بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Effects of some Botanical Extracts Against Cotton Mealy Bug (*Phenacoccus solenopsis* Tinsley)

تأثير بعض المستخلصات النباتية علي بق القطن الدقيقي(*Phenacoccus solenopsis* Tinsley)

A thesis submitted in partial fulfillment of the requirements for the M. Sc. degree in Plant Protection

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الآيــة

اللَّهُ لَا إِلَٰهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ * لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ* لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ * مَن ذَا الَّذِي يَشْفَعُ عِندَهُ إِلَّا بِإِذْنِهِ * يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ * وَلَا يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلَّا بِمَا شَاءَ * وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ * وَلَا يَؤُودُهُ حِفْظُهُمَا * وَهُوَ الْعَلِيُّ الْعَظِيمُ.

(البقرة/255)

DEDICATION

To my

Parents, wife, sisters and brothers

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All thanks are due to Almighty Allah (SWT) who gave me health and strength to produce this work. I am greatly indebted to my supervisor Dr. **Loai Mohamed Elamin** for his guidance, patience, keen interest and continuous participation throughout this study.

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Abstract

The experiments were conducted in the Laboratory of Entomology and Agricultural Zoology, Department of Plant Protection, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during May to August, 2018.The average temperature is between 27- 30°C and relative humidity (RH) is between 30 to 35 %..

The aim of this studyis to investigate the activity of some plant leaf extracts (Chewing tobacco *Nicotiana glauca*, Usher Plant *Calotropis procera* and Ghubaych plant *Guiera senegalensis*) against cotton mealy bug (*Phenacoccus solenopsis*) under laboratory condition.

In the first experiment four different concentrations of each plant extracts alone (2.5 %, 5 %, 7.5% and 10 %) were tested. In the second experiment we used the same concentrations of same plant extractions plus one drop of sesame oil and one drop of liquid soap as surfactant. In the third experiment sesame oil was replaced by surfactant material (Agral® - Alkyl Phenol ethylene oxide) at recommended dose 200ml\100L. Each treatment was replicated four times and the mortality % was recorded after 1day, 2 days, and 3days post treatment.

The results showed different insecticidal activity between the threeplant extracts alone and also between the other treatments (extracts plus Agral® and extracts plus sesame oil). The extracts of (Ghubaych, Usher and Tobacco) alone gave only mortality (%) of 10%, 26.7 and 50%, respectively, while the same extracts showed a significant insecticidal effect when mixed with Agral® and sesame oil and the mortality (%) reached (43.3% ,93.3% and 93.3%) and (50%, 73.3% and 90%), respectively after three days post treatment. These results provedthat sesame oil and Agral® substance have a synergistic action.Fromthe above mentioned results we conclude that Ghubaych have a little effect against cotton mealy bug when compared to Tobacco and Usher extracts which can be considered as promising botanical insecticides in the future.

ملخص البحث

أجريتالتجاربمختبر الحشراتوالحيوانالزراعي،قسموقايةالنبات،كليةالدراساتالزراعية (شمبات)،جامعةالسودانللعلوموالتكنولوجيا (SUST) ،خلالالفترةمنمايوإلىأغسطس2018.كانت درجةالحرارة تتراوح ما بين٢٧ - ٣٠درجةمئويةوالرطوبةالنسبية (RH) ما بين٣٠-٣٥٪.

الهدفمنهذهالدراسةهوالتحققمنفاعلية مستخلصات الأوراق لنباتات التبغ *Nicotiana glauca*،العشر *Calotropis procera*والغبيش *Guiera senegalensis*ضدحشرةبق القطنالدقيقي *Phenacoccus solenopsis*تحتظروفالمختبر.

فيالتجربةالأوليتمإستخدامأربعةتركيزاتمختلفةلكلمستخلصنباتي منفرداً(٢,٥٪، ٥٪، ٧,٥٪ و١٠٪). فيالتجربةالثانيةتمإستخدامنفسالتركيزاتمننفسالمستخلصاتالنباتيةبالإضافةإليقطرةواحدةمنزيتالسمسموقطرةوا حدةمنالصابونالسائلكمادةخافضةللتوترالسطحي.

فيالتجربةالثالثةتمإستبدالزيتالسمسمبمادةالأغرال® Alkyl phenol ethylene oxide -Agral بالجرعةالموصيبها(٢٠٠مل/١٠٠لتر) تمتكراركلمعاملةأربعمراتوتمتسجيل نسب الموتبعديوم،يومينوثلاثةأيام بعد المعاملة.

أظهرتالنتائجتأثيراتمختلفةبينالمستخلصات النباتية الثلاثة منفردةوأيضابينالمعاملاتالأخري (المستخلصاتبالإضافةلمادةالأغرالوالمستخلصاتبالإضافةإليزيتالسمسم) كمبيداتحشرية.

أعطتمستخلصاتالغبيش،العشروالتبغ بدون إضافات نسبةموت(١٠٪، ٢٦,٧٪ و ٥٠٪) على التوالي. بينماأظهرتنفسالمستخلصاتتأثيرإبادي

معنويعندمزجهابمادةالأغرالوزيتالسمسمحيثأعطتنسبةموت(٤٣,٣٪,٩٣,٣٪و٩٣,٣٪) و (٥٠٪,٧٣,٣٪و ٩٠٪) على التوالي بعدثلاثةأيام بعد المعاملة.

أكدتهذهالنتائجأنزيتالسمسمومادةالأغراللهماتأثيرتنشيطييزيدمنفاعليةالمستخلصات. منالنتائج أعلاهنستنتجانالغبيشلهتأثيرضئيلضدحشرة بق القطنالدقيقيعندمقارنتهبمستخلصاتالتبغوالعشروالتييمكناعتبارهما مصدراً لمبيداتحشرية نباتية واعدةفيالمستقبل.

CHAPTER ONE

INTRODUCTION

The mealy bugs are mostly polyphagous insects, affecting huge number of economicallyimportant horticultural crops including vegetables, fruits and ornamentals (Sinacori, 1995). They reproduce sexuallyas well as by parthenogenesis. More than 160 mealybug species have been identified as pestsworldwide and most of them are invasive species (Miller and Miller, 2002). They are sucking insect pestswith a short period of 30 days lifecycle in tropical areas (Buss and Turner, 2006) and oftenhave ability to build populations observed in shoots and apexes and become difficult tocontrol with foliar application of pesticide because of having waxy secretions on their bodysurface. Mealybugs do not only destroy the host plant by depletingthe plant sap but they are also responsible for transmitting viral diseases (Bertin *et al.*, 2010). Furthermore, theirexcreted honeydew on plant surfaces provides medium for growth of black sooty mold (Buss and Turner, 2006) which also disturb the photosynthesis process of the plant. Mostly mealybugs are not causing serious problems in their countries of originbecause endemic parasitoids and predators suppress the population naturally. The seriousoutbreaks often occur when mealybugs get introduced into new locations in the absence oftheir natural enemies (Dhaliwal *et al.*, 2010).

In Sudan mealy bugs in the past were classified as minor pest, but recentlymany species of mealy bugswere found to be serious pestsofmany valuable corps.

Classical biological control has been identified as thebest option to manage exotic mealybug species in different parts of the world (Abbas*et al.*, 2005). The chemical control provides rapid control of this pestbut also threatens

natural enemies (predators and parasitoids) and leaving hazardous effects for human, animal and environment.Sparks *et al.*, (1996) stated that insecticides provide high efficiency against pest control but it may cause resistance in insects. The use of conventional pesticides against mealy bug has been proved unsatisfactory and it is also difficult due to the presence of waxy layer covered the insect body (Joshi *et al*, 2010).The cotton mealy bugs due to thier high reproductive capacities and multiple generations per year are potentially capable of becoming resistant to pesticides on consistent exposures (Mark and Gullan, 2005).

Many plants produce secondary metabolites which possess insecticidal properties, therefore can be used as alternatives to synthetic insecticides.Accordingly scientists made a lot of efforts to find an alternative controlmeasures such as botanicals to manage agricultural and medical insect pests.

The objective of this study is to investigatethrough laboratory screening the activityof three botanicals Chewing tobacco or Tree tobacco (*Nicotiana glauca*), Usher plant (*Calotropis procera*) and Ghubaysh plant (*Guiera senegalensis*) water extracts againstthe Cotton mealy bug (*Phenacoccus solenopsis*).

CHAPTER TWO

LITERATURE REVIEW

2.1. Cotton Mealy bug (*Phenacoccussolenopsis*)

2.1.1.Classification

Kingdom :Animalia

Phylum :Arthropoda

Class :Insecta

Order :Homoptera

Family :Pseudococcidae

Genus : *Phenacoccus*

Spices : *solenopsis*(Tinsley, 1898)

2.1.2. Origin and Distribution

The Mealy bug species (*Phenacoccussolenopsis*) during the first decade of 21st century emerged as the most devastating pest of agricultural crops and ornamentals. These species are well spread over a wide range of tropical and subtropical countries.

The success of Mealy bug as a devastating pest of cotton owes to its wide range of Morphological traits and ecological adaptability (Hodgson *et al*, 2008). The pest status of these species was first time reported from Texas, America which later on spread throughout the world (Ben-Dov*et al.*, 2002).

2.1.3. Host Range

Cotton Mealy bug has a wide range of host plants ranging from herbaceous weeds to woody plants. *P. solenopsis*has been recorded as pest of 154 host-plant species out of which 20 field crops, 64 weeds, 45 ornamental plants and 25 shrubs and trees, belonging to a total of 53 plant families(Arif*et*

al., 2009) whereas(Ben-Dov*et al.,* 2002) recorded 174 host-plants belonging to 55 families.

2.1.4. Description

The cotton mealy bug belongs to new world and resembles to *P. solani* and *P. defectus* which are also native to the New World. However, live adult females of *P. solenopsis* generally possess paired dark spots or stripes on dorsal sides, whereas the other two species are uniformly white. *P. solenopsis* sexually dimorphic, having short-lived, winged males and longer-lived, wingless, larviform females.

2.1.5. Ecology

Cotton mealy bughas been reported in 202 plant species (55 families) in Africa, Asia, North America and South America and Oceanic regions.

Hodgson *et al*. (2008) reported a significant difference in the ecology of *P. solenopsis* from the hot, dry climate of southwestern USA which occurs primarily on the roots and underside of the foliage and stems, compared to the higher humid regions of India and Pakistan, where it is found almost entirely on the upper portions of the foliage, well above the soil line.

2.1.6. Biology

Mealy bug is soft body insect,it has shown sexual reproduction, producing live young ones instead of laying eggs by a phenomenon of ovoviviparity, reproduces mostly parthenogenetically, female lays eggs in ovisacs containing 150-600 eggs. Hatching takes place in 3-9 days into nymphs (Crawlers) which lasts for 22-25 days finally growing into adults in 25-30 days under optimum conditions. They can produce hundreds of nymphs in one generation with the capacity to lay up to 6000 eggs per generation(Abbas *et al.*, 2007).

2.1.7. Economic importance and damage

Thehigh reproductive rate and ability of overwintering (Egg and adult female stage) aid the insect to become a serious pest of many commercially important crops. It attacks host plants by sucking cell sap of the phloem tissue (Aijun*et al.*, 2004), andsecretes honeydew which makes sooty molds on the surface of the leaves, thus, ceasing the natural process of photosynthesis, ultimately resulting in the death of plant tissues(Dhawan*et al.*, 1980). The presence of large number of Mealy bug individuals on various parts ofthe host plantisone of the most important clue indicating pertinent crop losses. The major signs of cotton Mealy bug infestations are wrinkled leaves and shoots, distorted and bushy branches, white powdery substance on leaves, shoots and stem, presence of honey dew, less number of bolls, unopened flowers, chlorosis, stunting, deformation and death of plants (Nagrare*et al.*, 2011).

2.1.8. Control and management

The management of cotton mealy bug is crucial to save billions of dollars and control strategies seem inevitable to suppress the pest population under threshold levels. It is observed that larval stages of cotton Mealy bug are more vulnerable and are most likely to be affected by both biotic and abiotic factors. By applying control measures at this stage might provide radical success(Kumar *et al.*, 2013). The control of cotton Mealy bug includes cultural practices, use of biological control agents and use of pesticides. The life cycle and structural adaptability enable the Mealy bug to counter one type or other of these control strategies successfully.

2.1.8.1. Cultural Control

Proper destroying of the uprooted infested plats, weed hosts and management of irrigation and fertilizers are the effective cultural methods to prevent the mealy bug infestation (Fuchs *et al*., 1991).

2.1.8.2. Biological Control

The natural enemies of cotton Mealy bug has been reported from various parts of the world and are thought to be one of the most important control agent in mealy bug programs. Heavy infestation of cotton mealy bug may result from the absence of natural enemies on this invasive pest (Mahmood*et al.*, 2011). A majority of scientist has described the predatory potential of different predators and parasitoids. It was reported that biological control measures were proved efficient and non-hazardous to host crop. The serious outbreaks of mealy bugs results in the absence of their natural enemies and classical biological control has been considered as the most appropriate method for the management of many exotic mealy bug species in different parts of the world(Dhaliwal *et al.*, 2010).

The coccinellid beetles such as *Cheilomenes sexmaculata*, *Rodolia fumida*, *Scymnus coccivora* and *Nephus regularis* are important predators of mealy bug nymphs. Biological control by release of natural enemies has proved very successful.

2.1.8.3. Botanical insecticides used against mealy bug

Successful efforts were done by Nagrare*et al.*, 2011 using *Azadirachtaindica*(Neem) tree seed extraction equally effective in pest control, industries and medicines. The tobacco, datoora and Meethaneem*(Meliaazadirachta*) is also considered as non hazardous, economical and safest having no lethal effects with a high rate of efficiency against a variety of pest insects as described by Narwal*et al.,* 1997).

Botanicals compounds despite their environmental compatibility and non-hazardous effects for humankind and animals are used at quite lesser extent in comparison with other options of pest management(Nabil Elwakeil, 2013) The environmental concerns posed by the useof synthetic chemical compounds in pest management has leadto more regulated and documented use of pesticides banning several products, and thus promoting other methods of pest control including biological control agents and their products. Plant extracts are biodegradable, less toxic to a wide variety of life, cheaper and supportive to biodiversity conservation.

2.1.8.4 Chemical Control

Kamariya (2009) revealed that methyl parathion 0.05 per cent was found the most effective and economic insecticide for the control of mealy bug, *P. solenopsis* on cotton with 87 to 99 per cent mortality, followed by profenophos 0.1 per cent and dimethoate 0.03 per cent. In laboratory, the highest toxicity with longer persistency was found in methyl parathion 0.05 per cent followed by profenophos 0.1 per cent, chlorpyriphos 0.05 per cent, quinalphos 0.05 per cent and Malathion 0.1 per cent.

Singh and Dhawan (2009) revealed that profenophos 50 EC, acephate 75 SP, thiodicarb 75 WP, chlorpyriphos 20 EC, quinalphos 25 EC and carbaryl 50 WP @ 500 ml, 800 g, 250 g, 2000 ml, 800 ml and 1000 g per acre were significantly effective against the pest.

2.1.8.5. Integrated Pest Management (IPM)

A management strategy to control *P.solenopsis* in India that incorporates cultural, mechanical, biological and chemical control factors has recently been developed (Tanwar *et al*., 2008). They recommend a survey for the mealy bug prior to planting, targeting and chemically treating small populations, removal of

alternate host plants and ant colonies, using recommended insecticides for optimal effectiveness on the plants and around their root system, providing an attractive habitat for native and exotic natural enemies, and using a variety of sanitation methods to prevent spread of the pest to new fields.

2.2. Usher Plant (*Calotropis procera*)

2.2.1. Classification

Kingdom : Plantae
Division : Magnoliophyta
Class : Dicotyledoneae
Order : Asclepeadales
Family : Asclepiadaceae
Genus : Calotropis
Species : *Calotropis procera*(Aiton, 1810)

2.2.2. Description

A large Shrub or small tree of 2-4m height, with white latex and smooth, grey - green stems and thick, soft bark, deep tap root of 3 - 4 m length. The simple and opposite leaves are 8 - 25 cm long, 4 - 14 cm width, ovate, thick and waxy. They have a short pointed tip at the end and a heart - shaped base partly clasping the stem. The white and purple flowers have five lobes, are more or less tubular, and 2 - 3 cm in diameter.Fruits are grey - green, fleshy or dry capsules of 8 - 12 cm length and 6 - 8 cm width.They contain numerous small, brown and flattened seeds of 8 -10 mm length and 4-5 mm width, with long white hairs attached at one end (Weberg, 2003). "Shrubs, mostly less than 6 ft., but up to 15 ft. similar to *C.gigantea*, but leaves belong to elliptical corolla usually about in Across with lobes move erect, coronalobes glabrous or pubescent, and follicle 4 - 5 long (Bailey and Bailey, 1976).

2.2.3. Distribution

According to Erdman (1983) the Usher Plant has large broad leaves, ever green and grows abundantly in arid land semi-arid regions of the world without irrigation, fertilization, pesticides, or other agronomic practices.

According to Rahman and Wildcock (1991) *C. Procera* is native to West Africa as far south as Angola,East Africa, Madagascar, the Arabian Peninsula,

southern Asia, and In to China to Malaysia, central and South America and the Caribbean Islands.

Foster (1992) reported that *C.procera* is widely distributed in north tropical Australia. In Sudan it is spreading widely throughout Sudan, abundant and available the whole year round (Eltayeb, 2004).

2.2.4. Uses

This plant has been widely used in the Sudanese medicinal system (Ayoub and Kingston, 1981 and Ayoub and Srenden, 1981). The latex of the plant was reported having potential anti-inflammatory, antidiarrhoeal, analogesic, antipyretic and Schizonticida activities (Kumar and Basu, 1994).

Water containing latex of the plants was able to avoid adult females of *Anopheles Stephens* and *Culexfatigans* to oviposit in the water and the latex water could kill eggs and larvae of *A.Stephensi*, *C. Fatigans* and *Aedesaegypti*(Girdhar, *et al*., 1984).Also *Calotropis procera* kill the larvae of *Spodoptera litura* and coleopteran pests of stored rice (Bakavthiappan *et al*., 2012).

2.2.5. Chemical properties

The milky sap contains a complex mix of chemical some of which are steroidal heart poison known as (*cardiac aglycones*). These belong to the same chemical family as chemicals found in foxglove (*Digitalis Purpurea*).

The *Calotropis procera* also contains a toxic chemical compounds such as Calotropin, Vorusharin, Uscharidin and Calotoxin (Aiton, 2010).

2.3. Ghubaych plant (*Guierasenegalensis*)

2.3.1. Classification

Kingdom:Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Combretaceae

Genus: Guiera

Species:senegalensis(Gmelin, 1768).

2.3.2. Description

Guierasenegalensis is very well known in its native area, generallyoccursasashrubthatcangrow to a heightof 3 to5 m according to habitat. Its stem presents numerous knotsthat send out branches. The ash-grey stem and branches have fibrous or pubescent bark and bear opposing, shortpetiolated oval leaves, sometimes mucronate, sometimes even cordate at their base, about 2 to 4 cm long by 1 to 2 cm wide. These grey-green leaves, darker on their upper surface, display black spots on their lower surface and are slightly downy on both sides. These features lend the plant an overall silver green color (Silva*etal.*, 2008).

The fruit is an achene around 3 cm long brown or greenish,spindle-shaped,hairywithsides (Koumaré, 1968).

2.3.3. Distribution

Itiswidelydistributedinthesavannahregionofwestand central Africa, Nigeria, Senegal, Gambia, Mali, Niger, Burkina Faso and Ghana, (Zeljan *et al*.,1998).

2.3.4. Habitat

ItisaplantthatgrowsprimarilyinSudan and Sahelarea,onsandy soils, leached or exhausted, fallow and dry stations. Plant pioneer disseminated by cattle in the fallow land, it is also indicative of overgrazing. It is found from Senegal to Cameroon to Sudan (Somboro *et al.,* 2011).

2.3.5. Uses

Reportedly used to treat rhinitis, bronchitis and fever, and the rootstotreatdiarrheaanddysentery(Faye*et al.*, 1980).Itisrecognizedas being active against cough, respiratory congestion and fever, and is prescribed as an anti tussive (Negrevergne, 1968), to ease breathing and to treat lung and bronchial disorders (Sanogo*et al*., 1998). It is also used against malaria (Ancolio, *et al*., 2002). The syrup D2 from *G. senegalensis* has been screened for its anti tussive clinical essay (Dénou, 2008). Aqueous extracts from its roots and leaves have also been screened for toxicity (Koumaré, 1968). The galls of *G. senegalensis* are used in Burkina Faso as an ethno veterinary product to increase milk production in cows and to treat fowl pox infection in chickens (Fiot*et al*, 2006).

*Guierasenegalensis*leavesextract torefractionsmaybeused for the treatment of various disease caused by *Escherichia coli*, *Pseudomonas aeruginosa and Klebsiella pneumonia* (Azas *et al.,* 2002).

G. Senegalensis at lower doses is not harmful to the liver and therefore can be exploited as it is served in the treatments of some illnesses (Pousset, 1989). Treatment withwaterandmethanolextractsfrom*G.senegalensis*leaves resulted in endotheliotoxicity, hepatonephropathy and pancreatic hyperplasia (Nacoulma, 1996). Guieranone A from *Guiera senegalensis* showed a strong antiplasmodial activity associated with a high cytotoxicity toward

humanmonocytes (Fiot*et al.*, 2006). Methanol fraction from leaves extract only had antiplasmodial activity(Lamien*, et al.*, 2005).

2.3.6. Chemical properties

G. senegalensis contained harman, tetrahydroharman, harmalan and Guieranone (Fiot*et al.*, 2006). The plant has been found to contain carbohydrates, steroids, flavonoids, saponins, alkaloids, tannins and mucilage (Salihu and Usman, 2015).Oshobuand Geidam(2014) reported four flavonoids in the leaves of *G. senegalensis*, namely catechin, myricitrin, rutin and querterin. Some findings on the plant elsewhere showed the presence of alkaloids, solanine, tannins, terpenoids menthol, coumarins, saponins, flavonoids, quercetin, cardiotonics and cyanogenicheterosides which were assayed in various organs of the plant leaves, stem bark, fruits and roots (Fiot*et al.*, 2006 and Mohammed, 2013). The ashes are poor in alkali but rich in alkaline earth metals. Theyfound especially Mg, Ca, Sr, Ti, Fe, Al and in lesser amounts sometimes traces of Cu, Ni, Co, Zn (Jigam*et al*., 2011).

2.4. Chewing tobacco or tree tobacco (*Nicotiana glauca*)

2.4.1. Classification

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliopsida

Order : Solanales

Genus *: Nicotiana*

Species *: glauca* (Graham, 1836)

2.4.2. Description

Nicotiana glauca is a species of wild tobacco known by the common name tree tobacco. Its leaves are attached to the stalk by petioles(many other *Nicotiana* species have sessile leaves), and its leaves and stems are neither pubescent nor sticky like *Nicotiana tabacum*. It resembles jessamine*Cestrum parqui*but differs in the form of leaves and fusion of the outer floral parts. It grows to heights of more than two meters (Gilman, 2004).

2.4.3. Distribution

Locations within which *Nicotianaglauca* is naturalized include Australia, warmer parts of Europe, sub-Saharan Africa, temperate Asia, New Zealand, USA, Mexico and Hawaii (Henderson, 2001).

2.4.4Chemical properties

Tobacco contains the alkaloid nicotine, which is a stimulant, and harmala alkaloids. Also contains other compounds such as germacrene and anabasine and other pipssseridine alkaloidsvarying between species(Gilman, 2004).

2.4.5.Uses

Dried tobacco leaves are mainly used for smoking in cigarettes, cigars, pipe tobacco, and flavored shish a tobacco and use as insecticide. They can also be consumed as snuff, chewing tobacco, dipping tobacco and suns. Tobacco use is a risk factor for many diseases, especially those affecting the heart, liver, and lungs, as well as much cancer. In 2008, the world Health organization named tobacco as the world's single greatest preventable cause of death(Henderson, 2001).

Nicotine is an alkaloid and natural insecticide, in tobacco plants nicotine may constitute up to 3 % of the dry weight. The compound mimics the endogenous neurotransmitter acetylcholine and exhibits agonistic effects on most nicotinic acetylcholine receptors. Nicotine has been used as a synthetic insecticide and is the archetype for the large range of neonicotinoid insecticides(Buerge*et al*.,2008).

2.5. Agral® substance(Alkyl Phenol Ethylene Oxide)

Alkyl phenol ethylene oxide (APEO) is one of the most widely used classes of surfactants. Recently, approximately 500,000 tons have been produced world- wide annually (Renner 1997) and it makes APEO the world's third largest group of surfactants in terms of production and use (Ying *et al*, 2002). They can be used as detergents, wetting agents, dispersants, emulsifiers, solubilizers and foaming agents. APEO is important to a number of industrial applications, including pulp and paper, textiles, coatings, agricultural pesticides.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study location

The experiments were conducted in the Laboratory of Entomology and Agricultural Zoology, Department of Plant Protection, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during May to August, 2018.The average temperature is between 27- 30°C and relative humidity (RH) is between 30 to 35 %.

3.2. Insectcollection and rearing

The mealy bug (*P. solenopsis*.) was collected from infested okra plants grown in experimental farm, Agricultural Research Corporation (ARC), Shambat, North Khartoum, Sudan. . The insects were reared in plastic containers covered with muslin cloth (Plate 1).The insects were fed on untreated okra fruits. The food was continuously replaced by a fresh one every 48 hours. The culture was kept under laboratory condition as stock culture for running the organized experiments.

3. 3. Collection of plant materials

The leaves of the Usher Plant (*Calotropis procera*) (Plate 2) were collected from the experimental farm, College of Agricultural Studies, Shambat-North Khartoum, Sudan, and Tree Tobacco (*Nicotiana glauca*) (Plate 3), were collected from The Northern Darfur State, West of the Sudan and Ghubaych plant (*Guiera senegalensis*) (Plate 4)were randomly collected from around Ghubaysh village area of Western Kordufan State, West of the Sudan. The leaves of plants were washed and dried under shade for 5-7 days under room condition (Plate 5), and then powdered by using electrical blender. The prepared powder of each plant was kept safe in plastic bags until used.

3.4. Preparation and extraction method

Ten grams of powder of each plant (Tree Tobacco(*Nicotiana glauca),* Ghubaych(*Guierasenegalensis)* and Usher(*Calotropisprocera*))were mixed with 100 ml of water to obtain 10% concentration, also other concentrations (7.5%, 5% and 2.5%) were prepared by diluting the stock solution. The mixture was left for 24 hrs and then filtered and kept in plastic containers used for conducting the required treatments(plate 6).

3.5. The treatments

In the first experiment four concentrations of each plant extracts alone were used (2.5 %, 5 %,7.5% and 10 %). The obtained okra fruits soaked in different concentrations of each plant extracts for one minute and left on filter paper for 10 minutes and then used for treatments. Ten adults of *P. solenopsis* were placed inside plastic cups containing three pieces of treated okra fruits (plate 7).

In the second experiment the same concentrations (2.5 %, 5 %7.5% and 10 %) of same tested plant extractionsplus one drop of sesame oil(0.01ml) and one drop of liquid soap(0.01ml)were used as surfactant. In the third experiment sesame oil was replaced by surfactant material (Agral® - Alkyl Phenol ethylene oxide) according to recommended dose 200ml\100L(plate8). Each treatment was replicated four times. One day post treatment the insects provided with fresh okra fruits for feeding. The mortality data were recorded after 1day, 2 days, and 3days. Four plastic cups each contains 10 adults were used as untreated control.

3.6. Statistical analysis

The experiments were designed in a Complete Randomized Design (CRD) and the data were statistically analyzed according to analysis of variance (ANOVA) using Statistic 8.0 program. LSD test was used for means separation.

Plate1. Insect rearing cage

Plate 2. Usher plant

Plate 3.Tobacco plant

Plate 4.Ghubaych plant

Plate 5.Drying of plant leaves

Plate 6.Plant extracts

Plate 7.The treatment

Plate 8.Agral® substance

CHAPTER FOUR

RESULTS

4.1. Mortality (%) among cotton mealy bug*(Phenacoccus solenopsis*) treated with Tobacco tree (*Nicotiana glauca*) water extract alone.

Results in table (1) and figure (1) showed that tobacco water extract alone has a little effect against cotton mealy bug. The highest concentrations (7.5% and 10%) gave only (33.3% and 50%) mortality after three days post treatment. The statistical analysis showed that there areno significant difference between two lower concentrations(2.5% and 5%) and the control, while there are a significant different in the highest ones (7.5% and 10%).

4.2.Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Tobacco tree (*Nicotiana glauca*) water extract plus Agral® substance.

Results in table (2) and figure (2) showed that tobacco water extract plus Agral® substance was effective against cotton mealy bug, where the highest concentration (10%) caused 93% mortality three days post treatment. The mortality (%) was gradually increased by increasing of concentration and exposure time. The statistical analysis showed that there are no significant difference between the three lowest concentrations and the control, while the highest concentration (10%) showed a significant different when compared to control.

4.3. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Tobacco tree (*Nicotiana glauca*) water extraction plus one drop of sesame oil.

Results in table (3) and figure (3)showed that tobacco water extract plus one drop of sesame oil was very effective against cotton mealy bug. The mortality (%) ranged from 43.3 and 63.3 after only one day post treatment at

concentrations of 2.5% and 10% respectively. The highest concentrations (7.5% and10%) caused mortality 86.7% and 90% after three days post treatment. The mortality (%) was gradually increased by increasing of concentration and exposure time. The statistical analysis showed that there is a significant difference between all concentration used in this experiment and control.

Table 1.Mortality(%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Tobacco (*Nicotiana glauca*) water extract alone.

Concentration (%)	Exposure Period (days)		
	1st	2nd	3rd
2.5	6.7^{B}	6.7^{B}	6.7^{B}
5	6.7^{B}	10.0^{B}	10.0^{B}
7.5	20.0^{AB}	33.3^{A}	33.3^{A}
10	33.3^{A}	50.0^{A}	50.0^{A}
Control	0.0^{B}	0.0^{B}	0.0^{B}
CV%	3.48	3.2	4.2
SE±	10.0	8.8	8.8
LSD	23.1	20.3	20.3

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

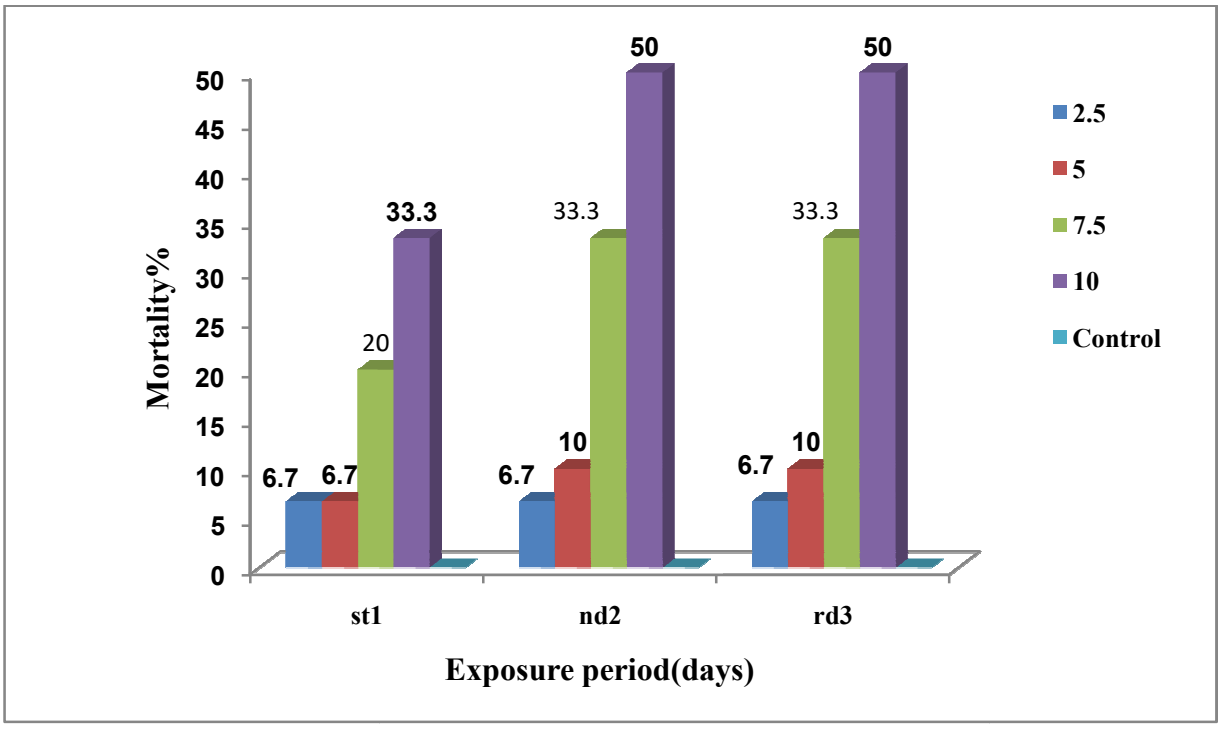

Figure 1. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis)* treated with Tobacco (*Nicotiana glauca*) water extract alone.

Table 2. Mortality (%) among cotton mealy bug*(Phenacoccus solenopsis)* treated with Tobacco (*Nicotiana glauca*) water extract plus Agral® substance.

Concentration (%)	Exposure Period (days)		
	1st	2nd	3rd
2.5	13.3^{C}	30.0^{B}	30.0^{B}
5	23.3^{BC}	30.0^{B}	30.0^{B}
7.5	53.3^{B}	63.3^{AB}	63.3^{AB}
10	86.7^{A}	90.0^{A}	93.3^{A}
Control	0.0^{C}	6.7^{C}	6.7^{C}
CV%	9.76	5.06	8.67
SE±	13.1	16.0	15.1
LSD	30.3	36.9	34.8

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

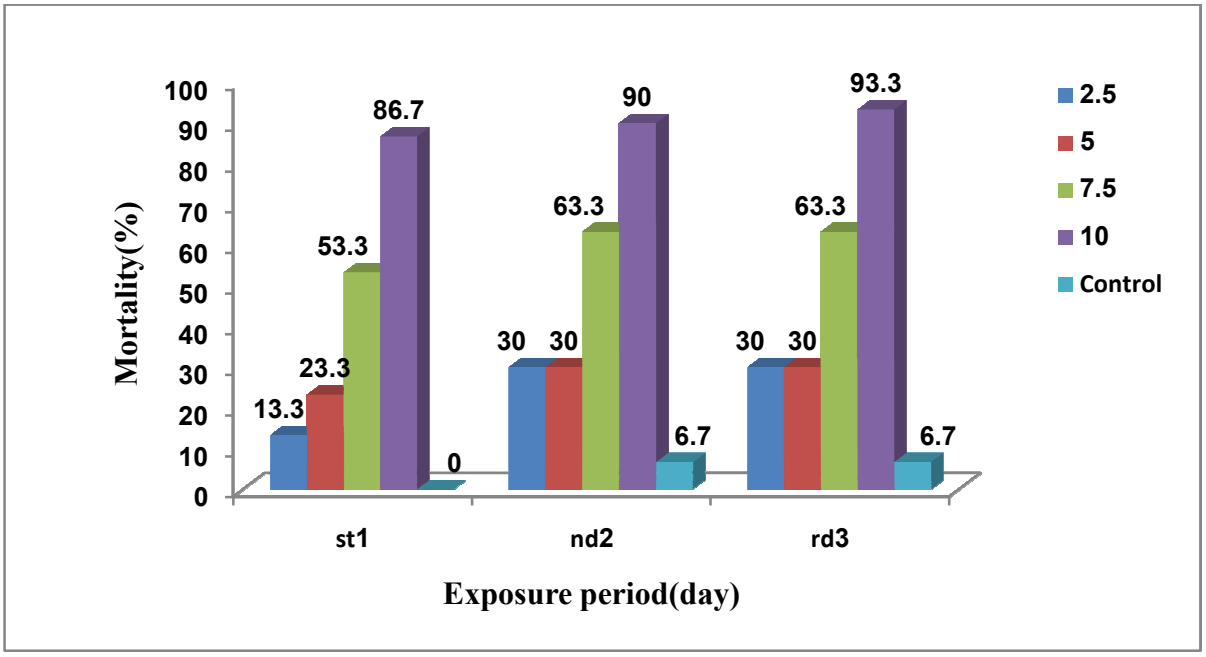

Figure 2. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis)* treated with Tobacco (*Nicotiana glauca*) water extract plus Agral® substance.

Table 3. Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Tobacco (*Nicotiana glauca*) water extract plus one drop of sesame oil.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	43.3^{A}	66.7^{A}	80^{A}
5	43.3^{A}	80^{A}	80^{A}
7.5	46.7^{A}	80^{A}	86.7^{A}
10	63.3^{A}	83.3^{A}	90^{A}
Control	13.3^{B}	13.3^{B}	13.3^{B}
CV%	5.3	16.7	12.1
SE±	16.997	10.541	8.5
LSD	39.195	24.3	19.6

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

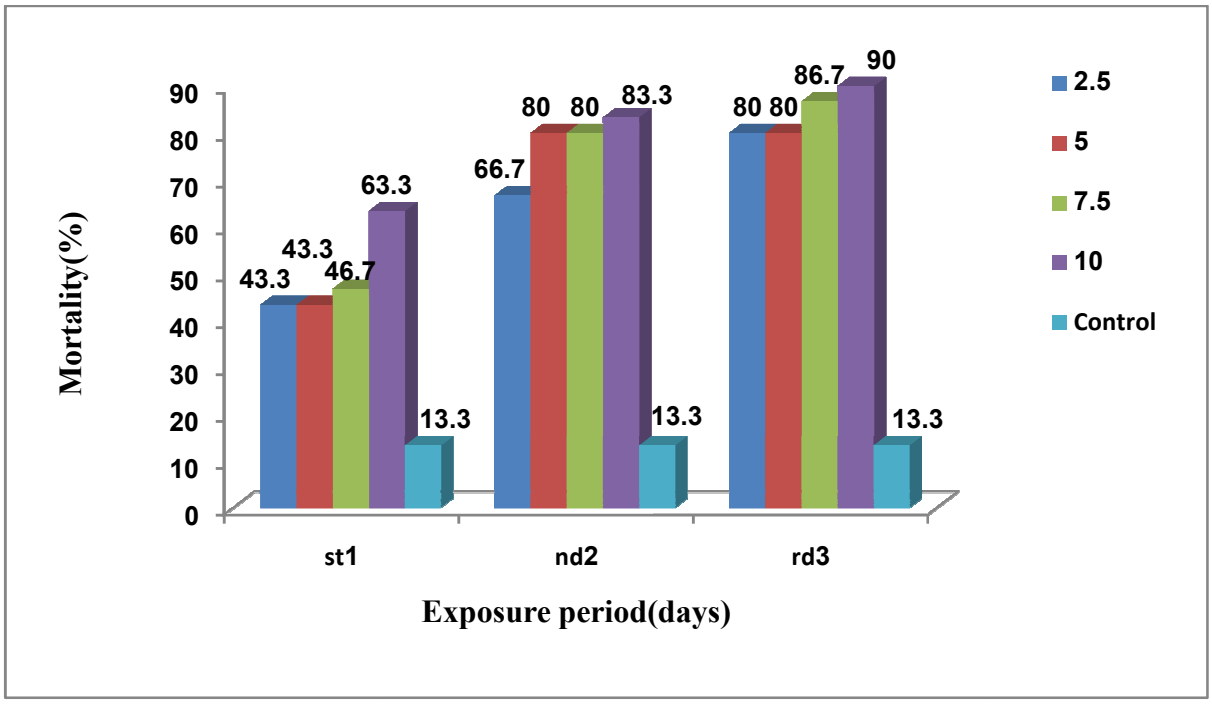

Figure 3. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Tobacco (*Nicotiana glauca*) water extract plus one drop of sesame oil.

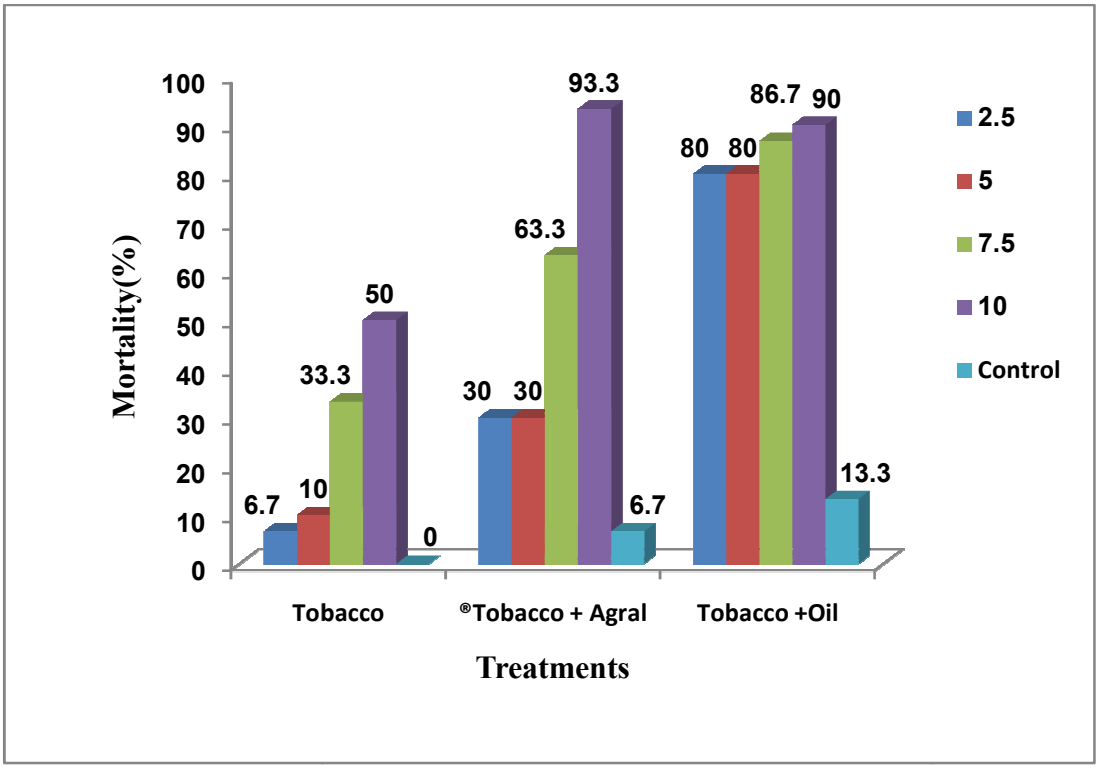

Figure 4. Comparison between the mortality (%) among cotton mealy bug (*Phenacoccus solenopsis*) treated with Tobacco (*Nicotiana glauca*) water extract alone, Tobacco plus Agral® and Tobacco plus one drop of sesame oil after three days post treatment.

4.4 Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Usher plant (*Calotropisprocera*) water extractalone.

Results in table (4) and figure (5) showed that Usher plant water extract alone has a little effect against cotton mealy bug. The highest concentration (10%) gave only (26.7%) mortality three days post treatment. The statistical analysis showed that there are no significant differences between the three concentrations (2.5, 5 and 7.5%) and control during the period of exposure.

4.5 Mortality (%) among cotton mealy bug*(Phenacoccus solenopsis*) treated with Usher plant (*Calotropis procera*) water extract plus Agral® substance.

Results in table (5) and figure (6) showed that Usher plant water extract plus Agral® substance was most effective against cotton mealy bug, when compared to usher extract alone. Thetwo highestconcentrations (7.5% and10%) caused mortality of 70%and 93% respectively three days post treatment. The statistical analysis showed that there are no significant difference between the three concentrations (2.5% and 5% and 7.5%) and control, while the highest concentration (10%) showed a significant different when compared to control.

4.6 Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Usher plant (*Calotropisprocera*) water extract plus one drop of sesame oil.

Results in table (6) and figure (7) showed that Usher plant water extract plus one drop of sesame oil was effective against cotton mealy bug. The two highest concentrations (7.5% and10%) caused mortality 63.3% and 73.3% respectivelyafter three days post treatment. The statistical analysis showed that there are a no significant difference among the four concentrations during all days of expose and there are a significant different between them and the control.

Table 4.Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Usher plant (*Calotropisprocera*) water extract alone.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	0.0B	0.0B	0.0B
5	0.0B	0.0B	0.0B
7.5	0.0B	3.3B	6.7B
10	10.0A	26.7A	26.7A
Control	0.0B	0.0B	0.0B
CV%	3.2	10.0	9.1
SE±	4.7	8.2	8.2
LSD	10.9	18.8	18.8

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

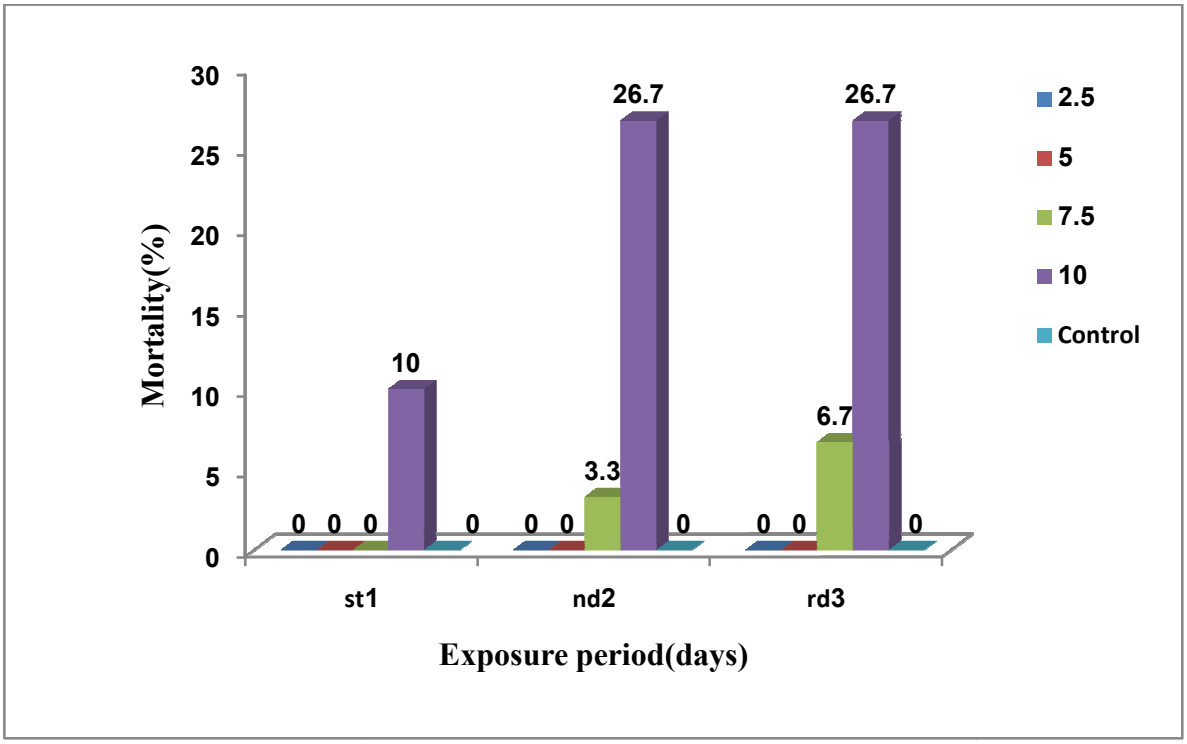

Figure 5. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Usher plant (*Calotropis procera*) water extract alone.

Table 5. Mortality(%) among cotton mealy bug*(Phenacoccussolenopsis)* treated with Usher plant (*Calotropisprocera*) water extract plus Agral® substance.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	3.3^{B}	13.3^{B}	13.3^{B}
5	6.7^{B}	16.7^{B}	20.0^{B}
7.5	46.7^{A}	70.0^{A}	70.0^{A}
10	50.0^{A}	90.0^{A}	93.3^{A}
Control	0.0^{B}	6.7^{B}	6.7^{B}
CV%	5.9	9.5	2.7
SE±	10.0	16.2	10.0
LSD	23.1	37.3	23.1

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

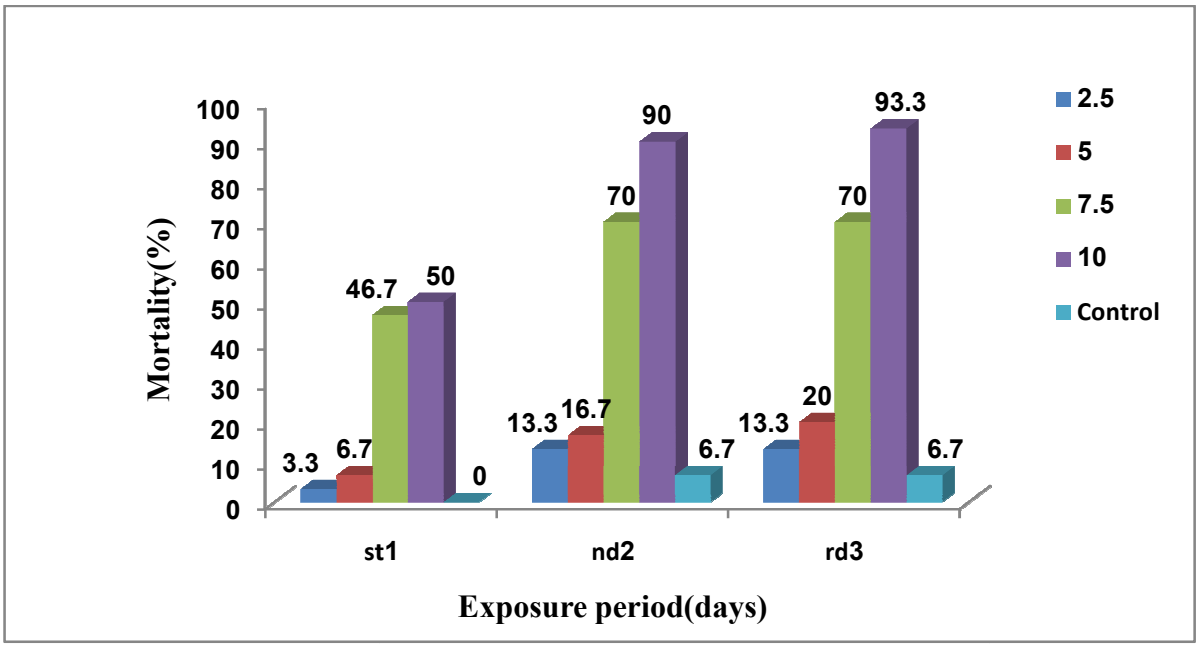

Figure 6. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis)* treated with Usher plant (*Calotropis procera*) water extract plus Agral® substance.

Table 6.Mortality(%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Usher plant (*Calotropisprocera*) water extract plus one drop of sesame oil.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	26.7^{A}	43.3^{A}	46.7^{A}
5	30.0^{A}	46.7^{A}	50.0^{A}
7.5	33.3^{A}	41.3^{A}	63.3^{A}
10	36.7^{A}	66.7^{A}	73.3^{A}
Control	13.3^{B}	13.3^{B}	13.3^{B}
CV%	9.7	7.2	6.0
SE±	10.0	15.5	16.7
LSD	23.1	35.6	38.4

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

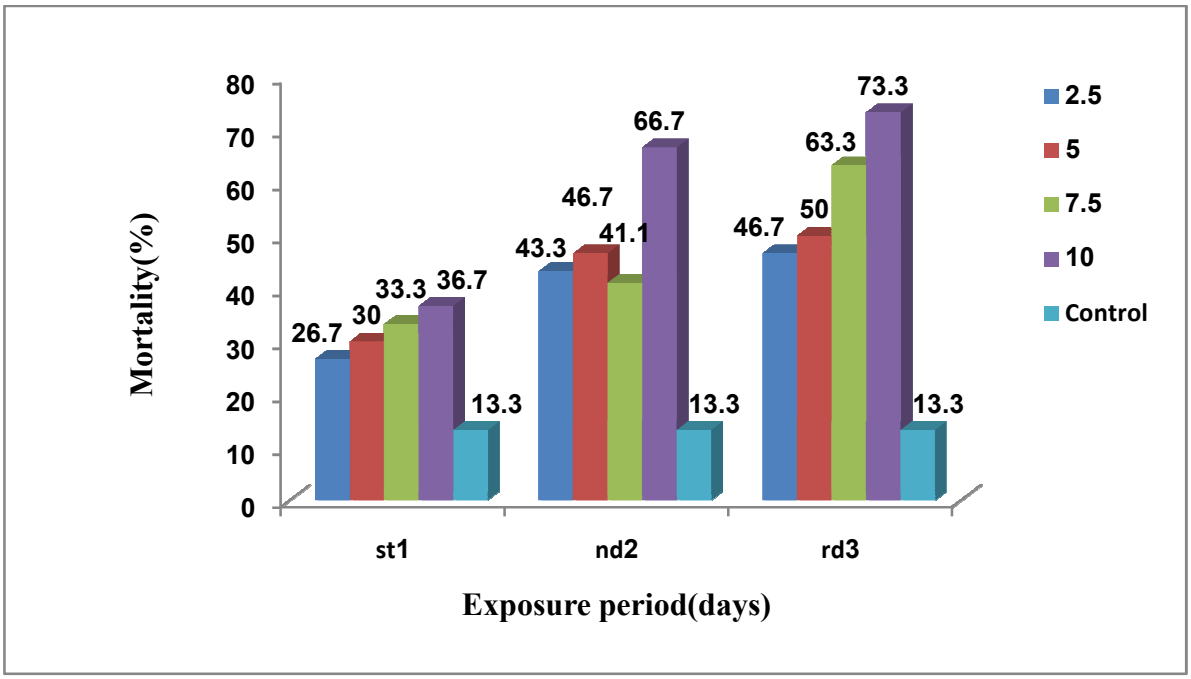

Figure 7. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Usher plant (*Calotropis procera*) water extract plus one drop of sesame oil.

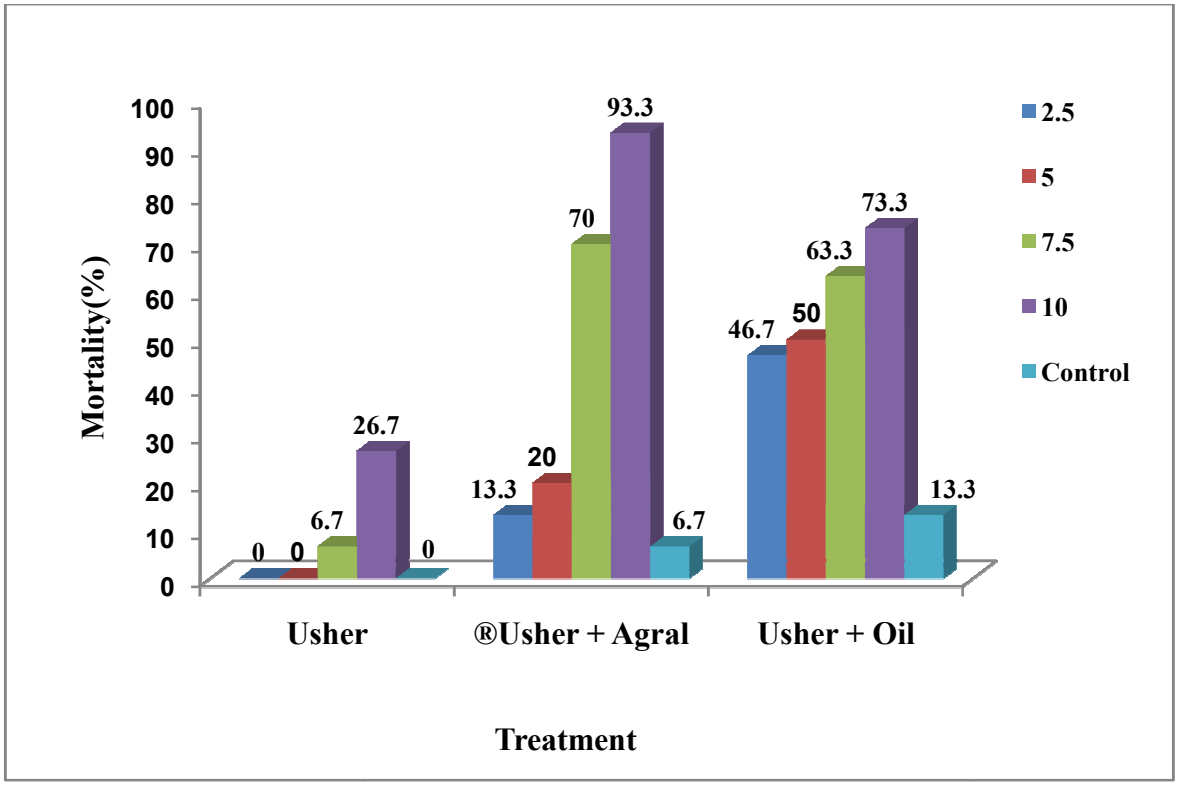

Figure 8. Comparison between the mortality (%) among cotton mealy bug (*Phenacoccus solenopsis*) treated with Usher plant (*Calotropis procera*) water extract alone,Usher plus Agral® andUsher plus one drop of sesame oil after three days post treatment.

4.7. Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Ghubaych plant (*Guierasenegalensis*) water extract alone.

Results in table (7) and figure (9) showed that the Ghubaych plant water extract alone was a less effective against cotton mealy bug. The highest concentrations (7.5% and10%) caused only mortality of 6.7%and 10% ,respectively after three days post treatment. The statistical analysis showed that there are no significant difference between all concentrations and control.

4.8. Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Ghubaych plant (*Guierasenegalensis*) water extract plus Agral® substance.

Results in table (8) and figure (10) showed that the Ghubaych plant water extract plus Agral® substance have a little effect against cotton mealy bug. The highest concentrations (7.5% and10%) caused mortality of 33.3%and 43.3% after three days post treatment. The statistical analysis showed that there is a no significant difference between the two lowest concentrations and control, while the highest concentrations showed a significant difference between them and control.

4.9. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Ghubaych plant (*Guierasenegalensis*) water extract plus one drop of sesame oil.

Results in table (9) and figure (11) showed the Ghubaychplant water extract plus drop of sesame oil was caused a moderate effect against cotton mealy bug. The concentrations (2.5% and10%) caused mortality of 60%and 50%, respectively after three days post treatment. The statistical analysis showed that there is a no significant difference among all concentrations and the significant different was reported between the concentrations (2.5%, 5% and 10%) and the untreated control.

Table 7. Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis)* treated with Ghubaych plant (*Guierasenegalensis*) water extract alone.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	0.0A	0.0A	0.0A
5	3.3A	3.3A	3.3A
7.5	3.3A	6.7A	6.7A
10	6.7A	10.0A	10.0A
Control	0.0A	0.0A	0.0A
CV%	7.7	4.3	4.3
SE±	3.7	4.7	4.7
LSD	8.1	10.5	10.5

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

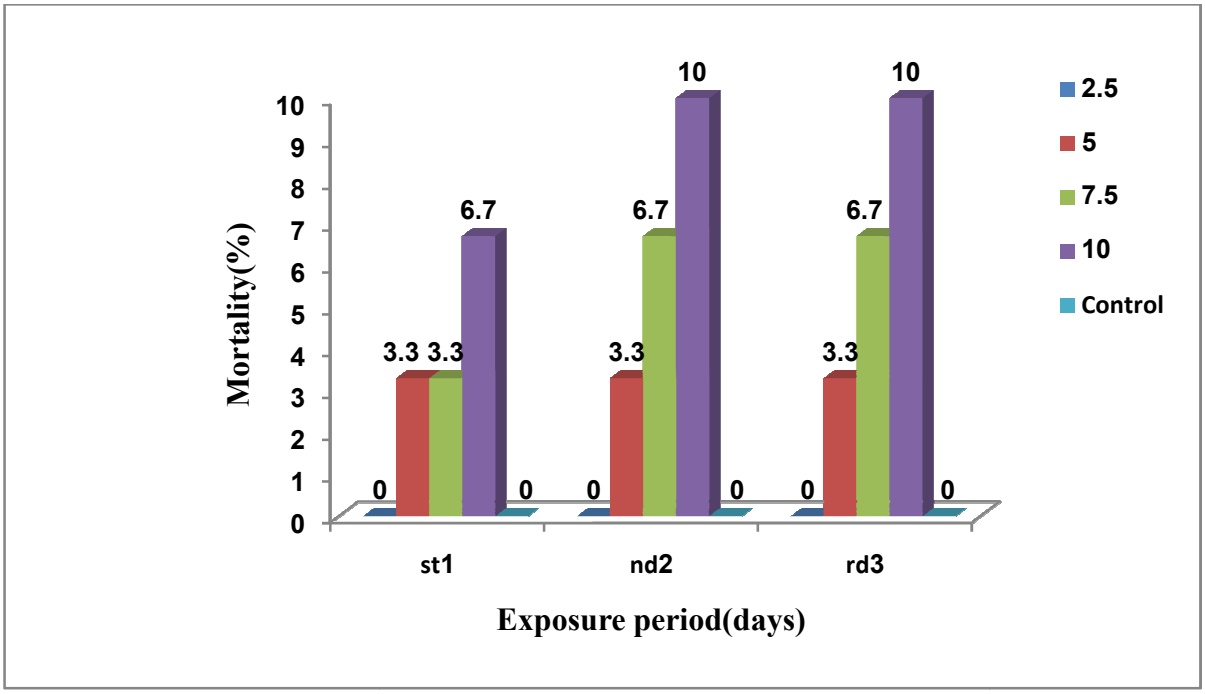

Figure 9. Mortality (%) among cotton mealy bug*(Phenacoccus solenopsis)* treated with Ghubaych plant (*Guiera senegalensis*) water extract alone.

Table 8. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis)* treated with Ghubaych plant (*Guiera senegalensis*) water extract plus Agral® substance.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	0.0^{C}	3.3^{B}	3.3^{B}
5	10.0^{BC}	10.0^{B}	10.0^{B}
7.5	20.0^{AB}	33.3^{A}	33.3^{A}
10	26.7^{A}	40.0^{A}	43.3^{A}
Control	0.0^{C}	6.7^{B}	6.7^{B}
CV%	7.0	8.2	3.6
SE±	6.7	9.2	8.2
LSD	14.9	20.5	18.2

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

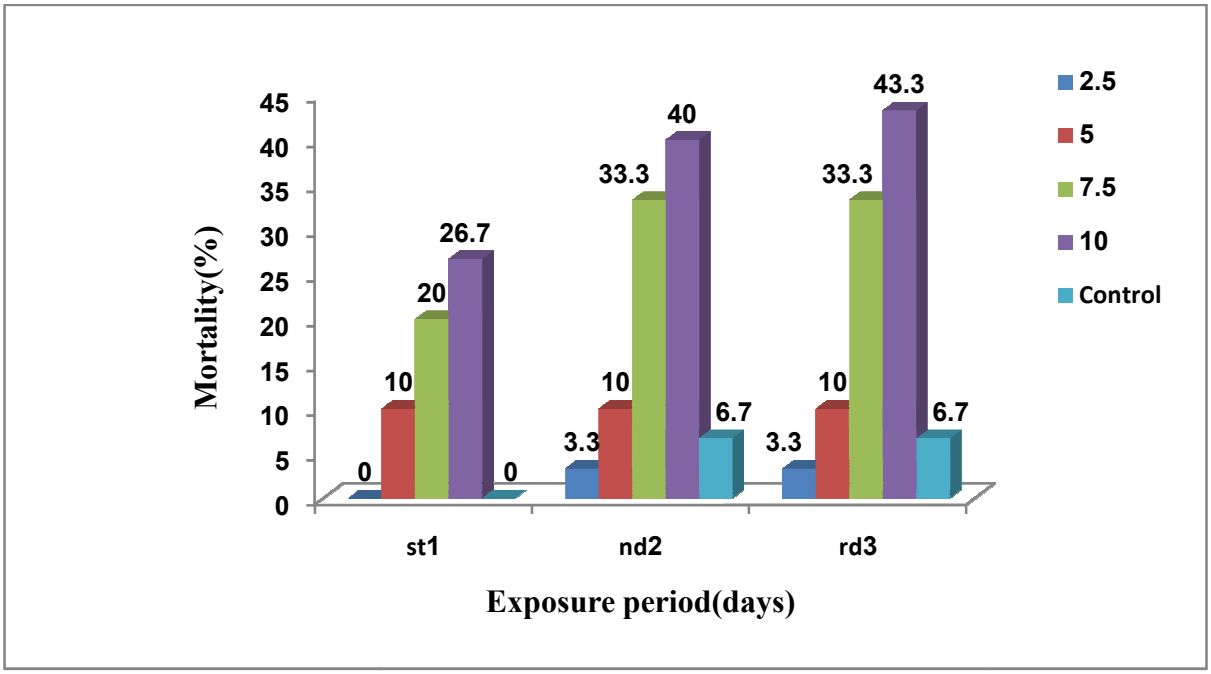

Figure 10. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis)* treated with Ghubaych plant (*Guiera senegalensis*) water extract plus Agral® substance.

Table 9. Mortality (%) among cotton mealy bug*(Phenacoccussolenopsis*) treated with Ghubaych plant (*Guierasenegalensis*) water extract plus one drop of sesame oil.

Concentration (%)	Exposure Period (day)		
	1st	2nd	3rd
2.5	40.0^{A}	60.0^{A}	60.0^{A}
5	36.7^{A}	50.0^{A}	50.0^{A}
7.5	36.7^{A}	36.7^{AB}	36.7^{AB}
10	40.0^{A}	46.7^{A}	50.0^{A}
Control	13.3^{B}	13.3^{B}	13.3^{B}
CV%	7.3	8.0	4.1
SE±	15.9	12.8	14.0
LSD	35.5	28.6	31.2

*** Means followed by the same letter (s) are not significantly different at (P<0.05).**

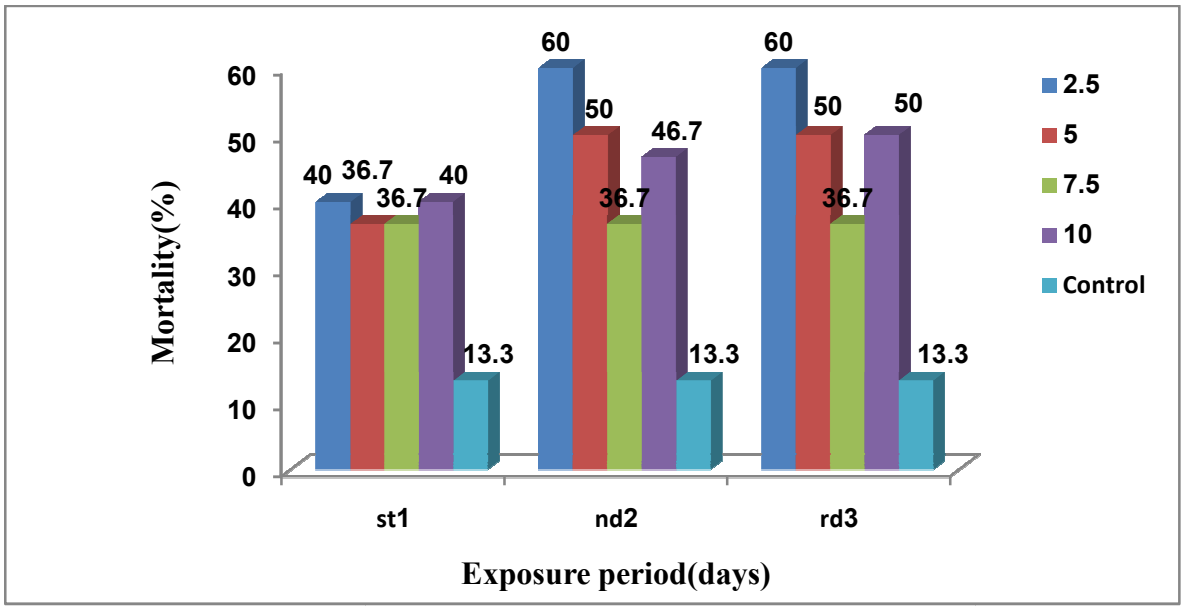

Figure 11. Mortality (%) among cotton mealy bug *(Phenacoccus solenopsis*) treated with Ghubaych plant (*Guiera senegalensis*) water extract plus one drop of sesame oil.

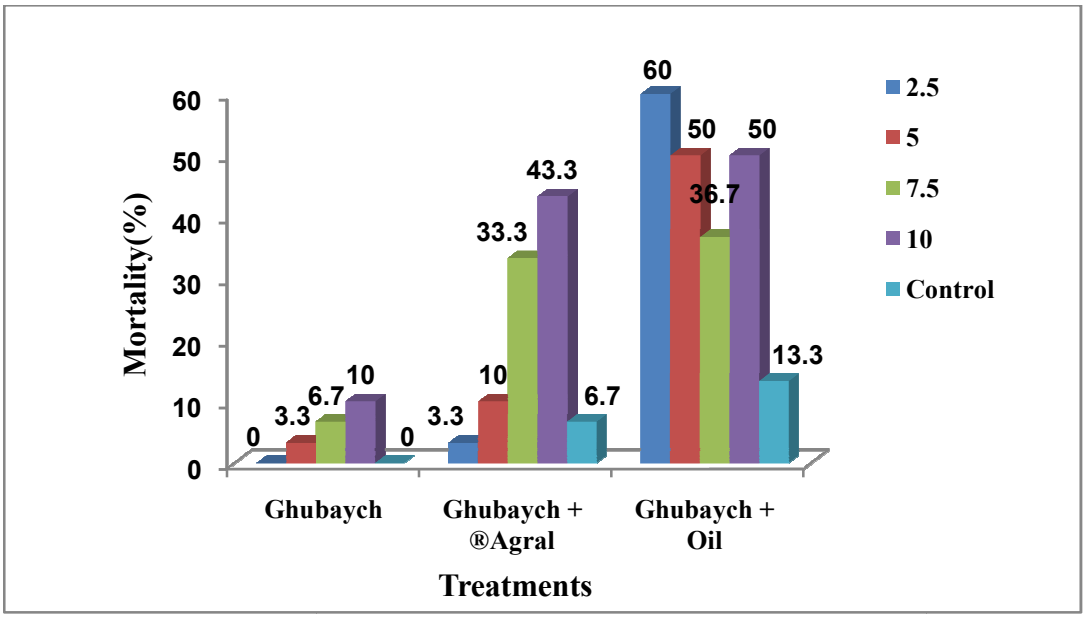

Figure 12. Comparison between the mortality (%) among cotton mealy bug (*Phenacoccus solenopsis*) treated with Ghubaysh plant (*Guiera senegalensis*) water extract alone, Ghubaysh plus Agral® and Ghubaych plus drop of sesame oil after three days post treatment.

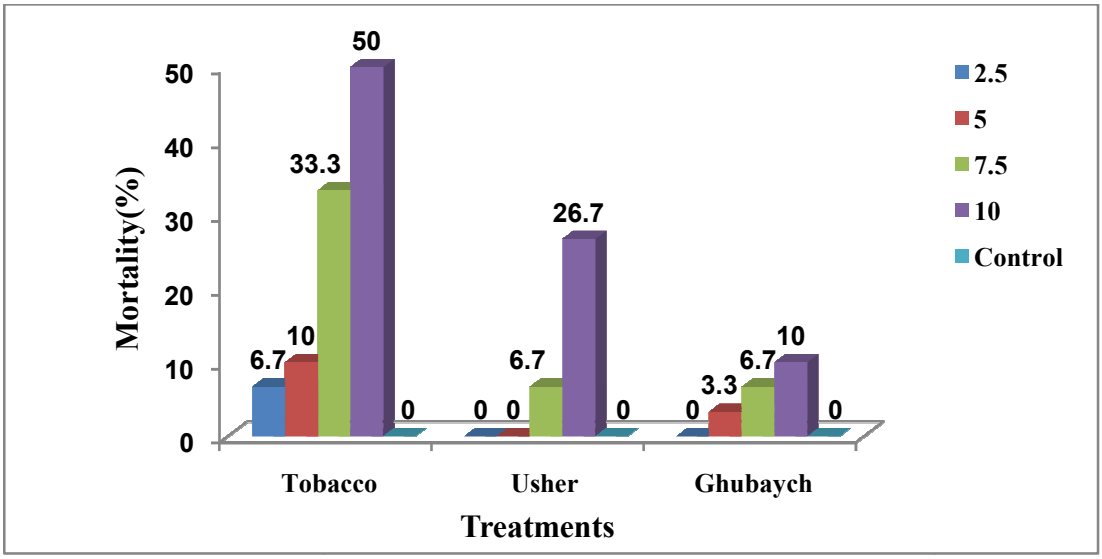

Figure 13. Comparison between the mortality (%) among cotton mealy bug (*Phenacoccus solenopsis*) treated with Tobacco tree, Usher plant and Ghubaych plant water extract after three days post treatment.

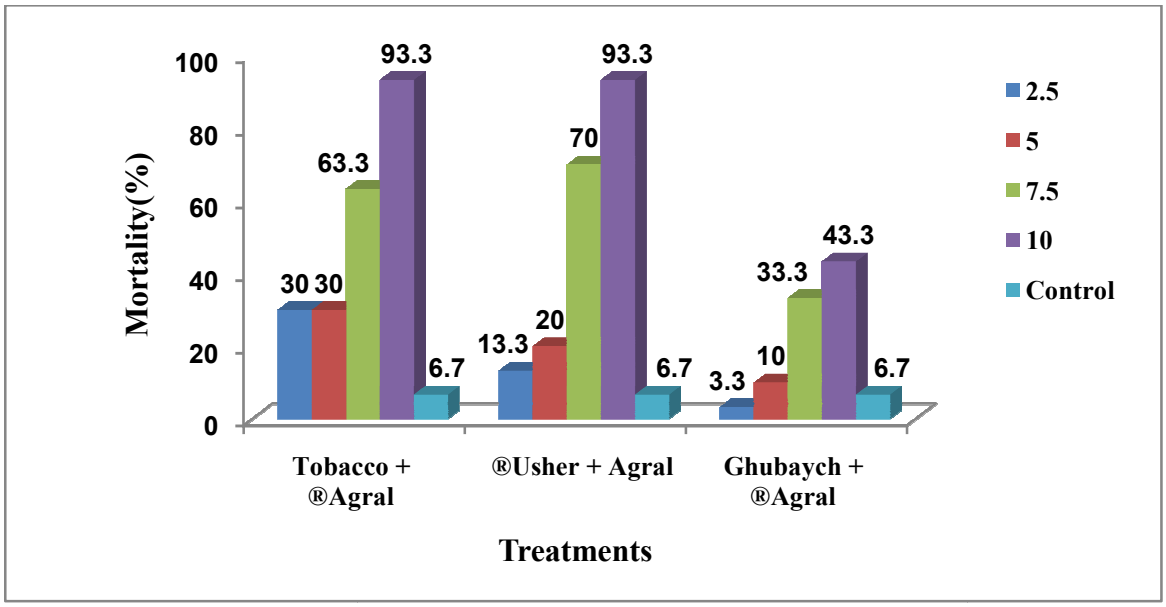

Figure 14. Comparison between the mortality (%) among cotton mealy bug (*Phenacoccus solenopsis*) treated with Tobacco, Usher and Ghubaych water extracts plus Agral® substance after three days post treatment.

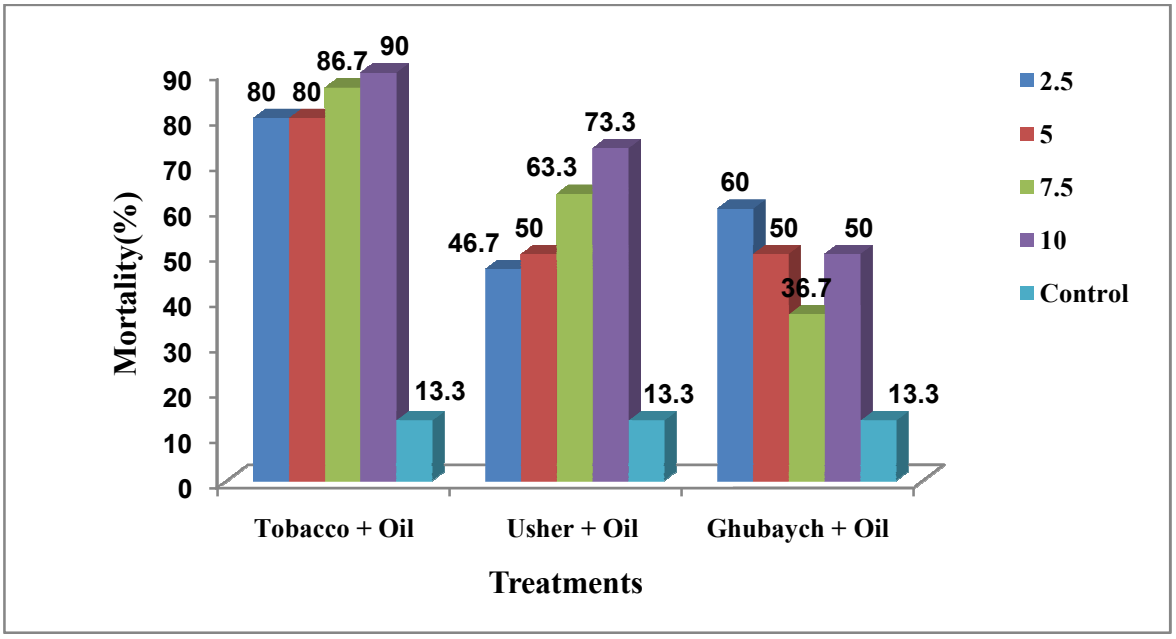

Figure 15. Mortality (%) among cotton mealy bug (*Phenacoccus solenopsis*) treated with Tobacco, Usher and Ghubaych water extracts plus drop of sesame oil after three days post treatment.

CHAPTER FIVE

DISCUTION

This study was carried out to investigate the effects of three botanicals, Tobacco, Usher and Ghubaych plant water extractalone, water extracts plus Agral® substance and water extracts plus one drop of sesame oil against cotton Mealy bug*(Phenacoccussolenopsis*) .

The two highest concentrations of Tobacco water extract plus Agral®substance (7.5% and 10%) gave a high mortality percentage of 63.3%, and 93% respectively, and also Tobacco water extract plus one drop of sesame oil gave mortality percentage of 86.7% and 90%, respectively.

The above mentioned results showed that, tobacco extracts have insecticidal activity against mealy bug, especially when mixed with other additive materials such as Agral®, sesame oil and etc., therefore other studies can be made to identify the synergistic action of other different oils and additive materials to improve and increase the efficacy of tobacco extracts against other insect pests.

These results agreed with (Steenkamp*et al*, 2002) who reported that alkaloid nicotine found in *N.glauca* is very toxic to insects and effect their nervous system and also various studies have been reported that *N.glauca* have insecticidal effect.

AlsoBenhissenSalih*aet al.* (2018) reported that *N. glauca* extract was highly toxic against mosquito larvae, the high rates of larval mortality observed at 62.4 g/l within 24 hours with LC50 value 26.87 g/l.

In this experiment Usher plant extracts showed a good bioactivity against mealy bug under laboratory condition. Usher plant water extract plus Agral® substance at 7.5 % and 10% gave mortality (%) of 70% and 93% and also

Usher water extract plus one drop of sesame oil highest concentrations 7.5% and 10% gave mortality (%) of 63.3% and 73.3%, respectively.

These results are in agreement with El Tayeb, (2004) Who reported that the insecticidal efficacy of water extracts of Hargal plant, *Solenostemmaargel*, and Usher, *C. procera*, leaves gave a good insecticidal effect against larvae of mosquito species, *Anopheles arabiensis* and *Culexquinquefasciatus*when compared with standard larvicide (Temphos®), and this finding also supported by Elimam (2009) who reported that aqueous extract of *Calotropisprocera*gave good results in controlling of *Anopheles arabiensis* and *Culexquinquefasciatus*. Also Ahmed*et al.,* (2005) found that extracts of*Daturaalba* and *Calotropisprocera* gave a good protection of sugarcane sets from termites.

The highest concentration of Ghubaych plant water extract plus Agral® substance and plus sesame oil at 10% gave a moderate mortality (%) 43.3% and 50%, respectively. These results are in agreement with Mukhtar (2004) who reported that the insecticidal efficacy of aqueous extracts of Ghubaych, leaves against Culex mosquito larva, gave a moderate mortality (%).

CONCLUSION

- Based on the above mentioned results, leaves powder aqueous extract of Tobacco tree (*Nicotiana glauca*)andusher plant (*Calotropisprocera)*were effective in controllingcotton mealy bug *(Phenacoccussolenopsis*) especially at concentration of 7.5% and 10%.
- The highest mortality (93%)were achievedwith Tobacco and Usher extracts plus Agral® substance
- The most effective treatments areTobacco plus sesame oil, Tobacco plusAgral® substance,Usher plus Agral® substance and Usher plus sesame oil.
- Ghubaych plant (*Guiera senegalensis*) extract was less effective when compared to Tobacco and Usher extracts, which gave a little mortality (%) against cotton mealy bug at all treatments.
- The sesame oil and Agral® substance have a synergistic action and increase the insecticidal activity of *N.glauca* and *C.procera* extracts.

RECOMMENDATIONS

- The leaves powder aqueous extract of Tobacco (*Nicotiana glauca*) plus sesame oil ,Tobacco plus Agral® substance and Usher plant (*Calotropisprocera)* plusAgral® substance, Usher plus sesame oilcan be considered as promising botanical insecticidesused to control cotton mealy bug*(Phenacoccussolenopsis*).
- Further comparative studies should be conducted to evaluate the effects of these plant extracts against other insect pests; alsoother organic solvent extracts can be tested.
- Additional field evaluation of such extracts will be tested in the future.

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