

Sudan University of Science and Technology
College of Graduate Studies

Insecticidal Effect of Five Botanical Plants Leaves
Extracts on the Cowpea Beetle *Callosobruchus maculatus*
(Coleoptera: Bruchidae)
in Northern Darfur State

التأثير الابادى لمستخلصات اوراق خمس نباتات على خنفساء اللوبيا فى ولاية شمال دارفور

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DEDICATION

TO SOUL OF MY FATHER AND MOTHER

TO MY BROTHER ABDELNEIUM

TO MY SISTERS HOWA AND FATAMA

TO MY WIFE ASHA

TO MY SON AHMED

FOR THEIR GREAT LOVE, SACRIFICE AND
ENCOURAGEMENT

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ABSTRACT

This work was carried out at Alfashir locality, North Darfur State, Sudan, during season 2017/2018 under laboratory condition and field surveying to find out alternative method for pesticide to protect cowpea seeds from infestation by the beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae) by using some botanical plants.

This study included a field survey to determine the presence, distribution and infestation of legumes seeds, cowpea, faba bean and kidney bean by the adults of cowpea beetle in three areas, crops market, Almoushi market and Nafasha market(locality Alfashir).

The study showed that the pest was present and distributed along the State specially in Alfashir areas, with high infestation rate (80%) in August on Faba bean at Almousha market and followed by (78%) in July on cowpea at Almousha market.

Laboratory studies were done in the lab. of Biological Science, Faculty of Education , Alfashir University to evaluate the efficacy of the five botanical leaves (powder, aqueous and ethanolic extracts) of *Ocimum basilicum*, *Azadirachta indica*, *Solenostemma argel*, *Datura stramonium* and *Nicotiana rustica* against adult of cowpea beetle, various concentrations of powder and aqueous extract (5%, 10% and 15%) and ethanolic extract (2%, 3% and 5%) were tested as potential source of control agent for adults of cowpea beetle. all treatments were arrange in Completely Randomized Design (CRD) and the data was subjected to Analysis of Variance (ANOVA) using SAS and SPSS computer software package (version21) at 0.05 Significant levels. Means were separated using Least Significant Difference (LSD).

The obtained data revealed promising activities of the five botanical plants leaves powder and aqueous, ethanolic extracts at different concentrations, powder aqueous(5%, 10% and15%) and ethanolic (2%, 3% and 5%) against the tested adult compared to the control for 24, 48 and 72 hours.the best result was obtained using ethanolic extracts of *Nicotiana rustica* in all concentrations caused high mortality to the tested adults.

Generally the result indicates that the lethal effect of ethanolic extracts of *N. rustica* was higher than that of Hargel followed by ocimum, neem and then datura leaves extract on adults of cowpea beetle.

The LD₅₀ of powder leaves of hargal, neem, ocimum, datura and tabocco were, 0.669,7.371, 96.94, 2.545 and3.180 respectively. Aqueous extracts were 3.679, 5.708,

2.72, 4.95 and 18.40, respectively. Ethanolic extracts were 2.482, 2.55, 3.107, 0.87 respectively.

The preliminary phytochemical analysis of (hargal, ocimum, neem, datura and tabocco) indicates the presence of different biochemical compounds. The major bioactive compounds according to their high peak areas were, *Ocimum basilcum* (pentamethyl pentaphenicylcyclophenasiloxane, peak area 3.63%), *Azadirachta indica* (5,10,15,20-teraphenyl[2-(2)HI](prophyrinalo)zinc(11), peak area 3.99%), *Solenostemma argel* (2-[2,6-Bis(hex-5-enyloxy)phenyl]-9-[2-(but-3-enyloxy)-6-hex-5-enyloxy)phenyl]-1,10-phenanthroline, peak area 3.66%), *Datura stramonium* (2-hydroxy-5,10,15,20-tetraphenylporphinato)copper(11), peak area 3.84%) and *Nicotiana rustica* (pyrrolidine N-(4-methyl-3-pentenyl, peak area 99.25%).

المستخلص

أجريت هذه التجارب بمدينة الفاشر ولاية شمال دارفور على احدى اهم الافات التى تصيب بذور البقوليات وخاصة اللوبيا فى المخازن وهى خنفساء اللوبيا *Callosobruchus maculatus* فى الفترة 2018/2017 لدراسة امكانية استخدام اوراق بعض النباتات المحلية ومستخلصاتها لمكافحة هذه الافة.

اشتملت هذه الدراسة على مسح للمخازن التى توجد فى اسواق المحاصيل لمعرفة درجة الاصابة وتواجد الافة وكذلك على دراسة معملية لتقييم التأثير الالابدى والكفاءة الحيوية للمستخلص الكحولى (الايثانولى) والمائى ومسحوق اوراق نباتات الريحان والنيم والحرجل والدتورا والتبغ.

اشتملت الدراسة ايضا على تحليل مسحوق اوراق النباتات باستخدام التحليل الكرمتوجرافى الغازى ذات التكتيف الطيفى الضوئى (GC-MS) لمعرفة المركبات الكيميائية.

اظهرت نتائج المسح تواجد وانتشار الحشرة فى كل المخازن الخاصة بتخزين بذور البقوليات فى مناطق الدراسة (سوق المواشى وسوق المحاصيل وسوق نيفاش) وكانت اعلى نسبة اصابة فى شهر اغسطس بلغت 80% فى بذور الفول المصرى و78% فى يوليو على بذور اللوبيا ايضا بسوق المواشى.

من خلال التجارب اظهرت النتائج بالنسبة للنباتات المستخدمة (مستخلص مائى ومسحوق الاوراق) للتركيزات 5% و10% و15% و (الايثانولى) 2% و3% و5% بان هنالك فروقات معنوية بنسبة للحشرات المعاملة والغير المعاملة.

بصورة عامة مستخلص الايثانولى لنبات التبغ كان اكثر تأثيرا فى الموت ثم يليه نبات الحرجل والريحان والنيم واخيرا الدتورا.

الجرعة القاتلة لمسحوق نباتات الحرجل والنيم والريحان والدتورا والتبغ كانت بتركيزات 7.371, 0.669, 96.94, 2.545 و 3.180 على التوالي والمستخلص المائى 3.679, 5.708, 2.72, 4.95 و 18.40 على التوالي و المستخلص الكحولى 2.482, 2.55, 3.107 و 0.87 على التوالي.

كما دلت نتائج الكرمتوجرافى الغازى (GC-MS) للنباتات على وجود مركبات كيميائية حيوية فعالة اعتمادا على معامل الاعاقة (RT) وقمة المنحنى Peak area الخاص بكل مركب كيميائى وهى كالاتى:

الريحان pentamethyl pentaphtaphenicyclopentasiloxane (peak area 3.63% والنيم
(2-teraphenyl[2-(2)HI]prophyrinalo)zinc(11) peak area 3.99% والحرجل (5,10,15,20-
[2,6-Bis(hex-5-enyloxy)phenyl]-a-[2-(but-3-enyloxy)phenyl]-1,10-phenathroline pea
area 3.66 والدتورا (2-hydroxy-5,10,15,20-teraphenylporphinato)copper(11) peak area
3.84% والتمباك (pyrrolidine N-(4-methyl-3-pentenyl) peak area 99.25).

CHAPTER 1

INTRODUCTION

Increased use of agrochemicals, however has caused considerable concern about their effect on health and natural environment as well as the agricultural products quality (Boon-long,1990). Although the use of pesticides gives important benefit both in agriculture and the field of public health but, no doubt, pesticides have polluted and our calm and sweet atmosphere.

We can't get a single gram of food stuff without residual toxicity and the problem extended to non target organisms like natural enemies, honey bees, livestock, soil microorganism, and aquatic organisms. In addition to this it leads to insect resurgence and create resistance to pests and diseases. More than that, frequent use of pesticides increases the farmers cost of cultivation and environment pollution.

It is known, pests are a serious problem causing annual yield loss and during periods of storage the seeds are subjected to damage by various stored product pests and the losses due to insect pests in developing countries ranged between 25-40% annually (Mohammed-Ahmed,2012). Many insects species caused damage to the stored products, of these is the cowpea beetle *Callosobruchus macultus* which belongs to the order Coleoptera, family Bruchidae, it rapidly reproduce and cause serious reduction in weight value of stored seeds (NRI,2003). The damage caused by this pest may happen in the field which range from 25% to 30% while in the

store it may reach 80% within 6-8 months in moderate areas of the world and complete losses obtained when the period of infestation is prolonged (Hill,1990). Humanity faces many problems that arise from its rapidly increasing population and is the provision of the population with good quality food that is accessible for all (FAO,2015).

There are various strategies that may be used to increase crop yield and improve food production, but various problems remain. Among them, the destruction of crops by pests is one of the most difficult, especially in developing countries, and although pests make up only a small percentage of insects, they cause significant losses to agricultural and forest crops, such as contributing to 20% annual loss of cereal crops (Sallam,2013).

The most voracious of the insect pests are Lepidoptera larvae, which have huge nutritional needs and are thus the most detrimental to food production (Nicholson,2007). Moreover, many insect species are vectors of diseases that lead to millions of human deaths each year, for example malaria. Therefore, the search for effective tools to control insect population is one of the most intensively developing fields of research. Presently, the most common way to control insect pests is through the use of synthetic pesticides, but they negatively impact the natural environment (Vander Gaag, 2000).

These compounds have a wide spectrum of activities against diverse groups of insects and can almost completely remove pests from agro ecosystems. However, although the immediate impacts are positive, there is no way to limit their action to only agricultural areas. The negative effects of synthetic insecticides are due to their insufficient selectivity, accumulation in the environment and food chains, long persistence, disturbance of the balance of ecosystems and high socio-economic costs (Mariyono, 2008; Damalas, 2011). Additionally, more selective pesticides are more expensive, so the inexpensive, non selective pesticides are primarily used in developing countries (Ecobichon,2001).

These problems have forced mankind to search for alternatives to these compounds, and the demands, of agriculture include inexpensive insecticides that cause the least amount of damage to the environment .Future agricultural and rural development is, to a large extent, influenced by the rapidly increasing food demand of 2.5 billion people expected to swell the world population (FAO,2015).

Achieving food sufficiency in a sustainable manner is a major challenge for farmers' agro – industries, researchers and governments (Schillhorn, 1999). The intensification of agriculture to fulfill food needs has increased the number of insect pest species attacking different crops and as a result the annual production losses of the standing crops. In the past, synthetic pesticides have played a major role in crop protection programmers' and have immensely benefited mankind.

Nevertheless, their indiscriminate use has resulted in the development of resistance by pests (insects, weeds, etc), resurgence and outbreak of new pests, toxicity to non – target organisms and hazardous effects on the environment endangering the sustainability of ecosystems (Jeyasankar and Jesudansan,2005). Due to environmental side effects and health concerns, many synthetic carbamate, organophosphate and organochlorine pesticides have been banned or are being under evaluation. On the other hand, industry does not equally sustain the economic cost of research and registration, of all pesticides chemical classes. The development of nematicides is rarely supported, even though in some cases such as in the Netherland, they represent more than 60% of the total pesticides used in agriculture (Chitwood, 2002).

All the above facts necessitate the urge for new and alternative pest control method. An interesting way of searching for biorational pesticides is screening naturally occurring compounds in plants (Isman,2006 and Isman 2008). Plants, as long – lived stationary organisms, must resist attackers over their life time, so they produce and exude constituents of the secondary metabolites, playing an important role in their defense mechanisms. In fact, the phytochemical, research has its roots in allelochemistry involving the complex chemical-mediated interactions between a plant and other organisms in its environment (Chitwood, 2002).

Botanical pesticides are an important group of naturally, often slow acting crop protectants that are usually safer to humans and the environment than conventional

pesticides, and with minimal residual effects. Moreover, the botanical pesticides contain mixtures of biologically active substances and no resistance is developed in pests or pathogens. Therefore, the use of plant pesticides has been recommended ever more as a suitable alternative of plant protection with minimum negative risks.(Isman,2006 and Pavela,2007). Especially botanical insecticides have long been a subject of conventional insecticides.

The use of plant insecticides has a long term tradition in Europe; the first known written references to application of plant extracts against pests come from Rome and date back to about 400 B.C. (Dayan *et al*, 2009). At present, several dozens of plant insecticides are used worldwide, based on various extracts especially of the families, Rutaceae, Lamiaceae, Meliaceae and Asterraceae.

Although plant pesticides have been studied in many laboratory tests (Chandler, 1951; Morgan, 2009), very few studies are available that present results from practical uses, and there is a great lack of biological efficiency comparisons of several products on multiple pest species at the same time. Botanical pesticides have a proven track record and long use as simple extractives for pest control and have spun off important groups of synthetic pesticides from phytochemical leads such as pyrethroids and neonicotinoids.

While botanicals are now a small part of the overall pesticide market due to replacement by synthetics, the new environmental movement has provided a

favorable environment for the rebirth of botanical insecticides. Public concern over use of synthetic insecticides growing this has led to the large growth in organic agriculture where the industry self regulates the use of products restricting synthetics but allowing some botanical pest control in many cities in Europe and North America. Cosmetic use of synthetics is now banned in urban areas. Public resistance to adoption of genetically modified organisms is another factor favoring alternative control measures such as bio pesticides, bio control and other methodologies. In reality however, botanicals have certain advantages but an equal number of drawbacks in practical use.

The advantages of botanical pesticides lie in their rapid degradation and lack of persistence and bio accumulation in the environment, which have been major problems in synthetic pesticides. For example DDT residues are still present in some sandy soils in Ontario decades after use was discontinued and contaminate some medicinal crops grown in these soils to levels which are a barrier to their export. Research with a number of experimental botanical pesticides as piperamides and alpherthienyl, shows they are degraded in the environment in hours or days.

Along history of safe use for some plant natural products provides also some confidence about low risk, although this cannot be assumed for new products. The diversity and redundancy of phytochemicals in botanical extracts is also useful. Redundancy, which is the presence of numerous analogs of one compound, is known to increase the efficacy of extractives through analog synergism, reduce the

rate of metabolism of the compounds and prevent the evolution of pesticide resistance when selection occurs over several generations from a research discovery point of view. The number of insect deterrents derived from plants seems endless as co adaptation appears to have produced a huge diversity of novel compounds across the plant kingdom and a remarkable redundancy of plant defenses within each plant species FAO, 2015.

General objective

The overall objective of this study was to assess the insecticidal effect of five plant leaves powder and extracts (tobacco leaves, neem leaves, datura leaves, basil leaves and hargel leaves) on the adult of cowpea beetle (*Callosobruchus maculatus*).

Specific objectives

- 1- To determine the situation of infestation and abundance of cowpea beetle on legumes crops seeds through a survey in North Darfur.
- 2- To make an experiment in host preference of cowpea beetle.
- 3- To investigate the insecticidal activity of powder, ethanolic extracts , and aqueous extracts against adults of cow pea beetle *Callosobruchus maculatus*.
- 4-To characterize partially the insecticidal compounds in the bioactive fraction by using mass chromatography.

CHAPTER 2

LITERATURE REVIEW

2.1. Cowpea beetle

2.1.1. Classification

Kingdam: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Bruchidae

Genus: *Callosobruchus*

Species: *maculatus*

S.N: *Callosobruchus maculatus* (Fabricius , 1775).

2.1.2. Distribution

Callosobruchus maculatus is a species of beetles commonly known as the cowpea weevil or cowpea seed beetle, It is a member of the leaf beetle family, Bruchidae. This common pest of stored legumes has a cosmopolitan distribution, occurring on every continent. except Antarctica (Fabricius, 1775) – The beetle most likely originated in west Africa and move around the globe with the trade of legumes and other crops Tran and Credland, (1995). As only a small number of individuals were likely present in legumes carried by people to distant places, the populations that

have invaded various multiple bottlenecks. Despite these bottlenecks populations persist. This ability to withstand a high degree of inbreeding has likely contributed to this species, prevalence as a pest.

2.1.3 Description of life Stages

Egg

Eggs of cowpea beetle are small, flat, oval or spindle shaped and white, only 0.75mm long, clear, shiny and firmly glued to the bean surface (Beck and Blummer,2009).

Larvae

Larvae are whitish in color, somewhat C-shaped with a small head. rarely seen as they feed within seeds. the first instar in the family Bruchidae is different from the three subsequent larval instars in possessing well formed thoracic legs, an H-shaped plate on the prothorax and a hatching spine on each lateral side of the first abdominal segment (Pajni,1987).

Adult

Adults of *C.maculatus* are reddish-brown, slightly elongate beetle, 3mm in length. They are not s elytra are marked with black and grey, with two black spots near the are short, leaving the last segment of the abdomen exposed, the last abdominal segment also has two visible black spots. Legs and antennae are long, the hind femurs are frequently enlarged. The most reliable genus and species indicators

spines and teeth on the underside of the hind femurs, *C. maculatus* have a single triangular spine at the apical end of both ventral femur ridges (Brier, 2008).

The males and females can be easily distinguished in the adult form; females possess dark stripes on the sides of the enlarged palps covering the tip of the abdomen and are dark brown or almost black in coloration compared to the light – brown males. Unlike other stored product insects, adults of this beetle can be found in two morphological body forms: one with wings and capable of flight and another without wings and flightless. The adult form is produced when larval rearing conditions are crowded, or in continuous light or dark (such as in storage), high environmental temperature, or low moisture content (Utida, 1972 and Beck and Blumberg, 2011).

2.1.4 Life Cycle

A female adult can lay over a hundred eggs, and most of them will hatch. She lays an egg on the surface of a bean, and when the larva emerges about 4 to 8 days later, it burrows into the bean (Raina, 1970).

During development, the larva feeds on the interior of the bean, eating the tissues just under the surface, leaving a very thin layer through which it exits when it matures. It emerges after a larval period of 3 to 7 weeks, depending on conditions. Larval crowding can occur when up to 8 or 10 larvae feed and grow within one bean. Crowding limits resources for each individual, leading to long development time, high mortality, small adult size, and low fecundity. Raina, (1970) reported that

once the beetle emerges as an adult, it may take 24 to 36 hours to mature completely. The life span is 10 to 14 days, the adult requires neither food nor water, sugared water, or yeast, it may consume it. A female given nutrients may lay more eggs. The beetle tolerates a range of humidity and temperature, making it adaptable to climates worldwide. Its developmental time varies with factors such as humidity, temperature, legume type, crowding and inbreeding levels in the population (Fox and Reed, 2011).

Bean that is too dry will be impossible for the larva to bore into, and wet beans may have fungal growth. In experiments, a humidity range of 25% to 80% was acceptable, with different optimal levels at each life stage. Most eggs hatched between 44% and 63% humidity, and 44% produced the highest survival. The adult lives longer at 81% to 90% (Schoof, 1941).

2.1.5 Site of infestation

Collosobruchus maculatus lays eggs on the pods of host legume as they approach maturity in the field but emergence usually occurs after harvest. In storage, *C. maculatus* lays eggs on the seeds. Larval development and pupation are completed entirely within a single seed (Booker, 1967). Messina, (1993) and Fox, (1993) reported that *C. maculatus* females and males live an average of 7 days under laboratory conditions and very few survive more than 2 weeks at 25 C⁰. The beetles have a short generation time (22 – 28) days at 28 C⁰. Relative humidity has little effect on longevity (Giga and Smith, 1983). Both sexes can mate soon after female

C. maculatus adjust oviposition rates to cope with changes in host availability, laying fewer eggs when host availability is low, and hence decreases larval competition.

However, females sometimes continue laying eggs on unsuitable substrates during host deprivation. This egg dumping behavior results in high mortality, but be a strategy for host range expansion if preferred hosts are unavailable (Wange and Horng, 2004; cheng *et al.*, 2008).

Edvardsson & Tregenza, (2005) reported that the mates of *C. maculatus* has spines on their intromittent organs that puncture the female reproductive tract during mating. Females kick their mates during copulation. If females are prevented from kicking the males, copulations last longer and the injuries females sustain are more severe.

Callosobruchus maculatus is acosmopolition pest of stored legumes (Fabaceae), particularly of the genus *vigna*. Females cement eggs to the surface of the host. Larvae burrow in to seeds where their entire development (four instars plus pupal stadium) is completed. Larvae cannot move among seeds and are thus restricted to the seed that, their mother has chosen for them. Beetles emerge from seeds reproductively mature. Emerging adults are well adapted to storage conditions requiring neither food nor water to reproduce because beetles most commonly occur in seed stores (Messina, 1991).

2.1.6. Damage of cowpea beetle

Cowpea production is affected by insect pests and disease infestation which lead to economic losses (plate, 7). Insect damage is the major constraint to cowpea grain production in most cowpea producing nations (Singh and Van, 1979). The major insect pests that cause economic losses are cowpea aphids (*Aphis craccivora*), leaf hoppers (*Empoasca* spp) and (*Coryna* spp), blister beetles (*Hycleu slugens*), green stink bugs (*Nesara viridula*) and cowpea beetle (*Callosobruchus maculatus*).

The cowpea weevil, *Callosobruchus maculatus* (F): Coleoptera: Bruchidae, is a cosmopolitan field – to – store pest ranked as the principal post harvest pest of cowpea in the tropics (Caswel, 1981). It causes substantial quantitative and qualitative losses manifested by seed perforation and reductions in weight, market value and germination ability of seed (Oluwafemi, 2012).

2.1.7. Control of cowpea pests

In order to reduce serious losses experienced during storage, various techniques and control methods have been developed and more are still being developed. Management of cowpea seed storage pests relies heavily on the use of chemical insecticides. However, most the small scale farmers have not adopted these techniques due to some financial and technical reasons. Chemical insecticides are widely used (Langyinto *et al.*, 2008). In Nigeria, the abuse and misuse of these chemical pesticide have several repercussions one of which is acute and chronic

poisoning in man (Akunne and Okonkwo, 2006). Others include sudden deaths, blindness skin irritation and pest resurgence in the ecosystem (Langyinto, *et al.*, 2008; Omologe, 2008). Furthermore, the development of resistant strains, killing of non target species, pollution of part of the ecosystem, toxic residue, workers unsafety and increasing costs are recorded as environmental repercussion of abuse and misuses of pesticides (Akunne and Okonkwo, 2006). Insecticides also have negative impact on the environment, humans and non – target organism. Therefore, there is a need to develop cheap, safe and easy methods of protecting stored cowpeas against cowpea beetle. resource- poor farmers in Africa employ a range of traditional methods such as use of ash, sand, dry pepper and botanical extracts. Naturally occurring plant products have been used to protect agricultural products against a broad range of pests.

Some of the techniques that can be explored include the use of plant products such as garlic, peppermint and chilies. Aromatic plant have both medicinal and aromatic properties and contain a variety of volatile oil which have insecticidal, antifeedant and repellent effects on insect pests. The chemical repellent hypothesis states that non – host plant odors repel herbivores by disrupting their ability to locate or feed on the host plant (Beizhou, *et al.*, 2012). There is limited information on the use of the plant products as an alternative control method for controlling weevils in storage. The use of plant products may offer a sustainable, environmentally friendly and safer alternative to synthetic insecticides.

Lienard, *et al.*, (1993) reported that, in developing countries small scale farmers mix the crushed leaves of *Cassia occidentalis* in to bean stores to deter the beetle. Other cassia is useful, as well. The powder leaves are effective, and a warm – water extract and essential oil from the seeds are better. Kestenholz, (2007) stated that the seed oil does not stop oviposition, but it increases the mortality of the eggs and the first instar larvae. The warm water extract deters the adult female from ovipositing.

Other botanical biological pest control agents tested include Nishinda (*Vitex negundo*), Tasmanian blue gum (*Eucalyptus globules*), Bankalmi (*Ipomoea sepiaria*), neem (*Azadirachta indica*), safflower (*Carthamus tinctorius*), sesame (*Sesamum indicum*), and gum Arabic (*Acacia nilotica* Syn. *Acacia arabica* (Rahman and Talukder, 2006).

Hermetic storage technologies like the purdue improved cowpea storage bags have also proven successful in controlling *C. maculutus*. These technologies work by separating the container environment from the surrounding air and forcing the insect inside to deplete the available oxygen inside the container not only does this ultimately kill the insects, but it also reduces the level of damage they inflict as active feeding ceases below a certain threshold of oxygen (Murdock *et al.*, 2012.,Murdock and Baoua, 2014). In laboratory trials *D. basalis* has totally eliminated the beetle (Kapila and Agarwal, 1995). *A. calandrae* and *U. mukerjii* may also prove useful with freezing the whole storage areas , a period of six to 24

hours at -18°C kills all the adults and larvae. If cooling is slow, the beetle can acclimatize, so longer freezing is required (Johnson and Valero, 2000).

2.2. Cowpea

2.2.1 Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order :Fabales

Family :Fabaceae

Sub family: Faboideae

Tribe: Phaseoleae

Sub tribe: Phaseolinae

Genus: *vigna*

Species: *unguiculata*

(Verdcourt, 1970; Marechal *et al.*, 1978)

2.2.2 Distribution and uses

Cowpea (*Vigna unguiculata*(L)Walp.) is grown in tropical Africa, Asia, North and South America mostly as a grain, but also as a vegetable and fodder crop. It is favoured because of its wide adaptation and tolerance to several Stresses. It is an important food source and is estimated to be the major protein source for more than 200 million people in sub Saharan Africa and is in the top ten fresh vegetable in China.

Cultivated cowpeas are grown as warm – season – adapted annuals in tropical and subtropical zones reported by (Hall, 2001). in all countries in sub – Saharan Africa and in Asia, South America, Central America, the Caribbean, the United States and around the Mediterranean sea. In subtropical zones temperatures are only suitable for cowpea in the summer, whereas temperatures are suitable year-round in tropical zones.

The vast majority of the worlds cowpea production cover 95% takes in sub Saharan Africa, with about 12 million hectares under cultivation worldwide in 2013 (Singh *et al.*, 2002, FAO Stat, 2015). Asia is second producing region representing less than 3% of the global production in average over the 1993 – 2013 period, most of it being cropped in Myanmar (FAO, 2015). In Africa, cowpea can be cultivated up to 1.800 m altitude but is mainly grown in the low lands. The centre of maximum diversity of cultivated cowpeas and land races is found in west Africa in a region comprising the Sudan Savannah zone of Nigeria, central Burkina Faso, Ghana, Togo, northern Benin and the north-western part of Cameroon (Padulosi and Ng, 1997) substantial cowpea cultivation also occurs in the semi-arid Sahelian zone which is a transition zone between the Sahara desert in the north and the Sudan Savannah zone in the south significant cowpea production also occurs in the Northern Guinea savannah zone and the forest and Southern Guinea savannah zones of west Africa.

Cowpea [*Vigna unguiculata* (L) Walp (Fab)], a dietary protein, is a staple food crop of significant economic importance in Nigeria and worldwide (Magloire, 2005., Emeasor *et al.*, 2007) people like vegetable dishes of young cowpea leaves, immature pods, or immature seeds (Emeasor *et al.*, 2007) cowpea seedpods and leaves are consumed in fresh form as green vegetables in some African countries (Ghaly and Alkoaik, 2010), while the rest of the cowpea plant after the pods have been harvested serves as a nutritious fodder for livestock (Abebe *et al.*, 2005) and also a source of cash in income (Dugje *et al.*, 2009).

The nutrition value of cowpea makes it an extremely important protein source to vegetarian and people who cannot afford animal protein (Adeyemi *et al.*, 2012) it can be referred to “protein source for all” because it is affordable for both the rich and poor citizens.

The production and storage of cowpea have faced so many constraints, throughout west Africa such as diseases and the limited use of fertilizers and irrigation in puts (Raguraman and Singh, 2000; Brisible *et al.*, 2011) but the major constraint is the insect pest known as *Callosobruchus maculatus* (Musa *et al.*, 2009) which infests it before and after harvest consequently leading to loss of economic value (Baidoo *et al.*, 2005) infestation, on stored grains may reach 50% within 3-4 months of storage (Oparaeke and Dike, 2005) in the bid to control the storage insects of cowpea.

2.2.3 Description of the plant

The cowpea *Vigna unguiculata* (L.) Walp is an annual herbaceous legume cultivated for its edible seeds or for fodder, cultivated cowpeas are herbaceous annuals that are either erect, prostrate or climbing annuals with a tap root and virtually all are glabrous. They are mostly grown for grain but a small proportion are grown as green leafy vegetables and fodder in Africa or as fresh pods in eastern Asia. (Boukar *et al.*, 2015).

Cowpea can grow up to 80cm and to 2m for climbing cultivars it has a well – developed root system, germination is epigeal with the first pair of true leaves being simple and opposite and subsequent leaves being trifoliolate with oval leaflets (6 – 15 cm long and 4 – 11cm broad) and the flowers are born on racemose inflorescences at the ends of peduncles that arise from leaf axils and can be white, yellowish, pale blue or violet. For each inflorescence, flowers are sequentially produced in alternating pairs on thickened nodes at the tip with cushion – like extra floral nectaries between each pair of flowers.

2.2.4 Economic importance

Cowpea is a multipurpose crop, providing food for human and feed for livestock and it is a cash generating commodity for farmers, small and medium-size entrepreneurs. It has a wide variety of uses namely as a nutritious component in the human diet as well as nutritious livestock feed. Cowpea can be used at all stages of

growth as vegetable crop. The tender green leaves are an important food source in Africa and prepared as a pot herb, like spinach, immature snapped pods are used in the same way as snap-beans, often being mixed with other foods.

Green cowpea seeds are boiled as a fresh vegetable, or may be canned or frozen, dry mature seeds are also suitable for cooking and canning, cowpea can also be used as cover crop (Timko and Singh, 2008; Langyintuo *et al.*,2003; Singh,2002).

The very early maturity characteristics of some cowpea varieties provide the first harvest earlier than most other crops during production period, this is an important component in hunger fighting strategy, especially in sub-Saharan Africa where the peasant farmers can experience food shortage a few months before the maturity of the new crop. Its drought tolerance, relatively early maturity and nitrogen fixation characteristics fit very well to the tropical soils where moisture, erosion and low soil fertility is factor in crop production (Hall, 2004, Hall *et al.*,2002).

In many areas of the world, cowpea is the only available high quality legume hay for live stock feed, cowpea may be used green or dry fodder, it can also be used intercropping with other main crops like pearl millet (*Pennisetum glaucum*), maize or sorghum, cassava. The protein found in cowpea is similar to the one from other legumes, rich in the essential amino acids Lysine and Tryptophan (Timko and Singh, 2008). The protein nutritive value of cowpea and other legumes is lower than that of animal proteins because they are deficient of sulfur amino acids and contain

a non-nutritional factors (phytates and polyphenols), enzyme inhibitors (against trypsin, chymolrypsin and R-amylase and hemagglutinins (Jackson, 2009).

Minerals and vitamins are the other nutritional important constituents of the cowpea seeds, it has been reported that folic acid, a vitamin B necessary during pregnancy to prevent birth defect in the brain and spine content is found in higher quantity in cowpea compared to other plant (Timko and Singh, 2008; Hell *et al.*, 2003). The total crude protein content in foliage ranges from 14-21% and in crop residues, it is 6-8%. The high protein content in all cowpea parts consumable by human and animal (leaves, stem, pods and seeds), is the key factor in alleviating the malnutrition among, women and children an improvement of healthy status of the livestock in resource. Limited households where regular access to animal protein is limited due to low economic status, different dishes can be prepared from cowpea. The young tender leaves can be cooked and eaten as vegetable, the green pods can be cooked and eaten just like green beans, the seeds can be cooked when fresh (semi-ripe) and, when fully matured and dry, eaten as pulses.

2.3. Plants used in this study

2.3.1 Argel

2.3.1.1 Classification

Class: Magnoliopsida

Order: Gentiales

Family: Asclepiadaceae

Genus: *Solenostemma*

Species: *argel*

2.3.1.2. Distribution

Hargel (*Solenostemma argel*) is a desert plant which is widely spread, it grows naturally in the northern part of Sudan. It is also widely distributed throughout North Africa, Egypt, Chad , Palestine, Saudi Arabia and central and northern parts of Sudan. Organgi, (1982) reported that Sudan is the richest source of this plant. The principle production are in the Sudan is the northern region that extends from Barber to Abu Hamad, especially Rubatab area. In Sudan and in other countries the plant is known as Hargel (EL-Kamali, 2001).

2.3.1.3. Botanical description

S. argel is an erect, perennial shrub; reaches up to 105- 200cm height, with small branches carrying opposite decussate leaves. These leaves are pale – grey – green, lanceolate to oblong – ovate with acute or sub-acute apex and guneate base (EL – Kamali,1991). The inflorescence is cymose bracts broad, linear – lanceolate, acute. flowers white fruit a follicle, 5cm long, 17–18 mm broad, ovid lanceolate and acuminate at the apex. It is very hard and dark purple (Plate, 8).

2.3.1.4 General uses

Solenostemma argel is a medicinal African herb that has long been used by African for its antispasmodic purgative, loss of appetite and carminative effects. It has also been known for its antifilamentary, Immunostimulatory as well as antimicrobial and fungicidal properties (Hahn *et al.*, 1998).

Hargal leaves are used as beverage and indigenous medicine for the treatment of allergies and some diseases of liver and kidney. It is effective remedy for bronchitis and is used to treat neuralgia and sciatica. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for suppurating wound.

The leaves are used to treat gastro intestinal, cramps, stomach-ache, colic, cold and urinary tract infections and is effective as an anti-syphilitic if used for prolonged periods of 40–80 days. Also, the extract from leaves of Hargal showed fungi toxic activity and stem extract exhibited antimicrobial activity against selected gram – positive and gram negative bacteria (Hamed, 2001).

2.3.1.5 Chemical Constituents

The family Asclepiadace is a rich source of indoline, alkaloids, steroidal, perogen – ine and their glucosides (Deepak *et al.*, 1989., Si – Qi *et al.*, 1993., Srivastava *et al.*, 1993). Its other chemical constituents are cyanogenetic (glucosides, saponin, tannins, cumarin, flavonoids, phenolic acids and trilerpenoides) (Aeri, 2007).

Murwan *et al.*, (2010) found that its leaves contained phytic acid and Tannin. Inno-cent *et al.*, (2005) found that its aerial parts contained two monoterpene, glucosides, apregnane glucoside, benzyl alcohol O – β apiofuranosyle (1 – 6) β gluco pyrano – side, 2– phenyl – ethyl O – a armino pyranosyl (1– 6) β gluco pyranoside, astragalin and kaempferol 3 – O – neohesperidose. Shafee *et al.*, (2012) isolated two new natural kaepferol glucosides namely kaempferol 3 – O – β – D – glucopyranosyl (1 – 2) β – D – xylopyranoside (S₂) and Kacmpferol 3 – O – α – 1 – arabinopyranosyl (1 – 2) β – D – glaclop – yranoside (1 – 2) β – D – glaclop – yranoside (S₃). Osborn, 1968 and El–Fishawy, (1976) reported, sitoslerols, choline, flavonoid, glycosides, argelin, argelosid and atriterpinoid saponin.

Previous studies have reported the presence of monoterpenes (Kamel *et al.*, 2000) pregnane glycosided (Hassan *et al.*, 2001; Hamed, 2001). Palaza *et al.*, (2003) reported the occurrence of norel pregnane glycosides namely argelosides from *S. argel* also monoterpene, glycosides, pregnane glycosides, flavonoids and tannins as well as other seroids and alkaloids were isolated and identified from different parts of the herb (Kamel *et al.*, 2000; Hamed, 2001).

2.3.1.6. Insecticidal activity of hargal plant

In the Northern State of Sudan farmers used hargal as traditional method to control insect pests on okra specially the boll worms. The farmers spread the vegetative parts of hargal plant in main irrigation canals. The aqueous extract of hargal is

treated with irrigation water to okra where it will be absorbed through the root and later by the larvae while feeding (Sir Elkhatim, 2005). A crude aqueous extract of dried fruit pericarp, flower, root and stem of the plant were tested against the third instar larvae of the mosquito *Culex quinquefasciatus* (Say). Extract of the fruit pericarp was found the most effective with LC_{50} of 0.49 g/m at 24 hrs (El-Kamali, 2001). Methanolic extracts of aerial parts of hargel were incorporated into rearing media of *Culex pipiens* L.

Results showed significant acute effects on oviposition, egg hatchability and larval viability effect with a respective LC_{50} values (%) after 1,2,4 and 7 days were 0.037, 0.0031, 0.0009 and 0.007ppm, The correspond LC_{95} for the same period were 0.0394, 0.293, 0.065 and 0.030 ppm, respectively. However, the ovicidal effect of hargel was relatively less pronounced, as 0.1% concentration reduced egg hatchability by 33.7% the morbidity of the newly hatched larvae was 100% after treatment by methanolic extract of Hargel, (AL – Doghairi *et al.*, 2009).

The aqueous filtrate of Hargal (5% 7.5% and 10%) was found effective against the cotton soil termite (*Microtermes toracalis*), (Sidahmed *et al.*, 2009). The insecticidal properties of Hargal plant were evaluated under Laboratory conditions against broad bean beetle (*B.incarnatus*). The results showed that the powder and aqueous extract were effective against *B. incarnatus* at the different concentration tested (2 – 5%, 5% and 10%) (Bakheit,2004). Sidahamed *et al.*,(2009) evaluated the efficacy of hargal plant shoot aqueous extract against the white scale insect *Parlatoria*

blanchardii. The results showed that the aqueous extract of hargel plants when used as spray at all tested rates increased the mortality rate of adult females compared to the untreated control at 7 days after application. El-Kamali., (2001) applied water extracts from the stems, roots, fruits and flowers of Hargal against *Culex* larvae and obtained LC₅₀ of 1, 0.8, 0.5 and 0.25mg/L respectively. It seems from these contrasting figures that, application of hargel extract of the whole plant is more potent as a biocide than the extract of each part alone.

Probit analysis also showed an LD₅₀ of 0.14 mg/L of hargel extract against anopheles larvae concerning the effects of usher water extract on both mosquito species, the probit analysis showed LD₅₀ of 0–108 mg/L and 0–263 mg/L for culex and anopheles larvae, respectively. These results are in the same trend with those of Ali, (2004), who obtained LD₅₀ of 122.29MG/L and 166.71 mg/L for culex and anopheles larvae, respectively.

2.3.2. Rehan

2.3.2.1 Classification

Order: Lamiales

Family: Lamiaceae

Genus: *Ocimum*

Species: *basilicum*

2.3.2.2. Distribution

Basil belong to genus *Ocimum* (Lamiaceae) contain up to 150 species of herb and shrub in tropical regions of Asia, Africa, and Central and South America. (Simon *et al.*, 1990).

2.3.2.3. Botanical description

This is an erect, small shrub with many branches, usually not more than 1m in height. The leaves are simple, lanceolate to oblong, up to 9 cm long and 4.5 cm broad with cuneate or unequal – sided base and toothed margins they are sparsely hairy on the undersurface and pitted with glands.

The veins are slightly hairy the herbs have an aromatic smell when crushed the flowers are creamy white or yellowish and appear in paniculate racemes about 12 cm long the calyx is small and 2 lipped, the upper lip is broadly oval in shape with a tiny short point. The lower lip is oblong and toothed, and the petals combine to form aliped tube. The fruits occur as small 4 – lobed capsules (Griffiths, 1959). (Plate,6).

2.3.2.4. Chemical constituents

Basil (*Ocimum* sp), is a pleasant by smelling, They are usually considerable variation in the contents of the major components within the species. Lawrence, (1988) founded that the main constituents of the essential oils of basil are produced

by two different biochemical pathways, the phenyl–propanoids (methyl chavicol, eugenol, methleu genol and methyl cinnamate) by the shikimic acid path way, and the terpenes (linalool and geraniol) by the mevalonic acid pathway.

Rosmarinic acid (RA) ca–caffeoyl–3–4– dihydroxyphenyl – acid) is one of the most abundant caffeic acid esters present in *Ocimum spp.* RA and its derivatives have been reported to have antioxidant, anti-HIV and anti – inflammatory or cyclooxygenase inhibitory activity, comparable to ibuprofen, naproxen, and aspirin (Mazumder *et al.*, 1997.,kelm *et al.*, 2000,). Similar to RA , lithospermic acid B (LAB) is known to be a common phenolic constituent in most members of lamiaceae family (Tada, *et al.*, 1996) .,(Tanaka *et al.*, 1989) and exhibits endothelium – dependent vasodilator and hypotensive effects (Kamata *et al.*, 1994; Hase *et al.*, 1997).

2.3.2.5. Pharmacological and insecticidal studies

The leaves yield a very aromatic volatile oil that consists mainly of thymol (32 – 65%) and cugenol the plant also contains xanthonnes, terpenes, and lactones. (Watt and Breyer, 1962) Thymol isolated from the plant has been shown to be antiseptic, antilussive ,and antispasmodic (Paris and Moyse,1971), some of the observed biological activities of the plant could be due to the presence of xanthonnes which have been associated with monoamine oxidase inhibition activity, the tetraoxygenated xanthonnes with anticonvulsant properties, and the secoiridoids,

which possess stimulant and antispasmodic properties (Oliver–Bever, 1986). A fraction of the crude extract has been shown to contract guinea pig ileum and rat colon and to raise rat arterial blood pressure (Onajobi, 1986). The volatile oil exhibited antimicrobial, insect repellent, and anthelmintic activity (Sofowora, 1982). Oral and topical formulations of the plant have been evaluated in Nigeria (Sofowora, 1993).

Basil leaf extract is effective in the treatment of malaria and Central Nerves System (CNS) diseases. Evaluation of the CNS activity was done using the open – field and rota tests, sleeping time induced by sodium pentobarbital and anticonvulsant activity. Essential oils obtained in each season were effective in increasing the sleeping duration and a preparation obtained in spring was able to protect animals against tonic seizures ((Kaou. *et al.*, 2008 and Freire *et al.*, 2007).

In each season, eugenol and 1, 8–cineol were the most abundant compounds, and in spring the essential oil presented the greatest relative percentage of sesquiterpenes, suggesting that these compounds could explain the differences observed in the biological activity in essential oils obtained in different seasons of the year (Tringali *et al.*, 2000).

Dalziel,(1937). reported that *Ocimum vividae*. Wild, has been named the mosquito plant and it was used to repel mosquitoes in West Africa. Guenther,(1961) reported that *O. vividae* was used as a mosquito repellent.

Malaka, (1972). Stated that a preparation of the leaves of *O. basilicum* L., has been used among many methods to protect yams before planting against termites. Deshpande and Tiphis, (1977). Obtained by TLC eight fractions from the essential oil of *O. basilicum*, they tested the activity of each fraction against stored grains insect pests, namely *Tribolium castaneum*, *Sitophilus oryzae*, *Stagobium paniceum* and *Bruchidius chinensis*. From bioassay tests methylcinnamate and methylcharicol were found to be the components mainly responsible for the insecticidal activity.

Pandey *et al.*, (1983) investigated the efficacy of certain plant extracts against brinjal *Aphis gossypii* Glov at different concentrations (0.1, 0.5 and 1.0%) and depending on concentration, an extract of mature seed of *O. basilicum* L. was shown to give 45.40-56.22% mortality.

Mansour *et al.*, (1986) investigated the effect of essential oils isolated from 4 species of the family Labiate on adult females of *Tetranychus cinnabrinus* in the laboratory. They showed that concentrations of the acetone solutions of the oils from 0.1 to 2% cause mortality and induce repellency within 48 hours of introducing adult females and consequently egg-laying was found to be reduced, and seven day old residues still had some activity, the most effective oils were *Lavandula anqustifolia*,L (EC₅₀, 0.094), *L. anqustifolia* (EC₅₀ 0.7%), *O. basilicum* (EC₅₀ 1.4%), *Salvia fructicosa* (EC₅₀ 1.4%) and rosemanry (EC₅₀ 2.2%).

Shaaya *et al.*, (1997). found its essential oils show fumigant toxicity to saw toothed grain beetle, *Oryzaephilus surinameniss*. Also Jacobson, (1989). reported that its whole plant extract contained juvocinine 1 and juvocinine-11 and analogue mimic toxicity against milk weed bug.

2.3.3. Datura

2.3.3.1. Classification

Order: Solanales

Family: Solanaceae

Genus: *Datura*

Species: *stramonium*

C.N: Jimson weed

2.3.3.2. Distribution

Dutura stramonium L. – named by Carl Linnaeus as published in species plantarum in 1733. The genus was derived from ancient Hindu word for plant, datura. The species name is from New Latin, stramonium, meaning thorn apple. Stramonium is originally from Greek; Strychnos (night shade) and manikos (mad) (Maibam *et al.*, 2011).

Datura stramonium is native to the Americas and has been introduced in many tropical, subtropical and even temperate regions. *D. stramonium* is native to

deserts of the North American Southwest, Central and South America, Europe, Asia, and Africa. It is mainly distributed in the Himalaya region from Kashmir to Sikkim up to 2700m, in the hilly district of central and south India (Khare, 2007).

2.3.3.3. Botanical description

Datura stramonium is a large and coarse shrub of about 3 to 4 feet in height. On rich soil, it may even reach the height of 6 feet. The root is large, whitish in color, with a tap root system giving off many fibers. Stem is green or purple, hairless, cylindrical, erect and leafy, smooth, branching repeatedly in a forked manner. leaves and branches the alternate leaves are ovate or ovate-cordate in outline, but pinnately lobed. These lobes are somewhat shallow and point at their tips, there are usually 2 to 3 of these lobes on each side of the leaf blade.

Leaves are cauline and ramal, existipulate, up to 8cm long and 6cm across, petiolate, simple, dissected, acute, glabrous, unicosted, and arranged in reticular venation. The upper surface is dark and grayish-green, generally smooth, whereas the underside is paler, and when dry, minutely wrinkled. Leaves, when bruised exude rank, heavy, and somewhat nauseating narcotic odor.

The flowers are bracteate, ebracteolate, pedicellate, actinomorphic, bisexual, complete, regular, pentamerous, except fourth whorl and are hypogynous—they are sweet-scented, and can produce stupor if breathed for a prolonged period of time, each flower is replaced by a hard fruit that dry and sping and spheroid-ovoid in

shape, underneath, each fruit is a truncated remnant of the calyx that curves sharply down. These fruits are initially green, but become brown with maturity, they divide into four segments to release the seeds. The seeds are dull, irregular, and dark-colored, their surface may be pitted or slightly reticulated (Preissel and Preissel, 2002; Das *et al.*, 2012). (Plate, 5)

2.3.3.4 General uses

Historic use of jimson weed and various other species of *Datura* has occurred for many purposes, throughout time. In Europe the plant was used for witch craft, in salves or ointments. Throughout most European countries the seeds were used to brew beer (Shaman Australia Ethno botanicals). The seed was dried and smoked; the users were left on a high which consisted of hallucination and total relaxation. Jimson weed was thought to cure those with deafness, so the insomniac, and release the heat of those with a fever.

Datura stramonium is thought to be one of two plants identified in 4,000-year-old rock paintings throughout the pecosriver region of Texas and northern Mexico, used by the Huichol Indians along with peyote to commune with the spirit world (Boyd and Dering, 2000). Hearne, (1975) reported that the decoction of leaves to the body for fever or administered as a suppository. The fruit and leaves were considered good for pain the chest if too much was taken; it was believed to cause insanity.

In northwestern New Spain, the Opata rubbed a leaf of Taquaro on the painful area for spleen disease. They believed it also matured tumors and abscesses (Nentwig, 1977). An ointment of the ground seeds and suet is rubbed on boils, pimples, and swellings, the powdered leaves are applied to hemorrhoids, and hot baths containing the plant give relief colds and diarrhea (Curtain, 1947).

Datura stramonium is now used to treat asthma, and gastrointestinal problems, also aches, abscesses, arthritis, boils, headaches, hemorrhoids, rattlesnake bites, sprains, swellings, and tumors (Sandoval, 1998). It acts as a sedative in large doses and as a stimulant and detteriant in high ones. *Datura* is an anodyne, antibiotic, antispasmodic and narcotic. Relieving the pains of rheumatism and sciatica when applied as an ointment and easing spasms of parkinsons disease are unproven accounts of the effects of jimson weed.

In the Northern part of Nigeria, the fresh leave, were used as insecticidal and repellent to mosquito. More recently, preparations from plants have been used as ingredients in the treatment of asthma. With this exception, however, the plant is generally considered too toxic for medical applications (Sofawora, 1993 and Afolabi *et al.*, 2007).

Most of the plant is used for medicinal reasons. Eating the seeds rapidly gets the plant to the nervous system, but also increases the risk of lethal overdose. The leaves can be dried and smoked to relax the bronchiol muscles of the throat, and

leaves are used also to line beds of those with insomnia. Annette Sandoval in home grow healing recommends using the fresh leaves, flowers, or seeds.

2.3.3.5 Active compounds

Datura stramonium contains hyoscyne, as well as atropine, hyoscyamine, apo-hyoscyne and meteloidine thus it is poisonous and hallucinogenic as well as acting as a pain killer (Ivancheva *et al.*, 2006).

The plant *D. stramonium* belonging to family Solanaceae contains psychoactive substance like alkaloids, atropine and scopolamine which are classified as detritant or anticholinergic (Giannini, 2002).

2.3.3.6 Phytochemicals

The medicinal value of a plant depends on its bioactive phytochemical constituents that produce definite physiological action in the body. The most important bioactive phytochemical constituents include presence of alkaloids, tannins, saponins, glycosides and flavonoids (Aderotimi *et al.*, 2006). The major tropane alkaloids such as hyoscyamine and scopolamine and several minor tropane alkaloids have been identified in *Datura* species.

Typical examples of minor alkaloids in *Datura stramonium* are tigloidin, aposcopamine, apoatropin, hyoscyamine, N-oxide and scopolamine N-oxide 17–20, ba-ditigloyloxytropane and 7-hydroxylhyoscyamine are reported for the first time

in this species (Das *et al.*, 2012). Distribution of hyoscyamine and scopolamine in *Datura stramonium* was studied. The production of hycyamine and scopolamine in *Dutua stramonium* has been investigated in the different plant parts, at different stages of their life cycle the maximum content were found in the stems and leaves of young plants, hyocyanine being always the predominate component.

These compounds were included in many pharmacopieas because of their anticholinergic activities (Goldfrank, and Flommenbaum, 2006). *Datura stramonium* contains variety of alkaloids including atropine, hyoscamine and scopolamine (Ivancheva *et al.*, 2006). Sixty-Four tropane alkaloids have been detected from *D.stramonium* two new tropane alkaloids, 3-phenylaceloxy-6, 7-epoxynortropane and 7-hydroxyapoatropine were tentatively identified the alkaloids scopoline, 3-(hydroxyaceloxy) tropane, 3-hydroxyl-6-) 2-methyl butyryloxy) tropance, 3, 7-dihydroxy-6-tiloyloxytropane, 3-tigloyloxy-b-propionyl oxytropane, 3phenylacetoxy - 6 ,7 - epoxytropane, 3 -phenylacetoxy-6-hydroxy tropane, aponor scopolamine, 3a, ba-ditigloyloxytropane and 7-hydroxyhyoscyamine are reported for the first time for this species.

Other alkaloids found in *D. stramonium* reported by Strahil *et al*, (2006) include hygrine,3a,ba-ditiglyoyloxy-7-hydroxytropane,6-hydroxhyoscyamine, pseudotropine, 3a-tigloyloxytropane, hydroxy-6-tigloyloxytropane, phenylacetoxyyropance, 3-acetoxy-6-isobutyryloxylopropan 3-(2-phenyl propionyloxy) tropane, littorine, b-hydroxyapoatropine, 3-tropoyloxy-6-

acetoxytropine, tropine, 3-acetoxytropine, 3-hydroxy-6-acetoxytropine, 3-hydroxy-6-methyl butyryloxytropine, 3-tigloxy-6-isobutyryloxytropine, aponorscopolamine, 7-hydroxyhyoscyamine, meteloidine 3a, batitigloyloxytropans.

The secondary metabolites identified in the plant matter showed antimicrobial activity (Banso and Adeyemo, 2006). However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalization and deaths.

2.3.3.7. Insecticidal properties

Acaricidal, repellent and oviposition deterrent properties

The ethanol extracts obtained from both leaf and seed in *D. stramonium* (Solanaceae) were investigated for acaricidal, repellent and oviposition deterrent properties against two adult spotted spider mites (*T. urticae* Koch (Acari: Tetranychidae) under laboratory conditions leaf and seed extracts, which were applied in 167.25 and 145.70 g/L concentrations, respectively (Using a petri leaf disc-spray tower method), caused 98% and 25% mortality among spider mite adults after 48 hours, these results suggest that *D. stramonium* extracts could be used to manage the two-spotted spider mite (Kurnal and Yalcin, 2009).

Larvicidal repellent activities

Ethanollic extracts of leaves of *D. stramonium* were evaluated for larvicided and mosquito repellent activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

The LD₅₀ values for larvicidal activity were found to be 86.25, 16.07 and 6.25 mg/L against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* respectively. The ethanolic leaves extracts of *D. stramonium* provide complete protection time (mosquito repellency) of 27, 71.7 and 117.7 min against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at higher concentration (Swathi *et al.*, 2012).

2.3.4. Neem

2.3.4.1. Classification

Order: Sapindales

Family: Meliaceae

Genus: *Azadirachta*

Species: *A. indica* (A. juss, 1830)

2.3.4.2. Distribution

The neem tree (*Azadirachta indica*) is native to tropical South East Asia. It is fast growing, can survive drought and poor soil and keeps its leaves all year round. It is a tall tree up to 30 metres high, with leafy spreading branches.

Many white flowers with smell of honey appear for the first time when the tree is 2 to 3 years old, and the tree bears fruit after 3 to 5 years. The ripe fruits are about 2 cm long and oval shaped. Inside the fruit there is a light colored seed about 1.5cm long.

Two species of *Azadirachta* have been reported, *Azadirachta indica* juss – native to Indian subcontinent and *Azadirachta excels kack* confined to Philippines and Indonesia (Jattan *et al.*, 1995; Hegde,1995). The Former grows as a wild tree in India, Bangladesh, Burma, Pakistan, Sri lanka, Malaysia, Thailand and Indonesia – presently neem tress can be seen growing successfully in about 72 countrics world wide in Asia ,Africa, Australia ,North, Central and South America (Ahmed *et al.*, 1989; Sidhu,1995, Sateesh, 1998, Fathima, 2004). They are an estimated 25million trees growing all over India (Rembold,1996) of which 5–5% are found in Karnataka and it is in the third place next to Ultar Pradesh (55.7%) and Tamilnadu (17.8%) occupying the first two places respectively.

2.3.4.3. Botanical description

Azadirachta indica is evergreen a shady tree with an; it grows up to 25m high in some places but occurs in West Africa mostly as a medium – size tree. It has rough, dark brown bark with wide, shallow longitudinal fissures separated by flat ridges. The bole is short and stout. It is easily confused with *Melia azedarach*, an Asian tree which has also been introduced to other tropical parts of the world, references to *A .indica* in very old literature should be viewed with caution. The leaves are

compound, imparipinnate, each comprising 5 – 15 leaflets. They are very diagnostic and measure about 6cm long and 2cm broad the tree bears many flowered panicles, mostly in the leaf axils, sepals are ovate. sub-or bicullar, about 1cm long, with sweet – scented white oblanceolate petals. It produces yellow drupes, which are ellipsoid, glabrous and 12 – 20 cm long (Irvine, 1961, Anon,1986).(Plate,7).

2.3.4.4. Uses of neem

Neem trees are grown commercially in plantations to produce azadirachtin, a chemical extracted from the seeds and leaves. Azadirachtin has been promoted as a new insecticide that is considered more environmentally friendly than synthetic insecticides. Plantations have been established in tropical to subtropical regions of the world, including semi-arid and wet tropic regions, from sea level to about 700 m elevation (IVRC, 1992).

After the oil has been pressed from the seeds, the residue (neem cake) can be used in cattle and poultry feed. Neem is also used in silviculture in India and for reforestation in Asia, Central America and sub-Saharan region. It has been planted as an ornamental and has been sold by commercial nurseries in Queensland (Lawson, 1997). In some towns and cities, it has been promoted as a street tree (Hearne, 1975).

Neem has a range of potential uses, most commercial interest lies in the pest control properties of neem extracts, while azadirachtin and other neem extracts have been

shown to have insecticidal properties (NRC,1992). (WHO/UnEP,1989) identified that neem as an environmentally powerful natural pesticide, is considered to be one of the most promising trees of the 21st century for its great potential in pest management, environment protection and medicine.

2.3.4.5. Chemical constituents

Major chemical constituents of neem are terpenes and limonoids. The major active components in the limonoide are azadirachtin, 3–deacetyl–3–cinnamoylazadirachtin, 1– tigloyl –3– acetyl–11–methoxyzadirachtin 22, 23–dihydro –23 β – methoxyzadirachtin, nimbanal, 3 – tigloylazadirachtol, 3–acetyl–salannov nimbidioV margocin, margocinin, margocilin and others (Ogbuewu,2008).

Terpenoids are isoazadirolide, bnimbocinolide, nimbanone, nibonolone, methylgrevillate and margosinone. Neem increases the production of glutathione – s –transferase thus improving the ability of the liver to detoxify itself of chemical contamination.

2.3.4.6. Pest management prospects

The dependency on synthetic chemicals during early and middle twentieth century has prompted the large scale synthesis of newer chemicals (pesticides) many a time, the side effects of the synthetic pesticides are more serious than problems themselves. They are also known to cause health problems in farmers of both developed and developing countries (Sateesh, 1998).

Neem based pesticides are found to be much safer in this regard. Today neem has gained importance internationally as all communities have inclined towards green technology. Neem products have no ill effects on humans and animals and no residual effects on agricultural products this make neem the best, reliable substitute to hazardous pesticides. The demand for chemical pesticides will be reduced by large scale use of neem based pesticides that will in turn reduce the load of synthetic chemicals in the environment.

Today, modern societies, finding themselves confounded in the web of their creation, are willing to revert to nature for remedies and neem tree provides a promising means in this matter. Neem wood is durable and termite resistant and thus used mulch materials. The pesticidal activity of neem span a wide spectrum, having repellent, phagodeterrent (antifeedant), insect growth regulatory. Anti – ovipositional, fecundity and fitness reducing properties on insects. Schmulterer and Singh, (1995) listed 413 insect pest species sensitive to neem products.

These principles act as ecdysteriod analogues, which affect corpus cardiacum and block reproductive and growth processes in most insects causing sterility in females and degenerative changes in male testis due to disturbance in insect metabolism Formulation like: Margosano ®, Neemix ^(Im), Azatin ® Nim-20 and NIM-76, gave negative results with respect to toxicity effects on mammals (Schmutterer, 1990; Govidachari *et al* ., 2000) Hence Neemix ^(TM), was registered for use on vegetables in U.A for its inherent safety.

In most tests, neem products performed equally sometimes better than synthetics like primiphos methyl (Actellic 25 EC) permethrin and linden CyBHC (Ogunwolu and Oddunlam, 1996., Lale and Mustapha, 2000). Furthermore, the oriental yellow scale insects *Aonidiella orientalis* threatens survival of this jewel in the savanna (Mahmood,1995). Through necrosis, chlorosis and scorching of the whole foliage as they inject toxic metabolites into the foliage.

Neem based pesticides are easy to prepare, cheap and highly effective and thus constitute an important source of pesticides for economically poor third world country farmers (Brahmachari, 2004) Neem bio–pesticides are systemic in nature and provide long term protection to plants against pests. Pollinator insects, bees and other useful organisms are not affected by neem based pesticides (Tanzubil, 1996).

2.3.4.7. Neem and ethnomedicinal uses

In West Africa, India, Burma, etc. both aqueous and alcohol extracts of bark and leaves of neem are effective antimalaria agents, particularly on chloroquine resistant strains (Badam *et al.*,1987, Udeinya *et al.*, 2008) one active component, gedunin gave significant control as effective as guanine on malaria (Khalid *et al.*, 1989; Subapriya and Nagini, 2005). The mechanism is possibly redox status of red blood cells on parasite. The plasmodial parasite generates oxidant, while neem extracts reduced the oxidized cells to destroy the malaria parasite.

Furthermore, neem barks and leaves possess strong antiseptic property warranting use as active ingredient in tooth paste in India and Germany. While aqueous extract of leaves exhibit laxative potentials by increased bowel movement (UKO *et al.*, 1995) over does could however produce severe abdominal cramps or rectal prolapse. (Kloos and McCullough, 1987). reported potency of neem seed oil on Snail fever (*Schistosomiasis*) with the active principle being mulluscicidal and ovicidal and cercariacidal.

2.3.4.8. Insecticidal effects

Neem products do not necessarily kill insect pests, they are not always biocides, but incapacitate them in several other ways, for example by interfering with development and growth of insects, act as anti-feedants on the host plant, or prevent them from depositing their eggs. often, the precise effect is unknown (Vijayalakshmi *et al.*,1995). Neem extracts effectively reduced pests damage leading to increased yields . Jackai and Oyediran, 1991. Neem products have shown efficacy againts pod borer (*Naruea vitata*), pod sucking bugs complex (*Clavigralla lomentosicollis* Saz) (Jackai and Oyediran, 1991) and other insect pests (Zongo *et al.*, 1993; Saxena,1981).

Schmutterer, (1990), Jacobson (1986) and Sexena, (1981), observed that neem products have shown activity on a wide range of insect pests. Ulrichs *et al.*, (2001)

discovered that commercial neem, neem AzaLs significantly reduced the number of *Aphis craccivora* in cowpea.

2.4.5. Tobacco

2.4.5.1. Classification

Order: Campanulales

Family: Solanaceae

Genus: *Nicotiana*

Species: *rustica*

2.4.5.2. Botanical description

Tobacco (*Nicotiana*) is a genus of 21 to 67 species of perennial herbs and shrubs, including many subspecies, strains, and cultivars, *Nicotiana rustica* is an annual herbaceous plant that grows up to 80 centimeters tall. The leaves are smaller and more round than those of *Nicotiana tabacum*. The flowers are yellow, shorter and smaller than those of its relatives. The plant flowers in June and July. The fruits are round capsules containing tiny red-brown seeds. Various species are used as ornamentals, insecticides and for smoking (Ratsch, 1998).

Alkaloids, with more than 10,000 described, are one of the most diverse and prominent groups of natural products with pharmacological and toxicological importance (Harbone, 1993). Plant extracts containing insecticidal alkaloids as

bioactive constituents have played an important role in the abatement of insects of agricultural and public health importance for countries.

While the direct use of these substances has recently diminished, they continue to serve as leads for synthetic analogs and are also indispensable biochemical tools in mode of action studies. In recent years, the function of these alkaloids in the host plant has begun to unfold. It is now generally believed that the ecological role of these compounds, often acting in concert with other nonalkaloid substances, is to provide a chemical defense through multiple biological mechanisms (Winkler, 1993; Brown and Trigo, 1995). (Plate, 4)

2.4.5.3. Plant uses

Nicotiana rustica is often used for entheogenic purposes by South American shamans (5). It contains up to nine times more nicotine than species of *Nicotiana* such as *Nicotiana tabacum* (common tobacco). Other reasons for its Shamanic use are the comparatively high levels of beta-carbolines, including the harmala alkaloids harman and norharman.

Most commonly, in South American ethnobotanical preparations, it is allowed to soak or be infused in water, and the water is then insufflated into the stomach in a preparation known as Singado or Singa; it is also smoked in (igare, used as an enema, made into a lickable product known as ambil, and made into a snuff with the bark of

a species of theobroma, creating nu – nu. In the southeast part of Turkey , people use this herb and ashes of some tree bodies to make a moist snuff called maras out.

They use this by putting the mixture under their lips like swedish snus or Afghan naswar it is also a common admixture of Ayahuasca in some parts of the Amazon. *N. rustica* is no longer cultivated in its native North America, as *N.tabacum* has replaced it (6). In Russia, *N. rustica* is called makhorka. Historically makhorka was smoked mainly by the lower classes.

Nicotine from *Nicotiana* sp in some countries is used as a pesticide, especially in gardens, when nicotine is still widely used in the developing world as a cheap and effective insecticide. For example, in Zambia nicotine is used as an acaricide, fungicide, insecticide and repellent. The farmers soak tobacco leaves in water, add soap , and the solution for spraying on plants. Nicotine solutions can also be prepared from cigarette ends as contain most nicotine of the cigarettes (Malaya and Banda,1995). Although used for quite awhile as pesticide, no insects have yet developed resistance against nicotine (Kutchan,1995).

2.4.5.4. Insecticidal alkaloids

Alkaloids, with more than 10.000 described, are one of the most diverse and prominent groups of natural products with pharmacological and toxicological importance (Harbone, 1993).

Plant extracts containing insecticidal alkaloids as bioactive constituents have played an important role in the abatement of insects of agricultural and public health. While the direct use of these substances has recently diminished, they continue to serve as leads for synthetic analogs and also indispensable biochemical tools in mode of action studies.

In recent years, the function of these alkaloids in the host plant has begun to unfold; it is now generally believed that the ecological role of these compounds often acting in concert with other non alkaloidal substance, is to provide a chemical defense against predators and pathogens in a sustained manner through multiple biological mechanisms (Wink, 1993a; Brown and Trigo, 1995). Pelletier, (1983) define the alkaloid as an alkaloid is acyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms.

Plant lectins are the insecticidal properties of many plants lectins (Sharma *et al.*, 2004; Vane Damme, 2008), lectins have been shown to reduce the performance of several insect species belonging to the orders Lepidoptera, Coleoptera, Diptera and Hemiptera (Vandenborre *et al.*, 2009).

Aqueous tobacco (*Nicotiana tabacum*, *N. glauca* or *N. rustica*) extracts containing the alkaloid nicotine have long been used to control crop insect pests (Schmeltz,1971). Nicotine exerts its insecticidal effect by mimicking acetylcholine and interacting with nicotinic acetylcholine receptors (nAChRs), a major excitatory neurotransmitter in the insect CNS. Unfortunately, nicotine is highly toxic to mammals and extreme care must be used since it is readily absorbed through the skin.

Metacalf *et al.*,(1961) reported that pyridine alkaloids found in *Nicotiana* sp, such as onabazine, anatabazine and nornicotine, also have insecticidal properties.

2.4.6. Current botanicals in use

At present there are four major types of botanical products used for insect control (pyrethrum, rotenone, neem and essential oils), along with others limited use botanical insecticides products include nicotine, rotenone, ryania and sabadilla, in addition plant extracts and oils used in various countries.

2.4.6.1. Pyrethrum

Pyrethrum refers to the oleoresin extracted from the dried flowers of the pyrethrum daisy *Tanacetum cinerariaefolium* (Asteraceae). The flowers are ground to a powder and then extracted with hexane or a similar non polar solvent; removal of the solvent yields an orange –colored liquid that contains the active principles (Casida, 1995; Glynn, 2001). These are three esters of chrysanthemic acid and three esters of pyrethric acid. Among the six esters, those incorporating the alcohol pyrethrolone, namely pyrethrins are the most abundant and account for most of the insecticides.

The insecticidal action of the pyrethrins is characterized by a rapid knock down effect, particularly in flying insects, and hyperactivity and convulsions in most insects. These symptoms are a result of the neurotoxic action the pyrethrins, which block voltage-gated sodium channels in nerve axons. The mechanism of action of pyrethrins is qualitatively similar to the DDT and many synthetic organochlorine

insecticides. Pyrethrins are moderately toxic to mammals. Pyrethrins are especially labile in the presence of UV component of sunlight, a fact that has greatly limited their use outdoors.

A recent study indicated that the half – lives of pyrethrins on field –grown tomato and bell pepper fruits were 2hours or less (Antonious, 2004). This problem created the impetus for the development of synthetic derivatives that are more stable in sunlight. The modern pyrethroids, developed in the 1970s and 1980s, have been highly successful and represent one of the rare examples of synthetic pesticide chemistry based on a natural product model.

For many years world production of pyrethrum was led by Kenya, with lesser quantities produced in Tanzania and Ecuador in the past five years, botanical resources Australia, with planting in Tasmania is qualitatively similar or that produced in East Africa and elsewhere.

2.4.6.2. Neem

Two types of botanical insecticides can be obtained from seeds of the Indian neem tree, *Azadirachta indica* (Meliaceae) (California Department of Pesticides Regulation, 2005). Neem oil obtained by cold – pressing seeds can be effective against soft–bodied insects and mites but is also useful in the management of phytopathogens.

Apart from the physical effects of neem oil on pests and fungi; disulfides in the oil likely contribute to the bioactivity of this material more highly valued than neem oil are medium, polarity extracts of the seed residue after removal of the oil, as these extracts contain the complex triterpene azadirachtin. Neem seeds actually contain more than a dozen azadirachtin analogs, but the major form is azadirachtin and the remaining minor analogs, likely contribute little to overall efficacy of the extract seed extracts include considerable quantities of other triterpenoids, notably salannin, nimbin and derivatives, role of these other natural substances has been controversial, but most evidence points to azadirachtin as the most important active principle (Isman *et al.*, 1996).

Azadirachtin has two profound effects on insect at the physiological level; azadirachtin blocks the synthesis and release of molting hormones from the prothoracic gland, leading to incomplete ecdysis in immature insects. In adult female insects, similar mechanism of action leads to sterility. In addition azadirachtin is a potent antifeedant to many insects. The discovery of neem by western science is attributed to Heinrich Schmutterer, who observed that swarming desert locusts in Sudan defoliated almost all local flora except for some introduced neem tree (N. R. C, 1992).

2.4.6.3. Plant essential oils

Insecticides effects of plant essential oils and individual terpenes against disease vectors and insect pests consists a well studied case (Isman, 2000). Specifically contact and fumigant activity of plant essential oils against stored product pests have been reported, but the relationship between their chemical composition and their activity is always needed to be determined.

Studies were performed aiming to evaluate the insecticidal activity (LC₅₀) of essential oils obtained from aromatic plants lavender (*Lavandula hybrida* Rev, Lamiaceae), rosemary (*Rosmarinus officinalis* L.,Lamiaceae) and eucalyptus (*Eucalyptus globules* Labill, Myrataceae) and their main constituents against *Acanthoscelides obtectus*, say (Papachristos *et al.*, 2004). Strong insecticidal activity was found. and oxygenated monoterpenes predominated over hydrocarbons. All essential oils tested exhibited strong activity against *A. oblectus* adults and variability in their activity and chemical composition due to different plant part, season insect sex was substantiated. Among 16 of the principal components of the essential oils tested the most active were teprinen – 4 – ol, camphor, 1,8 – cineol and verbenone.

2.4.6.4. Rotenone

As an insecticide, rotenone has been in use for more than 150 years, but its use as a fish poison date back even further. (Shepard, 1951). Rotenone is one of several isoflavonoids produced in the roots or rhizomes of the tropical legumes derris, lanchocarpus, and tephrosia. Extraction of the roots with organic solvents yields resins containing as much as 40% total rotenoids (Fang, 1998., Cabizza *et al.*, 2004). Rotenone is a mitochondrial poison which blocks the electron transport chain and prevents energy production (Hollingworth *et al.*, 1994). As an insecticide, it is considered a stomach poison because it must be ingested to be effective.

2.4.6.5. Nicotine

Nicotine, an alkaloid obtained from the foliage of tobacco plants (*Nicotiana tabacum*) Solasaneae and related species, has long history as an insecticide, nicotine is undoubtedly the one of the first molecules used as insecticide. It is an acetylcholine mimic binding to postsynaptic receptors and interfering with the transmission of signals in nerves, leading to a continuous firing of the neuroreceptor.

This over stimulation leads to depression the central nervous system. It acts predominately through the vapour phase and to a less degree through stomach and contact. Nicotine's high toxicity to humans limited its use as a pesticide (Regnault – Roger and Philogene, 2008). Biotransformation of nicotine involves activation reaction and detoxification mechanisms have led to neonicotinoids, representing the

current major class of insecticides of outstanding potency, systemic action and low toxicity to mammals (Tomizawa and Casida, 2008). Other alkaloids falling in the same category are veratine and ceratrine the major components of Sabadilla (*Schoenocaulon officinale* Grey) seeds, which are mainly used to control thrips, but recently resistance issues have broken up (Humeres and Morse, 2006).

Sabadilla alkaloids possess like pyrethrins, a neurotoxic activity by slowing the shutting of Na⁺ channels and disturbing membrane depolarization. They cause paralysis before death. They are contact and non systemic insecticides, readily degraded in air and sunlight and are not considered hazardous to non target organisms (Copping, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1. Field study and location

This study was conducted in North Darfur State, Latitudes 13 33 and Longitudes 164 N and 25 (Figs.3.1 and 3.2) in season 2017/ 2018. North Darfur State is located in western Sudan in the dry semiarid region. The mean annual temperature is 27 C⁰ with extreme temperature ranging between 9.9C⁰ in January and February and 38.8C⁰ in May and June. Mean relative humidity varies between 31% in December, to 58% during August. The rainy season usually starts in early May or early June and ends by the end of October. Main soil type is sand to sandy loam and clay soil.

During the course of study a number of regular surveys and general observations were conducted to investigate the abundance and infestation with *Callosobruchus maculatus* in the area of storage of pulse seeds in Alfashir locality. Three areas in Northern Darfur State (Alfashir twon) namely Almwashi market, Crops market and Nafasha market were selected for field study (figs.3.1 and 3.2)



Fig. (3.1) Northern Darfur State (Source google map)

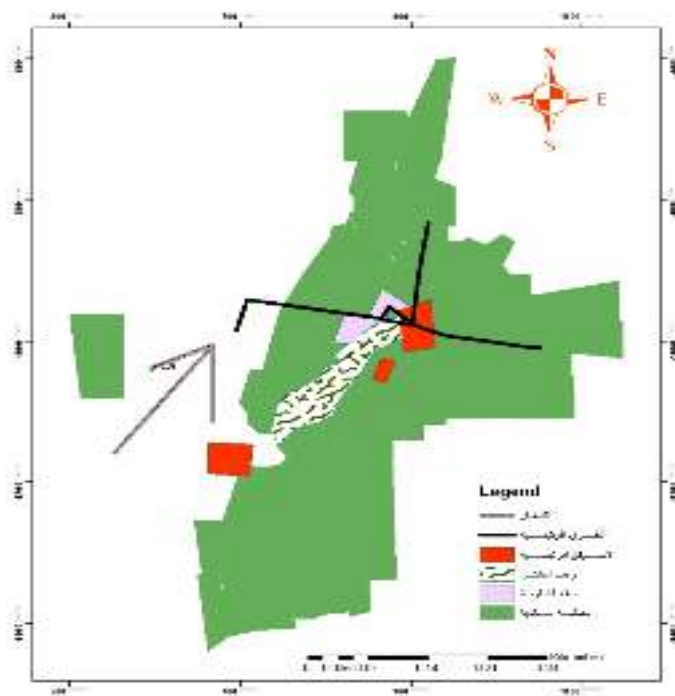


Fig.(3.2) Study areas in Alfashir town (Source google map)

3.2. Laboratory studies

Laboratory studies were conducted during the years 2017 – 2018 in Alfashir, (Northern Darfur State), Alfashir University, Faculty of Education, Department of Biological Science, to investigate the efficacy of leaves powder plus aqueous and ethanolic extracts of *Solenostemma argel* (Del), *Ocimum basilicum*, *Datura stamonium*, *Azadirachta indica* and *Nicotiana rustica*. against adult of cowpea beetle *Callosobruchus maculatus*. The average room temperature during the experimental period was between 35 C⁰ – 38 C^o.

3.2.1. Insect culture and rearing

The adult cowpea beetle (*C. maculatus*) was collected from local market (Alfashir crops market). The collected insects (infested cowpea seeds) were placed in glass and plastic container (plates.1, 2 ,and 3), each was covered with muslin cloth fixed in position with rubber bands (Rup and Chopra, 1984). Four weeks later the culture was sieved, sieving was done 24hrs prior to the test. The old adults were removed and newly emerged adult (0 – 24 hrs old) were collected and used for the bioassay.



Plate (1): Insect rearing cages



Plate(2):Infested cowpea seeds



Plate (3): Adults of *Callosobruchus maculatus*

3.2.2. Collection of plant materials

The five tested plants were collected from different areas on October 2017. The fresh and healthy leaves of tobacco, *Nicotiana rustica*, datura, *Datura stramonium*, basil, *Ocimum basilicum*, and neem tree *Azadirachta indica*, were collected from areas surrounded Al fashir town, while *Solenostemma argle* brought from Alfashir market (Plates 4, 5, 6, 7, and 8).



Plate (4): Plant of *Nicotiana rustica*



Plate (5): Plant of *Datura stramonium*



Plate (6): Plant of *Ocimum basilicum*



Plate (7): Plant of *Azadirachta indica*



Plate (8): Plant of *Solenostemma argel*

3.2.3. Preparation of plant materials

3.2.3.1. Preparation of Leaves powder

Collected Leaves samples were cleaned and washed using tap water, then spread to dry under room temperature. The dried leaves were first crushed by hand and then powdered by an electric blender (Moulinex type 719). The powder was stored in

tightly covered glass jars wrapped with aluminum foil until needed for preparation of extracts.

3.2.3.2. Preparation of leaves aqueous extracts

The aqueous solution of plants leaves powder was prepared by adding 200ml distilled water to 20 grams of the powder in a conical flask (500 ml) following the method of Ascher, (1981). The mixture was left to stand for 24 hrs at room temperature and shaken thoroughly by hand for 5 minutes every 8 hours for 24 hours. The mixture was then strained through a light cloth and then filtered through a Whatman filter paper No1 (24 cm). The stock solution (10% w/v) was kept in the refrigerator at 4 C° for further work.

3.2.3.3. Preparation of ethanolic leaves extract

Extraction processes were conducted at the chemistry laboratory, Faculty of Education AlFashir University. Ten grams of each of the previously prepared leaves powder of (neem, oicmum, tabocco, hargel and datura), were put separately in a thimble chamber of a soxhlet extractor apparatus (plate), and then extracted with 300ml ethanol 99.9% for each sample. The extraction continued for sixth hours and the ethanol solvent was removed off the crude extract. The obtained crude extract of each sample was stored for the experiment.

3.3. Experiments methods

3.3.1. Treatments

The testing insecticidal activity of hargel, neem, opicium, datura, and tobacco leaves on the adult of cowpea beetle *Callosobruchus maculatus*.

3.3.1.1 The powder of plant leaves

The method of Saramma and Verma, (1971) was followed Hundred intact seeds of cowpea were taken in each Petri – dish (15cm in diameter). The seeds were dusted with the leaves powder at 5%, 10% and 15% (w/w). The control Petri – dishes contained untreated seeds. Ten adults of *Callosobruchus maculatus* were introduced into each Petri–dish. The treatment replicated three times, the number of dead adults, in each petri – dish was recorded 24 hrs, 48 hrs and 72 hrs. The temperature and relative humidity were recorded three times every day, this procedure was conducted for each of the five tested plant powder.

3.3.1.2. Water extracts of plant leaves

Water extract of sample plants leaves at concentrations of 5%, 10% and 15% was used to spray intact tested seeds of cowpea. The control was sprayed with distilled water. Each Petri–dish (15cm in diameter) contained 100 seeds sprayed with 2ml of each concentration, the treated seeds were allowed to dry for one hour. Ten adults of *C. maculatus* were introduced into each Petri–dish. The treatment were replicated

three times. The number of dead adults in each petri-dish was counted every 24 hrs, 48 hrs and 72 hrs. This procedure was adopted for each of the five tested leaves aqueous extracts.

3.3.1.3 Ethanolic extracts of plant leaves

The method described by Undo and Epidi, (2009). was followed, Petri- dishes (9 cm in diameter) were used to confine insects during the experiment. Ethanolic extracts of plants leaves at concentrations 2%, 3% and 5% were used to spray intact tested seeds, the control was sprayed with ethanol. Each petri-dish contained 100 seeds sprayed with 2 ml of each concentration. Ten adults of *C. maculatus* were introduced into each Petri-dish. The number of dead insects in each Petri-dish was counted every day for three days. All treatments were arranged in Completely Randomized Design (C R D). This procedure was performed for each of the five tasted leave ethanolic extracts.

3.3.2. Host preference

The procedure described by Mohammed-Ahmed, (2004 and 2012) was adopted. Nine jars (15cm in diameter) were used for the three crops (cowpea, *p. valgaris* and faba bean) each jar contained 100 seeds of each crop. Then twenty of freshly emerged adults of *Callosobruchus maculatus* were introduced into each jar, each jar was covered with a piece of cloth and fixed with a rubber band. The jars were kept in the laboratory under room conditions, then left for four months June, July,

August and September. Observations and counted of damage seeds were carried out each month. Samples were taken from each treatment and replicated three times then the means of infested seeds was recorded at the end of the storage period.

3.4. Data and sample analysis

3.4.1. Statistical Analysis

Data were subjected to the analysis of Variance (ANOVA) using SAS and SPSS computer software package (Version 21) at 0.05 level of significant. Means were separated using Least Significant Difference (LSD).

3.4.2. Probit analysis

More to the statistical analysis Probit analysis was carried out according to Finney (1971).

3.4.3. Phytochemical analysis

Chromatography is a process in which a chemical mixture is separated into components based on differences between solutes (Sherma and Fried, 2003). Compound separation is based upon different components of the mixture traveling at different speeds due to affinity for the medium, solubility in the solvent, and molecular weight (Rouessac and Rouessac, 2000).

3.4.4. GC-MS analysis

In this study, the GS-MS carried out in National Research Centre, Cairo-Egypt to identify the unknown compounds. The GC-MS analysis was performed using

diethyl ether as solvent and a thermo Scientific, trace GC ultra/ ISQ single Quadrupole MS₁TG-5ms fused silica capillary column (30m,0,25.1mm,0.1mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1ml/min the injector and MS transfer line temperature was set at 280C° (Mclafferty and Turecek,1993).

CHAPTER 4

RESULTS

4.1. Field study

4.1.1. Survey of cowpea beetle in Alfashir town

The results of the field survey are shown in Table (1), figs. (4.1, 4.2, and 4.3). The survey carried out in Alfashir locality (crops market, Almwashi market and Nafasha market). These results showed that the adults of *C. maculatus*, infestation and abundance on cowpea (*Vigna unguiculata*), faba bean (*Vicia faba*) and (*Phaseolus vulgaris*). This varied with time and place, the highest infestation (80%) was recorded in August on faba bean at Almousha market followed by (78%) in July on cowpea at Almousha market, the lowest percentage shown on *V. vulgaris* . The early appearance of the pest was noticed in June at Almousha market on (*Vicia faba*).

Table (1) Percentage of infestation of *C. maculatus* in different areas in different pulse seeds

Months	Crops market			Al moushi market			Nafasha market		
	Cowpea	<i>V. vulgaris</i>	Faba bean	Cowpea	<i>V. vulgaris</i>	Faba bean	Cowpea	<i>V. vulgaris</i>	Faba bean
June	37	42	9	49	59	27	57	47	17
July	67	52	49	78	45	72	59	37	5
August	49	27	57	60	30	80	49	30	72
September	39	29	78	35	47	77	68	7	70

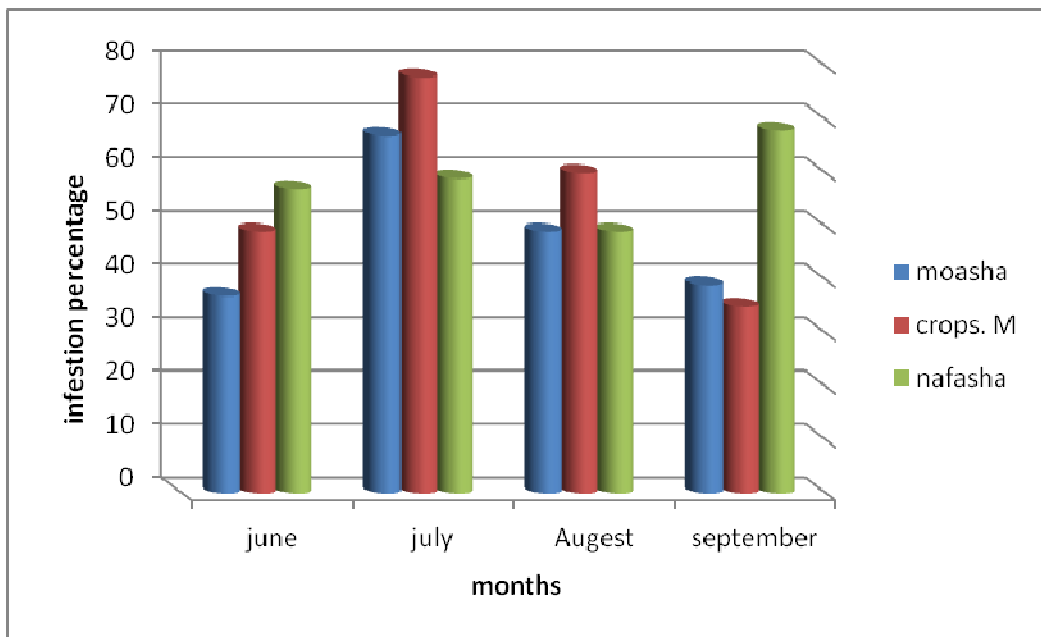


Fig. (4.1) Percentages of infestation of *C. maculatus* on cowpea seeds

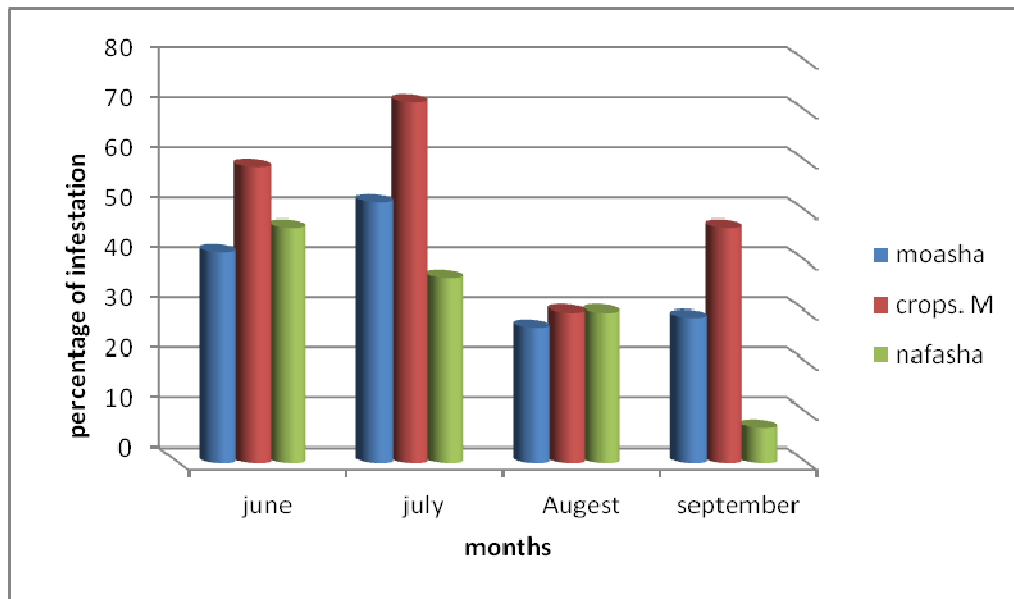


Fig. (4.2) Percentages of infestation of *C. maculatus* on faba bean seeds

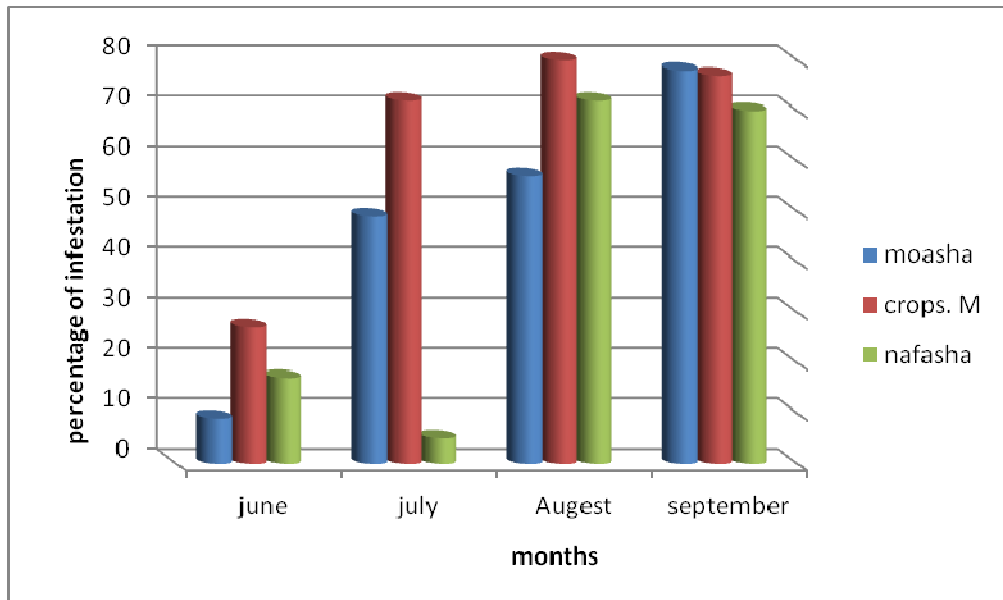


Fig. (4.3) Percentages of infestation of *C. maculatus* on *kidney bean* seeds

4.2.1 Laboratory studies

4.2.2. Effect of different botanical extracts on adults mortality of *C. maculatus*.

Various concentrations of powder, aqueous and ethanolic extracts of *Solenostemma argel*, *Ocimum basilicum*, *Nicotiana rustica*, *Datura stramonium* and *Azadirachta indica* were tested as potential sources of control agents for *Callosobruchus maculatus* adults. The results of these effects are can be presented as follows:

4.2.2.1. Effects of different concentrations of plant powders on adult mortality of *C. maculatus* 24, 48 and 72hours after treatment

Generally all adults of *C. maculatus* on cowpea seeds treated with plant powders exhibited high percent mortality compared to those on untreated seeds (tables, 2. 3. 4).

All tested concentrations of *A.indica*, *O. basilicum*, *S. argel*, *N. rustica* and *D. stramonium* caused significantly high ($p \leq 0.05$) mortality percent compared to the control.

Only the two lowest concentrations of *O. basilicum* gave 0% mortality and were not different from the control (after 24 hours) Table(2)

Only the two lowest concentrations of *D. stramonium* gave significantly high mortality percent than the other botanical concentrations (after 72 hours) Table(4) .

Table (2): Effects of different plant powders at different concentrations on adult mortality of cowpea beetle within 24hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
5%	1.666a	0.000b	2.666a	6.000a	2.000a
10%	1.000a	0.000ab	1.666a	2.000b	3.000a
15%	2.333a	1.000a	2.666a	2.666b	2.666a
Control	0.000b	0.000b	0.000b	0.000c	0.000b
Means	2.972	0.527	4.750	18.666	5.416
C.V	76.590	79.979	40.406	34.232	47.628
LSD	1.802	0.768	1.331	1.718	1.718
MSE	0.957	0.408	0.707	0.912	0.912

Mean having the same letters within the same column are not significantly different at $\rho=0.05$

LSD=least significant difference

C.V=Coefficient of variation

Table (3): Effects of different plant powders at different concentrations on adult mortality of cowpea beetle within 48hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
5%	2.000a	2.666a	4.000a	2.666a	1.667b
10%	2.333a	2.333a	4.000a	2.666a	1.333b
15%	2.666a	1.666a	4.333a	3.333a	5.000a
Control	0.000b	0.000b	0.000b	0.000b	0.000b
Means	4.305	4.222	12.750	6.555	13.555
C.V	46.656	51.961	29.606	46.153	64.549
LSD	1.537	1.630	1.718	1.882	2.430
MSE	0.816	0.866	0.912	1.000	1.290

Means having the same letters within the same column are not significantly different at $\rho=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

Table (4): Effects of different plant powders at different concentrations on adult mortality of cowpea beetle within 72hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
5%	2.000a	1.333a	2.333a	0.000b	3.000a
10%	0.666b	1.666a	2.667a	1.666a	2.333ab
15%	2.000a	1.333a	2.333a	0.666b	1.000bc
Control	0.000b	0.000a	0.000a	0.000b	0.000c
Means	3.000	1.638	4.555	1.861	5.416
C.V	89.214	92.307	81.818	69.985	48.237
LSD	1.959	1.882	2.824	0.768	1.438
MSE	1.040	1.000	1.500	0.408	0.763

Means having the same letters within the same column are not significantly different at $p=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

4.2.2.2 Effects of different concentrations of aqueous extracts on adult mortality of *C. maculatus* after 24, 48, 72 hours.

All tested concentrations of botanical aqueous extracts significantly increase the mortality percent ($p \leq 0.05$) compared to the control 24, 48 and 72 hours after treatment.

Only the lowest concentration of *O. basilicum* gave significantly high mortality percent than others botanical after 24 hours (Table (5)).

All concentrations of *A. indica*, *O. basilicum* and *S. argel* gave significantly high mortality percent than the control after 48 hours, and effects were dose dependent (Table 6).

Table (5): Effects of different plants aqueous extracts at different concentrations on adult mortality of cowpea beetle within 24hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
5%	1.666b	6.667a	2.000a	1.666a	1.000ab
10%	4.666a	4.333a	1.666ab	1.000b	2.000a
15%	2.000b	1.333b	1.333ab	1.666a	2.333a
Control	0.000b	0.000b	0.000b	0.000b	0.000b
Means	11.194	26.972	2.305	2.777	3.333
C.V	57.131	51.280	76.594	97.979	68.465
LSD	2.241	2.977	1.802	1.537	1.718
MSE	1.190	1.581	0.957	0.816	0.912

Means having the same letters within the same column are not significantly different at $\rho=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

Table (6): Effects of different plant aqueous extracts at different concentrations on adult mortality of cowpea beetle within 48hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
5%	2.000b	2.000b	2.333ab	1.333ab	2.666a
10%	1.333b	3.000b	4.000a	0.333bc	1.666a
15%	3.333a	5.000a	3.667a	2.000a	0.000b
Control	0.000c	0.000c	0.000b	0.000c	0.000b
Means	5.777	13.000	9.888	2.527	5.194
C.V	38.729	40.000	71.180	70.417	75.368
LSD	1.215	1.882	3.350	1.215	1.537
MSE	0.645	1.000	1.779	0.645	0.816

Means having the same letters within the same column are not significantly different at $p=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

Table (7): Effects of different plant aqueous extracts at different concentrations on adult mortality of cowpea beetle within 72hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
5%	1.000a	1.000ab	2.000ab	1.666b	2.333a
10%	0.333a	1.333ab	2.000ab	3.000a	2.000a
15%	1.666a	3.000a	4.000a	1.333b	2.333a
Control	0.000a	0.000b	0.000b	0.000c	0.000b
Means	1.638	4.666	8.000	4.555	3.777
C.V	158.698	94.372	93.541	43.033	24.494
LSD	2.241	2.369	3.522	1.215	0.768
MSE	1.190	1.258	1.870	0.645	0.408

Means having the same letters within the same column are not significantly different at $p= 0.05$

LSD=least Significant Difference

.V=Coefficient of Variation

4.2.1.3 Effects of different concentrations of ethanolic extracts on

adult mortality of *C. maculatus* 24, 48,72 hours after treatment.

All tested concentrations of *O. basilicum*, *S. argel*, *N. rustica*, *A. indica* and *D. stramonium* gave significantly high mortality percent $p \leq 0.05$ than control after 24, 48, 72hours.

All concentrations of *N. rustica*, *A. indica*, and *D. stramonium* gave highly significant ($p \leq 0.05$) mortality percent compare to the control after 24 hours(table(8)).

Only the lowest concentration of *S. argel* gave significantly high mortality than control after 24hours.

Generally all ethanolic extracts caused significantly high mortality compared to the control, and effects were dose and time dependent. This highest effects were noticed after 72 hours of exposure expect *N. rustica* showed after 24 hours.

Table (8): Effects of different plant ethanolic extracts at different concentrations on adult mortality of cowpea beetle within 24hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>N.rustica</i>	<i>D.stramonum</i>
2%	0.666a	1.666a	2.333a	10.000a	2.666a
3%	0.666a	1.333ab	2.666a	10.000a	1.666a
5%	0.666a	1.000ab	2.333a	10.000a	1.666a
Control	0.000a	0.000b	0.000b	0.000b	0.000b
Means	0.333	1.555	4.555	75.000	3.666
C.V	141.421	81.649	47.237	0.000	57.735
LSD	1.331	1.537	1.630	0.000	1.630
MSE	0.707	0.816	0.886	0.000	0.866

Means having the same letters within the same column are not significantly different at $\rho=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

Table (9): Effects of different plant ethanolic extracts at different concentrations on adult mortality of cowpea beetle within 48 hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>D.stramonum</i>
2%	2.000b	1.666a	1.333ab	2.667a
3%	1.333cb	1.666a	1.333ab	2.333ab
5%	4.000a	1.333a	3.333a	4.000a
Control	0.000c	0.000b	0.000b	0.000b
Means	8.333	1.888	5.666	8.305
C.V	41.659	42.857	74.535	61.530
LSD	1.438	0.941	2.105	2.606
MSE	0.763	0.500	1.118	1.384

Means having the same letters within the same column are not significantly different at $\rho=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

Table (10): Effects of different plant ethanolic extracts at different concentrations on adult mortality of cowpea beetle within 72 hours.

Treatment	<i>A.indica</i>	<i>O.basilicum</i>	<i>S.argel</i>	<i>D.stramonum</i>
2%	4.000a	2.000a	0.666b	3.000a
3%	2.000b	2.666a	1.333ab	2.666a
5%	1.333b	1.666ab	2.333a	2.333a
Control	0.000c	0.000b	0.000b	0.000b
Means	8.333	3.861	2.972	5.555
C.V	31.491	60.469	79.94	59.511
LSD	1.087	1.802	1.630	2.241
MSE	0.577	0.957	0.866	1.190

Means having the same letters within the same column are not significantly different at $p=0.05$

LSD=least Significant Difference

C.V=Coefficient of Variation

4.2.1.4. Calculation of the Lethal Dose (LD₅₀)

In Table (11) as shown the *Solenostemma argel* (powder and ethanolic extract) was the most potent as seen by its low LD₅₀ (0.669 and 2.545) followed by *Datura stramonium* (ethanolic and aqueous extracts) (0.87 and 5.45), *Azadirachta indica* (ethanolic and aqueous extract) (2.55 and 5.708), *Ocimum basilicum* (aqueous and ethanolic extracts) (2.72 and 3.107) and *Nicotiana rustica* (powder) (3.180) as shown in Figs. 13, 14 ,15 and 16) and Appendices (from 1 to 10).

Table (11): The lethal dose (LD₅₀) of different botanical extracts on adult mortality of *C. maculatus* after 72 hours.

extract	Hargal	Neem	Basil	Datura	Tobacco
Powder	0.669	7.371	97.94	2.545	3.180
Aqueous	3.679	5.708	2.72	4.95	18.40
ethanolic	2.482	2.55	3.107	0.87	-----

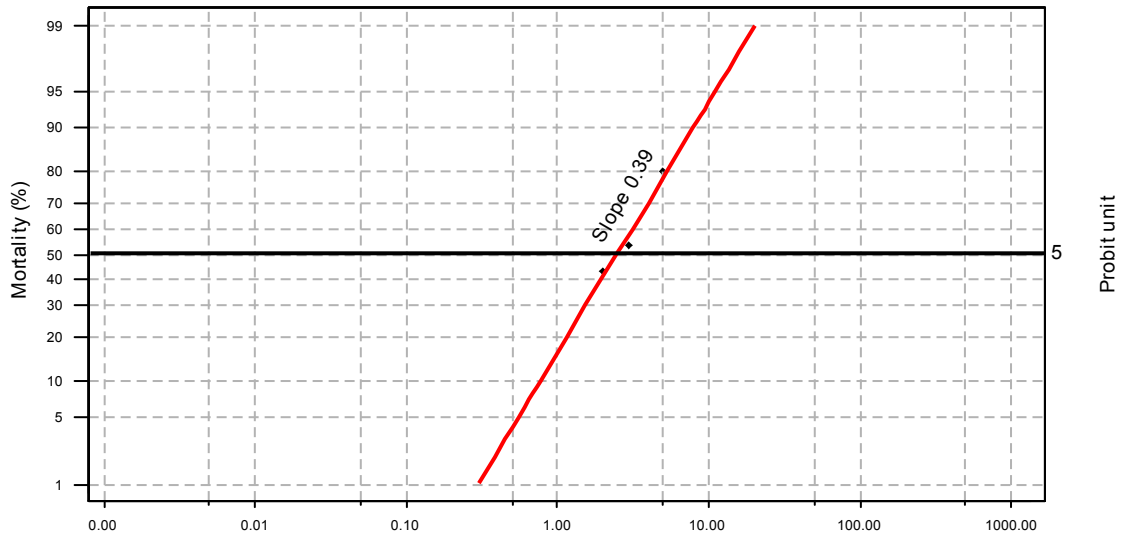


Fig. (1): Log dose probit regression line of *S. argel* (powder extracts)

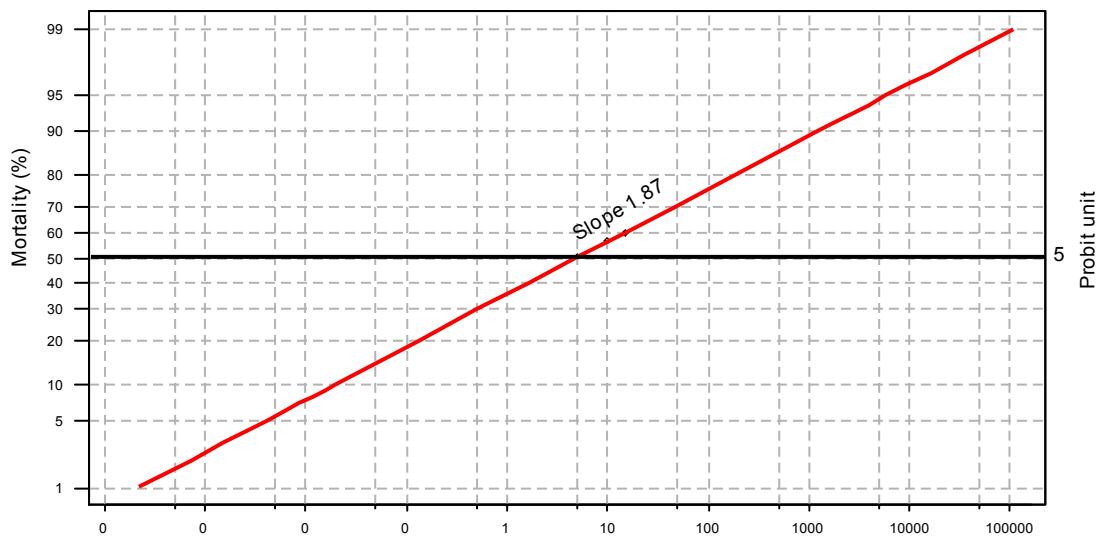


Fig. (2): Log dose probit regression line of *D. stramonium* (ethanolic extracts)

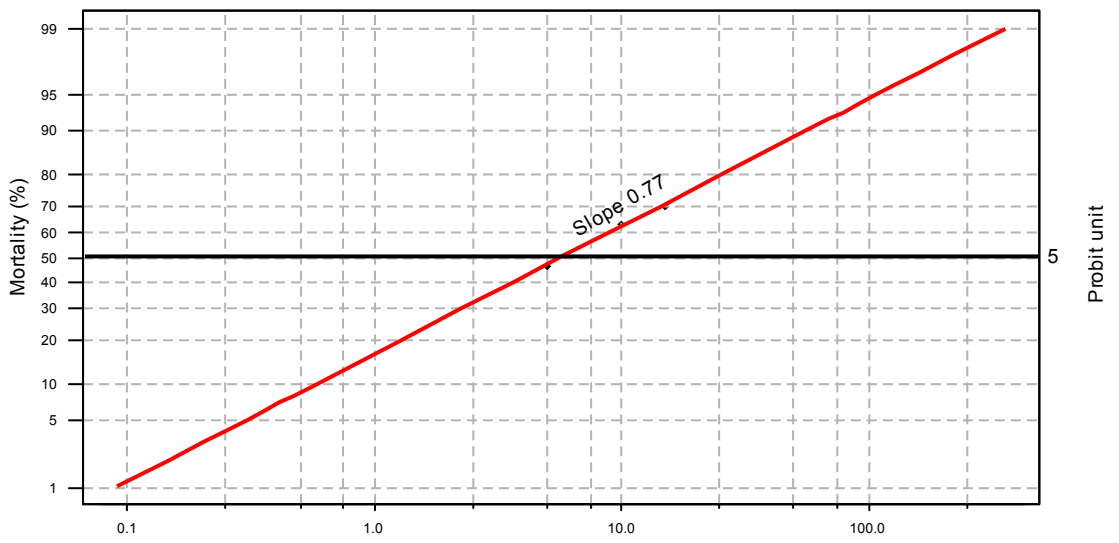


Fig. (3): Log dose probit regression line of *A. indica* (ethanolic extracts)

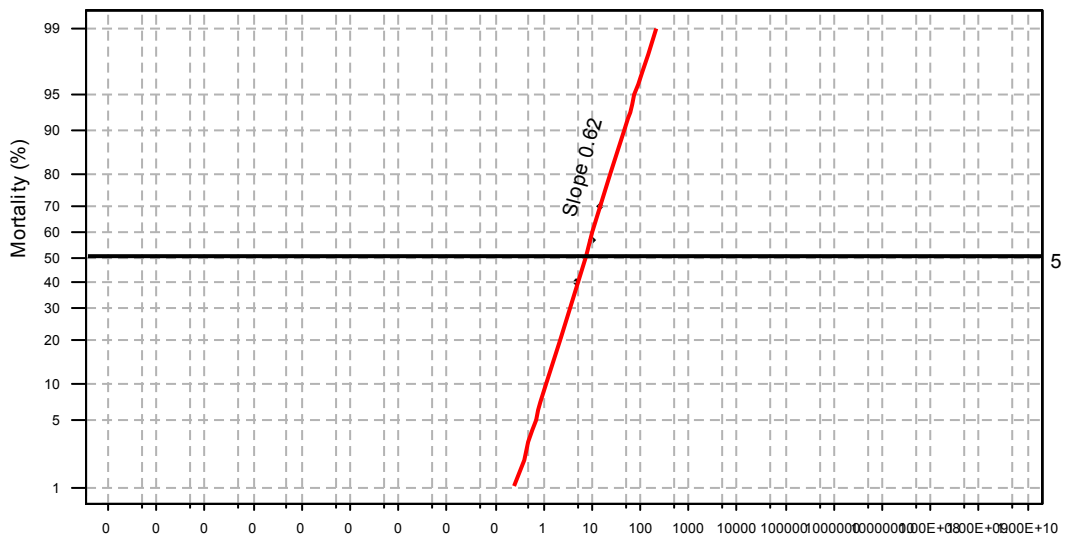


Fig. (4): Log dose probit regression line of *O. basilicum* (ethanolic extracts)

4.2.2.5. Phytochemical analysis

The quantification of all the identified components was investigated using a percent relative, peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the Nist, willy, as shown in Tables (12, 13, 14, 15, 16).

Table (12): The Gas Chromatography (MS) analysis of *O. basilicum*

s.n	RT	Peak areas%	Compound name	Molecular formula	MW
1	32.72	3.63	Pentamethyl pentaphenylcyclopentasiloxane	C ₃₅ H ₄₀ O ₅ Si ₅	680
2	39.28	3.57	Bis[3-methyl-1-(diphenylcarbamoyl)-1,2-butane diyl]-sulfone	C ₃₆ H ₃₂ N ₂ O ₄ S	588
3	52.99	3.44	2,3-Butanedione,dioxime(CAS)	C ₄ H ₈ N ₂ O ₂	116
4	43.09	3.10	N-butylpyrrolidine-N-carboxamide	C ₉ H ₁₈ N ₂ O	170
5	41.73	3.00	Methoxychromene precocene tetramer		

RT=Retention time

MW=Molecular weight

Table (13): The Gas Chromatography (MS) analysis of *A. indica*

s.n	RT	Peak area %	Compound name	Molecular formula	MW
1	40.79	3.99	(5,10,15,20-tetraphenyl[2-(2)H1]prophyrinalo)zinc(11)	C ₄₄ H ₂₈ N ₄ Zn	676
2	46.43	3.49	[ü6-1,3,5-tris(diphenyl phosphino)benzene]tricarbonylchromium(O)zinc(11)	C ₄₄ H ₃₃ CrO ₃ P ₃	766
3	34.60	3.94	Tetraphenyl porphyrinato dichlorotitanium(1v)	C ₄₄ H ₂₈ C ₁₂ N ₄ Ti	676
4	6.42	3.25	(25,3R,5S)-2-hexyl-3,5-bis[(trimethylsilyl)oxy]hehadecanoate	C ₃₁ H ₆₈ O ₄ Si ₃	588
5	28.96	3.03	3-Dehyddroyuna conitine dev.	C ₃₁ H ₅₁ AsNO ₉	656

RT=Retention time

MW=Molecular weight

Table (14): The Gas Chromatography (MS) analysis of *S. argel*

s.n	RT	Peak area %	Compound name	Molecular formula	MW
1	58.84	3.66	2-[2,6-Bis(hex-5-enyloxy)phenyl]-9-[2-(but-3-enyloxy)-6-hex-5-enyloxy]phenyl]-1,10-phenanthroline	C ₄₆ H ₅₂ N ₂ O ₄	696
2	43.13	3.58	[Tri[Titanium-pentamethylcyclopentadienyl(oxa)]cymethyl][N,N-diethylamino]	C ₃₆ H ₅₉ NO ₃ Ti ₃	697
3	57.93	3.38	1-(o-carbonan-9-yl)-2,3,4,5,6-pentaphenyl benzene	C ₃₈ H ₃₆ B ₁₀	602
4	57.72	3.37	Methyl-13-hydroxy-phacophorbide-a	C ₃₆ H ₃₈ N ₄ O ₆	622
5	56.55	3.20	1,2-D2-IMIDAZOLE	C ₃ H ₂ D ₂ N ₂	68

RT=Retention time

MW=Molecular weight

Table (15): The Gas Chromatography (MS) analysis of *D. stramonium*

s.n	RT	Peak area %	Compound name	Molecular formula	MW
1	10.88	3.84	(2-hydroxy-5,10,15,20-tetraphenylporphinato) copper(II)	C ₄₄ H ₂₈ CuN ₄ O	691
2	11.24	3.45	2(1H)-pyrimidinone,4-amino-(CAS)	C ₄ H ₅ N ₃ O	111
3	53.25	3.51	Dodecachloro-3,4-benzophenanthrene	C ₁₈ Cl ₁₂	636
4	10.88	3.84	(2-hydroxy-5,10,15,20-tetraphenylporphinato) copper(II)	C ₄₄ H ₂₈ CuN ₄ O	691
5	13.04	3.18	SALMOXANTHIN	C ₄₀ H ₅₆ O ₄	600

RT=Retention time

MW=Molecular weight

Table (16): The Gas Chromatography (MS) analysis of *N. rustica*

s.n	RT	Peak area %	Compound name	Molecular formula	MW
1	20.96	99.25	Pyrrolidine N-(4-methyl-3-pentenyl)	C ₁₀ H ₁₉ N	153
2	20.96	99.25	Benzoic Acid, 4[1-oxo-2-(1-pyrrolinyl)ethyl]amino-,methyl ester	C ₁₄ H ₁₈ N ₂ O ₃	262
3	31.83	0.48	Penten-1-ol,(2)-(CAS)	C ₅ H ₁₀ O	86
4	31.96	0.00	Cadaverine	C ₅ H ₁₄ N ₂	102
5	12.00	0.00	Vitamin B12	C ₆₃ H ₈₈ CON ₁₄ O ₁₄ P	1354

RT=Retention time

MW=Molecular weight

CHAPTER 5

DISCUSSION

The search for naturally-occurring pesticides for field crops and storage pests has been intensified. There is a continuous search for natural products that can reduce insect population in a manner that is less hazardous to the farmers and the environment. A number of investigations have identified and screened a variety of promising chemical compounds from leaves and seeds of many botanical families as insect feeding deterrents and growth inhibitors (Jacobson *et al.*, 1979; Jurd and Manners, 1980).

Without suspicion, cowpea which is called locally lubia, is an important crop used as food and as cash crop for the farmers in Al fashir locality Northern Darfur State, Sudan. One of the main problems of production of cowpea is the pests attack. According to the results obtained from previous study of Silim *et al.*, (1999). The cowpea beetle *Callosobruchus chinensis* breed almost exclusively in cow pea (*Vigna anguiculata*) as the most preferred pulse crop. It was reported to attack different leguminous seeds, it is primarily a pest of seeds of pigeon pea (*Cajanus cajana*), broad bean (*Vicia faba*), dry or garden pea (*Pisium sativum*), chick pea (*Cicer arietinum*), black eyed cowpea (*Vigna sinensis*), mung bean (*Vigna radiata*) and azuki bean (*Phaseolus angularis*). Few

investigations have been carried out on controlling this beetle with botanical materials in Sudan but where results are available they show that they are promising.

Field study

The present study showed that cowpea beetle (*Callosobruchus maculatus*) was present along the whole part of Darfur region especially in North Darfur State (Alfahir locality). This result agrees with Fabricius, (1775), who stated that, cowpea weevil common pest of stored legumes has a cosmopolitan distribution, occurring in every continent. Tran and Credland (1995) stated that the beetle most likely originated in West Africa and move around the globe with the trade of legume and other crops. The time of insect infestation to grain seeds of legumes (cowpea, faba bean and *V. vulgaris*) varied gradually from their areas (crops market, Almousha market and Nafasha market) The highest infestation (80%) during season was recorded in August on faba bean at Almousha market and followed by 78% in July on cowpea at Almousha market. The early appearance of the pest was noticed in June at Almousha on faba bean and this may be due to their optimum temperature required for multiplication. No previous studies were found for comparison.

Effects of different botanical extracts

The present study was carried out to investigate the insecticidal effect of five botanical leaves powder, aqueous and ethanolic extracts, do they all naturally grow in Sudan, on adults of *Callosobruchus maculatus*. The results obtained indicates that hargal plant

leaves (powder, aqueous and ethanolic extracts), effectively controlled the adults of *Callosobruchus maculatus* .

Solenostemma argel mortality effects, result shown in Tables(2, 5, 7) at concentrations of 5%, 10% and 15%, of aqueous extracts and 2%, 3% and 5% of ethanolic extract and 5%, 10% and 15% of powder revealed that adult mortality was not affected by increase in the concentration and this is probably due to antifeedant effect caused by hargal plant. Concentration 5%, 10% and 15% of hargal leaves powder have promising insecticidal activity against the tested adults for 72hours (the test period of study) when compared to the concentration 5%, 10% and 15% of Hargal aqueous extracts and concentration 2%, 3% and 5% of Hargal ethanolic extract. In a set of experiments El-Kamali, (2001) reported that the aqueous extract of leaves, flowers, roots and stem of *S. argel* was tested in alaboratory for activity against the third larval instar of mosquito *Culex quiquefsciatus*, and the result revealed that extract of the leaves was the most effective LC₅₀ of 0.5mg/ml at 24 hours.

In other studies Sidahmed *et al.*, (2009) reported that aqueous filtrate of Hargal 5%, 7.5% and 10% was found effective against the cotton soil termite (*Microtermes horacalis*).

Generally the mortality was dose and time dependent, the significant mortality was shown at all concentrations of powder, ethanolic and aqueous extracts. A high mortality rate was observed in a 15% aqueous extract after 72 hours of exposure.

Datura stramonium, the result of mortality data indicate that (ethanolic, aqueous extracts and powder) was most potent against *Callosobruchus maculatus* adults as showed by its lowest concentration (2% ethanolic and 5%, 5% powder and aqueous extracts), these findings agree with that reported by Kurnal and Yalcin, (2009) who found that ethanolic extracts obtained from both leaves and seeds, which were applied in 167.25 and 145.70g/l concentration caused 98% and 25% mortality among spider mite.

Swathi *et al.*, (2012) reported that *Datura stramonium* ethanolic extract of leaves has been used against different insect species, were evaluated for larvicidal and mosquito repellent activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

Generally the mortality was dose and time dependent, the significant mortality was shown at all concentrations of ethanolic, aqueous extracts and powder. A high mortality was observed in 15% powder after 48 hours of exposure.

In this study *Ocimum basilicum* (powder, aqueous and ethanolic extracts), caused moderate mortality effects. These results agree with that reported by Mansour, *et al.*, (1986) who investigated the effects of essential oils isolated from 4 species of the family Labiates on adult females of *Tetranychus cinnabrinus*. They showed that concentrations of the acetone solutions of the oils from 0.1 to 2% cause mortality and induce repellency effect of The same aauthors found that *O. basilicum* contains

methylcinnamate and methylchavicol component which induced moderate insecticidal activity against stored grains insect pests.

Generally the mortality was dose and time dependent, the significant mortality was shown at all concentrations of ethanolic, aqueous extracts and powder. A high mortality observed in 5% concentration (aqueous extract) after 24 hours of exposure, while 5% and 10% (powder) concentration showed no significant mortality.

Nicotiana rustica mortality effects agree with Malaya and Banda, (1995) who reported that nicotine from *Nicotiana sp* in some countries is used as pesticide, especially in garden. Also Metacalf *et al.*, (1962) who reported crude extracts from tobacco recommended for control of insect pests. This may be due to the presence of alkaloids such as nicotine, anabasine, anatabasine and nornicotine which have insecticidal properties.

Generally the mortality was dose and time dependent. The significant mortality was shown in all concentrations (powder and aqueous, ethanolic extracts). A high mortality rate was observed at 2%, 3% and 5% concentration of ethanolic after 24 hours of exposure.

Azadirachta indica powder, aqueous and ethanolic extracts treatments significantly increased the mortality of the adults of *Callosobruchus maculatus*. This is in conformity with Jackai and Oyediran, (1991) who stated that neem extracts effectively reduced pests damage leading to increased yields. Also Zongo *et al.*, (1993); Saxena,

(1981) reported that neem products have shown efficacy against pod borer (*Naruea vitala*), pod sucking bugs complex (*Clavigralla lomentosicoltis* Saz) and other insect pests. Kloos and Cullough, (1982), reported potency of neem seed oil on snail fever (Schistosomiasis) with the active principle being molluscicidal and ovicidal and cercarialicidal.

Generally the mortality was dose and time dependent, the significant mortality was shown at all concentrations of (aqueous, ethanolic extracts and powder). A high mortality was caused by 10% concentration of aqueous extracts after 24 hours of exposure while 2%, 3% and 5% concentrations of ethanolic extracts caused the least mortality after 24 hours of exposure.

Probit analysis

Probit analysis showed that the powder *Solenostemma argel* was most effective against the adults of *Callosobruchus maculatus*, as indicated by their low values of LD₅₀, the probit analysis showed LD₅₀ of 0.669 mg/L (powder) and 2.482 mg/L (ethanolic) was more effective against adult cowpea beetle. These results are in the same trend with those of Ali, (2004) who obtained LD₅₀ of 122.29mg/L (aqueous extract) for *Culex* and *Anopheles* larvae.

The slopes were steep indicating homogenous population, increase in concentrations increase the slope of regression line and caused progressive improvement of homogeneity.

Phytochemical analysis

Metabolite profiling in plant species was done by gas chromatography mass-spectrometry method for recent years, but only a limited number of plant research laboratories have gas chromatography. GC-MS analysis is an interesting tool to evaluate a number of active bio components used in cosmetic, pharmaceutical, insecticides and food industry (Gomathi *et al.*, 2015).

In the present study, the chemical profile of five botanical plants, *Ocimum basilicum*, *Azadirachta indica*, *Solenostemma argel*, *Datura stramonium*, , , and *Nicotiana rustica* using GC-MS methods was characterized.

Tables (12, 13, 14, 15 and 16), represent the gas chromatogram showing the relative concentration of various compounds evaluated as a function of retention time, the highest peak indicates, the relative concentration of the component present in plant.

The GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical and insecticidal value of that plant, thus, this type of GC-MS analysis of diethyl ether injected to leaves powder of these plants, GC-MS measured and identified many chemical compounds. The active principal components measured with retention time, molecular weight and percentage were also determined .

The GC-MS analysis of *Ocimum basilicum* showed the presence of five major peaks percentage and the components corresponding to the peaks Table(12) were determined, (pentamethylpentaphenylcyclotrioxane) peak 3.63%, Bis[2-methyl-1-(diphenylcarbamyl)-1,2-butanediyl]-sulfone) peak 3.57%, (2-3-Butanedione, dioxime (CAS)), peak 3.44%, (N-butylpyrrolidine-N-carboxamide), peak 3.10% and (methoxychromene precocene tetramer) peak 3.00%.

The quantitative analysis of *Ocimum basilicum* identified the various phytochemicals components such as essential oils (phenyl-propanoids), that revealed to Das *et al.*, (2012). Mazumder *et al.*, (1997) ; Kelm *et al.*, (2000), reported that the presence of antioxidant, anti HIV and anti-inflammatory.

The GC-MS analysis of *Azadirachta indica* identified the various components such as (5,10,20-tetraphenyl[2-(2) HI] porphyrinalo)Zinc(II) peak 3.99%, (tetraphenyl porphyrinato-dichlorotitanium(IV)) peak 3.94%, ([6-1,3,5-tris (diphenylphosphino) benzene]tricarbonylchromium(0)Zinc(II)) peak 3.49%, (bis[(trimethylsilyl)oxy]heptadecanoate) peak 3.25%, and (3-Dehydroaconitine dev.) peak 3.03%. that is revealed to (Subapriya and Nagini, 2005).

The quantitative analysis of *Solenostemma argel* identified the various phytochemicals components such as flavonoid, tannins, sterols, triterpene and vitamins that revealed to Dogharia *et al.*, (2004). Interpretation on mass spectrum GC-MS was conducted using the data base of NIST and Wiley library determined, (2-[2,6-Bis(hex-5-

enyloxy)phenyl]-a-[2-(but-3-enyloxy)-6-hex-5-enyloxy)phenyl]-1,10-phenanthroline) peak 3.66%, ([tri[titanium-pentamethylcyclopentadlenyl(oxa)]caemethyl][N,N-diethylamino)]) peak 3.58%, (1-(0-carbonan-9-yl)-2,3,4,5,6-pentaphenyl benzene) peak 3.38%, (methyl-13-hydroxy-phacophorbide-a) peak 3.37% and (1,2-D2-IMIDAZOLE) peak 3.20%, found as the major component according to their peak percentage.

The quantitative analysis of *Datura stramonium* identified the various phyto components such as alkaloids, hyoscyamine, tannins, scopolamine and tropane, that reported by Das *et al.*, (2012). Interpretation on mass spectrum GC-MS was conducted. The GC/MS analysis of diethyl ether extracts showed the presence of four major peaks and the components corresponding to the peaks were determined, (2-hydroxy-5,10,15,20-tetraphenyl porphinato)copper(II) peak 3.84%, (Dodecachloro-3,4-benzophenanthrene) peak 3.50%, (2,(1H)-pyrimidinone,4-amino-(CAS)) peak 3.45% and SALMOXANIN peak 3.18%.

The GC-MS analysis of *Nicotiana rustica* by injected diethyl ether to leaves powder in to the GC-MS measured and identified more than 35 alkaloids, that revealed to Harbone, (1993). The GC-MS analysis of the diethyl ether extracts showed the presence of five major peaks and the components corresponding to the peak were determined, (Pyrrolidine N-(4-methyl-3-pentenyl) peak 99.25%, (Benzoic Acid,4-[1-oxo-2-(1-pyrrolinyl) ethyl]amino-,methyl ester) peak 99.25%, was found as major compound and followed by (penten-101,(2)-(CAS)) peak 0.48%,(Cadaverine) peak 0.00% and Vitamin B12 peak 0.00%.

Generally this type of GC-MS analysis is a first step toward understanding the nature of active principal components in the plants.

Conclusion:

The present work is an attempt to add something new and useful to through use of five botanical plant leaves to control the cowpea beetle.

The study concludes that all these plant leaves (powder and extracts) were most effective as contact toxicity agents.

The study demonstrates that the ethanolic extract of *N. rustica* was highly effective as control contact toxicity agent.

These plants may be used as population controlling agents for *Callosobruchus maculatus* as they are cheap and biodegradable and can be prepared easily by farmers.

Recommendation:

- Use of alternatives of pesticides is step to save seeds from infestation because avoidance of chemical control is highly recommended in the stores.
- Future research is needed in toxicological properties of the active ingredients of the above mentioned extracts and powder by using phytochemical methods.
- The impact of these crude extracts on human and animal health and the non-target organisms should be studied.

- More research is needed to explore uses of traditional materials that could have effects on insect infestation in stores.

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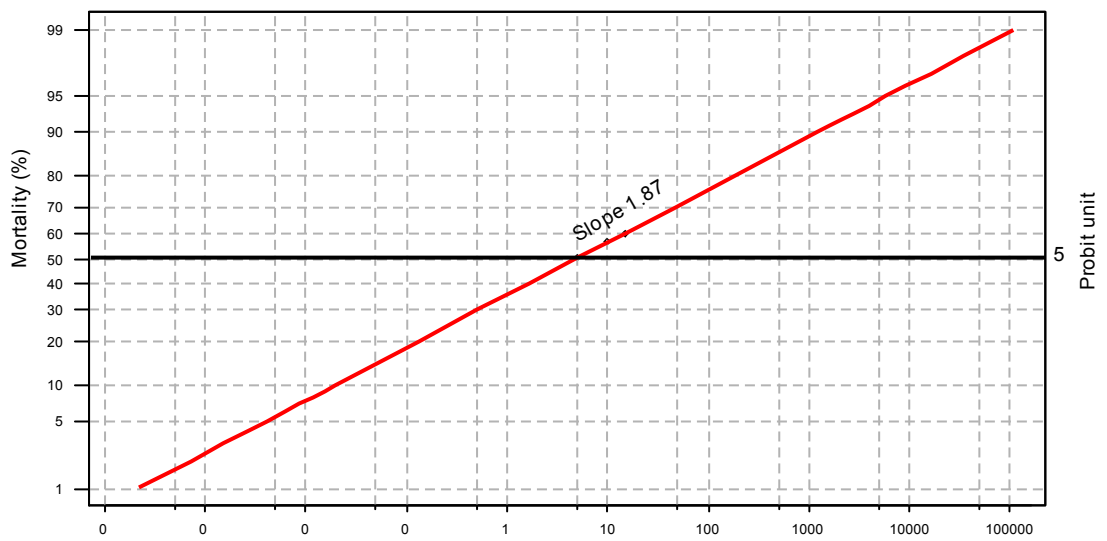
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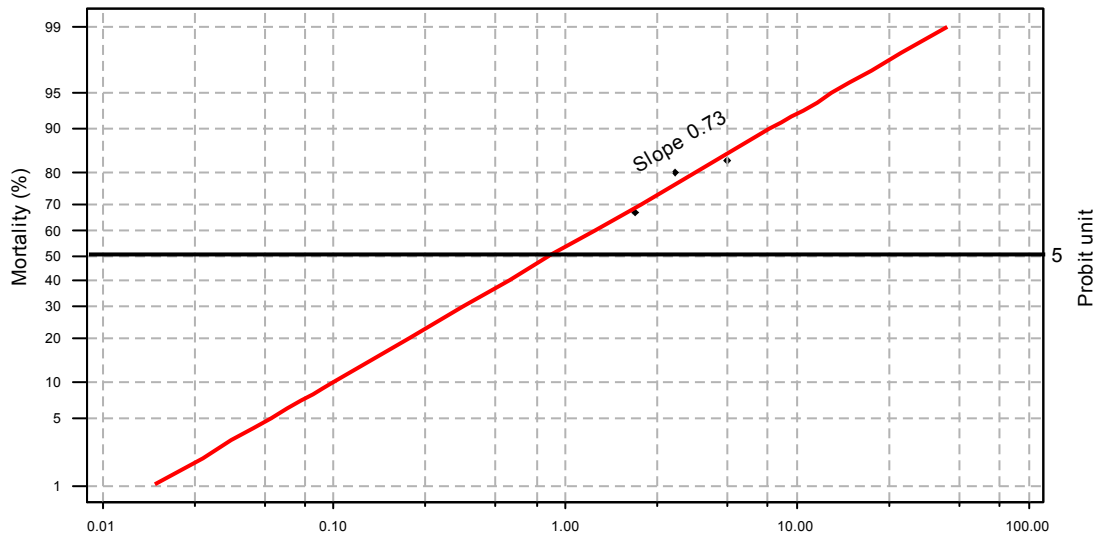
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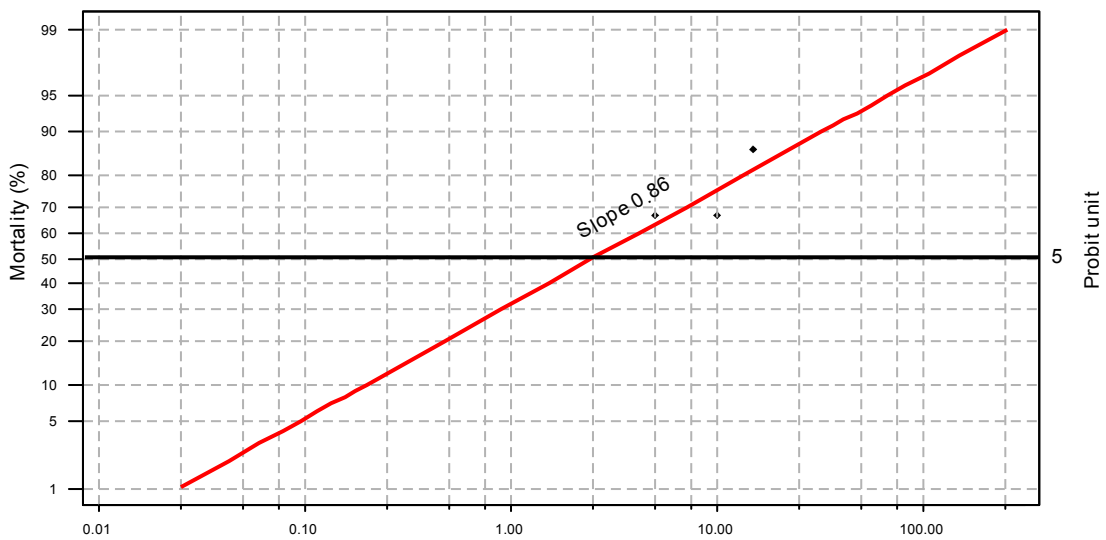
APPENDIES



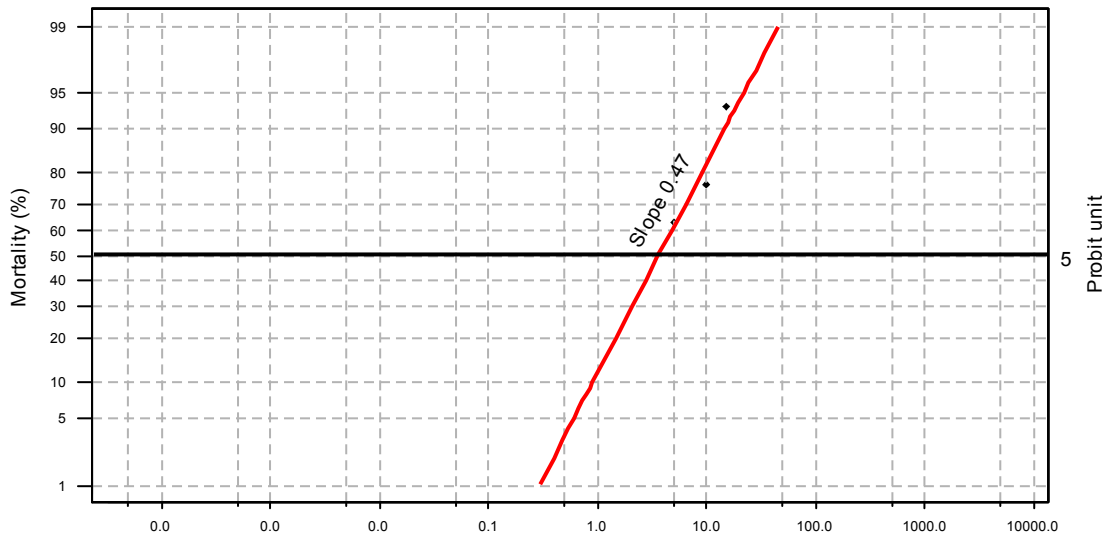
Appendix (1) Log dose probit regression line of *D. stramonium* aqueous extract (72hrs)



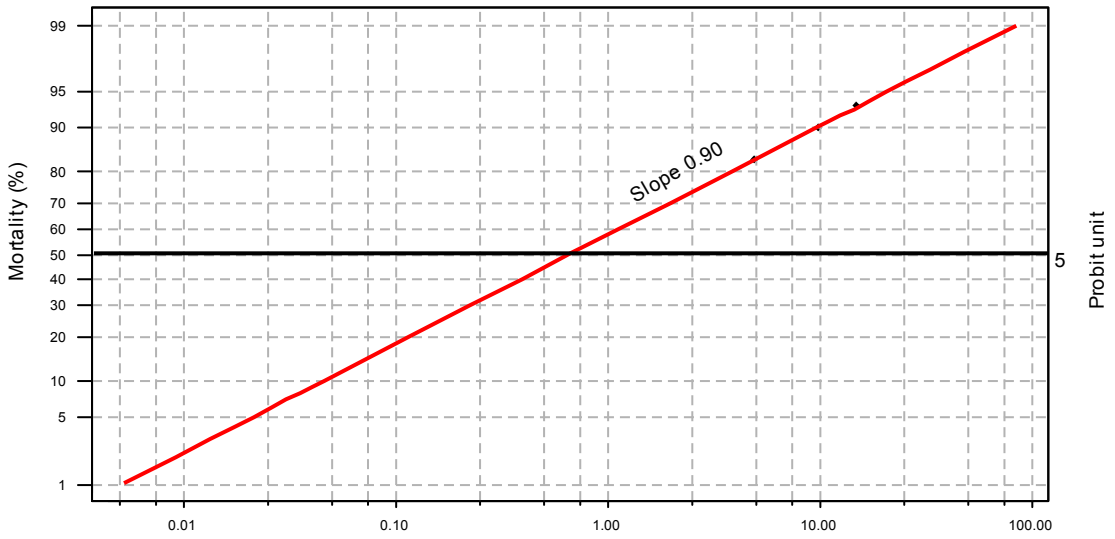
Appendix (2) Log dose probit regression line of *D. stramonium* powder extract(72hrs)



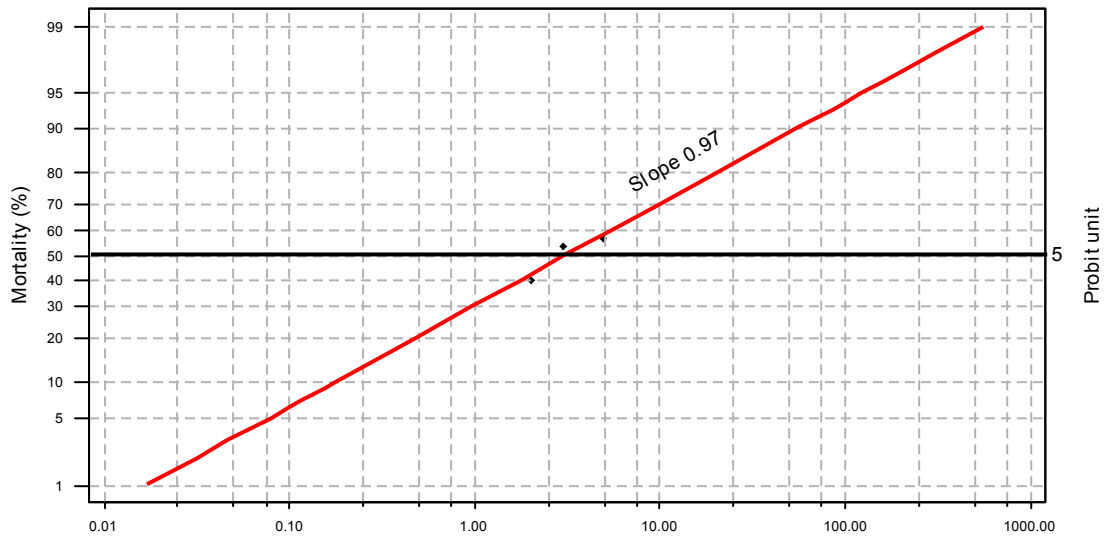
Appendix (3) Log dose probit regression line of *S. Argel* Aqueous extract(72hrs)



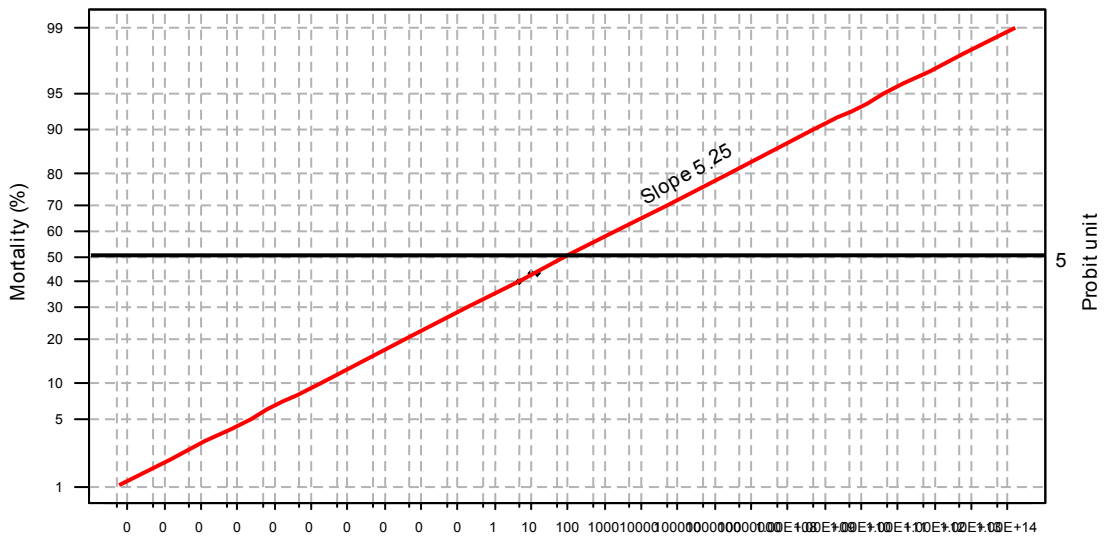
Appendix (4) Log dose probit regression line of *S. Argel* ethanolic extract(72hrs)



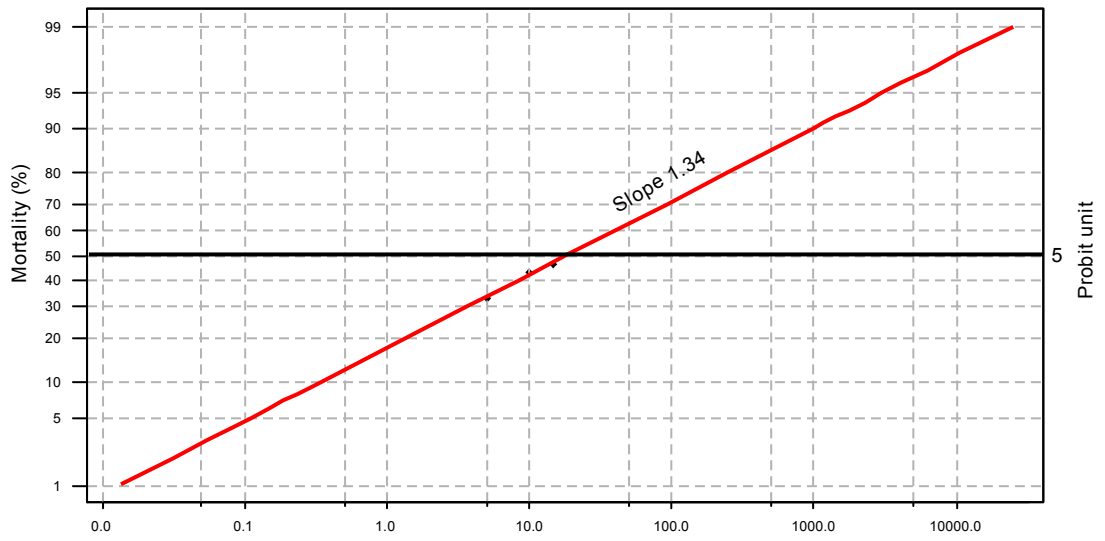
Appendix (5) Log dose probit regression line of *A. indica* Aqueous extract(72hrs)



Appendix (8) Log dose probit regression line of *O. basilicum* powder extract(72hrs)



Appendix (9) Log dose probit regression line of *N. rustica* Aqueous extract(72hrs)



Appendix (10) Log dose probit regression line of *N. rustic* powder extract(72hrs)