory conditions.

3. 4. 3. Host Feeding Preference of Cotton mealybug :

In this study, the method applied by Eickhoff *et al.*, (2005) was followed. Healthy twigs of the selected plant species with at least five tender leaves were collected, washed and dried to remove moisture from the surface of the leaves.

A plastic dish (34cm diameter by 13cm deep) was used in the experiment, eight holes, each 2cm in diameter, were drilled in the inner circle of the dish. The twigs were inserted in vials (2cm diameter by 9.3cm length) containing fresh organic peat moss wetted by water. The vials with different plants were randomly inserted in the holes of the dish (**Plate 4**).

A counted numbers of cotton mealybug (200 individual of each stage) in an open Petri-dish plate (9x2 cm) were put in the centre of the plastic dish, and the whole dish was covered with muslin cloth (**Plate 5**).

The food preference of *P. solenopsis* among the tested plant species was compared with the Chinese rose as a control plant (highly infested ornamental plant and available most of the year). The proportions of the mealybugs were determined. This was done by observing the tested plant species, and recording numbers of mealybugs on each plant after: 2 hours, 8 hours and 24 hours of release. This experiment was repeated 3times.

Plate 4: A plastic dish with 8 vials containing fresh organic peat moss (in which the selected plants were inserted).

Plate 5: the whole dish covered with muslin cloth to prevent scaping of mealybugs.

3. 5. Biology of the Predator, ' *Exochomus nigromaculatus'*

(Coleoptera : Coccinellidae)

3. 5.1. Rearing of Immature Stages:

Adults of *Exochomus nigromaculatus* were collected from the field, on a shrub, *Abutilon sp*. Twenty pairs of male and female beetles were selected and kept in separate Petri- plates (9x2 cm). The mealybugs (along with cotton leaves) were provided to each pair as food. The eggs laid by females in each plate were collected daily, and monitored until hatching. Hatching larvae were reared in plastic containers (11 cm longe ×9 cm diametere) at a temperature of 25 -28°C, with relative humidity (RH) of 28-35%.

Developmental times for eggs, larval instars, total larval stages, pupal stage, sex ratio, total developmental period and daily mortality of immature stages were recorded.

3. 5.2. Rearing of Adult Stages:

After eclosion, 13 pairs of males and females of *E. nigromaculatus,* were kept in separate Petri- dish plates (9x2 cm), and were also fed cotton mealybugs until development was completed. These experiments were made to study: longevity of males and females, the pre-ovipostion, inter- oviposion (number of days between two successive ovipositions) mention by Abdel-Salam *et al*. (2009), oviposion and post oviposion periods, the fecundity of females, fecundity rates (number of eggs /♀/ day), and the daily mortality were recorded.

3.5.3. Predation of *E. nigromaculatus* on Cotton mealybug:

The lady beetle, *Exochomus nigromaculatus,* a biological control agent of cotton mealybug, *P. solenopsis, was* examined for its ability to consume 3rd nymphal instar and adult stages of the cotton mealybug, under laboratory conditions. This was done for the purpose of identifying variations in the feeding potential of the predator's 4th instar grubs, females and males.

The mass cultures of the prey, cotton mealybug**,** *P. solenopsis,* were established from individuals collected during 2016 from Cotton fields (variety shambat B) grown at Shambat Research Station. The predator, *Exochomus nigromaculatus,* were collected from a shrub, *Abutilon sp., and* an ornamental plant, *Lantana camara,* at Sudan university farm. Cotton mealybugs were reared on Cotton leaves, and the ambient environmental conditions were, 25-30°C, 24-30% RH for rearing the mealybug and predator.

3.5.3.1. Prey Consumption of 4th Instar grubs of *Exochomus sp.*:

To find out the predatory potential of the 4th instar grubs of *E. nigromaculatus* on cotton mealybug, 18 larvae of newly emerged 4th instars of the predator were put separately in Petri- dish plates (9x2 cm). Each larva was provided daily with a mixed diet of 10 individuals of the 3rd instar nymphs and 10 adult females of the cotton mealybug.

The experiment was repeated three times. After 24 hour, number s of uneaten mealybugs were counted and deducted from the total number of mealybug supplied, to know the total number consumed daily, till the completion of the 4th instar stage (i.e., till pupation).

3.5. 3.2. Prey Consumption of the Adult Stage of *Exochomus sp.*:

The feeding potential of the adult Exochomus *sp.* were studied. Taking 10 adult males and 10 females of *Exochomus sp.*, and they were released separetly in a Petri –dish plates (9x2 cm), and each was provided with a mixed diet of 10 3rd instar nymphs and 10 adult females cotton mealybug, daily, the consumed numbers were recorded daily for 5 days. The remaining individuals of the prey were counted and removed daily before providing the fresh prey.

3. 6. Biology and Parasitization of the Parasitoid:

"*Aenasius arizonensis*"

The parasitoid, *Aenasius arizonensis* (Hymenoptera: Encyrtidae) was first observed in November 2015 at Algammuia scheme on Okra, later in 2016 observed on Abutilon and Cotton (varity shambat) in shambat research farm. Samples were taken to the Agricultural research corporation, Madani for identification.

A field survey was made to determine the seasonality of the Mealybug, *P. Solenopsis* and its parasitoid, *Aenasius arizonensis* during season 2015 - 2016, in three Agricultural Schemes namely; Soba, Gammuia and Selait.

Another field survey was carried out in April, 2019 to study the Infestation of *P. solenopsis* and parasitism by *A. arizonensis* at Soba Agricultural Scheme. Infested adults of *P. solenopsis* and its parasitized mummies were observed on three different families of weeds. These are locally known as Raba (*Trianthema brasilicum*), Lisan Al-tair (*Amaranthus virids*) and Hambouk (*Abutilon* spp).

An area of 2 feddan highly infested with Hambouk were divided into five sites. To determine parasitisation percentage, 50 apical shoots of Hambouk, (each 30 cm long) were collected from the five sites. Mean numbers of parasitoid mummies and percentage parasitism on different stages of *P. solenopsis* were calculated.

For studying biology of the parasitoid under laboratory conditions, Mealybug mummies were collected in plastic jars from ornamental plants, Lantana (*Lantana camara*) and hibiscus (*Hibiscus rosa-sinensis*) from the Horticulture Orchard, at the Faculty of Agriculture, Khartoum University, Shambat. They were reared in four transparent plastic jars under laboratory conditions of 28°C temperature and 45% relative humidity, to allow the emergence of the parasitoid.

Breeding colonies of *A. arizonensis* were established from individuals emerged from the dark mummies. One day old males and females of the emerged parasitoid were allowed to mate for 24 hrs. Then, they were transferred by an aspirator to a culture of 30 adult females of the Mealybugs, reared on Hibiscus *rosa-sinensis* in a plastic jar. A solution of 2% sugar mixed with water was supplied as a food source for the parasitoid adults by wetting a cotton swab, tied with thread and kept hanging in the jar.

After 24 hours, the pair of the parasitoid was taken out from the plastic jar. The exposed Mealybugs were checked daily until adult parasitoids emerged from the mummified Mealybugs (dark brown, barrel shape mummies). Then, the following parameters were recorded: mean developmental periods from oviposition to mummy formation, mummy formation to adult emergence, total life span of males and females, sex ratio and mean daily parasitisation. To get more accurate information, the experiment was repeated (3) times as prescribed by Bodlah, *et al.*, (2010).

3. 7. Effect of *Eucalyptus camaldulensis* extracts on *P.solenopsis:*

3. 7.1. Collection and Preparation of the Plant Material

The Kafur leaves were collected in December 2017 from the Horticulture Orchard of Faculty of Agriculture, University of Khartoum. The leaves were washed to remove dust and other pollutants, and placed in shade to dry at room temperature for 7-15days. The dried leaves were crushed with grinder to make fine powder.

3. 7.2. Extraction of Essential Oil:

Three extracts were prepared using Ethanol (98%), Petroleum ether and water as solvents.

Thirty grams of the leaves' powder were weighed and then passed to a cellulose extraction tube. Plant material was extracted by using 300 ml of the solvents; Ethanol, and Petroleum ether separately by the hydro distillation technique, in a Soxhlet apparatus at 50°C for 6-8 hours (until no more essential oil was obtained) **(Plates 6)**.

The extracts obtained were concentrated by a Vacuum Rotary Evaporator until solvents were completely evaporated. The flasks containing the dried materials were weighed. Weight of dried extracts was calculated by subtracting the already noted weight of the empty flask. (**Plate 7).**

The aqueous extract of *E. camaldulensis* was prepared by taking 30 gram of powder, weighed and mixed with 300 ml distilled water, agitated by magnetic stirrer for 3 hours, then kept in a refrigerator for 24 hours. The mixture was filtered; emulsion of 0.5% soap and molasses were added for a homogenous solution and kept as a stock solution.

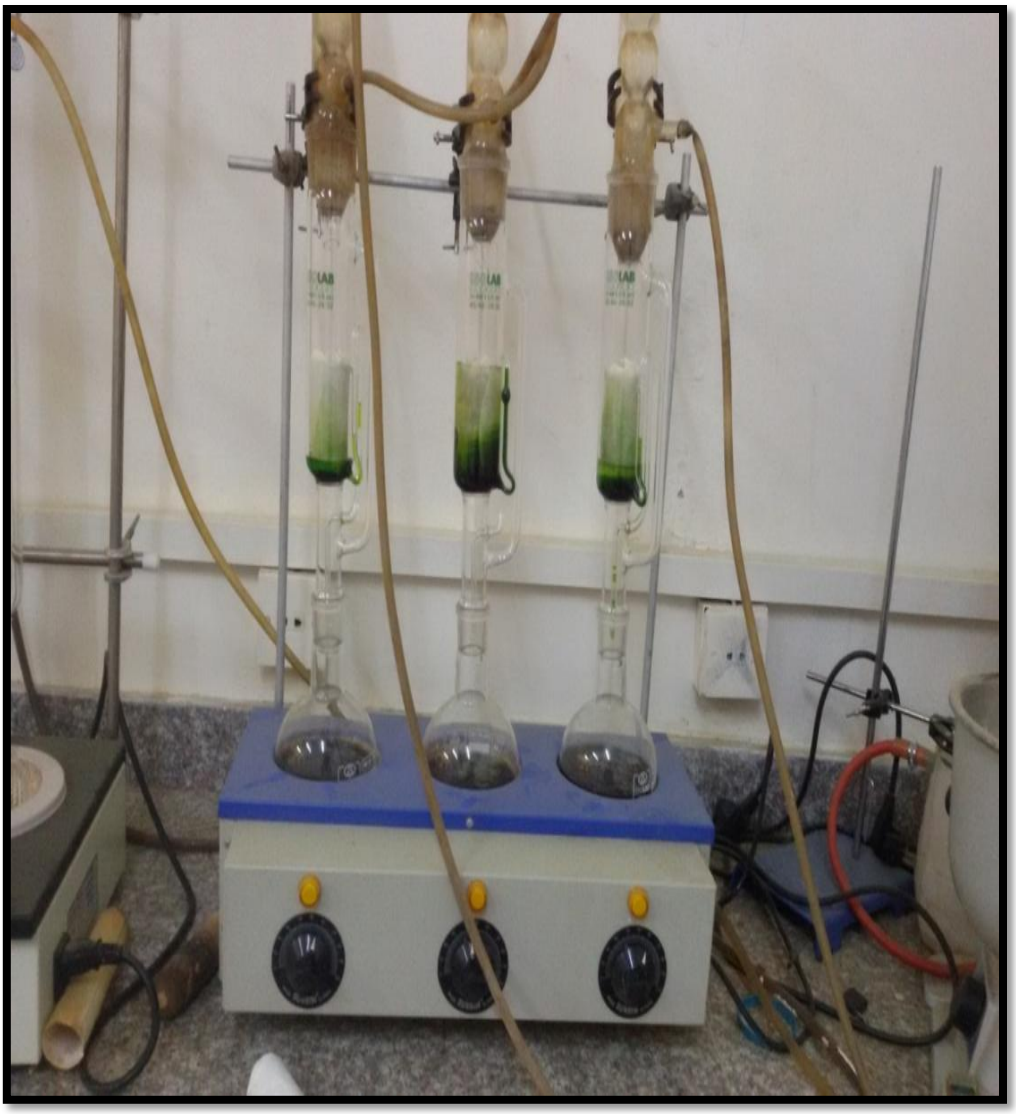

Plate 6. Soxhlet apparatus for extraction.

Plate 7. Vacuum Rotary Evaporator for separation of solvents.

3. 7. 3. Insect Culture:

Cultures of *P. solenopsis* were collected from Silait scheme and reared under laboratory conditions on cotton leaves, at a temperature of 25-30°C and relative humidity of 24-30%. The adult females used for laboratory assay were separated and kept in large plastic containers.

3. 7. 4. Efficacy of the Eucalyptus Oil against *P. solenopsis* :

3. 7. 4. 1. Leaf Dip Method:

Bioassay tests were conducted for the evaluation of bio- efficacy of aqueous leaf extract, ethanol and petroleum ether leaf extracts of *Eucalyptus camaldulensis* oils against adult females of cotton mealybug. Unsprayed fresh cotton leaves were collected, and washed thoroughly with tap water and completely air dried. Four, concentrations 2.5%, 5%, 7.5% and 10% were prepared from each of the three extracts. The cotton leaves were submerged in the different concentrations of each extract, separately for 5 minutes, and were allowed to air dry for 30 minutes. Another group of leaves were dipped in distilled water, ethanol, petroleum ether alone (as control), and others in Hitcel 44% EC (Profenofos 40% +Cypermethrin 4%), (as a standard), also for 5 minutes, and allowed to air dry for 30 minutes. The treated leaves were then placed, separately, in plastic Petri -dish plates (9 cm in diameter), and fifteen adults of the cotton mealybug were released in each Petri dish plate. This experiment was repeated 4times.Adult mortality was recorded after 24, 48 and 72 hours after exposure.

3. 7. 4. 2. Topical Application Method:

Another treatment by topical application method was made, using a Microapplicator syringe. One drop (0.5 nmm) of each of the prepared concentrations of each extract was applied on the thorax of each adult female mealybug. Then, fifteen treated adults by each concentration were released in a Petri – dish plate. In all treatments, adult females' mortality was recorded after 24, 48 and 72 hours after exposure. All treatments replicated four times.

Statistical Analysis:

Data regarding the field and laboratory experiments were statistically analyzed by using Software excel version 2007 and Statistics 8 (Analytical Soft Ware, 2003). Then, they were subjected to analysis of variance (ANOVA) under completely randomized block design (CRBD) and a completely randomized design (CRD) Means were compared following the least significant difference test (LSD test) at probability level of 5% to get mean values of observations and S.E.

CHAPTER FOUR

RESULTS

4. RESULTS

4.1. Seasonal Activity of *Phenacoccus solenopsis* and Host Plant Susceptibility:

The results regarding the population distribution of the cotton mealybug, *P. solenopsis* illustrated significant differences , at ($P<0.05$), of insect populations per plant twig and per 5 leaves at 4 locations in Khartoum State during two seasons : 2015/ 2016 and 2016/ 2017.

4.1.1. At Algammuia Scheme :

During the First Season (2015- 2016) the mean maximum population of mealybug was observed on the first fortnight of March 2016 on Tomato, with a mean population of 314.84 nymphs , 40.05 females per 30 cm apical shoot (twig), and 43.82 nymphs, 4.80 female per 5 leaves / plant, followed by Eggplant with a population of 33.29 nymphs, 6.06 female per 5 leaves / plant**. (Table 1& Plate 8)**.

In the Second Season (2016- 2017) the mean maximum population was on Kharif Okra, with a mean population of 14.08 nymph, 2.28 females / shoot and 10.8 nymph, 2.65 females per 5 leaves / plant. No infestation on Tomato and Eggplant during second fortnight of January 2017 up to 22 March 2017. **(Table 2)**

Table 1. Mean population of *P. solenopsis* on different crops

(Algammuia Scheme – First Season 2015/ 2016).

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		ANOVA
			Nymph	Female	Nymph	Female	
Algammuia **First Season** **4.12.2015 to 20.3.2016**	**4.12. 2015**	Okra	3.525 B	0.387 B	14.175 A	2.287 B	CV=396.08 SE= 3.1900 LSD = 6.2843
		Tomato	2.9125 A	0.2125 B	0.4375 B	0.0125 B	CV =749.90 SE =1.0597 LSD = 2.0877
	19.12. 2015	Okra	7.488 B	1.662 C	11.975 A	2.000 C	CV = 229.32 SE = 2.0963 LSD =4.1297
		Tomato	9.863 A	0.813 B	12.050 A	0.237 B	CV =432.83 SE =3.8470 LSD =7.5787
		Eggplant	0.013 B	0.138 B	20.863 A	0.425 B	CV = 1064.37 SE = 9.0193 LSD =17.768
	7.1. 2016	Tomato	3.3875 AB	0.5625 B	6.3500 A	0.0625 B	CV = 573.72 SE = 2.3500 LSD = 4.6296
		Eggplant	0.3250 B	0.0847 B	9.6875 A	0.9250 B	CV = 805.86
	21.1. 2016	Tomato	10.050 A	0.325 B	3.587 B	0.123 B	CV= 583.69
		Eggplant	0.875 A	0.113 B	13.800 A	0.763 B	CV = 659.51 SE = 4.0538 LSD = 7.9861
	4.2. 2016	Tomato	9.2875 A	0.4750 B	4.1250 AB	0.2375 B	CV = 505.70 SE = 2.8235 LSD = 5.5624
		Eggplant	0.988 B	0.263 B	20.675 A	1.025 B	CV = 545.56 SE= 4.9492 LSD = 9.7501
	19.2. 2016	Tomato	33.462 A	2.062 B	3.012 B	0.037 B	CV= 454.76 SE= 6.9342 LSD = 13.661
		Eggplant	0.2000 B	0.0375 B	4.3625 A	0.5250 B	CV= 396.92 SE = 0.8041 LSD = 1.5841
	5.3. 2016	Tomato	314.84 A	40.05 B	43.82 B	4.80 B	CV = 274.66 SE = 43.809 LSD = 86.305
		Eggplant	1.600 B	0.950 B	33.288 A	6.063 B	CV= 423.11 SE= 7.0077 LSD = 13.805
	20.3. 2016	Tomato	10.525 A	5.150 B	14.688 A	0.225 C	CV = 190.43 SE = 2.3024 LSD = 4.5358
		Eggplant	0.9872 C	1.6125 BC	5.3375 A	2.6500 B	CV = 187.35 SE = 0.7841 LSD = 1.5447

Table 2. Mean Population of *P. solenopsis* on different crops

(Algammuia Scheme – Second season 2016/ 2017)**.**

Area	**Date**	**Plant**	**No.of mealybugs/ 30cm long twig**		**No.of mealybugs/ 5 leaves**		**ANOVA**
			Nymph	**Female**	**Nymph**	**Female**	
Algammuia **second Season**	**19.10 .2016**	Okra	14.075 A	2.012 B	10.800 A	1.125 B	CV=292.01 SE= 3.2334 LSD= 6.3698
19.10.2016 to 22.3.2017	**4.11. 2016**	Okra	11.625 A	2.275 B	10.475 A	2.650 B	CV= 305.65 SE= 3.2651 LSD= 6.4323
	5.1. 2017	Tomato	9.5375 A	1.0875 B	1.4500 B	0.0125 B	CV= 275.20 SE= 1.3149 LSD= 2.5904
		Eggplant	0	0	0	0	0
	19.1. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	5.2. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	20.2. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	7.3. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	22.3. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0

4.1.2. At Silait Scheme :

During the First Season (2015- 2016) high mean population on leaves recorded from Kharif Okra, 25 nymphs and 3.16 females per 5 leaves per plant. The mean maximum population of mealybug was observed during first and second fortnight of March 2016, on Tomato with a mean population of 112 nymphs and 18.1 females per 30cm twig**. (Table 3 and plate 8).**

In the Second Season (2016- 2017) no infestation was observed on Okra, Tomato and Eggplant (zero).

Table 3. Mean Population of *P. solenopsis* on different crops

(Silait Scheme – First Season 2015/2016)

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		ANOVA
			Nymph	Female	Nymph	Female	
Silait first season 3.12. 2015 to 19.3. 2016	3.12. 2015	Okra	0.025 B	0.050 B	24.937 A	3.162 B	CV=534.96 SE= 5.9579 LSD= 11.737
	18.12. 2015	Okra	1.5875 B	0.0 B	17.100 A	0.3625 B	CV= 368.43 SE= 2.7744 LSD= 5.4656
	6.1. 2016	Tomato	3.5000 A	0.4000 B	0.3875 B	0.0 B	CV= 233.46 SE= 0.3957 LSD= 0.7795
		Eggplant	0.0 B	0.0 B	1.2625 A	0.0125 B	CV= 622.30 SE= 0.3136 LSD= 0.6179
	20.1. 2016	Tomato	2.9750 A	1.2750 B	0.4250 BC	0.0625 C	CV= 265.52 SE= 0.4972 LSD= 0.9795
		Eggplant	0.1375 B	0.1875 B	0.4500 A	0.1000B	CV= 286.35 SE= 0.0990 LSD= 0.1951
	3.2. 2016	Tomato	9.1125 A	0.9250 B	6.8750 AB	0.0875 B	CV= 565.69 SE= 3.8013 LSD= 7.4887
		Eggplant	0.0 B	0.1125 B	0.9375 A	0.3000 B	CV= 349.33 SE= 5.9167 LSD= 11.656
	18.2. 2016	Tomato	43.200 A	0.975 B	10.950 B	0.175 B	CV= 270.67 SE = 5.9167 LSD= 11.656
		Eggplant	0.1750 B	0.2875 B	1.0250 A	0.3125 B	CV= 299.46 SE= 0.2131 LSD= 0.4198
	4.3. 2016	Tomato	111.97 A	3.65 B	14.40 B	0.70 B	CV= 351.77
		Eggplant	0.6875 C	0.9125 BC	1.4500 A	1.2000 AB	CV= 141.93 SE= 0.2384 LSD= 0.4697
	19.3. 2016	Tomato	21.887 A	18.088 A	5.150 B	0.062 B	CV= 161.05 SE= 2.8766 LSD= 5.6670
		Eggplant	0.9625 AB	1.000 AB	1.7000 A	0.4625 B	CV= 230.14 SE= 0.3752 LSD= 0.7392

4.1.3. At Soba Scheme:

During the First Season (2015- 2016) maximum population of mealybug was observed on Kharif Okra during the first fortnight of December 2015 with a mean population of 46.46 nymphs, 6.79 females per 30cm twig, and 153.49 nymphs, 19.85 females per 5 leaves per okra plant, okra followed by Tomato 4.83 nymphs, 4.59 females per 30 cm twig during the second fortnight of March 2016 (**Table 4).**

In the Second Season (2016- 2017) low infestation on Kharif Okra and no infestation on Tomato and Eggplant was recorded in **(Table 5).**

Table 4. Mean Population of *P. Solenopsis* on different crops

(Soba Scheme - First Season 2015/ 2016)

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		ANOVA
			Nymph	Female	Nymph	Female	
Soba Scheme First Season 2.12.2015 to 18.3.2016	2.12. 2015	Okra	46.46 B	6.79 B	153.49 A	19.85 B	CV= 234.58 SE= 21.010 LSD= 41.390
	17.12. 2015	Okra	41.212 A	1.875 C	22.275 B	1.363 C	CV= 326.41 SE= 8.6091 LSD= 16.960
	5.1. 2016	Tomato	2.4125 C	0.0250 A	0.1750 A	0.0125 A	CV= 1372.44 SE= 1.4241 LSD= 2.8055
		Eggplant	0.0125 B	0.0 B	0.2375 A	0.0750 B	CV= 566.09 SE= 0.0727 LSD= 0.1433
	19.1. 2016	Tomato	0.0 A	0.1625 A	0.0125 A	0.0 A	CV=1420.13 SE= 0.0982 LSD= 0.1935
		Eggplant	0.0125 B	0.0 B	0.7625 A	0.1625 B	CV= 766.65 SE= 0.2841 LSD= 0.5597
	2.2. 2016	Tomato	0.2125 AB	0.0250 B	0.3750 A	0.0500 B	CV= 394.96 SE= 0.1034 LSD= 0.2038
		Eggplant	0.3750 A	0.2000 A	0.5375 A	0.0750 A	CV= 503.93 SE= 0.2365 LSD= 0.4660
	17.2. 2016	Tomato	0.5875 AB	0.8000A	0.0125 B	0.0125 B	CV=710.01 SE= 0.3964 LSD= 0.7810
		Eggplant	0.0 A	0.0250 A	0.0375 A	0.0125 A	CV= 723.66 SE= 0.0215 LSD= 0.0422
	3.3. 2016	Tomato	3.1375 A	1.9250 A	0.2000 B	0.2375 B	CV= 337.81 SE= 0.7344 LSD= 1.4468
		Eggplant	1.5250 A	0.5250 B	1.7375 A	1.7125 A	CV= 211.23 SE= 0.4592 LSD= 0.9047
	18.3. 2016	Tomato	4.8250 A	4.5875 A	0.1625 B	0.3500 B	CV= 275.79 SE= 1.0820 LSD= 2.1315
		Eggplant	2.5125 A	1.4000 A	2.0375 A	2.3000 A	CV= 207.65 SE= 0.6772 LSD= 1.3340

Table 5. Mean population of *P. solenopsis* on different crops

(Soba Scheme – Second Season 2016/ 2017)

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		ANOVA
			Nymph	Female	Nymph	Female	
Soba second season 20.10. 2016 To 26.3. 2017	20.10. 2016	Okra	0.2500 AB	0.1125 B	0..3375 AB	0.5000 A	CV= 274.46 SE= 0.1302 LSD= 0.2565
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	5.11. 2016	Okra	0	0	0	0	0
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	20.12. 2016	Okra	0	0	0	0	o
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	6.1. 2017	Okra	0	0	0	0	0
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	20.1.20 17	Okra	0	0	0	0	0
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	6.2. 2017	Okra	0	0	0	0	0
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	21.2 .2017	Okra	0	0	0	0	0
		Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	11.3. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0
	26.3. 2017	Tomato	0	0	0	0	0
		Eggplant	0	0	0	0	0

(1)

(2

(3)

(4)

Plate 8. Nature of damage by Cotton mealybug, *P. solenopsis* on

1. **Cotton leaf** (Shambat), 2. **Okra pods** and leaves (Gammuia)

3. **Egg plant** (Silait) 4. **Tomato** (Gammuia).

Maximum mean populations of *P.solenopsis* per 30 cm apical shoot (twig), were recorded on **Tomato** (March 2016) at **Gammuia** followed by **Silait** and **Soba**. Whereas, maximum populations on 5 leaves per plant were recorded on **Okra** (December 2015) at **Soba** followed by **Tomato, Eggplant** on March 2016 at **Gammuia,** and low population on **Okra** (December 2015) at **Silait.**

No infestations were recorded from **North Bahri** on the selected crops, during Seasons 2015/ 2016 and 2016/ 2017.

Infestation of cotton mealybug were observed on the Okra stem, pods and lower side of the leaves, whereas on tomato on tender shoots (nymphs) and leaves, and on Eggplant on the lower side of leaves and rarely on stem.

4. 2. Biology of *Phenacoccus solenopsis*:

Studies on the life history and biological activities of a pest are difficult under field conditions, because of the interference of biotic and abiotic factors; so, these studies were made under laboratory conditions. Studies were conducted using four main crops: Cotton, Okra, Tomato and Sweet pepper, with detailed observations on the reproductive and developmental stages of *P. solenopsis*.

4. 2. 1. Nymphal duration of *P. solenopsis*:

The results showed no significant differences in the mean duration of the 1st ,2nd , and the 3rd nymphal instars on the tested host plants (Cotton, Okra, Tomato and Sweet pepper **(Table 6).**

First instar nymphs:

Duration of the first instar nymphs on the tested plant species ranged between 4 to 6 days, with an average on Cotton 5.3±1.5 days, Okra 4.7± o.56 days, Tomato 4.8± 2.56 days and Sweet pepper 5.3± 0.56 days **(Table 6 and Appendix 8-11).**

The first instar nymphs were oblong in shape, dorsally convex, light yellow in colour with three pairs of legs and a pair of six segmented filiform antennae. No wax coating, they were very fast crawlers, searching for their food and settle on the host **(Plate 9).**

Second instar nymphs:

The mean duration periods of the second instar for all tested plants are similar with a range of 4 to 6 days **(Table 6).**

After the first moult, which is confirmed by the presence of exuivum on the leaf or on the posterior end of the nymphs, the second instar nymphs emerged, which are oblong, yellow in colour. They secreted white waxy powder on dorsal side after 24 hours of moulting.

Third instar nymphs:

The mean duration period of the third instar nymphs of female *P. Solenopsis* is longer on Tomato 5.3± 0.49 days followed by Cotton 4.8± 0.76 days, and sweet pepper 4.5± 0.5 days, with a range of 4 to 6 days, while it was lower on Okra 3.8± 0.38 days with a range of 3- 4 days **(Table 6 and Appendix 8-11).**

The third instar nymphs of females were oblong in shape and light to dark in colour, having two pairs of black strips on the dorsal side of the body, having seven segmented antennae **(Plate 10).**

Table 6. Developmental time (mean ± SE) of *Phenacoccus solenopsis* reared on four host plants.

Host plant	1st nymphal instars	2nd nymphal instars	3rd nymphal instars	♀ Total developmental time	♂ Total developmental time
Cotton	5.3±1.51a	4.2±0.90a	4.8±0.76a	36.2±3.18a	17.2±0.88a
Okra	4.7±0.56a	4.2±0.15a	3.8±0.38b	30.3±1.23b	16.2±1.03a
Tomato	4.8±2.56a	4.5±0.51a	5.3±0.49a	28.3±5.42b	16.8±0.99a
Sweet pepper	5.3±0.54a	4.3±0.54a	4.5±0. 5ab	26.8±3.15b	17±1.0a
CV	14.60	18.42	17.09	13.80	5.41
LSD	0.8864	0.9521	0.9521	5.0548	1.0939
SE	0.4249	0.4564	0.4564	2.4233	0.5244

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test.

Plate 9. First instar nymph and adult female of *P. solenopsis*.

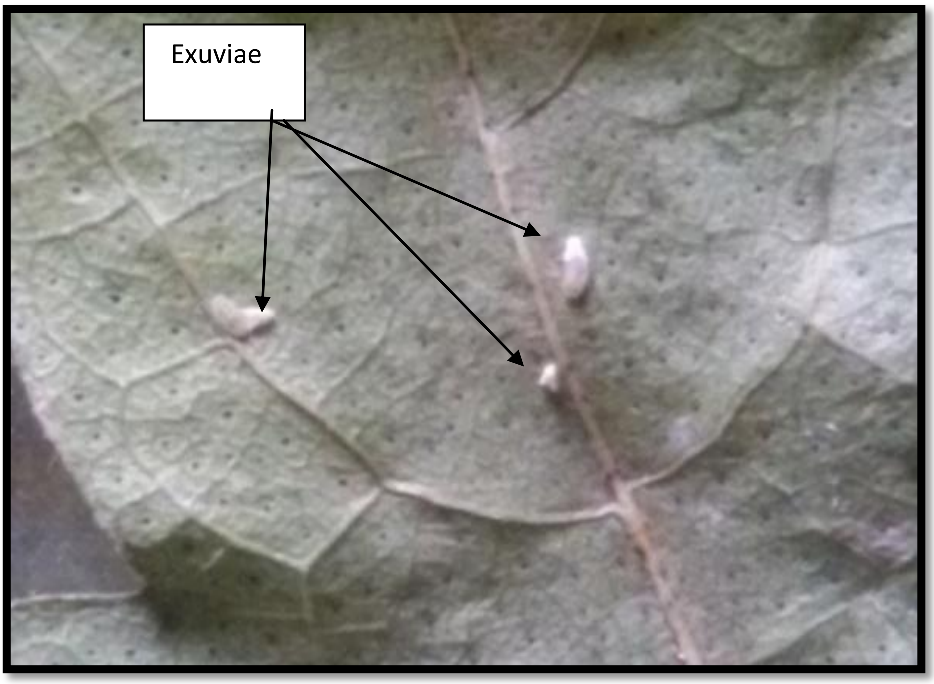

Plate 10. Moulting of the 2nd instar to 3rd instar of *P. solenopsis* confirmed by the presence of Exuviae on the leaf and posterior part of the nymphs.

Males and Females of *P. solenopsis*

The sexes become separable in the third nymphal instars, where the third instar females body are covered by mealy white wax. The third instar female moulted to a wingless adult female. The female nymphs moulted three times while males moulted four times.

4. 2 .2. Mode of reproduction of *P. solenopsis*:

Under laboratory conditions, the results showed that, , *P. solenopsis* reproduction is only sexual, where adult female reproduce only when fertilised by a male and produced crawlers not eggs in ovisacs , while un mated females failed to reproduce.

4. 2. 3. Life span of male *P. solenopsis:*

The results revealed no significant differences in the mean developmental period of adult male of *P. Solenopsis* for all the tested host plants **(Table 6).**

Male nymphs undergo a cocoon stage of fine wax filaments **(Plate 11).** Then, by the time, the cocoon fully develop to a small, winged adult male in a mean period of 5.4 days within 5- 6 days for all the tested plants (Appendices 8-11).

Adult males of *P. solenopsis* were delicate, slender, elongated in shape, the colour of the head, thorax, antennae and legs were yellowish brown whereas abdominal region pale yellow; with a pair of well developed thoracic, milky white wings and three pairs of well developed legs. The antennae were ten segmented and much longer than that of the female, and as long as the total body length of the male. Two pairs of waxy filaments were present at anal end of the body, with the inner pair longer than the outer pair **(Plate 12).**

After maturation, the male of *P. solenopsis* lived 2- 4 days on the tested host plants.

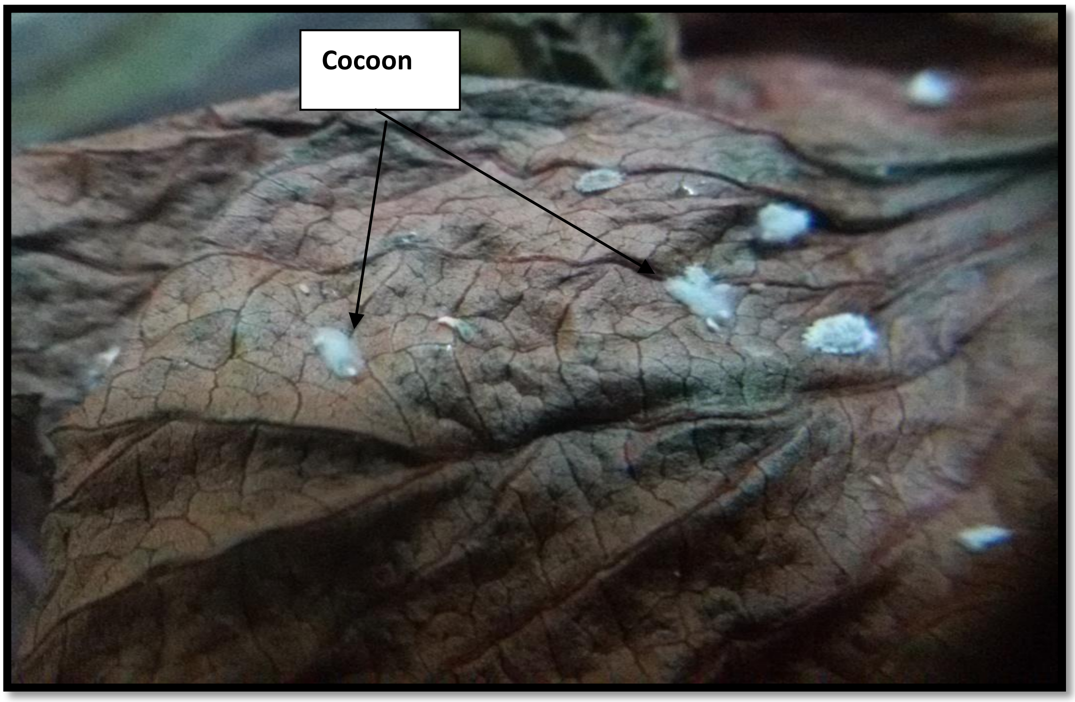

Plate 11. Male Cocoon (Pupal Stage) of *P. solenopsis*

Plate 12. The adult male of *P. solenopsis*

4. 2. 4. Life span of female *P. solenopsis* :

The results revealed that there are no significant differences in the mean developmental period of adult females of *P. solenopsis* when reared on Okra(30.3±1.23b), Tomato(28.3±5.42b), and Sweet pepper(26.8±3.15b), whereas, Cotton(36.2±3.18a) is significantly different from the other tested plants **(Table 6).**

Adult females of *P. solenopsis* were oblong in shape and light to dark in colour, having two pairs of black strips on dorsal side of the body, females were soft bodied covered with a powdery, wax secretion with six pairs of transverse dark bands that located across the pro- to meta- thoracic segments, it is also possessed a pair of brownish, short, 8-9 segmented filiform antennae and three pairs of red coloured legs and 18 pairs of lateral wax filament extended from around the margin of the body with a pair of terminal filaments longest **(Plate 13).**

Plate 13. Adult female of *P. solenopsis*

Pre- oviposition:

The mean Pre- oviposition period of *P. solenopsis* varies within the tested plants; it was the same for Cotton (8.7± 0.25 days) and okra (8.7± 0.35 days) with a range of 8- 9 days, and longer for tomato (11.8± 2.05 days) with a range of 9-13 days, and shorter for sweet pepper (6.2± 0.54) with a range 6- 7 days **(Table 7).**

Oviposition:

The mean oviposition period of *P. solenopsis* varies from plant to the anthor, but it is similar for Cotton (9.9± 2.42 days) and Okra (9.9± 2.55 days) with a range of 7– 12 days, and lower for Tomato (6.8±3.37 days) and Sweet pepper (6.6± 1.6 days) **(Table 7).**

After mating, females become relatively inactive, and anchor themselves by mouth parts to a single feeding site, while the fertilized eggs develop within their bodies. Females secreted a flexible, cottony sac of white wax filaments from the posterior part of the abdomen which extended to half her body length. In this Cottony ovisac, the female laid their crawlers **(Plate 14).**

Fecundity of *P. solenopsis*:

The average total fecundity of *P. solenopsis* among the tested plant species was high on Cotton (241.09± 34.88) with a range of 45- 439, followed by Okra (176± 89.96) with a range of 62- 364, Sweet pepper (123± 70.83) with a range of 20- 200, and lowest fecundity was on tomato (101± 78.20) with a range of 10- 263 (**Table 7).**

The average number of ovisac / female was high on Cotton (8.6± 3.05) with a range of 6- 15, followed by Okra (6.8± 2.24) with a range of 4-9, Sweet pepper (5.3± 1.55) with a range of 4- 6, and the lowest ovisac on Tomato (3.6± 2.68) with a range of 1-6.

Post oviposition:

The average post oviposition period is similar for cotton (2.5± 0.36 days) and Okra (2.5± 0.47 days) with a range of 2- 3 days, lower for Tomato (1.2± 0.67 days) with a range of 1- 3 days, while longer on Sweet pepper (2.8± 0.83 days) with a range of 1- 6 days **(Table 7 and Appendices 8-11).**

Table 7. Reproduction parameters (mean ± SE) of *P. solenopsis.*

Host plant	Pre-oviposition (days)	Oviposition (days)	Post oviposition (days)	Fecundity (Total no. of crawler/ ♀)	No.of ovisac/ ♀
Cotton	8.7±0.25	9.9±2.42	2.5± 0.36	241.09±34.88	8.6±3.05
Okra	8.7±0.35	9.9±2.55	2.5± 0.47	176±89.96	6.8±2.24
Tomato	11.8±2.05	6.8±3.37	1.2± 0.67	101±78.20	3.6±2.68
Sweet pepper	6.2±0.54	6.6±1.6	2.8± 0.83	123±70.83	5.3±1.55

Plate 14. The adult female of *P. solenopsis* with ovisac.

4.2.5. Sex ratio of *P. solenopsis*:

From the results, the sex ratio of female to male in the progeny were for Cotton(1:0.4), okra (1:0.04), tomato (1:0.11), and sweet pepper (1:0.12).

4.2.6. Nymphal mortality of *P. solenopsis*:

Mortality of the **1st** instar was high on Cotton (76.97%), followed by Tomato (19.43%) and Sweet pepper (17.86%), and low mortality on Okra (5.04%) (**Fig. 3 and Appendix 16**).

Mortality of the **2nd** instar of *P. Solenopsis* reared on the tested host plants was high in Tomato (56.45%), followed by Cotton (45.61%), Okra (21.21%) and sweet pepper (16.52%) **(Fig.3 and Appendix 16).**

High percentage mortality of the **3rd** instar was recorded on the pre-pupal stage of males on Okra (72.5%) and lower on sweet pepper (9.09%).

High mortality of the third instar female was recorded on Tomato (46.5%), followed by Sweet pepper (13.53%) and lower on Okra (5.22%).

In the Cocoon stage of male *P. solenopsis*, high mortality were recorded on Tomato (76.09%), followed by Okra (18.18%) and Sweet pepper (10%) **(Fig. 3 and Appendix 16).**

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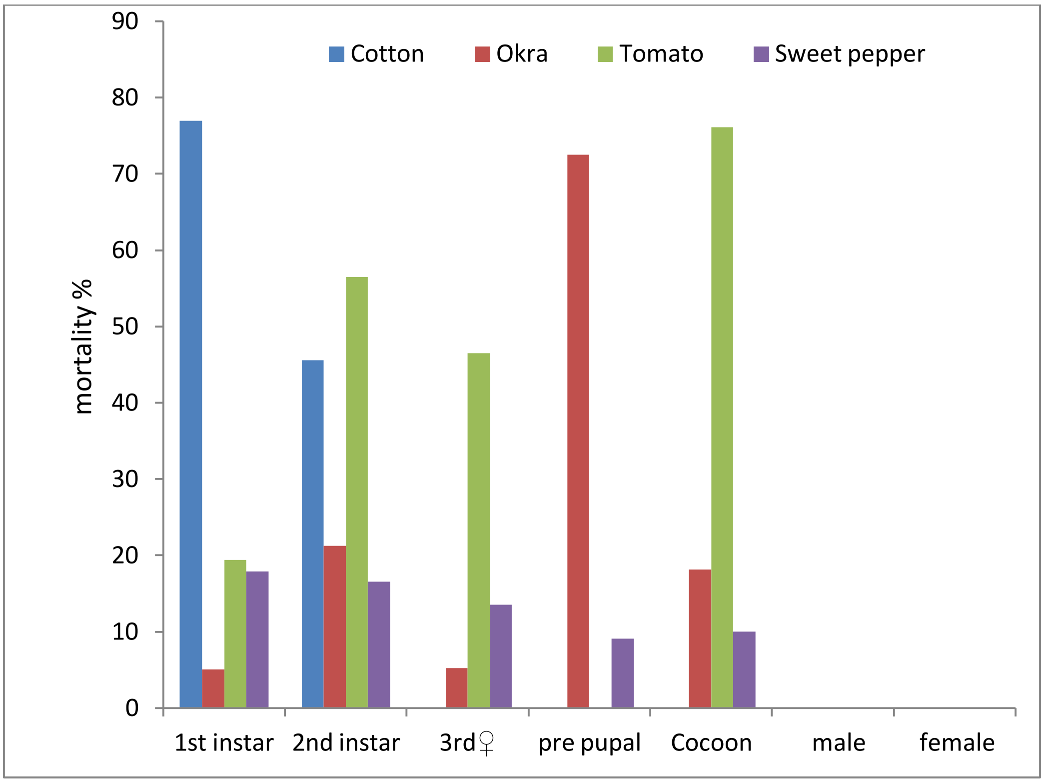

Figure 3. Natural percentage Mortality of *P. solenopsis* on different tested host plants.

4. 3. Host preference of *Phenacoccus solenopsis*:

This study revealed variation between the host plant species in regard to cotton mealybug, (*P. solenopsis*) attractiveness. However, the proportions of all nymphal instars varied significantly with respect to plants species and observation intervals (P value = 0.05). **(Tables: 8, 9, 10).**

After Two hours of release, the attraction of all the nymphal instars and adults of *P. solenopsis* revealed non-significant differences between the host plants as compared with the control (Chinese rose). The mean proportion of the 1st nymphal instar ranged from 1.3 to 25.3, on Raba and Hambouk respectively, where the attraction of the 2nd nymphal instar, ranged from 0 to 9 on Egg plant and Hambouk, respectively. The attraction of the third nymphal instar, displayed low levels on the tested plants, with a range of 0 to 2 on Eggplant and Lantana, respectively. For the attraction of the adults of *P. solenopsis*, low populations of the adult stage were recorded, which ranged from 3 to 0.67 on Egg plant and Cotton, respectively **(Table 8).**

Table (8): The average number of nymphal instars and adult females of Cotton mealybug **2 hours** after release.

Host plants	**Stages of Cotton mealybug**			
	1st nymphal instar	2nd nymphal instar	3rd nymphal instar	Adult Female
Hambouk	25.33 a	9 a	0.33a	1.67a
Lantana	19.33 a	8.67 a	2 a	1.33 a
Okra	18.33 a	5.33 a	1 a	2 a
Tomato	16.33a	5 a	1 a	1 a
China rose	15a	2 a	1 a	0.67 a
Cotton	11.67a	5.67 a	1.33 a	0.67 a
Egg plant	11.67a	0 a	0 a	3 a
Raba	1.33a	1.33 a	0.67 a	1.67 a
CV	94.64	106.57	109.09	96.23
LSD	33.571	11.754	2.3848	3.4422
SE	11.494	4.0242	0.8165	1.1785

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test

Eight hours after release, there was a highly significant difference between the two host plants (Lantana and Cotton) and Raba, where the proportions of the 1st inster of *P. solenopsis* ranged from 0 to 50 on Raba and Lantana, respectively. Lantana possessed high population proportion followed by Cotton 45. The proportions of the 2nd instar settled on the different host species ranged from 0 to 16.33 on Raba and Lantana, respectively, with no significant difference as compared with the control. Nevertheless, the proportions of the 3rd nymphal instar ranged from 0 to 11.33 on Raba and Okra, respectively, with a significant difference between them. Also, there were non-significant differences between the attractiveness of all tested plants and the control; where the proportions of the adult stage ranged from 0.33 to 5.67 on Raba and Cotton, respectively (Table **9).**

Table (9): The average Number of nymphal Instars and adult females of Cotton mealybug **8 hours** after release.

Host plant	Stages of cotton mealybug			
	1st nymphal instar	2nd nymphal instar	3rd nymphal instar	Adult Female
Hambouk	30a b	6.67a	3.67ab	4.67a
Lantana	50a	16.33a	9.33ab	3.67a
Okra	36.67 ab	13.33a	11.33a	3a
Tomato	35.33 ab	14.33a	5ab	3.67a
Chinese rose	26 ab	10.33a	4ab	2a
Cotton	45 a	15.67a	7.33ab	5.67a
Egg plant	21.33 ab	5a	3ab	3a
Raba	0 b	0a	0b	0.33a
CV	53.69	74.76	74.79	69.37
LSD	39.105	18.201	9.7359	5.3768
SE	13.388	6.2316	3.3333	1.8409

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test

At 24 hours after release, the occurrence of the 1st instar after 24 hours of release ranged between 0.33 to 62.33 on Raba and Okra, respectively, with a high significant differenc observed between Okra and the other three tested plants (Lantana , Eggplant and Raba). The proportions of the 2nd instar on the different host species ranged between 0.33 to 38.67 on Raba and Okra, respectively , with no significant difference between Okra(38.67a), Cotton (31.33ab) and Hambouk (27.33abc). High significant difference was noticed in the proportions of the 3rd instar on Okra compared with the other plants and the control, which ranged between 0.33 and 43.67, on Raba and Okra, respectively. For the Adult stage, its population on Okra was significantly different compared with the other host plants, where the adult population ranged between 0 to 33 on Egg plant and Okra, respectively **(Table 10).**

Attractiveness of the mealybug's stages to the different host plants is also represented in **Figures: 4, 5, 6 and 7.**

Table (10): Average Number of nymphal Instars and adult females of cotton mealybug 24 **hours** after release.

Host plant	Stages of Cotton mealybug			
	1st nymphal instar	2nd nymphal instar	3rd nymphal instar	Adult Female
Hambouk	38.33abc	27.33abc	28ab	8.33b
Lantana	15.67bc	5 cd	2.67c	3b
Okra	62.33a	38.67a	43.67a	33a
Tomato	26.67abc	12.67bcd	11.67bc	2.67b
China rose	26abc	10 bcd	5.67c	2b
Cotton	55ab	31.33ab	16bc	10.33b
Egg plant	10c	4.67cd	1.33c	0b
Raba	0.33c	0.33d	0.33c	1.33b
CV	60.29	60.37	56.44	86.51
LSD	42.133	23.397	18.396	15.646
SE	14.418	8.0104	6.2981	5.3567

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test

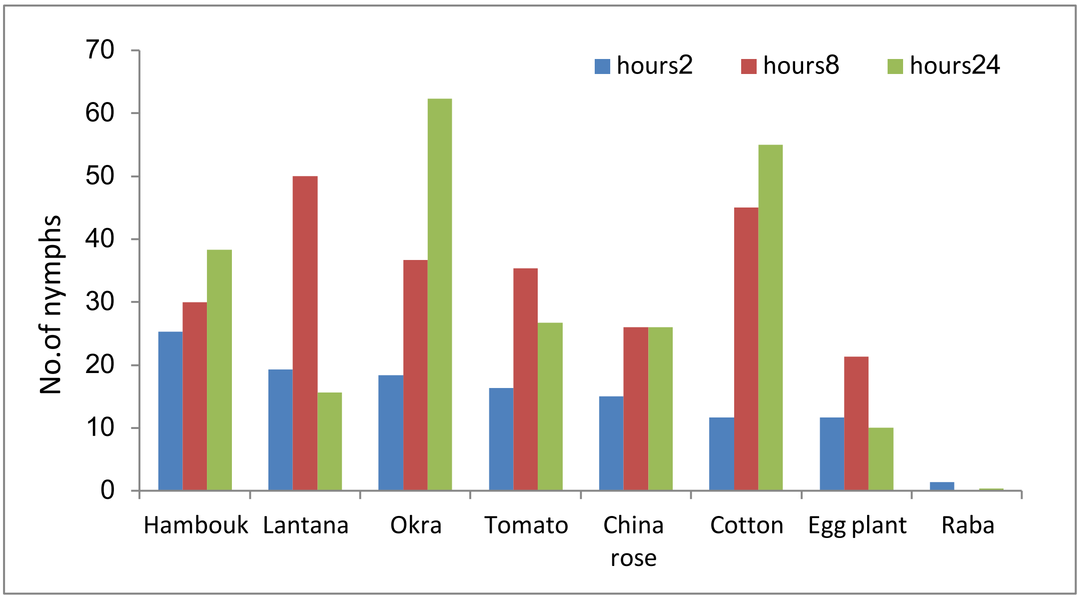

Figure (4): number of the 1st nymphal instars of cotton mealybug on host plants, at different intervals after release.

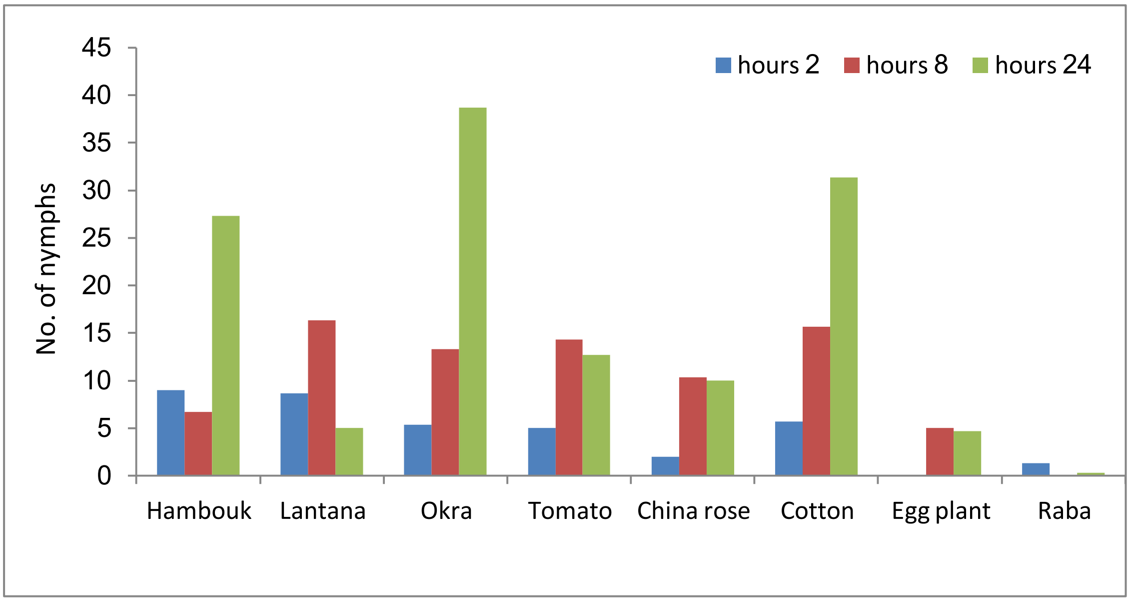

Figure (5): number of the 2nd nymphal instar of cotton mealybug on host plants, at different intervals after release.

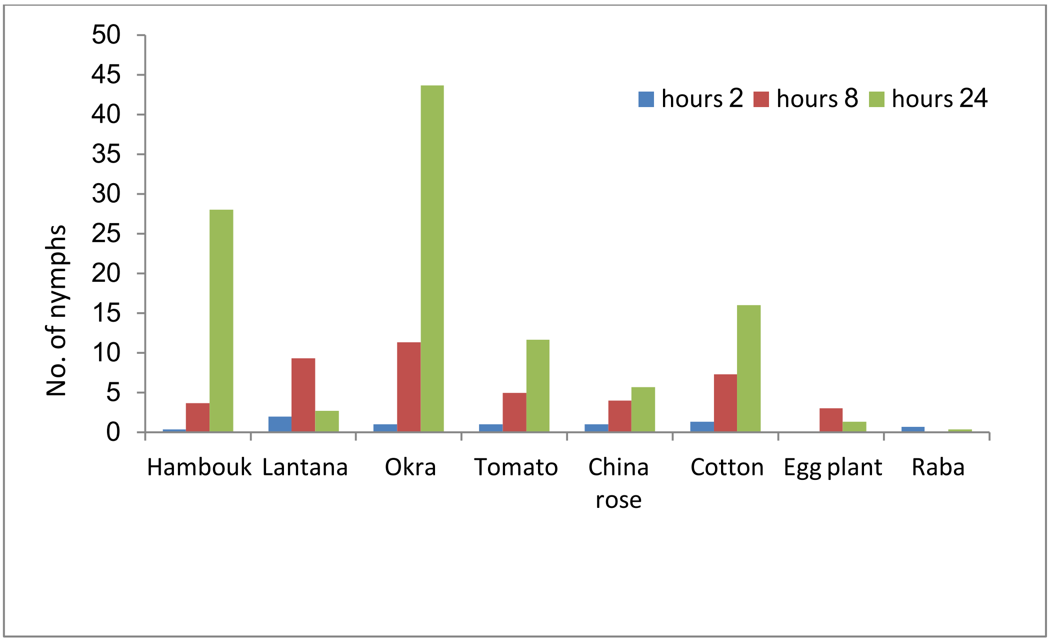

Figure (6): number of the 3rd nymphal instar of cotton mealybug on host plants, at different intervals after release.

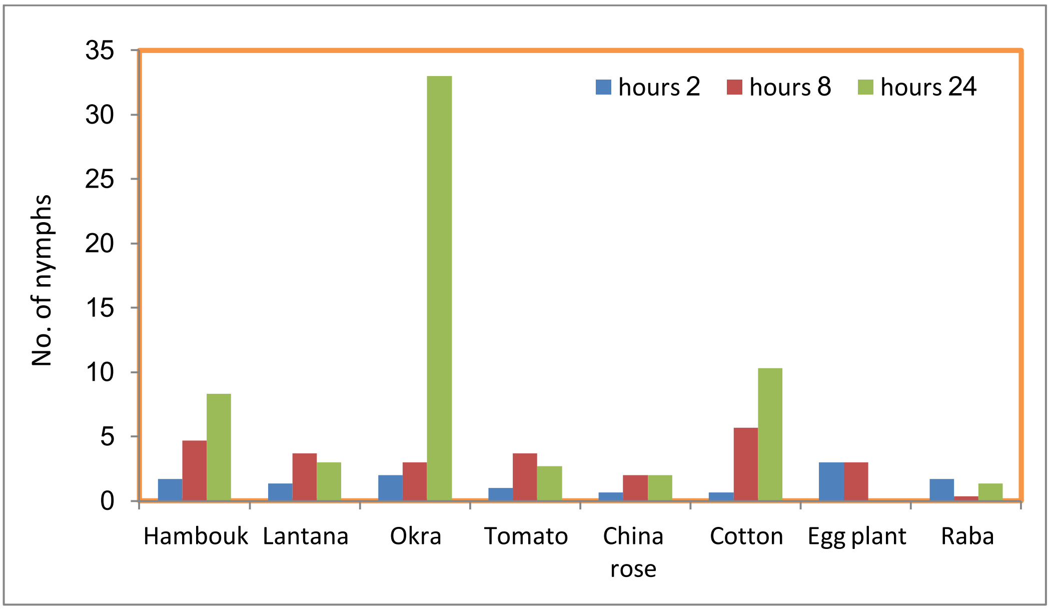

Figure (7): number of Adult females of the cotton mealybug on host plants, at different intervals after release.

4. 4. Biological control of *Phenacoccus solenopsis* :

Field surveys in Khartoum State showed that, nine natural enemies were associated with cotton mealybug, *P. solenopsis*, which include eight predators and one parasitoid. The predator species found were : *Exochomus nigromaculatus* (Goeze) (Coleoptera :Coccinellidae), striped beetle, *Brumus sutuaralis* F. (Coleoptera : Coccinellidae), Zigzag beetle, *Menochilus sexmaculatus F*. (Coleoptera : Coccinellidae), spotted beetle, *Hippodamia variegata* (Coleoptera : Coccinellidae), *Chrysoperla sp*. (Neuroptera : Chrysopidae), larvae of *Chryptolaemus montrouzieri*, (Converent lady beetles) (Coleoptera : Coccinellidae) , larva and Adult of *Hyperaspis trifurcata* (Coleoptera : Coccinellidae) and *Scymnus spp*. (Coleoptera : Coccinellidae). The parasitoid species found was identified by Entomology Unit , ARC, Wadi Medani, as *Aenasius arizonensis* (Hymenoptera : Encyrtidae). All species of natural enemies were abundantly found on Abutilon, cotton, Chinese rose and *datura stramonum*. (Plates: 15- 18)

Plate 15. Zigzag beetle, *Menochilus sexmaculatus*

Plate 16. Lace wing larva *(Chrysoperla carnea*)

Plate 17. Striped beetle, *Brumus sutuaralis* F.

Plate 18. *Hyperaspis trifurcata* larva and Adult

4. 4. 1. Biology of the Predator, *Exochomus nigromaculatus* :

Developmental time of immature stages, adult stage, fecundity and mortality percent of *Exochomus nigromaculatus* were studied on cotton mealybug *P. solenopsis,* as prey, at a temperature of 25°C-28°C , with a relative humidity of 28-35%.

The results showed that, there were significant variations in the developmental time of immature and adult stages of the predator.

Egg stage:

The eggs are usually elongate, oval with bright yellow transparent colour, they are laid either upright, or on their sides in clusters or batches or groups but sometimes individually, with a mean developmental period of 4.83± 0.45 days (**Table 11 and Plate 19**).

Table 11. Life cycle and reproductive parameters of predator, *Exochomus sp* feeding on *Phenacoccus solenopsis*.

Stages	Range	Developmental time (days) Mean±SE
Eggs		4.83±0.45
Nymph		
1st instars grub		2.55 ±0.18
2nd instars grub		2.22 ±0.15
3rd instars grub		2.62±0.14
4th instar grub		4.61±0.34
Pre- pupa		1.42±0.14
pupa		4.67±0.17
Adult		
Male	28-60	50.42± 2.55
Female	23-59	50.67±2.97
Female		
Pre-oviposition	8-17	10.67 ±0.83
oviposition	11-37	31.73 ±2.25
Inter-oviposition	1-2	1.52±0.07
Post oviposition	0-16	9.75±1.37
Total life cycle		
Male		73.34± 2.55
Female		73.59±2.97
fecundity	232-479	367±24.37

Plate 19. Cluster of eggs of *Exochomus sp.*

Larval stage:

After hatching from eggs the grubs (or larvae) pass through four instars. Grubs hatched from eggs as 1st instar with a mean developmental period of 2.55 ± 0.18 days. They are black grey in colour, with spiny abdomen (**Plate 20**). The larvae increased gradually in size, and moulted to reach the 2nd , 3rd and 4th instars, with mean developmental periods of 2.22 ± 0.15 days, 2.62 ± 0.14 days, and 4.61 ± 0.34 days, respectively(**Table 11**). Because of moulting and increase in size at every stage, the colour of the grubs turn to yellow with black brown spines on the abdomen (**Plates 21 & 22 & 23**).

No differences in the mean duration time of the 1st , 2nd , and 3rd instar grubs, while the 4th instar was quite different from the three previous stages (Table 11).

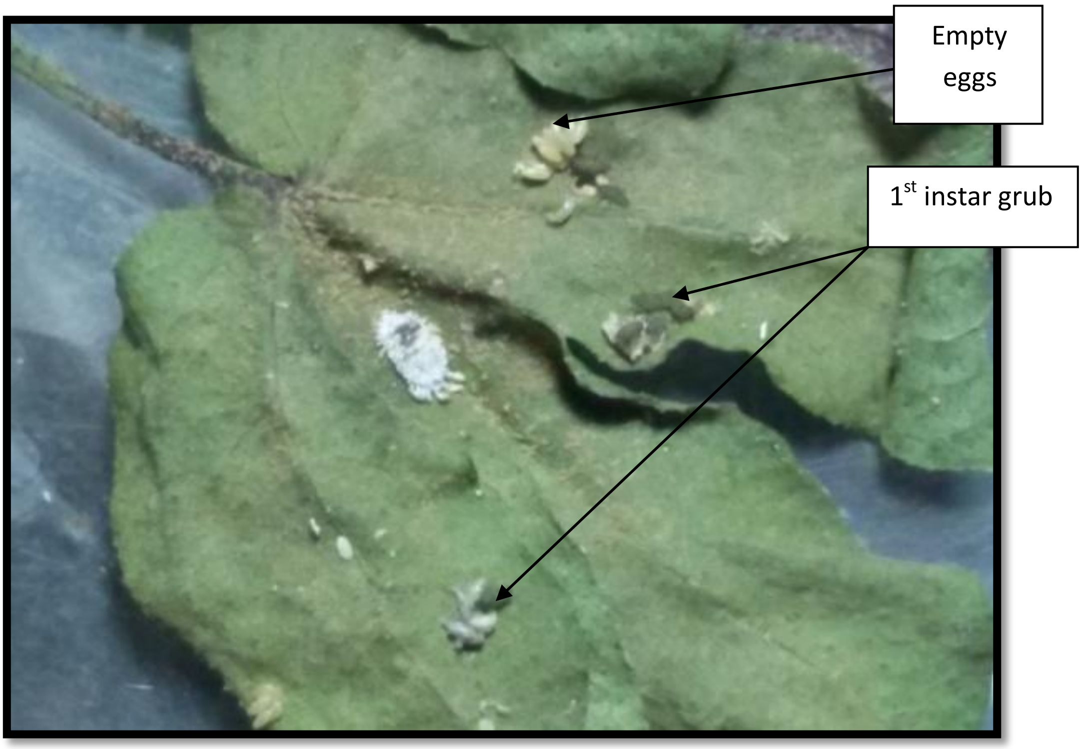

Plate 20. First instar grub of *Exochomus nigromaculatus*.

Plate 21. Second instar grub of *Exochomus nigromaculatus.*

Plate 22. Third instar grub of *Exochomus nigromaculatus*.

Plate 23. Fourth instar grub of *Exochomus nigromaculatus*.

Pre- Pupal Stage:

The last 4^{th} instar grub stop feeding, attaches itself by the tip of its abdomen (anal organ) to a substrate and prepares for pupation, and are particularly immobile (**Plate 24**). The mean period of this stage takes 1.42± 0.14 days.

Pupation Stage:

The larval cuticle ruptures on the dorsal side, and split length wise forming a protective chamber around the pupa (**Plate 25**). Male and female pupa cannot be distinguished by their external morphology. Females tend to be larger and heavier than males in the pupal stage. The mean pupal period was **4.67± 0.17 days (table 11).**

Adult Stage:

Oviposition period ranged between 11-37 days, and a mean of 31.73±2.25 days, with inter oviposition period ranging between 1-2 days and 1.52 ± 0.07 days as the mean. The last reproduction period, called the post ovipostion, range between 0-16 days with a mean of 9.75±1.37 days. The 0 value when the females die during the reproductive phase. The fecundity ranged between 232-479 eggs, with a mean of 367±24.37 eggs per female during its all life cycle. The mean average total life cycles for a female and a male **(Plates 26 and 27)**, were 73.59±2.97 days, 73.34±2.55day respectively (**Table 11**).

Plate 24. Fourth instar grub stop feeding, attached to leaf of China rose as pre-pupal stage.

Plate 25. Pupal Stage of *Exochomus sp.*on lantana.

Plate 26. Adult emergence from the pupa.

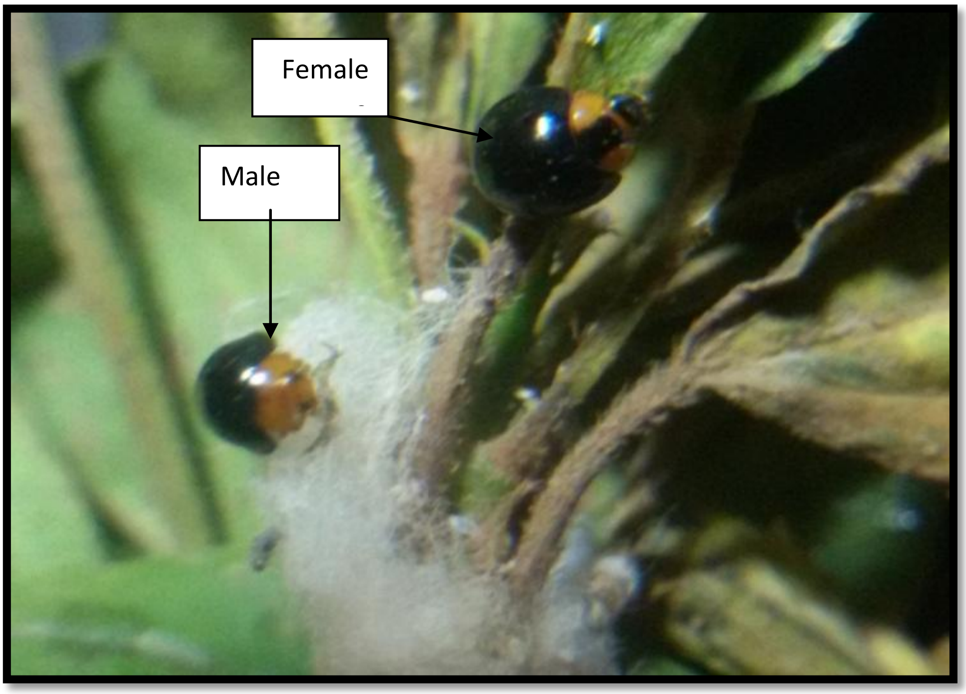

Plate 27. Adult male and Female of *Exochomus sp.*

4. 4.1.1. Prey Consumption of the 4^{th} Instar *E. nigromaculatus:*

Feeding potential of the 4^{th} instar grub of *Exochomus sp.,* on a mixed diet of the 3^{rd} nymphal instars and adult females of the cotton mealybug *P. solenopsis,* was studied under laboratory conditions at a temperature of 21°C-28°C, with a relative humidity of 28-35%.

The results showed that, the 4^{th} instar grub takes 5 days before entering into the second stage (pre-pupa). The prey consumption increases towards the 4^{th} day, then decreased in the 5^{th} day (**Fig. 8)**.

The mean numbers of the prey consumed by the 4^{th} instar grub of *E.nigromaculatus* in the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} day were (3.7±0.52 + 1.8±0.36) , (4.16±0.21 + 3.79±0.35), (6.28±0.46 + 6.33±0.73), (6.87±0.36 + 5.75±0.69) and (5.38±0.47 + 3.15±0.82), of the mixed diet of 3^{rd} nymphal instars and adult females of the cotton mealybug , respectively (**Table 12** and **Fig. 8**).

4. 4. 1. 2. Prey Consumption of Adult *E. nigromaculatus:*

The mean number of the prey consumed by the adult females of the predator in the 1^{st}, 2^{nd} ,3^{rd} , 4^{th} , and 5^{th} day were 6.8 ± 0.20 + 2.3±0.01 , 8 ± 0.56 + 1.2 ± 0.96 , 7 ± 0.06 + 3.2±1.04 , 7.2 ± 0.003 + 2 ± 0.03 and 8.25 ± 0.82 + 2.18±2.04 (**Table 13 and Fig. 9**).

Table 12. Feeding potential of the 4^{th} instar of *Exochomus sp.* on a mixed diet of the 3^{rd} and adult stage of *P. solenopsis*

Days of feeding	Total prey consumed by *Exochomus sp* (mean ±SE)	
	3^{rd} instar nymph of *P. solenopsis*	Adult ♀ of *P. solenopsis*
1^{st} day	3.7±0.52	1.8±0.36
2^{nd} day	4.16± 0.21	3.79±0.35
3^{rd} day	6.28± 0.46	6.33±0.73
4^{th} day	6.87±0.36	5.73±0.69
5^{th} day	5.38±0.47	3.15±0.82

Table 13. Feeding potential of the adult female *Exochomus sp.* on a mixed diet of the 3^{rd} and adult stage of *P.solenopsis*

Days of feeding	Total prey consumed by *Exochomus sp* (mean ±SE)	
	3^{rd} instar nymph of *P. solenopsis*	Adult ♀ of *P. solenopsis*
1^{st} day	6.8 ± 0.20	2.3± 0.014
2^{nd} day	8± 0.56	1.2±0.96
3^{rd} day	7 ±0.06	3.2±1.04
4^{th} day	7.2±0.003	2±0.03
5^{th} day	8.25± 0.82	2.18±2.04

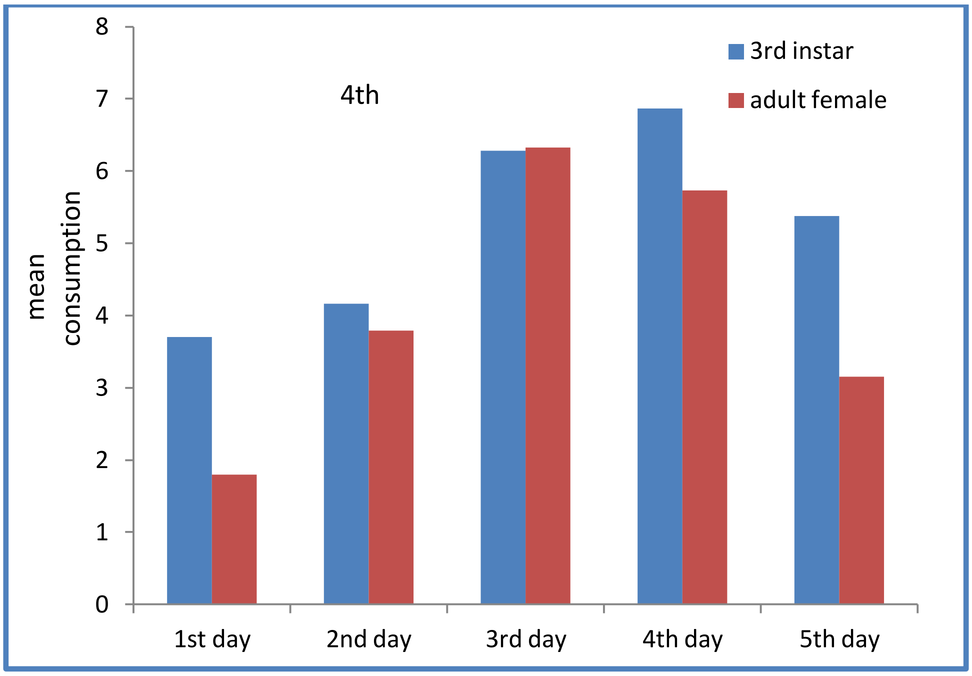

Figure 8. Prey consumption of 4th instar grub *Exochomus* sp., on a mixed diet of the 3rd and adult stage of *P. solenopsis*.

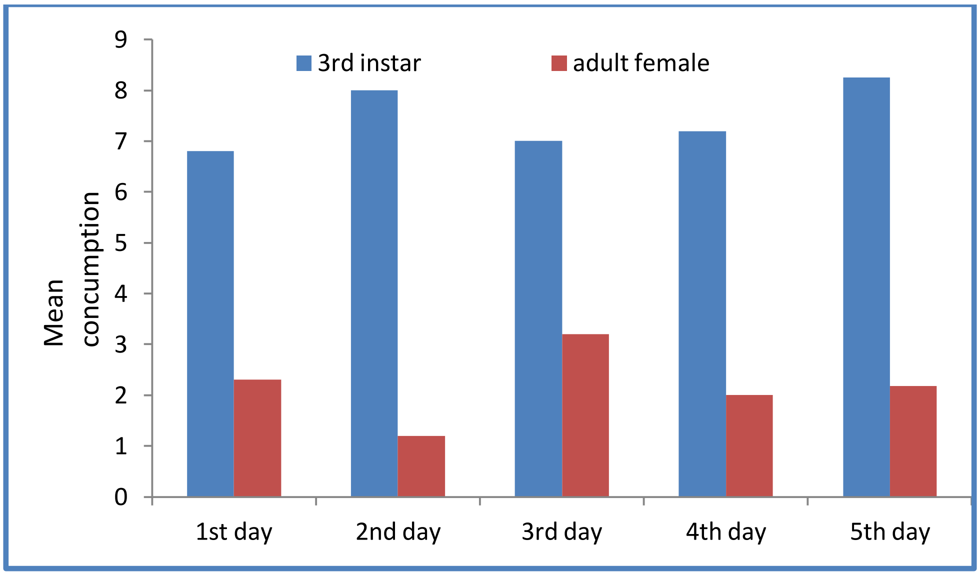

Figure 9. Prey consumption of adult female *Exochomus sp.*, on a mixed diet of the 3rd and adult stage of *P. solenopsis*.

For the adult males, the mean numbers of the prey consumed were 7.33± 0.14 + 0.42± 2.56 , 6.36 ± 0.37 + 2.27± 0.06 , 6.73± 0.06 + 2.64 ± 0.38 , 7.45± 0.23 + 2.73 ± 0.50 and 7.97± 0.8 + 2.82± 3.5 , respectively (**Table 14 and Figure 10**).

[Appendices Number 18 and 19: show Feeding of *E. nigromaculatus* 4th Instar grubs and adults on the mealybugs].

4.4.1.3. Mortality of the Predator, *E. nigromaculatus*:

The results revealed higher mortality on the 3rd instar grub of *E. nigromaculatus* 25.39 , followed by the 4th instar 21.27 , 1st instar 18.28 , Eggs 17.84 , pupal stage 12.33, 2nd instar 11.88 , and low mortality on the pre- pupal stage 1.35 , and no mortality was recorded on the adult male and female stages **(Table 15 & Fig. 11).**

Table 14. Feeding potential of adult male *Exochomus sp.*,on mixed diet of the 3rd and adult stage of *P.solenopsis*.

Days of feeding	Total prey consumed by *Exochomus sp.* (mean ±SE)	
	3rd instar nymph of *P. solenopsis*	Adult ♀ of *P. solenopsis*
1st day	7.33±0.14	0.42±2.56
2nd day	6.36±0.37	2.27±0.06
3rd day	6.73± 0.06	2.64±0.38
4th day	7.45±0.23	2.73±0.50
5th day	7.97± 0.8	2.82±3.5

Table 15. Mortality of the predator, *Exochomus sp* feeding on cotton mealybug, *Phenacoccus solenopsis*.

Developmental stage	Number alive	Mortality factor	Number dying	Percent mortality
eggs	213		38	17.84
1st instar grub	175	Unknown	32	18.28
2nd instar grub	143	Unknown	17	11.88
3rd instar grub	126	Unknown	32	25.39
4th instar grub	94	Unknown	20	21.27
Pre- pupation	74		1	1.35
Pupation	73		9	12.33
Adult male (♂)	30		0	0
Adult female(♀)	34		0	0

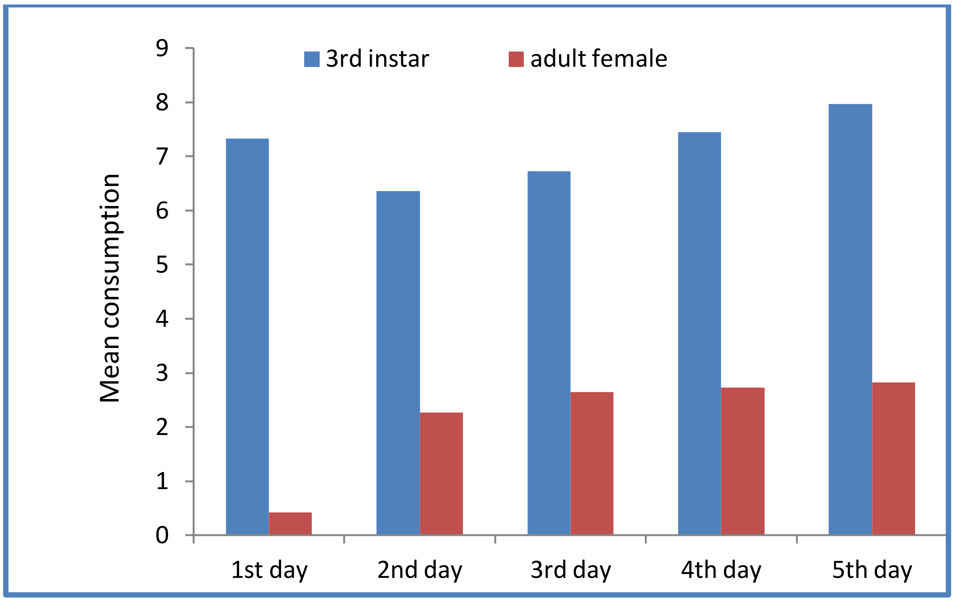

Figure 10. Feeding potential of the adult male *Exochomus sp.* on a mixed diet of the 3^{rd} nymphal instars and adult females of *p. solenopsis* .

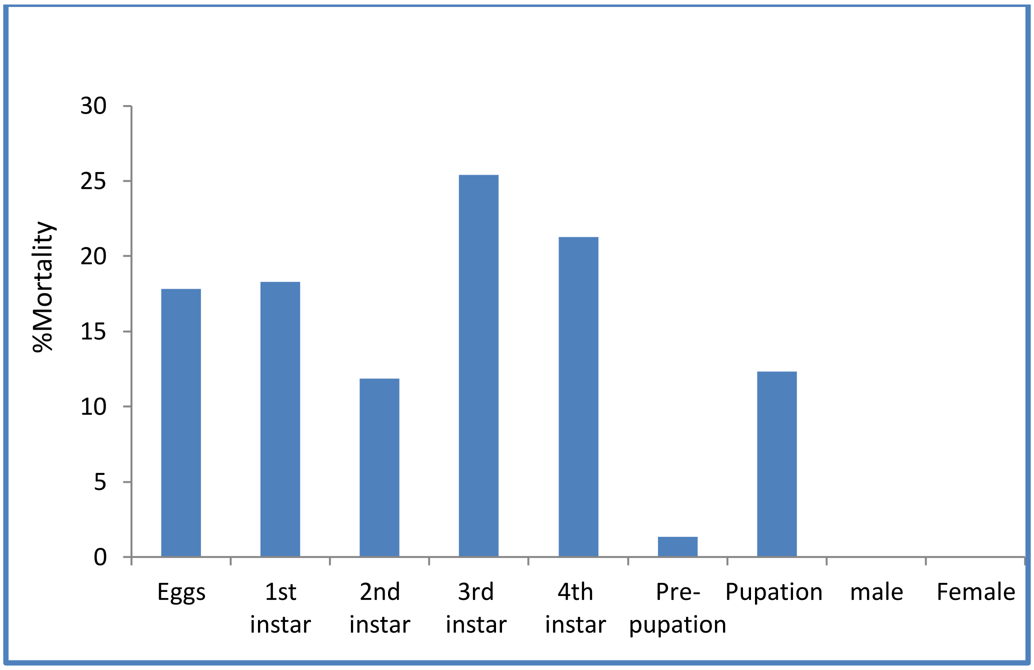

Figure 11. Percentage mortality of predator, *E. nigromaculatus* reared on *P. solenopsis.*

4. 4. 2. Distribution and Biology of Parasitoid, *Aenasius arizonensis:*

4. 4 .2. 1. Distribution of the Parasitoid:

A preliminary survey of the parasitoid, *A. arizonensis* in the field showed that, it is available most of the year round on some host plants **(Table 16).**

4. 4. 2. 2. Biology of the Parasitoid:

The biology of the wasp was studied under laboratory conditions**.** The observations showed that, after mating the females search for mealybugs and insert the ovipositer through ventral side of the body of mealybug. The females oviposit fertilized eggs within 24 hours inside the mealybug. The parasitized mealybug sheds its wax, swollen and shows very poor movement after 2-4 days of oviposition then hardens into a leathery, brown coloured structure, which transformed into a barrel shaped, dark brown mummy within a week **(Plates 28 and 29).**

From the results it was observed that, adults emerged from mummies by cutting a circular hole after a pupal period; the emergence holes were always found on the posterior – dorsal part of the dorsum of the mummified *P. Solenopsis*. Only one parasitoid adult emerged from each mummy of the host, i.e., the parasitoid female lay a single egg in its host.

Table 16. Seasonality of the parasitoid, *A. arizonensis* on different host Plants under field conditions (Season: 2015- 2016)

No.	Month	Host plant species
1	October	Okra
2	November	Okra
3	December	Okra - Lantana
4	January	Okra- Hambouk
5	February	China rose – Hambouk
6	March	Hambouk
7	April	Hambouk
8	May	Ramtouk- Hambouk
9	June	Datura- Hambouk- china rose
10	July	China rose- hambouk
11	August	-
12	September	-

Plate 28. Parasitized mummies of *P. solenopsis* on lantana

Plate 29. Parasitized mummies of *P. solenopsis* on china rose.

The Developmental Parameters of *A. Arizonensis:*

The results of this study are shown in (**Table 17).** These results indicated that, no difference in the mean developmental periods from ovipostion to emergence of the parasitoid females (13.09 ± 1.02 days) and males (13.29± 1.12 days), with a range of 10-17 and 10-16 days, respectively. The males died within 9.71± 1.21 days from emergence, with a range of 5-11 days and a pre- oviposition period less than one day.

The mean longevity period (from egg to death) for adult females was 23.37 ±1.89 days, and 23± 2.33 days for males, with a range of 13-29 and 13-25 days for females and males, respectively. The number of parastized mealybugs per day ranged from 20- 24 with a mean of 22.5± 0.96 (**Table 17**).

The offspring include males and females, has a sex ratio of 1:1.6. Newly emerged wasps were identified as female and males with shiny black body, the males differ from female in that, it is generally smaller in size.

[Appendices number 20 and 21: show Plates of pupae and emerging adult female of the parasitoid].

Table 17. Developmental parameters of *A. arizonensis* on *P. solenopsis* under laboratory conditions (Season: 2015-2016).

Biological characteristics	**Mean ± S.E** (days)	**Range** (days)
Oviposition to mummy formation	3 ± 1.16	2- 4
Adult male emergence after oviposition	13.29 ± 1.12	10- 16
Adult female emergence after oviposition	13.09 ± 1.02	10-17
Emergence to death for male	9.71 ± 1.21	5- 11
Emergence to death for female	10.28 ± 0.87	9- 14
Pre- oviposition period	<1	<1
Male longevity	23 ± 2.33	13- 25
Female longevity	23.37 ± 1.89	13- 29
Mean of Parasitization (no. of parasitized host female/ day).	22.5 ± 0.96	20- 24
Sex ratio of progeny : (male: female)	1: 1.6	

4.4.2.3. Parasitism of *Aenasius arizonensis* on *P.solenopsis* in the field:

For field surveys, high infestations of the 3rd nymphal instars and adult females of *P. solenopsis* by the parasitoid were recorded on Hambouk. The results of the survey revealed a significant difference between the mean number of adult female's mummies per 30 cm apical shoot (17.9 ± 0.85) and that of 3rd instars mummies (4.24 ± 0.85) **(Table 18).**

These results showed that, the total parasitism on Hambouk was 31.26%, which expressed as 25.02% on adult females and 6.24% on the 3rd instar. No parasitism was observed on the first and second instars nymphs.

During this study, activities of ants as predators of mummified mealybugs were observed, and also observed carrying about young mealybug crawlers, aiding in their dispersal **(Plate 30).**

Table 18. Mean numbers of parasitoid mummies and percentage parasitism on different stages of *P. solenopsis* (April 2019)

Stages	Mean no. of mummies per 30cm shoot	percent parasitism	Total percent parasitism
Adult Female	17.908 A	25.02	31.26
third Nymphal instar	4.24 B	6.24	
CV	54.14		
SE	0.8479		
LSD	1.986		

Plate 30. Active ants, seeking for honey dew on a colony of cotton mealybug, *P. solenopsis*.

4.4. 3. Efficacy of *Eucalyptus camaldulensis* against *P. solenopsis*

Laboratory experiments were carried out to evaluate the efficacy of Petroleum ether, Ethanol and water leaves extracts of *Eucalyptus camaldulensis* , at different concentrations, against the cotton mealybug. From each extract, four concentrations (2.5%, 5%, 7.5%, and 10%) were prepared for the treatments.

The results of the experiments showed that, the mean mortalities of the mealybugs increased as the oil concentration increased from 2.5% to 10%, and as the time increases from 24 hours to 72 hours for all concentrations.

Genaraly, percentage mortalities of the adult females increased for all concentrations with passes of time, reaching the maximum after 72 hours of exposure at extract concentration 10%. The solvents alone (control) exhibit mortalities which should be taken in consideration.

Leaf dip method:

Data in (**Tables 19 and 20),** showed that Petroleum Ether Extract 10% exhibit more mortality at intervals 24 hours (1.5) and 48 hours (3.5) than Ethanol Extract 10% (1.0), (2.5), respectively, for the same intervals **.** In contrast, after 72 hours, Ethanol Extract showed higher mortality (8.5) **(Table 20)**. Zero mortality was recorded for water extracts **(Table 21).**

leaf dip method recorded lowest percent mortality for all the concentrations of solvent extracts at intervals 24 hours and 48 hours, while at interval 72 hours, ethanolic extract 10% have high mortality (56.7 %) than petroleum ether extract 10%(38.3%) **(Fig.12 and 13)** and the aquous extract 10% (1.67%)**.**

Table 19. Mean mortalities of *P. solenopsis* by Petroleum ether extracts of *Eucalyptus camaldulensis* (leaf dipping method).

Treatment and concentrations	**Mean mortalities by leaf dipping method**		
	24 hours	**48 hours**	**72 hours**
Hitcel 44% (Standard)	13.500 A	15.000 A	15.000 A
P. ether ext. 10%	1.500 B	3.500 BC	5.750 BC
P. ether ext. 7.5%	0.250 BC	1.000 CD	2.2500 DE
P. ether ext. 5%	0.000 C	0.500 D	1.500 DE
P. ether ext. 2.5%	0.000 C	0.000 D	1.500 DE
P. ether (control)	0.750 BC	2.500 BC	6.000 BC
CV	77.30	66.05	73.34
SE	0.33	0.82	0.79
LSD	0.67	1.65	1.6

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test

Table 20. Mean mortalities of *P. solenopsis* by Ethanol extracts of *Eucalyptus camaldulensis* (leaf dipping method) .

Treatment and concentrations	**Mean mortalities by leaf dipping method**		
	24 hours	**48 hours**	**72 hours**
Hitcel 44% (Standard)	13.500 A	15.000 A	15.000 A
Ethanol ext.10%	1.000 BC	2.500 BC	8.500 B
Ethanol ext. 7.5%	1.000 BC	1.2500 CD	4.250 CD
Ethanol ext.5%	0.750 BC	0.750 D	1.750 DE
Ethanol ext.2.5%	0.250 BC	0.250 D	0.500 E
Ethanol (control)	0.500 BC	0.750 D	1.750 DE
CV	77.30	66.05	73.34
SE	0.33	0.82	0.79
LSD	0.67	1.65	1.6

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test

Table 21. Mean mortalities of *P. solenopsis* by Water extracts of *Eucalyptus camaldulensis* (leaf dipping method).

Treatment and concentrations	Mean mortalities by leaf dipping method		
	24 hours	**48 hours**	**72 hours**
Hitcel 44% (Standard)	13.500 A	15.000 A	15.000 A
Water ext. 10%	0.000 C	0.000 D	0.250 E
Water ext. 7.5%	0.000 C	0.000 D	0.000 E
Water ext. 5%	0.000 C	0.000 D	0.000 E
Water ext. 2.5%	0.000 C	0.000 D	0.000 E
water (control)	0.000 C	0.000 D	0.000 E
CV	77.30	66.05	73.34
SE	0.33	0.82	0.79
LSD	0.67	1.65	1.6

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test

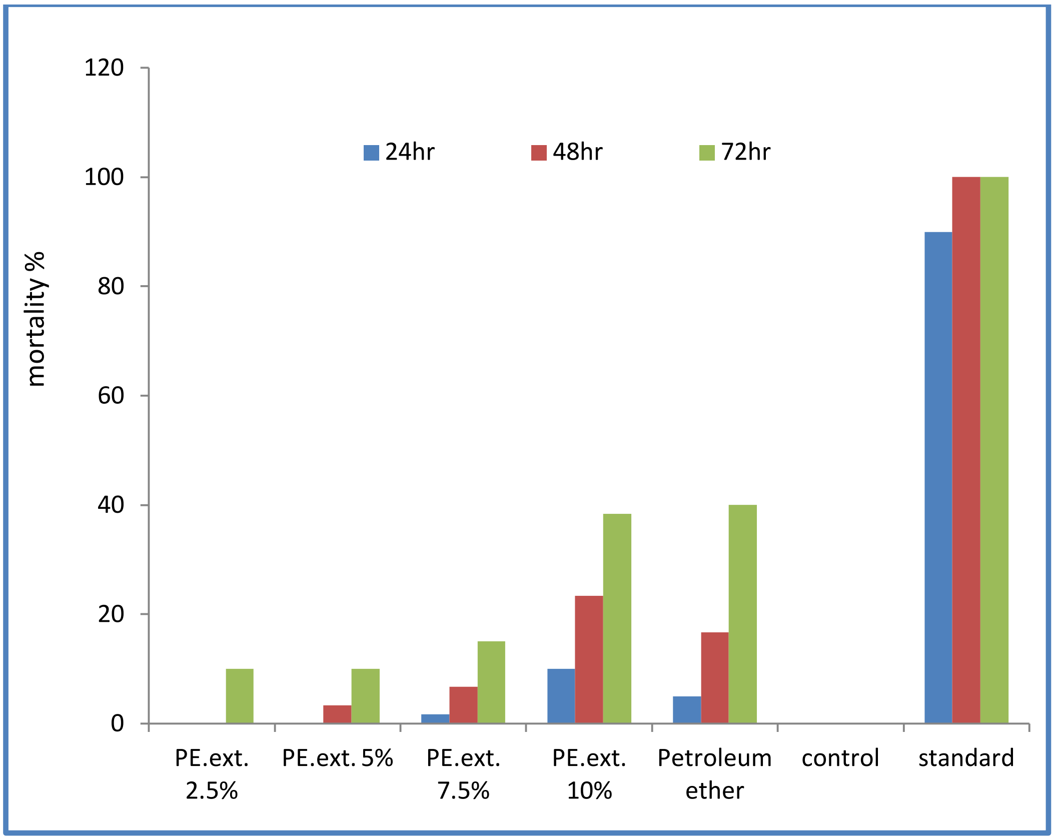

Fig.12: Mean mortality (%) of *Phenacoccus solenopsis* treated by Petroleum ether extracts of *E. camaldulensis* (leaf dipping method).

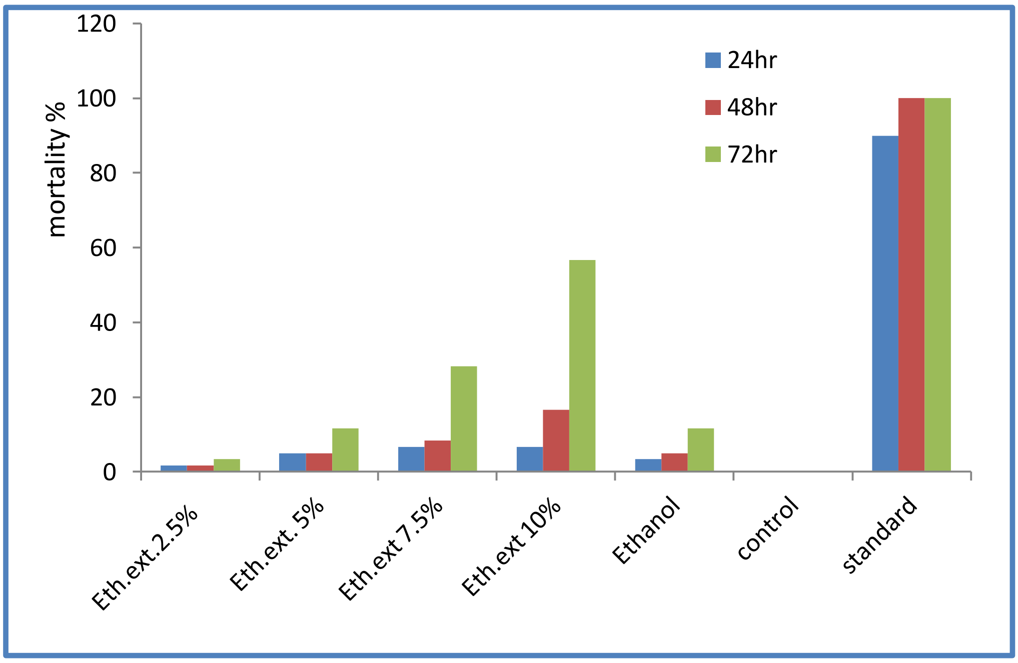

Fig.13: Mean mortality (%) of *Phenacoccus solenopsis* treated by Ethanol extracts of *E. camaldulensis* (leaf dipping method).

Topical application method:

All concentrations of Ethanol extract, 10%, 7.5%, 5%, and 2.5% have very high mean mortalities (15) **(Table 22),** followed by Petroleum ether extract 10% and its concentrations (7.5%,5% and 2.5%) **(Table 23)** , with a significant differences compared with the control (3 solvents) at 24, 48, and 72 hours of exposure. In contrast, the aqueous extract exhibit low mortalities at all concentrations with no mortality at concentration 2.5% **(Table 24).**

From the results , very high percentage of mortality was observed with ethanolic extracts which gave 100% mortality at all concentrations and intervals, while mortalities increases in case of ether extracts as concentrations increases from 2.5% to 10% and at interval of 72 hrs **(Figures 14 and 15).**

The aquous extracts showed lower percent mortalities compared to the other solvent extracts **(Fig.16).**

Table 22. Mean mortalities of *P. solenopsis* by Ethanol extracts of *Eucalyptus camaldulensis* (topical method).

Treatment and concentrations	**Mean mortalities by Topical method**		
	24 hours	**48 hours**	**72 hours**
Hitcel 44% (Standard)	15.000 A	15.000 A	15.000 A
Ethanol ext.10%	15.000 A	15.000 A	15.000 A
Ethanol ext. 7.5%	15.000 A	15.000 A	15.000 A
Ethanol ext.5%	15.000 A	15.000 A	15.000 A
Ethanol ext.2.5%	15.000 A	15.000 A	15.000 A
Ethanol (control)	1.500 E	1.750 DE	2.500 C
CV	6.77	8.55	7.93
SE	0.38	0.51	0.49
LSD	0.76	1.03	0.98

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test.

Table 23. Mean mortalities of *P. solenopsis* by P. ether extracts of *Eucalyptus camaldulensis* (topical method).

Treatment and concentrations	**Mean mortalities by Topical method**		
	24 hours	**48 hours**	**72 hours**
Hitcel 44% (Standard)	15.000 A	15.000 A	15.000 A
P.ether ext. 10%	14.500 A	15.000 A	14.750 A
P.ether ext. 7.5%	13.250 B	14.000 A	14.250 A
P.ether ext. 5%	11.750 C	12.750 B	13.250 B
P.ether ext. 2.5%	8.250 D	11.000 C	12.500 B
P.ether (control)	2.000 E	2.500 D	3.000 C
CV	6.77	8.55	7.93
SE	0.38	0.51	0.49
LSD	0.76	1.03	0.98

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test.

Table 24. Mean mortalities of *P. solenopsis* by Water extracts of *Eucalyptus camaldulensis* (topical method).

Treatment & concentrations	**Mean mortalities by Topical method**		
	24 hours	**48 hours**	**72 hours**
Hitcel 44% (Standard)	15.000 A	15.000 A	15.000 A
Water ext. 10%	0.5000 F	2.000 D	2.500 C
Water ext.7.5%	0.000 F	0.750 EF	1.000 D
Water ext.5%	0.000 F	0.250 F	0.250 DE
Water ext.2.5%	0.000 F	0.000 F	0.000 E
water (control)	0.000 F	0.000 F	0.000 E
CV	6.77	8.55	7.93
SE	0.38	0.51	0.49
LSD	0.76	1.03	0.98

*Mean sharing similar letters did not differ significantly from each other at (5%) level of probability using LSD test.

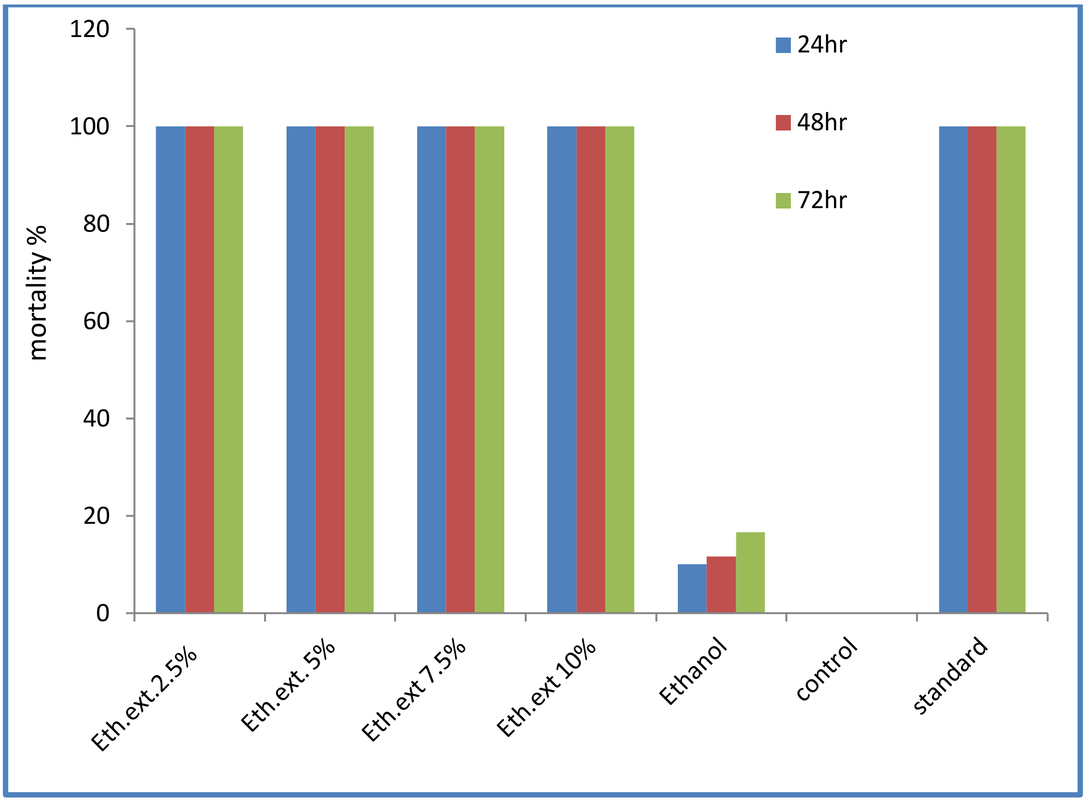

Fig.14. Mortality % of *Phenacoccus solenopsis* treated by Ethanol extracts *E. camaldulensis* (topical method) at different concentrations and intervals.

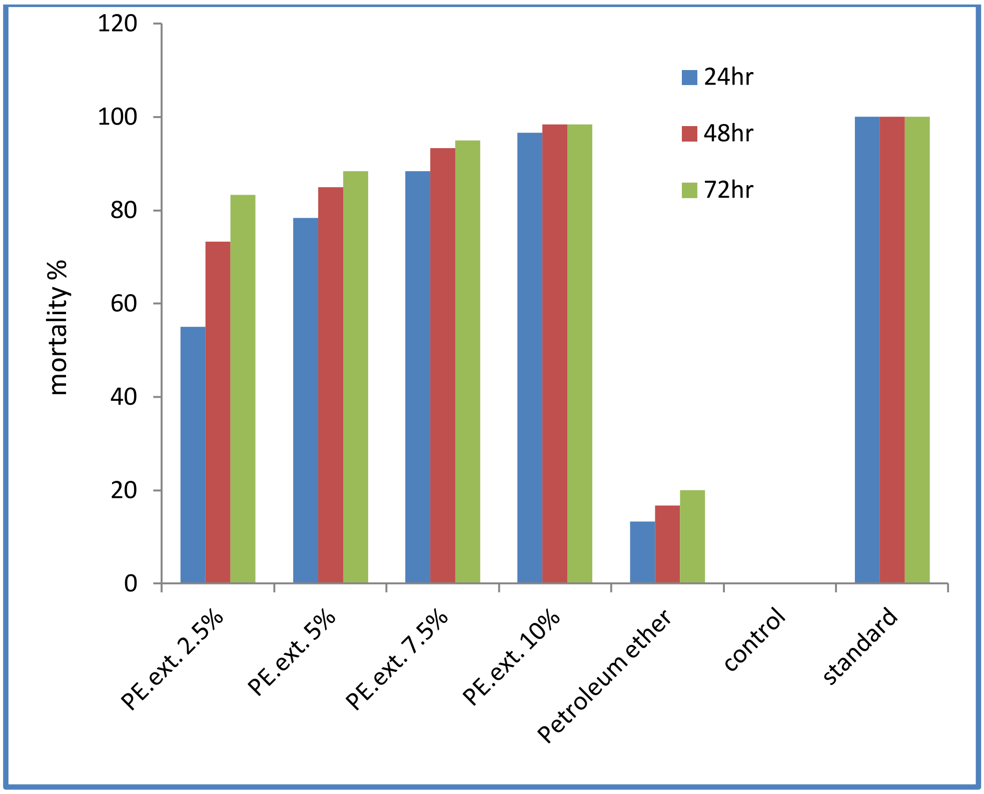

Fig.15. Mortality % of *Phenacoccus solenopsis* treated by petroleum ether extracts of *E. camaldulensis* (topical method) at different concentrations and intervals.

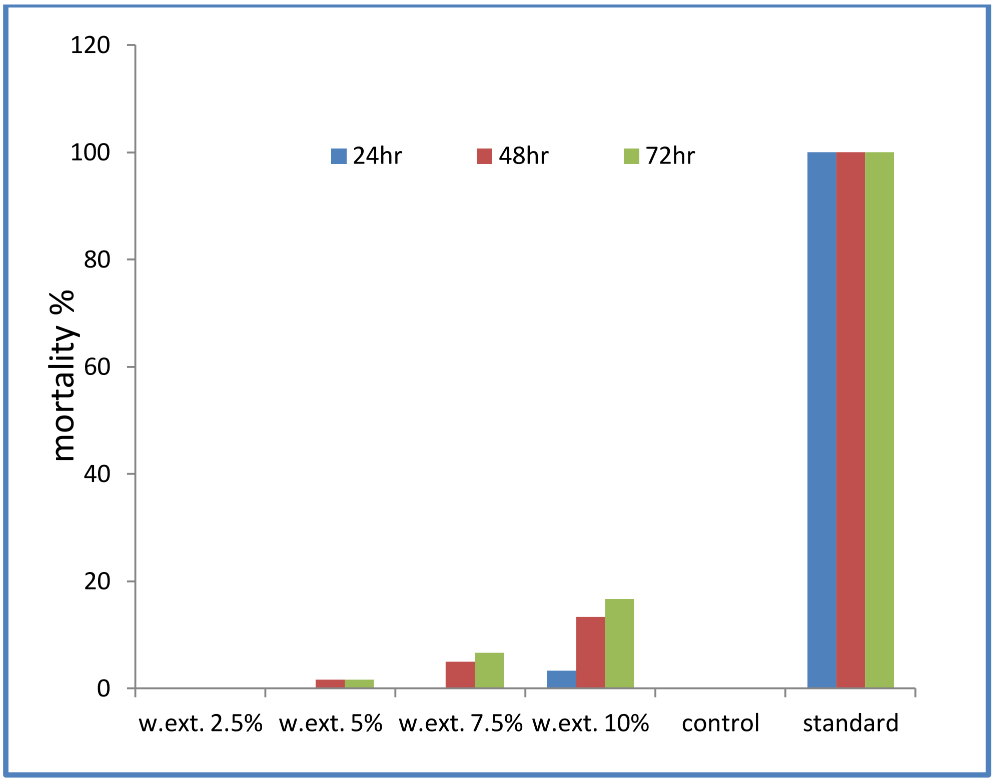

Fig.16. Mortality % of *Phenacoccus solenopsis* treated by aquous extracts of *E. camaldulensis* (topical method) at different concentrations and intervals.

CHAPTER FIVE

DISCUSSION

5. DISCUSSION

5. 1. Seasonal activity and host plant susceptibility of mealybugs:

Investigation on the seasonal abundance and peak activity of mealybugs on various host plants throughout the year was necessary in order to give awareness to the farmers for their timely management before the pest reach the economic injury levels on crops.

The study area included three main Agricultural schemes (Gammuia, Silait and Soba) and the area of North Bahri (**Figure2**). The schemes have three main growing seasons: Summer, Kharif and winter, the main crops are fodder and vegetables. Availability of water throughout the year adapted growth of a number of weeds like Raba, Lissan tair, Hambouk, Rantouk, and Nageel, which were observed highly infested with mealybugs, and dominant on the crops grown.

Results of the survey revealed that, mealybugs remain active throughout the year (except the rainy season and flooding) on various plant species; vegetables, fruits, weeds and ornamental plants. Its activity was observed after the rain, during the months of October, November and December (season 2015) on Okra grown after Kharif, and remained on Okra and weeds to attack the winter crops,

where it reached the peak on tomato, eggplant on March 2016 (**Appendices 2,4,6**).

Population of cotton mealybug varied significantly between the tested host plant species, study areas, and also during different months of the year (Tables 1- 6) and (**Appendices 2-7**). Maximum mean population of *P.solenopsis* per 30 cm apical shoot (twig), was recorded on **Tomato** (314.84nymphs , 40.05 females) at **Gammuia** site (**table1**) followed by **Tomato** (112 nymphs, 18.1 females) at **Silait** (March 2016)(**table 3**). Lower on **Okra** (46.46 nymphs, 6.79 females**) at Soba** site during December 2015(**table 4**), whereas, maximum population on 5 leaves per plant was recorded on **Okra** (December 2015) at **Soba** (153.49 nymphs, 19.85 females) (**table 4**) followed by **Tomato** (43.8nymphs, 4.8 females) and **Eggplant** (33.29 nymphs, 6.06 females) on March 2016 at **Gammuia** (table1) Low population was observed on **Okra** (25 nymphs, 3.16 females) during December 2015 at **Silait** (**table 3**).

As indicated above, the incidence of mealybugs was high in March 2016 on Tomato (season 2015/ 2016) at Gammiua and Silait sites (**table1 and 3**). This was mostly due to the moderate rainfall in August (32.9 mm), September (13.9 mm) and October (29.3 mm), with relative humidity in August (41%), September (37%) and October (27%), and a temperature ranging from 26.4- 39.7ºC with no rains until March 2016 (**Appendix 1**). On the other hand, the incidence of mealybug was low in winter crops (Season 2016/ 2017) due to high rain fall in August (52.6 mm), which resulted in flooding of channel in all the growning areas, with high relative humidity in August (52%), September (38%) (**Appendix 1**). This is supported by a group of researchers, Suresh and Kavitha (2008) and Joshi *et al.*, (2010), who concluded that, temperature and sunshine hours have a positive influence, whereas maximum relative humidity and rainfall have a negative influence on mealybugs , and adversely affects the spread and reduces the intensity of the pest. Also, Other laboratory studies by Hameed *et al*.,(2012) and Ali *et al*.,(2012) on the effect of temperature and relative humidity on Cotton mealybug, *Phenacoccus solenopsis* revealed that, the pest is able to develop and build up its population in different ecological zones within a temperature of 20-40ºC and relative humidity 70- 40%, respectively.

From the results of this study, it was clear that the infestation of *P. solenopsis* was transmitted from Kharif Okra and the different types of weeds to tomato and eggplant. The mean population of *P. solenopsis* for winter crops fluctuated, increasing and decreasing, during season 2015/ 2016 and reaching the maximum population (peak) on March 2016, at a temperature of 24.3- 39.6ºC, (**Appendices 2,4 and 6**).

High infestation was recorded on the winter crops grown on the south side of okra, at Gammuia (tomato and eggplant) and Silait (tomato) was due to the effect of winter wind (North to South). Whereas, at Soba,

tomato and eggplant were grown on the East side of okra (hence low infestation was observed) (**Tables 1, 3 and 4**). Also, from the results, it was observed that availability of favorable food and climatic changes from winter to summer season accelerates the development, survival and reproduction of various insect pests. Infestations of cotton mealybug were observed on okra bared parts of stem, pods and lower side of the leaves, whereas on tomato on tender shoots (mainly nymphs) and leaves, on eggplant on the lower side of leaves and rarely on stems. cotton mealybug can complete 15 generations per year in the field as reported by Tanwar *et al.,* (2007) and Aheer *et al.*, (2009), fluctuating populations according to the changes of seasons.

No mealybug infestation was observed at Northern Bahari area.

5.2. Biology of cotton mealybug '*Phenacoccus solenopsis*:

The present study identified mealybug species found on field crops, vegetables, ornamental plants and weeds in a number of sites in Khartoum State and some other states as Cotton mealybug, *Phenacoccus solenopsis*.

A series of experiments were conducted to study the biology of *P. solenopsis* on four main host plants of economic importance; cotton (*Gossypium sp*), okra *(Abelmoschus esculentus*), tomato (*Lycopersicon esculentum*) and sweet peper (*Capsicum annuum*) under laboratory conditions.

Effects of various host plants on the mortality and biological parameters of *P. solenopsis* have been reported by various workers from different parts of the world (e.g., Aheer *et al.,* 2009; Rashid; *et al.*, 2012Arif *et al.*, 2013) which play an important factor in the development of *P. solenopsis*, as they provide food source to the feeding population and having a significant effect on fecundity and other biological features (Abbas *et al.*, 2010).

Duration of the 1st ,2nd and 3rd instar nymphs for the tested plant species lasted 4 to 6 days (except 3rd instar on Okra 3-4day) (Appendices 8,9,10 &11) which match the finding of a group of researchers (e.g., Nikam *et al.*, 2010; Vennila *et al.*, 2010; Abbas *et al.*, 2012; Sahito and Abro, 2012; Arif *et al.*, 2013; Prithvi, 2016). Females had three nymphal instars, where male has two instars and a pupation period, which match the findings of Hodgson *et al*., (2008), Rashid *et al.*, (2012), and Muthulingam and Vinobaba (2013). Longer duration of males compared to females was due to an additional moulting and prepupal processes.

In the present study, the longest total developmental period of the female was observed on nymphs reared on Cotton, followed by Okra, Tomato and Sweet pepper, while in male, the duration is longer on Cotton, followed by Sweet pepper, Tomato and Okra (Table 7). The mean total fecundity was high on Cotton (241.09 ±34.9), followed by Okra (176± 89.96), Sweet pepper and the lowest on Tomato (Table 8); this match the findings of Abbas *et al*., (2012) who found that, under field conditions, Cotton was the most favoured food source, enabling the pest to produce the maximum number of offsprings (122.5 crawler/ female), followed by Okra (111.9 crawlers/ female) and supported by findings of previous researchers (i.e. Abbas *et al.*, 2010; Javaid *et al.*, 2012; Arif *et al.*, 2013and Dogar *et al*., 2018) , that the host plant play a significant role in the growth and spread of *P.solenopsis* and affect the life history parameters (i.e. greater reproductive rates of insect pests on a particular host indicate the higher suitability of the host).

The average pre- oviposition period on cotton and okra ranged between 8- 9 days (**Appendices 8 and 9**) which is in accordance with the results of Nikam *et al., (*2010) and Muthulingam and Vinobaba (2013). In the present results, male adult lived 2-4 days after emergence (**Appendices 8, 9, 10 and 11**), which confirmed the results of Sahito and Abro (2012) and Abbas *et al*., (2012).

P. Solenopsis reproduced sexually, where adult female reproduce only when fertilised by a male and produced crawlers not eggs in an ovisacs while the un mated females failed to reproduce, this is confirmed by Rashid *et al*., (2012), Abbas *et al.,* (2012), and Huange *et al.,* (2013) who recorded that no progeny could be produced by virgins

Most species of scale insects (Hemiptera: Coccoidea) reproduce sexually, although several types of parthenogensis have been described in Coccoidea, including obligate and facultative parthenogensis

(Huange *et al.*, 2013). Vennila *et al*. (2010) reported that, parthenogensis of *P. solenopsis* with ovoviviparity was dominant over oviparous mode of reproduction in open field. However, several hemipteran insects show alternative reproductive modes, i.e., one species could have two reproductive avenues either facultative or cyclical (Normark, 2003).

In the this study, all the tested host plants showed variation in mortality of the first, second and the third instar of cotton mealybug, *P. Solenopsis;* highest mortalities were recorded on the 1^{st} and 2^{nd} instar of *P. solenopsis* when reared on cotton followed by tomato (in all nymphal stages due to fungal contamination) (**Fig.3 and Appendices 12, 14**). This was supported by Abbas *et al*., (2012) who recorded that, most mealybugs die in the crawler stage due to the crowded conditions, competition for feeding sites, honey dew fouling and fungal infection. On contrast, adult females were observed to be more resistant to environmental stresses such as, high temperature, rain fall, starvation, physical disturbance and pesticides.

Muthulingam and Vinobaba (2013) recorded, under natural field conditions, higher mortality of the crawlers, longer effective reproductive period and increased longevity of adult females, along with expected natural mortality factors such as, predation, parasitisation and abiotic factor on crawlers and adults.

5.3. Host preference of *Phenacoccus solenopsis*:

This study revealed variation between the host plant species in regard to cotton mealybug, *P. solenopsis* attractiveness. However, the proportions of all nymphal instars varied significantly with respect to plants species and observation intervals (**Tables 8, 9 and 10**).

From figures (**4, 5, 6 and 7**) it is clear that, the settlement of all stages (1^{st}, 2^{nd}, 3^{rd} nymphal instar and adult females) of *P. solenopsis* increased with the pass of time on the host plant species belonging to the family Malvaceae. These observations are in agreement with the finding of Abbas *et al*., (2010), who studied under field conditions, the effect of host plant species and season on the fecundity of *P.solenopsis* in three locations in Pakistan (Alipur, Multan, Faisalabad) , using 10 alternative host plants to determine their relative preference and suitability as feed. Their results indicated that, the host plant species belonging to the family Malvaceae (cotton, okra and china rose) have higher number of crawlers per female which varies significantly between different months according to the temperature and relative humidity. On contrast, locality has no significant effect on fecundity.

Vennila *et al*. (2010) stated that, once the mealybug attached to the host, it inserts its proboscis and starts feeding and remains attached on the plant, therefore its numbers increase arithmetically with the passage of time.

Okra appeared to be highly preferred over the selected host species compared with control (Fig. 4, 5, 6 and 7), followed by cotton and Hambouk, except the 3rd nymphal instar where Hambouk was preferred more than cotton (Fig.6). This finding disagrees with Shahid *et al*., (2014) who studied host preference of *P. solenopsis* on 25 plant species, and recorded that, the proportions of the 1st and 3rd instars were maximum on cotton. Other studies by Ben- Dov *et al*. 2008; Aheer *et al*., 2009; Arif *et al*., 2009, and Shahid *et al*.2012 reported that, the most favorable host plants of *P. solenopsis* are cotton, eggplant, sunflower, chinese rose and lantana.

This study showed that, the first instar of *P. solenopsis* moves quickly towards the vials containing the host plant species more than other stages, where the mean population of the first instar was high in all observation intervals as compared with other developmental stages (Table 8 and Fig.4)**.** These results are in confirmatory with the findings of Shahid et *al*., (2017) who studied host plant preference and mortality of *P. solenopsis* on different plant species.

In addition, both tomato and egg plant are belonging to the same family (*Solanaceae*), but tomato is more preferred by the pest than egg plant, because the first plant is more succulent. This is supported by the findings of Shahid *et al*., (2017), who reported that sucking insects, including mealybug, are commonly attracted towards succulent plants that enriched with chlorophyll.

The response of the pest to the various host species is significantly different, the more suitable and preferred host, the more is the fecundity of the pest in the locality and the same environmental conditions (Abbas *et al*., 2010). In addition, Shahid *et al*., (2012) reported that, the insect selection and utilization of a host plant depends upon both biophysical and biochemical factors.

Quality and quantity of the food affects the food selection behavior, survival and reproduction of phytophagous insect pests. The selection of host plant by the pest is often divided into 'host plant finding' and 'host plant acceptance' where the volatile chemicals guide phytophagous insects to their host plants (Finch and Collier, 2000). The insects assess the plant with respect to its suitability as host species and also its nutritional suitability. However, the plant secondary compounds play a significant and dominant role in host plant selection by a behavioral response of the insects to these chemicals (Chapman, 2009).

5.4. Biology of the predator '*Exochomus nigromaculatus*':

In Sudan, a diversity of parasitoids and predators has been associated with mealybugs. These indigenous natural enemies can provide basic information for developing reliable and cost effective management method for the pest (Mohamed *et al*., 2015). Among these, the coccinellid species *Exochomus nigromaculatus* (Goeze) has been observed as common predator, showing ability to reduce pest populations to significantly low levels.

Studying the biology of predator, *Exochomus nigromaculatus* revealed that, the mean duration of egg , 1st, 2nd ,3rd, 4th larval instars, pupal stage , pre- oviposition , oviposition, total longivity and fecundity (**Table 11**) match the findings of; Abdel- Salam *et al*., (2009) and Atilhan and Özgökçe (2002).

The percent mortality of different developmental stages, 1st to the 4th larval instar grub of *Exochomus sp.* (**Table 15 and Fig. 11**) were similar to the findings of Nazari (2008), who studied the survival rate of the pest on Aphid, *Aphis nerii* at temperature of 35ºC.

The development of *E. nigromaculatus* is possible at temperatures ranging from 15 to 40º C and the maximum survival rate is observed at 30ºC (Nazari, 2008), while Atlihan and Kaydan (2001) recorded high mortality at temperature of 35ºC of all stages and mortality of the predator adults toward the end of the oviposition period.

Significant variations were recorded in the prey consumption of the 4th instar grub and the adult males and females of *Exochomus sp* when fed on a mixed diets of the 3rd instar and adult females of cotton mealybug under laboratory conditions, at temperature 25◦C-28◦C with relative humidity 28-35% (**Fig. 8, 9 and 10**).

Predaceous coccinellids are important entomophagous predators of arthropod pests, including white flies, aphids, mealybugs, scales, psyllids and mites (Arif *et al*, 2011); Coccids are the dominant prey group for 36% of Coccinellid species. Prey quality depends on its host plant, the physiological and developmental stages of the insects, but, mixed diets involving different developmental stages of the prey species may be superior for coccinellids over homogeneous one

(Hodek and Honek, 2009).

In this study, the total prey consumed of the 3^{rd} instar nymph is significantly higher than that consumed of the adult females of *P. solenopsis* (Fig. 8, 9 and 10). This confirm the findings of Fandi *et al*., (2010) who studied the predatory performance of four coccinellid predators on *Phenacoccus solenopsis*, the grubs of all the four predators preferred to prey upon second instar mealybugs followed by third instars and lastly the adult stage.

From this study, predatory adult female beetle (for five days interval) consumed higher prey followed by male and lastly the 4^{th} instar grub (**Tables 12, 13 and 14**). This is confirmed by another study by Uygun and Atilhan (2000) on coccinellid females of *Scymnus levaillanti* which consumed more prey than larvae and males.

The prey consumption by the 4^{th} instar grub increased towards the 4^{th} day , then decreased in the 5^{th} day (**Fig 8**) due to the fact that the larva stop feeding and attached to a substrate for pupation, while the prey consumption by the female and male predator increases towards the 5^{th} day (**Fig 8 , Fig 9 and Fig 10**).

Consumption rates may be higher in the laboratory than in nature, because the predator was confined with its prey in a small area and search time was minimal (Atlihan and Özgökçe, 2002).

5.5. Biology and parasitism of the parasitoid *Aenasius arizonensis*:

The preliminary survey of the parasitoid, *A. arizonensis* during 2015- 2016, showed that, it is available most of the year round on some seasonal host plants (**table 16**).

The biology of the wasp was studied under laboratory conditions**.** The observations showed that, after mating the females oviposit fertilized eggs within 24 hours inside the mealybug. The parasitized mealybug transformed into a barrel shaped; dark brown mummy within a week. This agrees with the findings of Solangi, and Mahmood (2011) and Prithvi and Patro, (2018) , who showed that, the parasitized mealybug take a week to transform into mummy feature (**Plates 28 and 29**).

Only one parasitoid adult emerged from each mummy of the host, i.e., the parasitoid female lay a single egg in its host; which agree with the finding of Solangi, and Mahmood (2011).

Considering **Table (17)**, the results of the study indicated that, no difference in the mean developmental periods from ovipostion to emergence of the parasitoid females and males. The males died within 9.71± 1.21 days from emergence, with a range of 5-11 days and a pre-oviposition period of females is less than one day, which was similar to the findings of Abdin *et al*., (2012) ; and Aga *et al*. (2016) .

The mean longevity period (from egg to death) for females and males (Table 18) corresponds to the findings of Abdin *et al*., (2012). The number of parastized mealybugs per day ranged from 20- 24 (Table 18), that varied with the findings by Aga *et al*., (2016) , who mentioned the number ranged from 2- 19.

Regarding **Table (18)**. the results showed the total parasitism of mealybug was high on adult females and low on the 3^{rd} instar and no parasitism was observed on the first and second instars nymphs, This is supported by a group of researchers (Liu, 1985; Islam, 1997; Aga *et al*., 2016) who recorded that, the parasitoid preferred large, late stage host for development, which suffer lower mortality and contain adequate food sources to generate superior offsprings.

Many studies were made on ants association with mealybugs infestation (Braybrook , 2012 and Mohamed, *et al.*,2017), which supported our findings that ants aggravate mealybug populations and act as a predators of mummified mealybugs which could be a delimiting factor and need to be considered in mass rearing of the wasp.

5.6. Efficacy of Kafur leaves extracts against *P.solenopsis*:

The plant used in the present study, *Eucalyptus camaldulensis* is indigenous and abundantly available in Sudan. It has promising essential oils that have fumigant and toxicant activities with repellent effects against different insect species (Siramon *et al*., 2008; Izakmheri *et al*., 2013 and Roonjho *et al*., 2013). The pesticidal activity of Eucalyptus oils has been due to the components such as "1, 8- cineol "which have a potential insecticidal activity against adult *Phenacoccus solenopsis* (Alvijeh *et al*., 2014).

In this study, chemical profile of *Eucalyptus camaldulensis* obtained by extraction with organic solvents was tested for mortality effects on cotton mealybug, *Phenacoccus solenopsis* by two methods, dipping and topical application. A comparison of the mean mortalities of Cotton mealybug, *Phenacoccus solenopsis,* showed that, mortality increased with increasing concentrations of all extracts of E. *camaldulensis* and exposure time, i.e., there is a relation between mortality and concentrations (**Tables 19 -24 and Figs.12-16**).

Dipping method recorded lowest mortality, where ether extract 10% has high mortality of *P. solenopsis* than ethanolic 10%, and aqueous extract 10% compared to control at interval of 24 hours and 48 hours, while at interval of 72 hours ethanolic extract 10% have high mortality (56.7 %) than ether extract 10 % (38.3%) (**Tables 19, 20 and 21**).

Complete mortalities (100%) were recorded from Topical application method in all concentrations of ethanol extracts , followed by ether extract compared with control (**Figs.14 and 15**).

The results showed that, Ethanol extract of *Eucalyptus camaldulensis* was the most effective against cotton mealybug having highest percent mortalities after 72 hours of exposure.

In all the treatments, the lowest mortalities of cotton mealybug females were observed in all concentrations of aqueous extract at all intervals.

This study, show the efficiency of *E. camaldulensis* extracts in controlling *P. solenopsis* adult stage, which is in accordance with those of Ahmadi *et al*. (2012) and Majeed *et al*. (2018) who found the highest mortality of citrus mealybugs with Eucalyptus extracts.

In addition, numerous studies confirmed the pesticidal activities of this plant against many insect pests (Al- anany *et al*., 2017).

CONCLUSION

CONCLUSION

Cotton mealybug, *Phenacoccus solenopsis* Tinsley, become an important pest in many crop systems, due to its broad host range and polyphagous nature causing severe losses to different crops worldwide.

In this study, field surveys were conducted to identify the species of mealybugs infesting vegetables, field crops, ornamentals and weeds. Efforts were made to study the distribution and Seasonal fluctuation of the mealybug and its natural enemies. In addition, laboratory studies were carried out to study the biology of mealybug, *P. solenopsis*, and the efficiency of a predator, *Exochomus nigromaculatus* and a parasitoid, *Aenasius arizonensis* as biological control agents of the mealybug. Besides, the efficacy of a botanical extract, *Eucalyptus camaldulensis* was also tested in the suppression of the mealybug. The results of these investigations are summarised as follows:

1. Population dynamics of *P. solenopsis* indicated that, mealybug population started appearing on Okra after the rainy season (in October) which gradually increased attacking winter crops and reached its peak in March in the first year of the study (2015/ 2016). In the second year (2016/ 2017) Population was low due to the heavy rains.
2. Total life cycle of *P. solenopsis* female ranged between 21 to 39 days and that of the male between 16- 18 days, according to the host plant.

3. All plant species which belong to the family Malvaceae such as okra, cotton and hambouk are the most favorable to cotton mealybug, and Plants species belonging to the families Verbenaceae (lantana) and Solanaceae (tomato) appeared to be an intermediate hosts, while the egg plant (Solanaceae) and raba (Aizoaceae) appeared to be the least preferred host plants.
4. According to the results of this study, it was confirmed that the food plant source play an important role in pest maturity and oviposition; however, cotton and okra (Malvaceae) favoured the fecundity and life span of *P. solenopsis*, while tomato and sweet pepper (Solanaceae) were less preferred, which may be related to antagonistic effects.
5. Based on data obtained about reproduction and prey consumption, *Exochomus sp* have a shorter developmental time of immature stages, therefore, it has a good potential for mass rearing , as a promised biological control agent that could be manipulated in an integrated pest management programmes.
6. The wasp, *Aenasius arizonensis* is an aggressive parasitoid, and is considered as the main factor for declining the mealybug populations in the field due to the fact that it is available most of the year round on some seseaonal host plants, and has better searching ability and parasitize mealybug colonies, as well as the scattered forms.

7. The results of biocide tests, confirmed the importance of using essential oils of *Eucalyptus camaldulensis* as a promising alternative for control of adult *P. solenopsis*.
8. Control by botanical extracts, Eucalyptus, predator, *Exochomus sp.*, and the parasitoid, *Aenasius arizonensis* can play a significant role in the management of mealybugs and other hemipterous pests as being substitute of toxic and hazardous synthetic chemicals in future pest management programmes.

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RECOMMENDATIONS

RECOMMENDATIONS

1. Regular monitoring for mealybug, *Phenacoccus solenopsis* infestation.
2. Further studies are essential on food preference and attitude of *P. solenopsis* under different climatic conditions, to avoid damaging to economic crops, and help in management strategies.
3. Studies on the indigenous parasitods and coccinellid predators are needed, which would ultimately help in designing effective IPM strategies to control the damaging pests in Sudan.
4. A programme of mass rearing of these bio-control agents can help in effective strategic management of *P. Solenopsis* in the field.
5. Efforts should be geared up at characterizing the entire bioactive agents present in *Eucalyptus camaldulensis* for its full utilization.
6. Studies on the different formulations of the botanical extracts may lead to improvements in the effectiveness against insects and to be considered as a useful alternative to chemical insect control.

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REFERENCES

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APPENDICES

Appendix I: Meteorological data of Khartoum State

Khartoum rain fall in MM

Year	Jan	Feb	Mar	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2015				0.0	1.4	32.9	13.9	29.3	0.0	0.0
2016	0.0	0.0	0.0	0.2	14.2	52.6	12.0	0.0	0.0	0.0
2017	0.0	0.0	0.0							

Khartoum Maximum Temperature In C°

Year	Jan	Feb	Mar	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2015				41.8	40.9	38.3	39.6	39.7	33.7	28.5
2016	28.5	33.1	39.6	41.2	37.7	36.2	38.7	39.7	36.7	33.0
2017	34.5	31.5	36.4							

Khartoum Minmum Temperature In C°

Year	Jan	Feb	Mar	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2015				29.2	28.7	26.4	27.0	28.5	21.7	15.6
2016	14.9	17.9	24.3	29.2	26.7	26	26.8	27.1	23.4	19.6
2017	18.8	16.6	20.4							

KHARTOUM MONTHLY RELATIVE HUMIDITY IN %

Year	Jan	Feb	Mar	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2015				20	27	41	37	27	21	23
2016	22	20	13	27	43	52	38	24	23	28
2017	24	16	11							

Appendix 2: population of mealybug, *P. solenopsis* infesting different crops along with weather parameters during 2015/ 2016 at Gammuia.

Area	Date	Plant	Twig		Leaf		Temp.°C		RH %	Rainfall
			Nymph	Female	Nymph	Female	min	max		
Gammuia First season 4.12 20.3.2016	4.12.2015	Okra	3.525 B	0.387 B	14.175 A	2.287 B	15.6	28.5	23	0.0
		Tomato	2.9125 A	0.2125 B	0.4375 B	0.0125 B				
	19.12.2015	Okra	7.488 B	1.662 C	11.975 A	2.000 C				
		Tomato	9.863 A	0.813 B	12.050 A	0.237 B				
		Eggplant	0.013 B	0.138 B	20.863 A	0.425 B				
	7.1.2016	Tomato	3.3875 AB	0.5625 B	6.3500 A	0.0625 B	14.9	28.5	22	0.0
		Eggplant	0.3250 B	0.0847 B	9.6875 A	0.9250 B				
	21.1.2016	Tomato	10.050 A	0.325 B	3.587 B	0.123 B				
		Eggplant	0.875 A	0.113 B	13.800 A	0.763 B				
	4.2.2016	Tomato	9.2875 A	0.4750 B	4.1250 AB	0.2375 B	17.9	33.1	20	0.0
		Eggplant	0.988 B	0.263 B	20.675 A	1.025 B				
	19.2.2016	Tomato	33.462 A	2.062 B	3.012 B	0.037 B				
		Eggplant	0.2000 B	0.0375 B	4.3625 A	0.5250 B				
	5.3.2016	Tomato	<u>314.84 A</u>	<u>40.05 B</u>	<u>43.82 B</u>	4.80 B	24.3	39.6	13	0.0
		Eggplant	1.600 B	0.950 B	33.288 A	<u>6.063 B</u>				
	20.3.2016	Tomato	10.525 A	5.150 B	14.688 A	0.225 C				
		Eggplant	0.9872 C	1.6125 BC	5.3375 A	2. 500 B				

Appendix 3: population of mealybug, *P. solenopsis* infesting different crops along with weather parameters during 2016/ 2017 at Gammuia.

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		Temperature °C		RH %	Rain fall
			Nymph	Female	Nymph	Female	Min	Max		
Gammuia second season 19.10.2016 to 22.3.2017	19.10.2016	Okra	14.075 A	2.012 B	10.800 A	1.125 B	27.1	39.7	24	0.0
	4.11.2016	Okra	11.625 A	2.275 B	10.475 A	2.650 B	23.4	36.7	23	0.0
	1.12-31.12.2016	Tomato	0	0	0	0	19.6	33	28	0.0
		Eggplant	0	0	0	0				
	5.1.2017	Tomato	9.5375 A	1.0875 B	1.4500 B	0.0125 B	18.8	34.5	24	0.0
		Eggplant	0	0	0	0				
	19.1.2017	Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	5.2.2017	Tomato	0	0	0	0	16.6	31.5	16	0.0
		Eggplant	0	0	0	0				
	20.2.2017	Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	7.3.2017	Tomato	0	0	0	0	20.4	36.4	11	0.0
		Eggplant	0	0	0	0				
	22.3.2017	Tomato	0	0	0	0				
		Eggplant	0	0	0	0				

Appendix 4: population of mealybug, *P. solenopsis* infesting different crops along with weather parameters during 2015/ 2016 (Silait).

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		Temperature°C		RH %	Rain fall
			Nymph	Female	Nymph	Female	Min	Max		
Silait first season 3.12.2015 to 19.3.2016	3.12.2015	Okra	0.025 B	0.050 B	24.937 A	3.162 B	15.6	28.5	23	0.0
	18.12.2015	Okra	1.5875 B	0.0 B	17.100 A	0.3625 B				
	6.1.2016	Tomato	3.5000 A	0.4000 B	0.3875 B	0.0 B	14.9	28.5	22	0.0
		Eggplant	0.0 B	0.0 B	1.2625 A	0.0125 B				
	20.1.2016	Tomato	2.9750 A	1.2750 B	0.4250 BC	0.0625 C				
		Eggplant	0.1375 B	0.1875 B	0.4500 A	0.1000B				
	3.2.2016	Tomato	9.1125 A	0.9250 B	6.8750 AB	0.0875 B	17.9	33.1	20	0.0
		Eggplant	0.0 B	0.1125 B	0.9375 A	0.3000 B				
	18.2.2016	Tomato	43.200 A	0.975 B	10.950 B	0.175 B				
		Eggplant	0.1750 B	0.2875 B	1.0250 A	0.3125 B				
	4.3.2016	Tomato	111.97 A	3.65 B	14.40 B	0.70 B	24.3	39.6	13	0.0
		Eggplant	0.6875 C	0.9125 BC	1.4500 A	1.2000 AB				
	19.3.2016	Tomato	21.887 A	18.088 A	5.150 B	0.062 B				
		Eggplant	0.9625 AB	1.000 AB	1.7000 A	0.4625 B				

Appendix 5: population of mealybug, *P. solenopsis* infesting different crops along with weather parameters during 2016/ 2017 Silait)

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		Temperature°C		RH %	Rain fall
			Nymph	Female	Nymph	Female	Min	Max		
Silait second season 21.10.2016 to 21.3.2017	21.10.2016	Okra	0	0	0	0	27.1	39.7	24	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	6.11.2016 21.11.2016	Okra	0	0	0	0	23.4	36.7	23	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	7.12.2016 22.12.2016	Okra	0	0	0	0	19.6	33	28	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	6.1.2017 21.1.2017	Okra	0	0	0	0	18.8	34.5	24	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	7.2.2017 22.2.2017	Tomato	0	0	0	0	16.6	31.5	16	0.0
		Eggplant	0	0	0	0				
	6.3.2017 21.3.2017	Tomato	0	0	0	0	20.4	36.4	11	0.0
		Eggplant	0	0	0	0				

Appendix 6: population of mealybug, *P. solenopsis* infesting different crops along with weather parameters during 2015/ 2016 (Soba).

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		Temperature°C		RH %	Rain fall
			Nymph	Female	Nymph	Female	Min	Max		
soba First season 2.12.2015 To 18.3.2016	2.12.2015	Okra	46.46 B	6.79 B	153.49 A	19.85 B	15.6	28.5	23	0.0
	17.12.2015	Okra	41.212 A	1.875 C	22.275 B	1.363 C				
	5.1.2016	Tomato	2.4125 C	0.0250 A	0.1750 A	0.0125 A	14.9	28.5	22	0.0
		Eggplant	0.0125 B	0.0 B	0.2375 A	0.0750 B				
	19.1.2016	Tomato	0.0 A	0.1625 A	0.0125 A	0.0 A				
		Eggplant	0.0125 B	0.0 B	0.7625 A	0.1625 B				
	2.2.2016	Tomato	0.2125 AB	0.0250 B	0.3750 A	0.0500 B	17.9	33.1	20	0.0
		Eggplant	0.3750 A	0.2000 A	0.5375 A	0.0750 A				
	17.2.2016	Tomato	0.5875 AB	0.8000A	0.0125 B	0.0125 B				
		Eggplant	0.0 A	0.0250 A	0.0375 A	0.0125 A				
	3.3.2016	Tomato	3.1375 A	1.9250 A	0.2000 B	0.2375 B	24.3	39.6	13	0.0
		Eggplant	1.5250 A	0.5250 B	1.7375 A	1.7125 A				
	18.3.2016	Tomato	4.8250 A	4.5875 A	0.1625 B	0.3500 B				
		Eggplant	2.5125 A	1.4000 A	2.0375 A	2.3000 A				

Appendix 7: population of mealybug, *P. solenopsis* infesting different crops along with weather parameters during 2016/ 2017(Soba).

Area	Date	Plant	No.of mealybugs/ 30cm long twig		No.of mealybugs/ 5 leaves		Temperature°C		RH %	Rain fall
			Nymph	Female	Nymph	Female	Min	Max		
soba second season 20.10.2016 to 26.3.2017	20.10.2016	Okra	0.2500 AB	0.1125 B	0..3375 AB	0.5000 A	27.1	39.7	24	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	5.11.2016	Okra	0	0	0	0	23.4	36.7	23	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	20.12.2016	Okra	0	0	0	0	19.6	33	28	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	6.1.2017 20.1.2017	Okra	0	0	0	0	18.8	34.5	24	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	6.2.2017 21.2.2017	Okra	0	0	0	0	16.6	31.5	16	0.0
		Tomato	0	0	0	0				
		Eggplant	0	0	0	0				
	11.3.2017 26.3.2017	Tomato	0	0	0	0	20.4	36.4	11	0.0
		Eggplant	0	0	0	0				

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Appendix 8: Life cycle and reproductive parameters of *Phenacoccus solenopsis* reared on cotton leaves.

Stage	No.	Duration(days) Range	Mean±S.E
Nymph			
1st instars	378	5 - 6	5.3±1.51
2nd instars	114	4 - 6	4.2±0.90
3rd instars	62	5 – 6	4.8±0.76
Cocoon male	17	5 - 6	5.4±0.56
Adult			
Male	17		
Female	45		
Female			
Pre-oviposition		8 - 9	8.7±0.25
Oviposition		7 - 12	9.9±2.42
Post oviposition		2 - 3	2.5±0.36
Total life cycle			
Male		16 - 18	17.2±0.88
Female		30 - 39	36.2±3.18
Male after maturity		3 - 4	3.3±0.50
Fecundity (No.)		45 - 439	241.09±34.88
No. of ovisac / female		6 - 15	8.6±3.05
Sex ratio (♀: ♂)			1: 0.4

Appendix 9: Life cycle and reproductive parameters of *Phenacoccus solenopsis* reared on Okra leaves.

Stage	No.	Duration(days) range	Mean±S.E
Nymph			
1st instars	695	4 - 6	4.7±0.56
2nd instars	660	4 - 6	4.2±0.15
3rd instars	520	3 – 4	3.8±0.38
Cocoon male	22	5 - 6	5.4±0.53
Adult			
Male	18		
Female	412		
Female			
Pre-oviposition		8 - 9	8.7±0.35
Oviposition		7 - 12	9.9±2.55
Post oviposition		2 - 3	2.5±0.47
Total life cycle			
Male		16 - 18	16.2±1.03
Female		29 - 31	30.3±1.23
Male after maturity		3 - 4	3.8±0.76
Fecundity (No.)		62 - 364	176±89.96
No. of ovisac / female		4 - 9	6.8±2.24
io(♀:♂)		1: 0.04	

Appendix 10: Life cycle and reproductive parameters of *Phenacoccus solenopsis* reared on Tomato leaves.

Stage	No.	Duration(days) Range	Mean±S.E
Nymph			
1st instars	664	4-6	4.8±2.56
2nd instars	535	4 - 5	4.5±0.51
3rd instars	279	4 – 6	5.3±0.49
Cocoon male	46	5 - 6	5.4±0.88
Adult			
Male	11		
Female	100		
Female			
Pre-oviposition		9 - 13	11.8±2.05
Oviposition		2 - 12	6.8±3.37
Post oviposition		1 - 3	1.2±0.67
Total life cycle			
Male		16 - 18	16.8±0.99
Female		21 - 38	28.3±5.42
Male after maturity		3 - 4	3.8±0.46
Fecundity (No.)		10 - 263	101±78.20
No. of ovisac / female		1 - 6	3.6±2.68
ex ratio(♀:♂)		1: 0.11	

Appendix 11:Life cycle and reproductive parameters of *Phenacoccus solenopsis* reared on sweet pepper leaves.

Stage	**No.**	**Duration(days)**	
		Range	**Mean±S.E**
Nymph			
1st instars	280	5 - 6	5.3±0.54
2nd instars	230	5 - 6	4.3±0.54
3rd instars	192	4 – 6	4.5±0. 5
Cocoon male	20	5 - 6	5.4±0.52
Adult			
Male	18		
Female	147		
Female			
Pre-oviposition		6 - 7	6.2±0.54
Oviposition		4 - 8	6.6±1.6
Post oviposition		1 - 6	2.8±0.83
Total life cycle			
Male		16 - 18	17±1.0
Female		22 - 34	26.8±3.15
Male after maturity		2 - 4	3.1±0.36
Fecundity (No.)		20 - 200	123±70.83
No. of ovisac / female		4 - 6	5.3±1.55
ratio(♀:♂)		1: 0.12	

Appendix 12: Mortality percent of different developmental stages of *P. solenopsis* reared on Cotton leaves.

Developmental stage	**Number alive**	**Mortality factor**	**Number dying**	**mortality Percent**
1st instar	378	Unknown	264	76.97
2nd instar	114	Unknown	52	45.61
3rd instar	45♀/17♂	Unknown	0	0
Cocoon	17	Unknown	0	0
Adult male (♂)	17	-	0	0
Adult female (♀)	45	-	0	0

Appedix 13: Mortality percent of different developmental stages of *P. solenopsis* reared on okra leaves.

Developmental stage	**Number alive**	**Mortality factor**	**Number dying**	**mortality Percent**
1st instar	695	Unknown	35	5.04
2nd instar	660	Unknown	140	21.21
3rd instar	440♀/80♂	Unknown	23♀, 58♂	5.22♀, 72.5♂
Cocoon	22	Unknown	4	18.18
Adult male (♂)	18	-	0	0
Adult female (♀)	412	-	0	0

Appendix 14: Mortality percent of different developmental stages of *P. solenopsis* reared on tomato leaves.

Developmental stage	Number alive	Mortality factor	Number dying	mortality Percent
1st instar	664	Unknown	129	19.43
2nd instar	535	Fungus contamination *Sperigillus niger*	302	56.45
3rd instar	187♀ 46♂	Fungus contamination *Sperigillus niger*	87	46.5♀
Cocoon	46	Fungus contamination *Sperigillus niger*	35	76.09
Adult male (♂)	11	-	0	0
Adult female (♀)	100	-	0	0

Appendix 15: Mortality percent of different developmental stages of *P. solenopsis* reared on sweet pepper leaves.

Developmental stage	Number alive	Mortality factor	Number dying	mortality Percent
1st instar	280	Unknown	50	17.87
2nd instar	230	Unknown	38	16.52
3rd instar	170♀/22♂	Unknown	23♀, 2♂	13.53♀,9.09♂
Cocoon	20	Unknown	2	10
Adult male (♂)	18	-	0	0
Adult female(♀)	147	-	0	0

Appendix 16: Mortatity percenr of nymphal instars and adult of *P. solenopsis* on tested host.

Plant species	Mortality %						
	1st instar	**2nd instar**	**3rd instar ♀**	**3rd instar ♂**	**Cocoon**	**Adult male**	**Adult female**
Cotton	76.97	45.61	0	0	o	0	0
Okra	5.04	21.21	5.22	72.5	18.18	0	0
Tomato	19.43	56.45	46.5	0	76.09	0	0
Sweet pepper	17.86	16.52	13.53	9.09	10	0	0

Appendix 17: Feeding potential of the adult female and male of *Exochomus nigromaculatus* feeding on a mixed diet of the 3rd and adult stage of *phenacoccus solenopsis* .

Stages of *Exochomus nigromaculatus*	**average prey consumed (mean ±SE)**	
	third instar nymph of *P. solenopsis*	**Adult ♀of *P. solenopsis***
♀	7.25±0.26	2.18±0.41
♂	6.97±0.26	2.02±0.54

Appendix 18: Feeding of the 4th instar grub of *Exochomus sp* on adult mealybugs

Appendix 19: Feeding of adult *Exochomus sp* on adult mealybug

Appendix 20: Pupae of *Aenasius Arizonensis*

Appendix 21: Emerging Adult female of *A. Arizonensis*