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Sudan University of Science and Technology

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Effect of aqueous Extract of Some Plants on Mortality of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

أثر المستخلص المائي لبعض النباتات على موت خنفساء ثاقبة الحبوب الصغرى *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

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

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الآيــة

1- (مَّثَلُ الَّذِينَ يُنفِقُونَ أَمْوَالَهُمْ فِي سَبِيلِ اللّهِ كَمَثَلِ حَبَّةٍ أَنبَتَتْ سَبْعَ سَنَابِلَ فِي كُلِّ سُنبُلَةٍ مِّئَةُ حَبَّةٍ وَاللّهُ يُضَاعِفُ لِمَن يَشَاء وَاللّهُ وَاسِعٌ عَلِيمٌ) (البقرة/261)

2- (قالَ تَزْرَعُونَ سَبْعَ سِنِينَ دَأَبًا فَمَا حَصَدتُّمْ فَذَرُوهُ فِي سُنبُلِهِ إِلاَّ قَلِيلاً مِّمَّا تَأْكُلُونَ) (يوسف/47)

Dedication

To my

Parents, sisters and brothers

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Yours Elzain Adam

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Abstract

Laboratory experiments were conducted at the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology. This study aimed to manege of lesser grain borer *Rhyzopertha dominica* using two botanicals as the natural products in relative to the insecticides. Were used three concentrations of each plant extract 30%, 20% and 10% plus untreated control.

The results revealed that the two highest concentrations of leaves powder aqueous extract of Usher used in this study (30% and 20%) gave the highest mortality percentage of 80%,66.6% respectively followed by Cafur 30 % which gave 63.3% mortality after 72 hours.

The concentrations which kill 50% of population (LC_{50}) of Cafur Extract was 157.177 after 24 hours, 109.526 after 48 hours and 94.425 after 72 hours.

The concentrations which kill 50% of population (LC_{50}) of Usher Extract was 22.842 after 24 hour, 18.187 after 48 hour and 11.926 after 72 hours.

The above results showed that Usher Extract gave the lowest LC_{50} 11.926 after 72 hour.

Therefore Usher extract could be considered as promising and safe alternative for control of lesser grain borer in stores using contact application methods.

ملخص البحث

اجريت تجارب معملية في قسم وقاية النبات, كلية الدراسات الزراعية, جامعة السودان للعلوم و التكنولوجيا. هدفت هذه الدراسة للعمل علي إدارة خنفساء ثاقبة الحبوب الصغرى *Rhyzopertha dominica* باستخدام نباتين كمنتجات طبيعية علي هيئة مبيدات حشرية. أستخدمت ثلاث تركيزات من كل مستخلص نباتي 30% ,20 % 10 % بالاضافه للشاهد .

اظهرت النتائج أن اعلى تركيزين لمستخلص بدرة اوراق العشرالمائي المستخدمة في هذه الدراسة (30% و20%) اعطت نسبة الموت الاعلي 80% و 66.6% علي التوالي ثم تبعه الكافور (البان) اعطى نسبة موت 63.3% بعد مرور 72 ساعة.

كانت التركيزات النصفية القاتلة و التى تودى الى موت 50 % من اعداد الافة من مستخلص الكافور بالنسبه للحشرة الكامله بعد 24 ساعه157.177 وبعد 48 ساعه 109.52 و بعد 72ساعه 94.425. بينما كانت التركيزات النصفية القاتلة و التى تودى الى موت 50 % من اعداد الافة من مستخلص العشر بالنسبه للحشرة الكامله بعد 24 ساعه 22.842 وبعد 48 ساعه 18.187 و بعد 72ساعه 11.926.

أظهرت النتائج أعلاه إن مستخلص العشر اعطي اقل تركيز نصفي قاتل 0.014 بعد 72 ساعه. عليه يمكن إعتبار مستخلص العشر وسيلة واعدة وبديلاً لمكافحه خنفساء ثاقبة الحبوب الصغري في المخازن بطريقه المعاملات السطحيه.

CHAPTER ONE

INTRODUCTION

In most countries, cereal grains such as wheat, corn, and rice form the principle sources of dietary energy. Sorghum is the staple food in many African countries including the Sudan (Shazali and Ahmed, 1998). It is the staple food in most regions of this country. It contains a reasonable amount of proteins, ash, carbohydrates, oils and fiber (Drich and Pran, 1987).In Sudan, sorghum is widely grown in areas of sufficient rainfall or under irrigation and is the most popular food grain (Elhag,1992, Shazali *et al*., 1996).

Farmers suffer heavy losses of stored grains due to insect pests. There are about thirty nine species of pests, which attack the stored grains and grain products. According to FAO estimates, about 10-25% of the world harvested food is destroyed by insects and rodent pests (Anonymous, 1980).

These grains are highly susceptible to infestation by stored product insects such as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Jood *et al*., 1996). *R. dominica* is one of the most injurious pest of stored grains both in larval and adult stage. It also infest other stored food items like beaten rice (Poha), dry fruits etc., (Kuzumenov *et al*., 1984; Raju, 1984). The adults are sturdy fliers, which fly from warehouse to warehouse, causing severe infestation and convert the stored grains to mere frass (Frenmore& Prakash, 1992). This and other stored product insects can cause significant economic losses in terms of grain mass (Subramanyam and Hagstrum, 1996) and nutrient depletion (Jood *et al*., 1996; Girish *et al*., 1975), and pose a public health risk from contamination by allergens, such as uric acid (Swaminathan, 1977; Jood and Kapoor, 1993). Insect infestations in stored grains during warm weather can also lead to undesirable taste and off-odors that can render the product inedible (Vassanacharoen *et al*., 2008). *Rhyzopertha dominica*is a cosmopolitan insect pest of stored raw grains. This species is well adapted to dry conditions (Emekci *et al*., 2004). Also, consumers generally prefer reduced pesticide residues on all agricultural products, including raw stored commodities (Arthur, 1996).

The current trend in stored-product pest management is to use reduced-risk or low-toxicity insecticides as a replacement for conventional organophosphates. Several problems have emerged with the use of conventional chemical control methods. These include resistance by many species of stored-product pests, including *R. dominica* (Subramanyam and Hagstrum, 1996). Uncontrolled use of insecticides and pesticides results in the evolution of resistant strains in addition to environmental contamination and health hazards (Prakash & Rao, 2006; Rahman *et al*., 2009). In such situations a safe method is to use the bioactive pesticides for protection of stored grains (Tapondjou *et al*., 2001; Boeke *et al*., 2004; Talukdar *et al*., 2004; Tooba *et al*., 2005; Epidi *et al*., 2008). Unlike insecticides, which mostly kill insects, plant ingredients are known to suppress the feeding and breeding behaviour of insects in many ways in addition to direct mortality (Jilani, 1984). Use of plant parts with insecticidal properties have been reported from all over the world as they are convenient, less expensive, highly effective and safer for the humans and environment. Plants such as *Azadirachta indica (A. juss)*, *Cassia fistula (L.)*, *Calotropis procera (Ait)*, *Lantana camara (L.) and Chrysanthenum coronarium (L.)* have shown insecticidal, antifeedant, repellant and growth regulating properties against various stored grain pests like *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (Fabr.), *Trogoderma granarium* (Everts.) and *Callosobruchus chinensis* (L.) (Gautam *et al*., 2003; Deka& Singh, 2005; Singh & Singh, 2005; Hazan *et al*., 2006; Prakash & Rao, 2006; Kestenholz *et al*., 2007; Neoliya *et al*., 2007; Sankari&Swamy, 2007). They are widely used as traditional stored grain protectants in powder form, crude mixtures or extracts due to their easy accessibility and biodegradable nature (Dwivedi& Garg, 2003).

The main objectives of this study was manege of lesser grain borer *Rhyzopertha dominica* using two botanicals as the natural products in relative to the insecticides.

The spescefic objectives:

1- To evaluate the effect of Usher aqueus extract on the mortality of lesser grain borer *R. dominica.*
2- To evaluate the effect of Cafur aqueus extract on the mortality of lesser grain borer *R. dominica.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum:

2.1.1 Classification

Order: Poales

Family: Poaceae

Genus: Sorghum

Scientific name: *Sorghum bicolor*

Common names: Grain sorghum, Mabele, Amazimba, Amabele

2.1.2 Description

Sorghum is an upright, short-day, summer annual that is a member of the Poaceae family. The grass blades are flat, stems are rigid, and there are no creeping rhizomes. Sorghum has a loose, open panicle of short, few-flowered racemes. As seed matures, the panicle may droop. Glumes vary in color from red or reddish brown to yellowish and are at least three quarters as long as the elliptical grain. The grain is predominately red or reddish brown (Kearney and Peebles, 1969; Barkworth, 2003).

Sorghums exhibit different heights and maturity dates depending on whether they are grain sorghums (*Sorghum bicolor* ssp. *bicolor*), forage sorghums *(Sorghum bicolor)*, Sudan grass *(Sorghum bicolor* ssp. *drummondii*), or sorghum-Sudan grass hybrids *(Sorghum bicolor* x *Sorghum bicolor var.*

sudanense). Growth characteristics also vary depending on the location grown, inputs, and agronomic practices. In general, forage sorghums are taller plants with later maturity dates and more vegetative growth than grain sorghums. Sudan grass and sorghum-Sudan grass hybrids fall in between grain sorghums and forage sorghums in height (Undersander, 2003).

2.1.3 Economic importance

Sorghum (*Sorghum bicolor* (L.) Moench) is the fourth most important cereal crop following wheat, rice and maize. It is a food staple for more than 500 million people in the semi-arid tropics of Africa and Asia and more than 80% of the world area of production is confined to these two continents. In sub-Saharan Africa, over 100 million people depend on sorghum as staple food (GIZ, 2014).

Sorghum is widely produced in the Sudan by the traditional, semi-mechanized and large commercial farms. Technology is developed and supplied by the Agricultural Research Corporation and the universities, and the extension is responsible for the dissemination part. Up until recently, this process was informal and in-efficient but of late efforts are being made to formalize the linkages, and the research and extension have come to the extent of organizing common platforms to expedite the technology promotion process (Reda, 2014).

The technology and input supply and distribution system is fairly well developed in the country and it is fully liberalized. There are a host of private companies with agents in the districts handling seed and input sales and direct delivery. Cooperatives are also involved in purchasing from companies and distributing to their member farmers. However, access to input credit is limited because it does not come on time and the process is time consuming. Sudan Agricultural Bank, Farmer commercial bank are involved in credit provision. Although, there is a big commercial company called Shekan with crop insurance provisions very few take benefit of the privilege because of lack of awareness, religious and other reasons(Reda, 2014).

The harvest is estimated to result in a national surplus of 0.98 million MT of sorghum. The government has lifted bans on exports to neighboring countries, and exports have begun to Ethiopia and Eritrea. However, formal exports have not resumed to South Sudan, given ongoing border tensions, though informal flows continue. In most years, the Sudan has a surplus of sorghum and a balance of millet, but has a great wheat deficit. Sorghum is the staple food for most people living in the Sudan, except for the two northern states where wheat is the traditional staple. It is consumed in a number of ways, most notably as a flat bread or pancake known as "*kisra"* and as a pudding known as "*acida*". Large quantities of sorghum, particularly in the western and southern states are made into beer known as "*marisa*". The total population in Sudan is estimated at 30 million, consuming about 3.1million tons of sorghum annually (2.7 million tones for human consumption and400, 000tones for animal feed). This represents a daily human consumption rate ofabout 250 g. of sorghum in one form or another (Mahgoub, 2014).

2.2.3 Main stored grain pests in Sudan

The khapra beetle, *Trogoderma granarium*Everts, is the most destructive insect in the Sudan, particularly in the semi-arid hot dry northern part (Darling 1959). In the Red Sea coastal area, particularly at Port Sudan, severe infestations by many insects especially *Trogoderma granarium*and *Tribolium castaneum* Herbst were reported to occur throughout the year (Saad, 1978). *Rhizopertha dominica*is also an important grain pest particularly in central Sudan where most of the sorghum is produced and stored. *Sitophilus oryzae* L. which is not found in northern Sudan, is of great importance in the southern parts of central Sudan probably due to the prevailing high humidity during the rainy season (SeifElnasr 1992).

In the Sudan, *T. castaneum*is more common than *T. confusum* (Khalil, 1970), perhaps an indication that the latter species is less tolerant to hot conditions (Good, 1933 and SeifElnasr 1992). Other important insects include the Angoumois grain moth *Sitotroga cerealella* and the rice moth, *Corcyra cephalonica*. Neither *Sitophilus oryzae*n nor *Sitotroga cerealella* were found in underground pits (matmoras) (Itto- 1987; Shazali 1987).

Sitotroga cerealella is usually important in out – door or open storage, particularly sorghum stored on the heads or in loosely stacked sacks, because of its inability to penetrate deep into bulk stored grains. The population of all stored grain insects usually increases steadily during the rainy season, (June – October) with maximum numbers at the end of the season. The duration of storage also affects the relative abundance of insects as well as their succession (Shazali 1982).

2.3 Lesser grain borer

2.3.1 Classification

Order: Coleoptera

Family: Bostrichidae

S.N: *Rhyzopertha dominica*

(Darling, 1959)

2.3.2 Description

Potter (1935) provided a detailed description of all life stages of *R. dominica*. The egg is typically white when first laid, turning rose to brown before hatching. The egg is ovoid in shape, 0.6 mm in length, and 0.2 mm in diameter. There are usually four larval instars. The larvae are scarabaeiform, the first two instars are not recurved, the third and fourth instars have the head and thorax recurved towards the abdomen. The widths of the head from the first to the fourth instar are 0.13, 0.17, 0.26 and 0.41 mm, and the lengths of the larvae are 0.78, 1.08, 2.04 and 3.07 mm, respectively. The pupae are 3.91 mm in length, with 0.7 mm between the eyes. At the end of the abdomen, the male pupae have a pair of 2-segmented papillae fused to the abdomen for their entire length, whereas female papillae are 3-segmented and project from the abdomen. Adults are 2-3 mm in length, reddish-brown and cylindrical. The elytra are parallel-sided, the head is not visible from above, and the pronotum has rasp-like teeth at the front.

2.3.3 Distribution

Rhyzopertha dominica is thought to originate from the Indian subcontinent, but now has a cosmopolitan distribution. It is a serious pest of stored products throughout the tropics, Australia and the USA. It is also found in temperate countries, either because of its ability for prolonged flight or as a result of the international trade in food products.

2.3.4 Life cycle

Females of *R. dominica* lay between 200 and 500 eggs in their lifetime. The eggs are laid loose in grain. The lowest temperature at which *R. dominica* can complete development is 20°C; at this temperature, the development from egg to adult takes 90 days. The fastest rate of development occurs at 34°C; at this temperature the egg takes 2 days, the larvae 17 days, and the pupae 3 days to complete development. *R. dominica* is unable to complete development between 38 and 40°C. Adults live for 4-8 months. Under optimal conditions of 34°C and 14% grain moisture content, there is a 20-fold increase in the population of *R. dominica* after 4 weeks. It can successfully infest grain at 9% moisture content, but has higher fecundity, a faster rate of development, and lower mortality on grain of a higher moisture content. Adult males produce an aggregation pheromone in the frass that attracts both male and female adults. Adults are good flyers, and can be trapped in pheromone-baited flight traps placed several kilometres from grain stores. Adults can bore into intact kernels. The larvae of *R. dominica* are mobile.

2.3.5 Habitat

Rhyzopertha dominica is found mainly in cereal stores, and food and animal feed processing facilities. It has also been trapped using pheromone-baited flight traps several kilometers from any food storage or processing facility (Fields *et al*., 1993).

2. 3.6 Damage

Rhyzopertha dominica is a major pest of wheat (Flinn *et al*., 2004) and rice (Chanbang *et al*., 2008) around the world. Both larvae and adult produce frass and cause weight losses by feeding on grains. *R. dominica* infestation can reduce rice to dust (Emery and Nayak, 2007).

There are three aspects of the impact of *R. dominica* infestation: loss in the quantity of stored grain, loss in quality of stored seeds (Sánchez-Mariñez *et al*., 1997) and the cost to prevent or control infestations (Cuperus *et al*., 1990; Anonymous, 1998).

On wheat and rice, larvae consume both germ and endosperm during their development in grain and thus produce more frass than *Cryptolestes ferrugineus* and *Sitophilus granarius* (Campbell and Sinha, 1976). *R. dominica* is also capable of damaging grain, causing weight losses of up to 40%, compared to 19%, 14% and 10% for *S. oryzae*, *Tribolium castaneum* and *Ephestia cautella*, respectively (Sittusuang and Imura, 1987). Weight loss from individual kernels has also been reported with different varieties of triticale, a wheat-rye hybrid (Baker et al., 1991), and in rice infested with *R. dominica* (Nigam *et al*., 1977). *R. dominica* feeding on seed germ reduces germination rates and vigour of the grains and may be followed by secondary pests and fungi (Bashir, 2002).

2. 3.6.1 Food production and nutritional value

Rhyzopertha dominica infestation of wheat, maize and sorghum grains resulted in substantial changes in the contents of calcium, phosphorus, zinc, iron, copper and manganese (Jood and Kapoor 1992). also observed a reduction in the starch digestibility of maize, rice and sorghum in response to *R. dominica* infestation. Single or mixed populations of *Trogoderma granarium* (Khapra beetle) and *R. dominica* resulted in substantial reductions in the contents of total lipids, phospholipids, galactolipids and polar and nonpolar lipids of wheat, maize and sorghum (Jood *et al*., 1996). *R. dominica* has also been reported to decrease vitamin contents of grain; 75% level of infestation of cereal grains caused losses of 23 to 29% (thiamine), 13 to 18% (riboflavin) and 4 to 14% (niacin) (Jood and Kapoor, 1994).

Chapatis prepared from flours with more that 50% *R. dominica* and *T. granarium* infestation level tasted bitter (Jood*et al*., 1993). At 75% infestation level there was a significant reduction in protein nitrogen and true protein contents of three cereal grains (Jood and Kapoor, 1992).

2. 3.6.2 Economic Damage

It is difficult to estimate the actual costs incurred for the control of *R. dominica* because it is generally found in mixed population with other stored-product insect pests that also cause damage. Laboratory experiments have estimated that one *R. dominica* consumes 0.15 g of wheat in its life time (Campbell and Sinha, 1976; Storey et al., 1982). If all *R. dominica* completed their life cycle from 1976 to 1979 in the USA, they would have consumed 300,000 metric tonnes of wheat annually, or 0.5% of the total of stored wheat.

2. 3 .7 Host Range

Adults and larvae of *R. dominica* feed primarily on stored cereal seed including wheat, maize, rice, oats, barley, sorghum and millet. They are also found on a wide variety of foodstuffs including beans, dried chillies, turmeric, coriander, ginger, cassava chips, biscuits and wheat flour. There are several reports of the lesser grain borer being found in or attacking wood (Potter, 1935), as is typical of other Bostrichidae. *R. dominica* has been reported to produce progeny on the seeds of some trees and shrubs (acorns, hackberry [*Celtisoccid entalis*] and buckbrush [*Symphoricarposor biculatus*]) (Wright *et al*., 1990).

2.3.8 Control

2.3.8.1 Physical control

Physical control of *R. dominica* involves the manipulation of the temperature, relative humidity, atmospheric composition (air gases composition), sanitation, ionizing radiation and the removal of adult insects from the grain either by sieving or air. All these practices may be helpful in eliminating or reducing insect pest infestations to a tolerable level (Jayas *et al*., 1995).

2.3.8.2 Sanitation and hermetic sealing

Cleaning during grading operations, drying, cool storage and hermetically sealed packaging can all play an effective role in conserving the seed viability with residue free pest control.

Grain packaging in airtight structures is one of the most important physical methods controlling *R. dominica*. These structures may range from well-sealed barrels holding several kilograms to 100-t capacity metal bins. The structures should be pressure-tested to confirm airtightness. Portable hermetic storage bags are also available (Garcia-Lara *et al*., 2013).

Removing insects by sieving is not equally effective for all species as several insect species, including *R. dominica*, spend most time of their life cycle remaining inside the grain or kernel. Impacting the grain, either by moving the grain using a pneumatic conveyer or dropping the grain onto a spinning, studded disc, can reduce *R. dominica* populations by over 90%. Good sanitation, particularly the removal of spilt grain around storage facilities, is a preliminary step in reducing insect populations that can infest grain in storage.

2.3.8.3 Aeration and drying

One of the more effective non-chemical control methods is to cool the grain with aeration fans, which gradually suppresses insect population growth in the storage period. The Kansas State extension program has advocated early aeration as the best non-chemical insect suppression method; a study conducted in Kansas, USA, exhibited that aeration starting from harvesting, using automatic fan controllers, allowed safe storage of grain for several months (Reed and Harner, 1998).

A moisture content (mc) of 25% is not uncommon in newly harvested grain in humid regions, but grains with 14% mc can be safely stored for 2-3 months. For longer storage periods, from 4-12 months, the moisture content must be reduced further. Reducing grain moisture content reduces the number of eggs produced and the survival of offspring and adults. There are 3 types of drying: ambient air drying, sun drying and mechanical drying. In ambient air drying system, air is heated and passed through grain to produce a relatively high vapour pressure gradient between the moisture in the grain and the moisture in the drying air. This gradient causes moisture to move from the grain into the air, where it is then exhausted from the grain bulk to the outside atmosphere (Jones *et al*., 2012). In many countries in Asia, Africa, and Latin America grain drying is achieved by spreading a thin layer of grain in the sun, on the threshing floor or on rooftops. A mechanical way to remove the water from wet grains is by blowing (heated) air through the grain. Mechanical drying of wheat grain is not practiced in many the developing countries, which largely rely on sun drying. At 34°C and 14% moisture content there were 109 *R. dominica* adults produced per female per generation; 10 adults at 10% moisture content, 0.3 adults at 9% and none at 8% moisture content (Birch, 1953).

2.3.8.4 Radiation

Radio-frequency heat treatment is increasingly used as a new thermal method for the disinfection of post-harvest insect populations in agricultural commodities (Tang *et al*., 2000). The application of this method leaves no chemical residue and provides acceptable product quality with minimal environmental impacts (Wang *et al*., 2003). Janhang et al. (2005) evaluated the efficiency of radio-frequency heat treatment against *R. dominica* both on the seed surface and inside the seed. The rice kernels with 10.4% moisture content and 93% germination rate were treated with radio frequency heat treatment at 27.12 MHz at 70, 75, 80 and 85 °C for 180 seconds. 100% mortality of *R. dominica* was achieved in all treatments; however, the rice seed quality was also decreased at higher temperatures. Phosphine-resistant adults were found to be more tolerant than phosphine-susceptible adults toward soft-electron and gamma radiation (Hasan *et al*., 2006).

More recently, a flameless catalytic infrared emitter was used to disinfest hard red winter wheat containing different life stages (eggs, larvae, pupae and 2-week-old adults) of *R. dominica* (Khamis *et al*., 2010). Approximately 94% mortality of all *R. dominica* life stages occurred when 113.5 g of wheat was exposed for 60 s at a distance of 8.0 cm from the emitter, resulting in wheat temperatures that ranged between 107.6 ± 1.4 and 113.5 ± 0.5°C. These findings suggested this technology as a promising tool for disinfestation of stored wheat (Khamis et al., 2010).

2.3.8.5 Controlled atmosphere

Reducing temperatures to below 34°C reduces the rate at which the population of *R. dominica* increases. *R. dominica* can not complete its life cycle below 20°C. In temperate countries, grain temperatures can be reduced by forcing air from outside through the grain, especially in winter. Grain can also be cooled by aeration using refrigerated air. Commercial units are available for both types of cooling. Increasing grain temperature to above 34°C also reduces the rate at which the population of *R. dominica* increases.

Although *R. dominica* is one of the most heat tolerant of all stored grain insect pests, it can be controlled by heating the grain to 65°C in 4 minutes, and rapidly cooling it to below 30°C. Commercial units that can handle 150 t of grain/h have been developed in Australia. The running costs of these units are comparable to those of chemical control. Care must be taken to ensure that the commodities in storage are only heated briefly so that the quality of the grain is not reduced. Heat disinfestation of grain has the potential for higher market acceptance than chemically treated grains. (Beckett and Morton, 2003).

Two interrelated ways have been used to make the method more affordable: one is to decrease typical grain treatment temperatures and hold these temperatures for a time sufficient for disinfestation; the other is to increase the rate of heating to induce physiological heat shocks, thereby bringing about faster insect mortality. Using a spouted bed, the rates of heat-tolerant species *R. dominica* mortality were recorded over a range of grain temperatures (50°C to 60°C) at 12% moisture content. It was observed that when the initial rate of heating was increased by increasing the air inlet temperature from 80-100°C, the time required for a given level of mortality was significantly decreased. Moreover, decreasing the target grain temperature and increasing the treatment period accounted for additional cost savings. For instance, at the most rapid rate of heating, grain that reached 60°C required 0.73 min of heat soak for 99.9% mortality and cost a theoretical US$ 2.72/t, while grain that reached 55°C required 23.62 min for the same level of mortality and cost US$ 1.87/t. By 50°C, 22 h were required, but the theoretical running cost was reduced to US$ 1.25/t (Beckett and Morton, 2003).

Manipulation of storage temperature is a relatively new technology that may be used to a greater extent in the future. The manipulation of gases (nitrogen (N_2), oxygen (O_2) and carbon dioxide (CO_2) within storage structures has been widely studied for the control of insect infestations. The two main approaches involve increasing CO_2 concentration and reducing oxygen in the storage vicinity. To control the insect infestations, oxygen levels must be maintained below 1% for 20 days, or carbon dioxide levels maintained at 80% for 9 days, 60% for 11 days or 40% for 17 days. The storage structures should be sealed properly before the addition of gases (Annis and Graver, 1990).

The effectiveness of CO_2 at different temperatures (20, 25, 30, 35 and 40°C) and exposure intervals (6, 12, 18, 24, 30, 36, 48, 54 hr) was tested against the life stages of different stored grain insects including *R. dominica*. The eggs of *R. dominica* were particularly tolerant at 20°C, which required extended exposure to treatment (54 and 48 hr, respectively) to prevent the egg survival. The adults were highly susceptible and a 24-hr exposure at 20°C or 6-hr at temperatures of >30°C were enough to achieve 100% mortality (Locatelli and Daolio, 1993). The combination application of carbon dioxide (5-20%) with the fumigant ethyl formate significantly enhanced the effectiveness of the fumigant against *R. dominica* and living stages of some other stored grain insect species (Haritos *et al*., 2006).

2.3.8.6 Inert dusts

Inert dusts have been used as a traditional method of insect control for thousands of years (Glenn and Puterka, 2005). Stored grain insects are more vulnerable to these dusts as they feed upon dry grains and possess relatively larger surface to volume ratio (Stathers *et al*., 2004). There are several types of inert dusts being used in insect control programs, such as ash, lime, clay, diatomaceous earth (DE) and silica aerogel. The most effective inert dusts are DE and silica aerogel. Silica aerogels are man-made powders with smaller and more uniform particle sizes than DE. A major portion of silica aerogel and DE is made up of silicon dioxide, which dehydrates the insect body by both cuticle lipid absorption and abrasion (Quarles and Winn, 1996). Although DEs have low mammalian toxicity (Athanassiou *et al*., 2004), most DE formulations are used at considerably high application rates for the effective insect pest control (Vayias *et al*., 2006). At high concentrations, DE reduces grain bulk density by 9% and flow rate by 39% (Jackson and Webley, 1994), which is considered unacceptable for many large-scale commercial farms. These levels of loss may be more acceptable for households or subsistence farms.

Diatomaceous earth from different geological sources have different efficacies (Nwaubani *et al*., 2014), and the concentrations required to control infestations must be assessed before use. Other factors determining the efficacy of any DE formulation include: test insect population (Wakil *et al*., 2013), test insect species (Vassilakos*et al*., 2006), exposure interval (Baldassari *et al*., 2008), dose rate (Wakil *et al*., 2010) and temperature and relative humidity (Chanbang *et al*., 2007).

Rhyzoperthadominica is relatively tolerant to DE, and concentrations between 500 and 1000 ppm are required to control populations (Subramanyam *et al*., 1994). However, by the introduction of enhanced DE formulations, an effective control of this insect species is possible, even at a dose rate of 50 ppm (Wakil *et al*., 2011; Riasat *et al*., 2013). Furthermore, the combined use of DE with other insect control methods could provide effective control of *R. dominica* (Lord, 2005; Athanassiou *et al*., 2008; Wakil *et al*., 2012; 2013).

2.3.8.7 Host plant resistance

Although there are substantial differences in the resistance of host varieties to *R. dominica* (Kishore, 1993; Cortez-Rocha *et al*., 1993), the use of resistant varieties has not been exploited as a method of control. Resistant varieties often do not prevent insect infestations, but reduce the rate at which infestations develop, and increase mortalities. Host resistance would enable the crop to be stored for a longer period before extensive damage is caused by insect populations. 28 different varieties of short, long and medium size rice kernel exhibited variable level of resistance to *R. dominica*, as measured on the Dobie Index of susceptibility (Chanbang *et al*., 2008).

Adult *R. dominica* mortality due to DE treatment was generally greater on more resistant varieties of rough rice compared to less resistant varieties (Chanbang *et al*., 2008).

The susceptibility of six milled rice varieties (IR-64, Ciherang, Membramo, Cibogo, Sembada, and Intani-2) to *R. dominica* was studied under laboratory conditions (Astuti *et al*., 2013). The susceptibility was measured on the basis of number of eggs laid by female insects, the number of F1 progeny emerged, the weight loss of infested samples and also using the Dobie index of susceptibility. Milled rice varieties with high phenolic content and hardness may show resistance to *R. dominica* infestation.

However, caution is needed with regard to the introduction of resistant varieties as a method of control of *R. dominica*; the insect may overcome host plant resistance, as it has developed resistance to insecticides, and the development of further resistance management strategies would be required.

2.3.8.8 Botanical insecticides

Over the last 15 years, due to environmental concerns and insect pest resistance to conventional chemicals, interest in botanical insecticides has increased. Botanical insecticides are naturally occurring insecticides which are derived from plants (Golob *et al*., 1999; Isman, 2000). Compared to synthetic compounds they are less harmful to the environment, generally less expensive, and easily processed and used by farmers and small industries.

Botanical insecticides are used in several forms, such as powders, solvent extracts, essential oils and whole plants, these preparations have been investigated for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators (Weaver and Subramanyam, 2000).

The introduction of powdered leaves of *Salvia officinalis* L. and *Artemisia absinthium* L. to wheat grains was very effective in reducing population size and delaying development time of *R. dominica* (Klys, 2004).

Natural feeding inhibitors found in either wild or cultivated plants are usually alkaloids and glycosides. The mode of action of these compounds is complex and poorly understood, although it is found that insects exposed to such substances usually stop feeding, resulting in a decreased body weight or even death if the insects fail to feed for a long period of time.

Plant essential oils and solvent extracts are the most studied botanical methods of controlling stored grain insect infestations (Stoll, 2000; Shaaya *et al*., 2003; Moreira *et al*., 2007; Rozman *et al*., 2007; Rajendran and Sriranjini, 2008). The essential oils obtained from different plant species repel several insect pests and possess ovicidal and larvicidal properties. Although they are considered by some as environmentally compatible pesticides (Cetin *et al*., 2004), some botanicals, especially essential oils, are toxic to a broad range of animals, including mammals (Bakkali *et al*., 2008). Suthisut *et al*. (2011a, b) found that some natural products were actually more dangerous to use than the commercial insecticides, because much more of the product is needed to control the insects than the fumigant or the synthetic contact insecticide.

Moreira *et al*. (2007) reported that *R. dominica* was more susceptible than *Sitophilus zeamais* and *Oryzaephilus surinamensis* to hexane crude extract of *Ageratum conyzoides*, experiencing more than 88% mortality after 24 hr of exposure.

Plant oils are also used for their fumigant activity against *R. dominica* (Lee *et al*., 2004) on the basis of their efficacy, economic value and use in large-scale storages.

In spite of the wide-spread recognition of insecticidal properties of plants, few commercial products obtained from plants are in use and botanicals used as insecticides presently constitute only 1% of the world insecticide market (Rozman *et al*., 2007).

2.3.8.9 Biological control

The use of natural enemies to control *R. dominica* and other stored grain insects has been limited in developed countries because of the low tolerance (0-2 insects/kg grain) of insects in stored grain. However, because of the interest in controlling insect pests without the use of insecticides, there has been renewed interest in predators and parasites (Brower *et al*., 1991). Despite this, research on the potential use of bio-control agents of stored grain insects has been limited to a small number of species.

2.3.8.9.1 Predators

There have been several laboratory studies on the use of predators of *R. dominica* (Brower *et al*., 1991). *Teretriosoma nigrescens* is a histerid beetle that is found in Central America, where it primarily feeds on *Prostephanus truncates*, a species closely related to *R. dominica*. It is able to feed on *R. dominica*. However, the ability of *T. nigrescens* to significantly reduce *R. dominica* populations has yet to be determined (Markham *et al*., 1994).

Xylocoris flavipes (Hemiptera: Anthocoridae) is a predator of many stored product insect pest (Rahman *et al*., 2009). The cadelle *Tenebroides mauritanicus* also feeds on grain, mites and stored-product insect eggs, including *Rhyzopertha* (Bousquet, 1990). The predatory mites *Cheyletus eruditus* and *Pyemotes ventricosus* feed on a wide variety of stored product insect eggs (Asanov, 1980; Brower *et al*., 1991), but their effect on populations in the field has not been determined. Among the four *Cheyletus* species found in storage structures of Central Europe, only *C. eruditus* is employed for the biocontrol of stored grain insect pests (Lukáš *et al*., 2007).

2.3.8.9.2 Parasites and parasitoids

Most of the parasitoids that attack the primary beetle pests are in the families Pteromalidae and Bethylidae. These hymenopteran parasitoids are very small, do not feed on the grain and can easily be removed from the grains by using normal cleaning processes. *Choetospila elegans* is a small pteromalid wasp that attacks *R. dominica* and certain other coleopteran and lepidopeteran insect pests. The wasp normally parasitizes larvae that are feeding inside the grain. At 32°C, a wasp takes approximately 15 days to complete its development on *R. dominica*; the generation time of *C. elegans* is almost half that of *R. dominica.* In the presence of hosts, female wasps live for 10-20 days at 32°C. A single female *C. elegans* is capable of parasitizing up to six *R. dominica* per day.

During a field study, Flinn (1998) observed that *C. elegans* suppressed *R. dominica* by 91% in 27 ton bins of stored wheat compared with control bins. In another study it was found that suppression of *R. dominica* population growth by parasitoid wasps was significantly higher at 25°C than at 32°C and that 25°C resulted in a very high level of population suppression (99%) compared to the control (Flinn, 1998). Another hymenopteran parasitoid, *Anisopteromalus calandrae*, is effective at reducing *R. dominica* populations.

The hymenopteran parasitoid *Anisopteromalus calandrae* suppressed *R. dominica* populations in all types of storage bag except those made of polythene. The highest percentage (81%) suppression occurred in calico bags and the lowest suppression (57%) occurred in polypropylene bags (Mahal *et al*., 2005).

The egg parasitic mite *Acarophenax lacunatus* significantly reduces the population of *R. dominica* (Faroni *et al*., 2000; Gonçalves *et al*., 2004).

2.3.8.9.3 Entomopathogens

The use of entomopathogenic fungi has been evaluated extensively in laboratory and field studies against *R. dominica*. The pathogenicity of entomophaghous fungi depends upon various physical (temperature, relative humidity, application time of fungal insecticide, dark and light period etc.) and biological factors (the specific host species, host pathogen interaction etc.). Unlike other microbial control agents, fungi possess the ability to infect the insects through cuticle (Boucias and Pendland, 1991; Thomas and Read, 2007). *Beauveria bassiana* (Ascomycota: Hyphomycetes) and *Metarhizium anisopliae* (Ascomycota: Sordario) are the most extensively studies fungal species in this regard (Lord, 2005; Vassilakos *et al*., 2006; Athanassiou *et al*., 2008; Wakil and Ghazanfar, 2010).

More recently, various native entomopathogenic fungi, isolated from different components of the maize agroecosystem, he shown virulence against *R. dominica* and two other stored maize insect species. *Paecilomyces* and *Metarhizium* were the most abundant genera isolated from the soil, wheras the isolates of *Purpureocillium lilacinum* were the best in controlling target insect species (Barra *et al*., 2013).

Entomopathogenic fungi have also been tested in combination with other control tactics: for example, Isariafumos orosea with enhanced diatomaceous earth and the plant extract bitterbarkomycin *B. bassiana* and enhanced diatomaceous earth (Wakil *et al*., 2011); *B. bassiana* admixed with a diatomaceou s earth formulation (Riasat *et al*., 2011); and *B. bassiana* with thiamethoxam and a diatomaceous earth formulation (Wakil *et al*., 2012). The results demonstrated that such combined controls could be an effective strategy to control *R. dominica* in stored wheat.

Bacillus thuringiensis var. tenebrionis has been investigated for the control of *R. dominica* (Keever, 1994; Mummigatti *et al*., 1994). Most *B. thuringiensis* varieties are ineffective against beetles; however, *R. dominica* was one of the more susceptible beetles to *B. thuringiensis* var. tenebrionis, with more than 75% mortality in 17 days at 250 ppm (Mummigatti *et al*., 1994). Toxins of 36 available subspecies of *B. thuriengensis* were evaluated against larvae and adults of *R. dominica*. The spore crystal complex of *B. thuringiensis darmstadiensis* obtained from Germany was the most effective against larvae, but the same subspecies from USA and Japan could not effectively control *R. dominica* (Beegle, 1996).

In a recent study, the combination of Cry3Aa protoxin and protease inhibitor (potato carboxypeptidase) resulted in delayed development, increased mortality and progeny suppression of *R. dominica* (Oppert *et al*., 2011).

Entomopathogenic nematodes (EPNs) are endoparasites of insects (Gaugler, 2002), that enter into host through natural body openings and release mutualistic bacteria inside the host's body that kills it within 24-48 hours. Their low toxicity to vertebrates (Boemare *et al*., 1996), exemption from registration in the USA by the Environmental Protection Agency (Kaya and Gaugler, 1993), commercial availability (Grewal, 2002) and ability to seek their host actively (Campbell and Lewis, 2002) make EPNs potentially good biological control agents for stored-product pests. However, they have not proved very effective against *R. dominica*; *R. dominica* suffered only 35% adult mortality to two EPN species (in Heterorhabditidae and Steinernematidae) (Ramos-Rodríguez *et al*., 2006). Similarly, at 20^{0}C, the mortality of adult *R. dominica* in wheat treated with *Steinernema feltiae* and *Steinernema carpocapsae* (at 20,000 infected juveniles per ml) did not exceed 23 and 42%, respectively (Athanassiou *et al*., 2010a).

2.3.8.10 Chemical control

The insecticidal efficacy of different group of insecticides varies with the surfaces on which they are applied, as insecticides degrade faster on concrete than on wood or metal (Collins *et al*., 2000). Deltamethrin was more effective on plywood than on concrete or tile surface against *R. dominica* and *Tribolium* spp. released for 21 weeks (Arthur, 1997).

Chlorpyrifos-methyl and pirimiphos-methyl, although effective against most stored grain insect pests, are relatively ineffective against *R. dominica*.

R. dominica was the most susceptible species among the four stored grain insects (*R. dominica*, *T. castaneum*, *Sitophilus oryzae* and *Lepinotusre ticulatus*) to spinosad applied on wheat or maize (Athanassiou*et al*., 2010b). These findings coincide with that of Vayias *et al*., (2009), who stated that *R. dominica* mortality were extremely high on wheat, corn, barley and rice, even when treated low levels of spinosad.

Insect growth regulators (IGR) have low toxicity to mammals, but take longer to control insect populations and are more expensive than other insecticides. They can be sprayed or dusted directly onto the grain, and protect grain from infestation from two weeks to over a year. Methoprene is an IGR commercial formulation labelled in the USA for direct use on to stored grains. In addition to externally feeding stored-grain insects, it is also an effective grain protectant against *R. dominica* (Arthur, 2004). The combination of methoprene and DE not only eliminated progeny production of *R. dominica* on stored rice but it also provided a measure of adult control (Chanbang *et al*., 2007). Kavallieratos *et al*. (2012) found that IGRs, including two juvenile hormone analogues, four chitin synthesis inhibitors, one ecdysteroid agonist and one combination of chitin synthesis inhibitors and juvenile hormone analogues, tested against *R. dominica* in wheat resulted in >88.5% suppression of progeny. The highest level of suppression was at 5 ppm. A juvenile hormone analogue (pyriproxyfen) and chitin synthesis inhibitor (lufenuron) suppressed progeny production 100% when applied at 1 ppm, and the highest values of parental mortality were observed in wheat treated with combination of chitin synthesis inhibitors and juvenile hormone analogues (lufenuron + fenoxycarb).

Neonicotinoids are broad spectrum insecticides that can be applied in different ways, such as foliar, soil drench, seed treatment and stem applications, and to various crops (Schulz et al., 2009; Jeschke and Nauen, 2010). Imidacloprid and indoxacarb can control *R. dominica,* but these chemicals are not yet registered for use on stored grains (Daglish and Nayak, 2012).

The integration of different control measures has been suggested to be one of the most promising approaches to insect management. Daglish and Wallbank (2005) used methoprene + diflubenzeron on stored sorghum and found 98.5-100% reduction in the progeny production of *R. dominica* four weeks after exposure. On the other hand, the combination of spinosad + *s*-methoprene could not control methoprene-resistant strain of *R. dominica* (Daglish, 2008). A combination of thiamethoxam and DE also exhibited promising insecticidal potential against *R. dominica*in laboratory studies (Wakil *et al*., 2012).

Phosphine is commonly used as a fumigant, is used to control insect infestations in stored commodities. Although it is effective, commodities can become re-infested once the fumigant has dissipated. Phosphine is also highly toxic to humans and should be handled with extreme care. It is usually applied to the grain as aluminium phosphide pellets or tablets, although magnesium phosphide is also available in some countries. Some countries allow phosphine to be delivered to the grain from compressed gas cylinders. A system has been developed in Australia in which phosphine mixed with carbon dioxide is delivered to the grain at low concentrations and continuous flow for several weeks. At high temperatures and humidity regimes, phosphine is corrosive to copper and can cause damage to electrical systems.

After treatment with an insecticide, grain often must be held for a certain period of time before it can be processed or used as animal feed. The period of protection is dependant upon the commodity treated, the temperature, grain moisture content and the insecticide used. In general, temperatures must be over 15°C for effective control; higher temperatures cause more rapid control but also cause faster degradation of the insecticide. High moisture content also reduces the duration of protection. Many of these insecticides can be used as a structural treatment to eliminate residual insect populations from empty silos and buildings.

2.4. Extracts

2.4.1 Cafur plant *Eucalyptus camaldulensis*:

2.4.1.1 Classification

Class: Dicotyledonae

Order: Myrtales

Family: Myrtaceae

Genus: Eucalyptus

Species: Eucalyptus

S.N*: Eucalyptus camaldulensis*

Notes: *Eucalyptus camaldulensis* exhibits considerable morphological variation throughout its range, and consequently a number of infra specific taxa have been described. Var. *camaldulensis*is the most widespread, and the only one occurring in the Murray-Darling Basin. For further discussion on morphological variation, Brooker *et al.*, (2002). Chemical and genetic variation has also been recorded in *E. camaldulensis* (Doran and Brophy, 1990; Stone and Bacon, 1994; Butcher *et al.*, 2001).

2.4.1.2 Description

Australia, *E. camaldulensis* commonly grows up to 20 m tall and rarely exceeds 50 m, while stem diameter at breast height can reach 1-2 m or more. In open woodlands it usually has a short, thick bole which supports a large, spreading crown. In plantations, it can have a clear bole of up to 20 m with an erect, lightly-branched crown. The bark is smooth white, grey, yellow-green, grey-green, or pinkish grey, shedding in strips or irregular flakes. Rough bark may sometimes occupy the first 1-2 m of the trunk on *E. camaldulensis var. camaldulensis*. This species is described in many texts including Boland *et al.*, (1984), Brooker and Kleinig (1983; 1990; 1994), Doran and Turnbull (1997). Juvenile leaves are petiolate, ovate to broadly lanceolate, up to 26 cm long and 8 cm broad, green, grey-green, or blue-green, slightly discolorous. Adult leaves are lanceolate to narrowly lanceolate, acuminate, lamina 8-30 cm long, 0.7-2 cm wide, green or grey-green, concolorous; petioles terete or channelled, 1.2-1.5 cm long. Inflorescence axillary, 7-11 (sometimes up to 13)-flowered; flowers white, peduncles slender, terete or quadrangular, 6-15 mm long; pedicels slender, 5-12 mm long. Buds pedicellate; hypanthium hemispherical, 2-3 mm long, 3-6 mm wide, operculum globular-rostrate (typical) ovoid-conical (var. obtusa) or, in subsp. simulata, horn-shaped like E. tereticornis, 4-6 mm long (up to 13 mm long in subsp. simulata), 3-6 mm wide. Fruits are hemispherical or ovoid, 5-8 mm long and wide; disc broad, ascending; 3-5 exserted valves.

2.4.1.3 Life form

*Eucalyptus camaldulensis*is a perennial, single-stemmed, largeboled, medium-sized to tall tree to 30 m high (Bren and Gibbs, 1986), although some authors (Boland, 1984; Brooker *et al.*, 2002) record trees to 45 m. According to Jacobs (1955) river red gum could reach ages of 500 to 1000 years. Brooker *et al*. (2002) for further descriptive information.

2.4.1.4 Uses

River red gum forests are historically and culturally important due to the number of significant Aboriginal sites they contain. Common relics include

Canoe and shield trees. Such trees show scars where the bark was removed. The wood has been used for heavy construction, railway sleepers, flooring, framing, fencing, plywood and veneer manufacture, wood turning, firewood and charcoal production (Boland, 1984). Useful for shade, shelter and in windbreaks; deep-rooted, which allows grass growth right up to the base; bark is resistant to stock damage. For southeastern Australia, *E. camaldulensis* along with *Eucalyptus melliodora* (Yellow Box) and *Acacia melanoxylon* (Blackwood) are considered superior shade trees. It is also attractive as an ornamental for acreage plantings.

2.4.3.5 Medical use

Volatile oils which are introduced into medical use contain 55- 70% lineol, plus lesser amounts of volatile aldehydes (Varro, *et al*., 1981). Essential oils of *Eucalyptus* spp. were used as an antibacterial, antimicrobial and acaricidal agent (Bagherwl, 1999; Harkenthal, *et al.*, 1999; and Lisin, *et al.*, 1999).
The antioxidant activities of the volatile oil and the ethanol extract as well as that of the tree bark were evaluated by the thiocyanate method. The ethanol extract of *Eucalyptus* fruit exhibited considerable activity compared with butylatedhydroxyanisole and tertiary butylated hydroquinone. The high inhibitory effect of the fruit ethanol extract on linoleic acid after 12 days might be related to the higher ellagic acid content. (Al-Ghorab, *et al*., 2002).

2.4.3.6 Insecticidal activity

The toxicity of leaves oil obtained from *Eucalyptus camaldulensis* by steam distillation was tested against the fourth larval instar of *Anopheles stephensi*. The LD50 for the larvae was 113 ppm (Kumar and Dutta, 1987). *E. Camaldulensis* reduced the number of galls of the nematode *Melodogyne arenaria* and eggs masses by 70 – 85 and 81-89 %, respectively (Ibrahim, *et al.,* 1998). Also *E. camaldulensis* suppressed egg hatching of *M. arenaria* to varying extents, depending on the concentration used (Shahda, *et al.,* 1998). *E. camaldulensis* powder mixed with rice at a rate of 1% byweight was effective in reducing the number of adults of *Sitotroga cerealella* emerging per 100g rice to 77 compared with 369 in the untreated rice; and prevented cross-infestation by *Rhyzopertha dominica* (Dakshinamurthy, 1988). In Sudan Mohagir, (2000) studied the effect of application of *Eucalyptus camaldulensis* on tree locust and it reduced the frequency of molting, gave high mortality, deformation and antifeedent effect. *E. camaldulensis,* also was installed in 8 areas of termite activity in different ecological zones during 1985 – 1986; observations recorded after a few months indicated that *E. camaldulensis* was very effective against termites (Hanif, *et al*., 1988).

2.4.2 Usher Plant *Calotropis procera*

2.4.2.1 Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Dicotyledoneae

Order: AsclePeadales

Family: Asclepeadaceae

Genus: Calotropis

Species: *Calotropis procera*

2.4.2.2 Description

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

2.4.2.3 Distribution

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

According to rahman and wildcock (1991). *C. Procera* is native to West Africa as for south as Angola.North and East Africa, Madagascar, the Arabian Peninsula, southern Asia, and In to China to Malaysia, central and South America and the Caribbean Islands.

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

2.4.2.4 Used of Usher Plant

This plant has been widely used in the Sudanese medicinal system (Ayoub and Kingston, 1981; Ayoub and Srenden, 1981). The latex of the plant was reported having potential anti in flammatory, antidiarrhoeal, analogesic, antipyretic and Schizonticida activities (Kumar and Basu, 1994; Dewan, *et al;* 2000, Kumar *et al;* Sharma and Sharma, 2000). Bioactivities of the plant such as insecticidal (Jacoband Sheila, 1993; khan and Siddiqui, 1994; Moursy, 1997), a caricidal (ChungSamanyart, *et al*., 1994), rematocidal (Rakesh, et *al*., 2001), molluscidal (Hussein *et al*., 1994), had been reported.

Water containing latex of the plants was able to avoid adult females of *Anopheles Stephens* and *Culexfatigans* to oviposit in the water and the latex water could kill eggs and larvae of *A.Stephens*, *C. Fatigans* and *Aedesaegypti*, (Girdhar, *et al*., 1984).

Calotropis Extracts has been reported (Deka and Singh, 2001; Singh *et al*., 2002). Plant parts and plant extract can be used effectively because these are less expensive and biodegradable, hence environmentally suitable – many farmers in Asia and Africa had been using plant extracts such as Neem, wild tobacco, dried chillies, *calotropis procera* and wood ash etc. For controlling and repelling termites (Anonymous, 2000).

2.4.2.5 Chemical Properties

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

The steroidal component includes and hydroxyl group in the C3β position, as second attached to the carbon, a C/D – Cis ring junction and a, β – unsaturated – γ – Lactone in the (17 position. in the plants, the steroidal component commonly attached via a glycosidic link to 2-desoxy or a 2.6 didesoxy sugar molecule (Aiton, 2010).

The features described are those required for toxicity but in addition there can be other substitutions into the steroid nucleus. These can be a C19 – aldehyde in place of the more usual methyl group in position as well as additional hydroxyl functions and sometimes epoxide structures. In the case of the *calotropis* glycoside, their names are calotropin, calotoxin, uscharidin and vorusharin (The latter involve rare sugars with nitrogen and sulphur in the structures). The steroidal moiety (known as "calotropagenin", Formula, $C_{23}H_{32}O_2$) has one more unusual structure the C-19 formyl (CHO) group is present and there is an additional secondary alcohol as well as the common C3 and C14 hydroxyle Functions. The position of this third hydroxyl function remains in some doubt it was apparently established by the Swiss group under the dues Rechistein as being in the C2 position with an equatorial configuration. However, this assignment does not explain some of the non-features and behaviors of this molecule, in particular the absence of spin – spin coupling of the two axial protons associated with their geminal hydroxyl group and failure react iodateinacleavadge reaction which presence of such ariscinal 1, 2 diol would require (Aiton , 2010).

CHAPTER THREE

MATERISLS AND METHODS

A study was conducted at the laboratory of Entomology, College of Agricultural Studies, Sudan University of Science and Technology (SUST), Shambat, Sudan, during August to November 2016. To investigate the effect of two botanicals Cafur plant (*Eucalyptus camaldulensis*) and Usher plant (*Calotropis procera*) water extract against adults of lesser grain borer *Rhyzopertha dominica*.

3.1 Insects culture

Parent adults of tested insects were obtained from stock cultures maintained at grain Storage from laboratory conditions at Saiga laboratory at Al Bagair Office. The lesser grain borer, *R. dominica* were reared at the rate of 10 adults in jars containing 250 g of Sorghum grains. The jars were covered with muslin cloth tied with rubber bands and kept under laboratory conditions. (Plate 2)

3.2 Collection of plant materials

The leaves of the Usher Plant (*C. procera)* were collected from Al Bagair, and Cafur (*E. camaldulensis),* were collected from the experimental farm, college of agricultural studies, Shambat- North Khartoum, Sudan. The plants were identified and confirmed at herbarium, at Botany Department, Faculty of agriculture, Khartoum University, Sudan. The plants were washed and dried under shade for 5-7 days under room condition, and then powdered by using electrical blender. The prepared powder of each plant was kept safe in plastic bags until used.

3.3 Method of preparing the dilutions

The following master stock solutions were prepared:

About 30 g of powder for each plants (Cafur, and Usher) were added to 100 ml of tab water. Soaked in tap water, thoroughly agitated, left for 24 hours and finally filtered by a Buchner funnel. For lethal concentration (LC) determinations, two concentrations were prepared by further diluting the stock solutions. The serial dilutions started from the highest to the lowest concentration at dilutions ranging from 30 to 10 ml (plate 1 (a)&(b)).

Stock solution 30% concentration

To made the concentration 20% from 30% we used the dilution equation

$C_1*V_1 = C_2*V_2$

Such as

$C_{1=}$ concentration one

C_2 = concentration required

$V_{1\,=}$ volume one

$V_{2\,=}$ volume require

3.4 Botenicals application

The experiment was conducted in the laboratory at room temperature. The experimental unit consisted of petri dish each petri one contains 10 Insects and 10 grains of sorghum in every petri dish. The tested insects were assigned randomly with grains. Each petri was individually sprayed with 2.5 ml of each botenical concentration and tap water for the control, using perfume sprayer. Mortality was recorded after 24, 48 and 72 hr following treatment (plate 4).

3.5 Experiment design and Data analysis

These experiments were designed in a Complete Randomized Design.The obtained data was statistically analyzed according to analysis of variance (ANOVA); (plate 3). The data were analyzed using GenStat (Computer Program) Version4 and the means were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ (Gomez and Gomez, 1984).

3.7 Probit analysis

Environmental Protection Agency (EPA) Probit analysis program was used for calculating LC/ EC and LC_{50} values version 1.5 software for each plant extract.

Plate 1: (a) Extract of Cafur **(b) Extract of Usher**

Plate 2: insects' culture

Plate 3: Experiment Layout

Plate 4: Botenical application showed dead insects

CHAPTER FOUR

RESULTS

4.1 Effects of aqueous extract of Cafor and Usher plants against adult stage of *R. dominica* after 24 hours.

The best result was given by concentration 30 % Usher which gave 63.3 % adult mortality followed by concentration 20 % of same plant, which gave 40 % mortality .Cafur concentration 30 % which resulted in 36.6% mortality while a mortality of 33.3% adult was obtained by Cafur extract at 20%.The least percentage of mortality of 23.3 % given by Usher at concentration 10 % . All treatments are not significantly different from each other but significantly different from untreated control (Table 1, Fig 1, Appendix 1.4).

Table 1: Effects of aqueous extract of Cafor and Usher plant against adults stage of *R. dominica* after 24 hour.

Treatment	Concentrations	Mortality (%)
Cafor leaves powder	10	26.6 (29.6) b
Cafor leaves powder	20	33.3 (34.9)ab
Cafor leaves powder	30	36.6 (36.8)ab
Usher leaves powder	10	23.3 (27.2)b
Usher leaves powder	20	40 (39.1)ab
Usher leaves powder	30	63.3 (53)a
Control	-	0.0 (0.0)c
C.V(%)	-	17.23
SE±	-	0.5193

-Means at the same column with the same letter (s) are not significantly different at P≤0.05.

-Values between brackets were transformed using Arcsine.

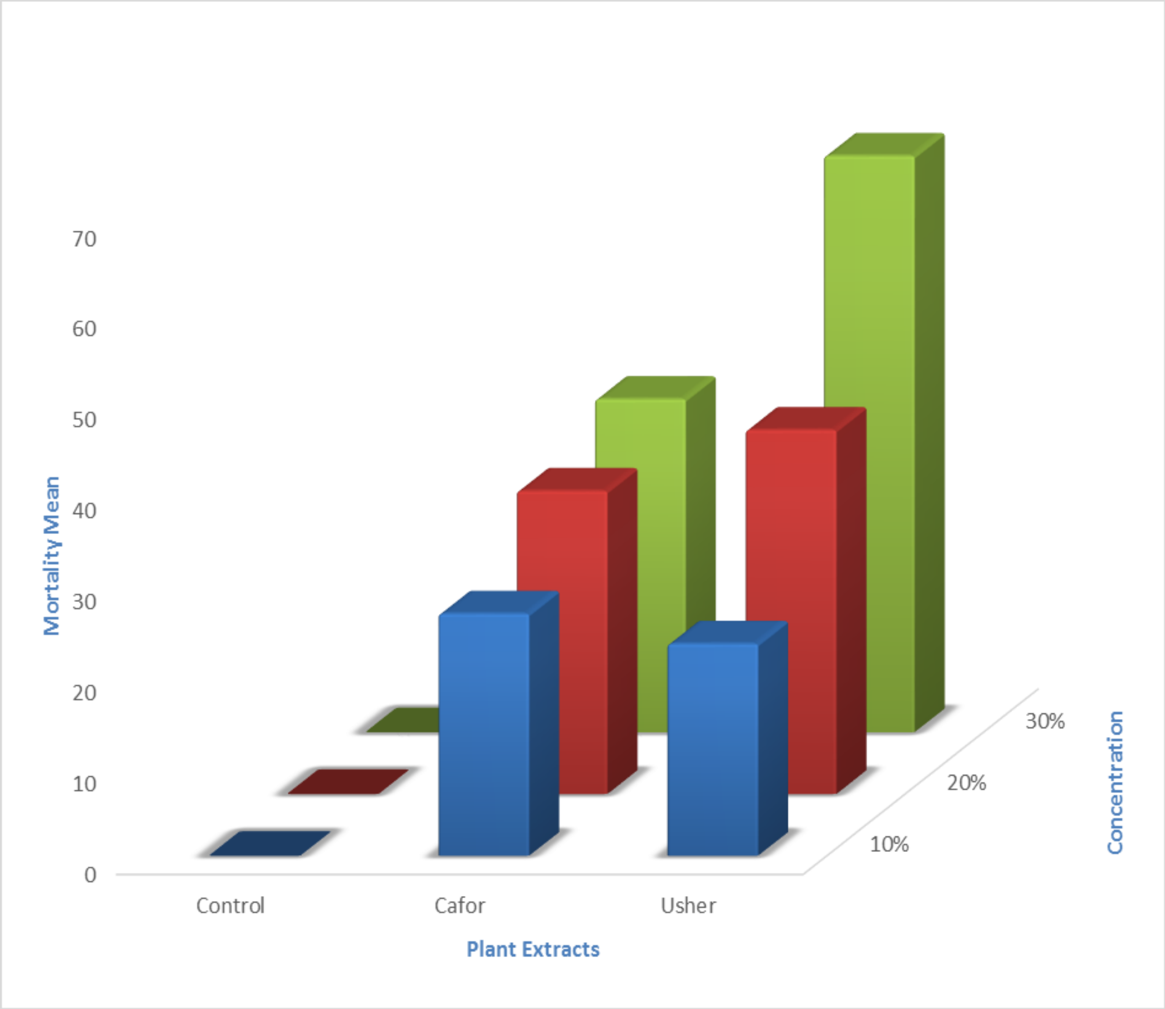

Fig 1: Effects of aqueous extract of Cafor and Usher plant against adult stage of *R. dominica* under laboratory conditions after 24 hour.

4.2 Effects of aqueous extract of Cafor and Usher plants against adult stage of *R. dominica* after 48 hours.

The best result was given by concentration 30% Usher which gave 66.6% adult mortality followed by concentration 20% of same plant, which gave 46.6% mortality .Cafur concentration 30 % which resulted in 40% mortality while a mortality of *36.6*% adult was obtained by Cafur extract at concentration 20% and Usher at concentration10%.The least percentage of mortality of 33.3 % given by Cafur at concentration 10%. All treatments are not significantly different from each other but significantly different from untreated control (Table 2, Fig 2, and Appendix 2.5).

Table 2: Effects of aqueous extract of Cafor and Usher plant against adult stage of *R. dominica* after 48 hours.

- Treatment	- Concentrations	- Mortality (%)
Caforleaves powder	10	33.3 (34)b
Caforleaves powder	20	36.6 (36.8)ab
Caforleaves powder	30	40 (39)ab
Usherleavespowder	10	36.6 (36.8)ab
Usherleavespowder	20	46.6(44.9)ab
Usherleavespowder	30	66.6 (55.0)a
Control	-	0.0 (0.0) c
C.V (%)	-	14.69
SE±	-	0.4925

-Means at the same column with the same letter (s) are not significantly different at P≤0.05.

-Values between brackets were transformed using Arcsine.

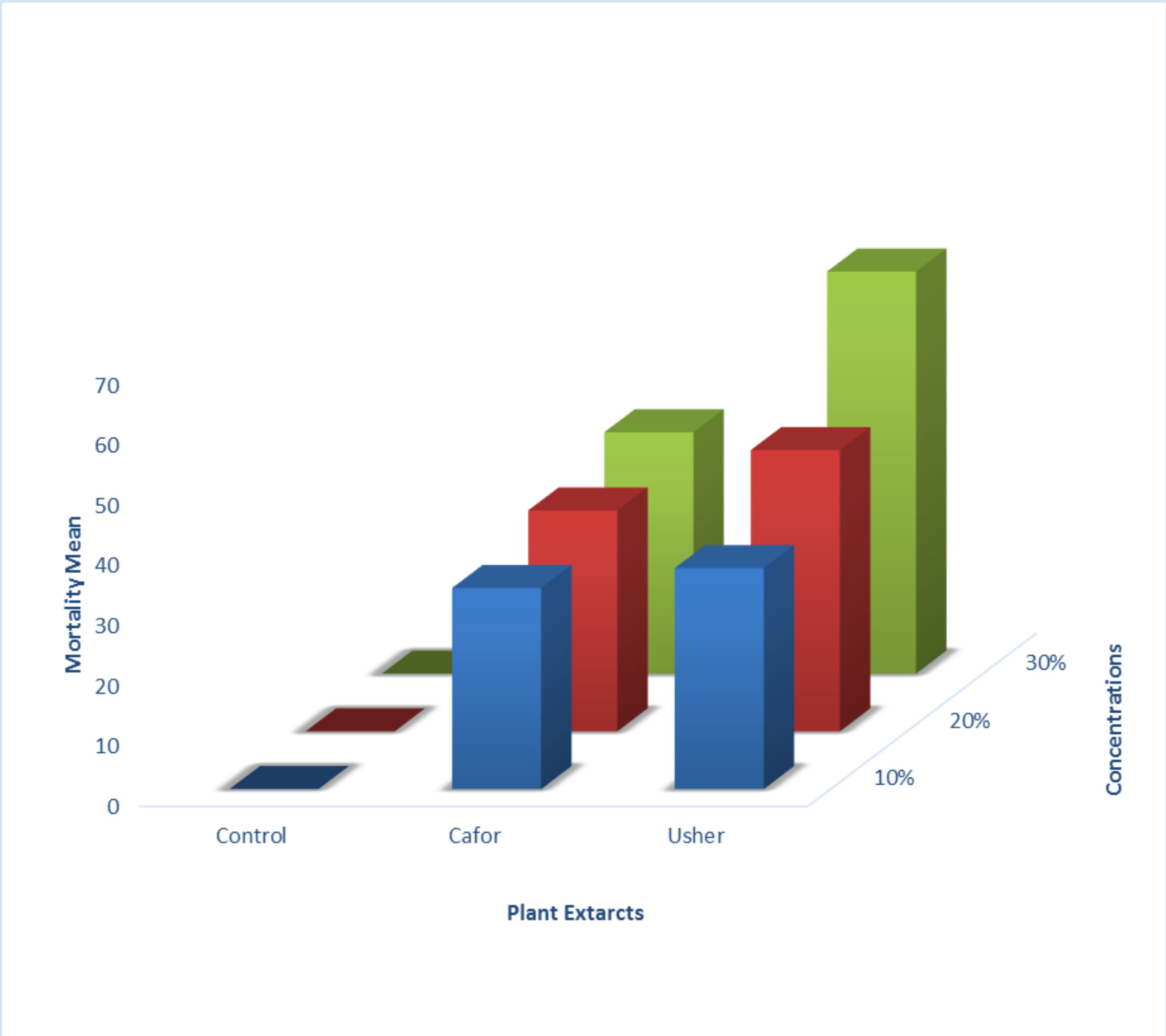

Fig 2: Effects of aqueous extract of Cafor and Usher plant against adult stage of *R. dominica* after 48 hours.

4.3 Effects of aqueous extract of Cafor and Usher plant against the adult stage of *R. dominica* after 72 hours.

The best result was given by concentration 30% Usher that gave 80% adult mortality followed by concentration 20% of same plant, which gave 60% mortality. Then followed by Usher at concentration 10 %, which gave 46.6% adult mortality. Cafur concentration 30 % which resulted in 43.3% mortality while a mortality of *40*% adult was obtained by Cafur extract at concentration 20%.The least percentage of mortality of 36.6% given by Cafur at concentration 10%. All treatments are not significantly different from each other but significantly different from untreated control (Table 3, Fig 3, and Appendix 3.6).

Table 3: Effects of aqueous extract of Cafor and Usher plant against adult stage of *R. dominica* after 72hours.

Treatment	Concentrations	Mortality (%)
Cafor leaves powder	10	36.6 (36.1)b
Cafor leaves powder	20	40 (38.8)b
Cafor leaves powder	30	43.3 (41.03)b
Usher leaves powder	10	46.6 (43.03)ab
Usher leaves powder	20	60 (50.8)ab
Usher leaves powder	30	80 (63.8)a
Control	-	0.0 (0.0) c
C.V(%)	-	17.23
SE±	-	0.5193

-Means at the same column with the same letter (s) are not significantly different at $P \leq 0.05$.

-Values between brackets were transformed using Arcsine.

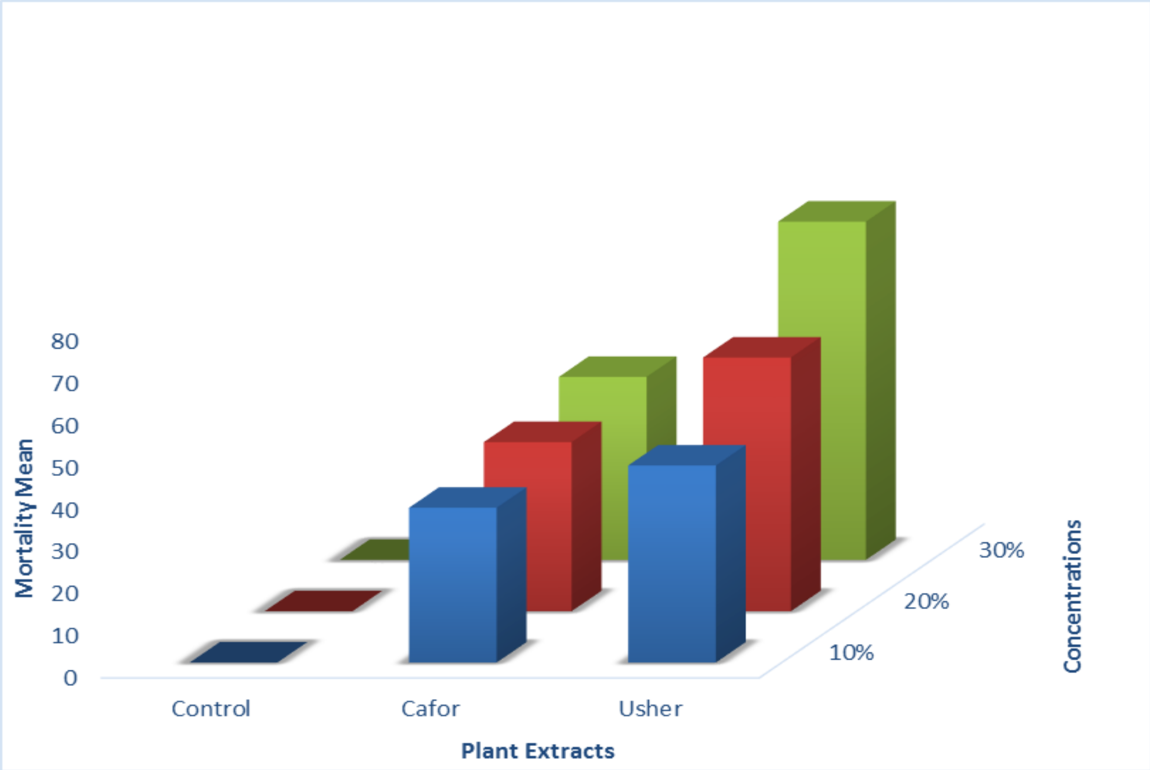

Fig 3: Effects of aqueous extract of Cafor and Usher plant against adult stage of *R. dominica* after 72 hours.

4.4 Probit Analysis:

4.4.1 Probit Analysis of Cafur and Usher Extracts:

Table 4, 5 and 6 showed that LC_{50} were 157.177, 109.526 and 94.425 after 24, 48, and 72 hours respectively.

Table 7, 8 and 9 showed that LC_{50} were 22.842, 18.187 and 11.926 after 24,48, and 72 hours respectively.

Table 4: LC_{50} values of effect of aqueous extract of Cafur on mortality of adult stage of *R. dominica* after 24 hours.

Botanical extract	Slope	Intercept	Chi-square	95% Confidence Limits		LC 50.00
				Lower	Upper	
leaves powder aqueous extract of Kafur	0.595730	3.785000	0.002	-0.785791	2.013199	157.177

Table 5: LC_{50} values of effect of aqueous extract of Cafur on mortality of adult stage of *R. dominica* after 48 hours.

Botanical extract	Slope	Intercept	Chi-square	95% Confidence Limits		LC 50.00
				Lower	Upper	
leaves powder aqueous extract of Kafur	0.364551	4.199303	0.006	-0.983831	2.475815	109.526

Table 6: LC_{50} values of effect of aqueous extract of Cafur on mortality of adult stage of *R. dominica* after 72 hours.

Botanical extract	Slope	Intercept	Chi-square	95% Confidence Limits		LC 50.00
				Lower	Upper	
leaves powder aqueous extract of Kafur	0.354865	4.299110	0.006	-0.979561	2.594723	94.425

Table 7: LC_{50} values of effect of aqueous extract of Usher on mortality of adult stage of *R. dominica* after 24 hours.

Botanical extract	Slope	Intercept	Chi-square	95% Confidence Limits		LC 50.00
				Lower	Upper	
leaves powder aqueous extract of Usher	2.187057	2.028353	0.473	0.774353	0.205052	22.842

Table 8: LC_{50} values of effect of aqueous extract of Usher on mortality of adult stage of *R. dominica* after 48 hours.

Botanical extract	Slope	Intercept	Chi-square	95% Confidence Limits		LC 50.00
				Lower	Upper	
leaves powder aqueous extract of Usher	1.530103	3.072444	0.642	0.182542	1.352364	18.187

Table 9: LC_{50} values of effect of aqueous extract of Usher on mortality of adult stage of *R. dominica* after 72 hours.

Botanical extract	Slope	Intercept	Chi-square	95% Confidence Limits		LC 50.00
				Lower	Upper	
leaves powder aqueous extract of Usher	1.806601	3.055171	0.697	0.435478	1.326482	11.926

CHAPTER FIVE

DISCUSSION

The current trend in stored-product pest management is to use reduced-risk or low-toxicity insecticides as a replacement for conventional organophosphates. Several problems have emerged with the use of conventional chemical control methods. These include resistance by many species of stored-product pests, including *R. dominica* (Subramanyam and Hagstrum, 1996). Uncontrolled use of insecticides and pesticides results in the evolution of resistant strains in addition to environmental contamination and health hazards (Prakash & Rao, 2006; Rahman *et al*., 2009).
This study was to investigated the effect of two botanicals Cafur plant and Usher plant water extract against adults of lesser grain borer *R. dominica*.

The two highest concentrations of leaves powder aqueous extract of Usher used in this study (30% and 20%) gave a high mortality percentage of 80%, and 66.6% respectively followed by Eucalyptus 30 % which gave 63.3% mortality and were significantly different from untreated control after 72 hours of exposure.

The present results are in agreement with El Tayeb. *et al.,* (2004) Who reported that the insecticidal efficacy of water extracts of Hargal plant, *Solenostemma argel* Del Hyne, and Usher, *C. procera*, leaves against the larvae of two mosquito species, *Anopheles arabiensis* and *Culex quinquefasciatus*. The highest concentrations of the two plants gave the best and comparable results with that of standard insecticide, (Temphos). Hargal extract showed the best LC_{50} against *Cx. quinquefasciatus* (0.006ml/L) and *An.arabiensis* (0.140ml/L), as compared with Usher extract (0.108 and 0.263 ml/L, respectively). The present results also supported by Elimam (2009) who reported that aquatic extract of *Calotropis procera* gave good results in controlling of *Anopheles arabiensis* and *Culex quinquefasciatus*. In addition, these results in line with Ahmed, *et al.,* (2005) who found that extracts of *Datura alba* and *Caltropis procera* gave protection of sugarcane sets from termites.

The present results are in agreement with Dakshinamurthy, (1988) who found that E. *camaldulensis* powder mixed with rice at a rate of 1% by weight was effective in reducing the number of adults of *S. cerealella* emerging per 100g rice to 77 compared with 369 in the untreated rice; and prevented cross-infestation by *R. dominica.*In Sudan Mohagir, (2000) studied the effect of application of *E. camaldulensis* on tree locust which reduced the frequency of molting, gave high mortality, deformation and antifeedant effect. *E. camaldulensis,* also was installed in 8 areas of termite activity in different ecological zones during 1985 – 1986; indicated that *E. camaldulensis* was very effective against termites (Hanif, *et al*., 1988).

The present study are in disagreement with Adam (2016) who found that effects of leaves powder aqueous extract of Mesquite, Kafor and Damas against both the adults and larvae of *Tribolium castaneum*. For adult : The two highest concentrations of leaves powder aqueous extract of Mesquite used in this study (20% and 30%) gave 92 %,96% respectively followed by Eucalyptus 30 % which gave 80% mortality and were significantly different from both liquid soap and untreated control after 24 hours of exposure. Such disagreement may be due to different species of insect (*T. castaneum* and *R. dominica).* Also may due to different seasons where the experment conducted.

Probit Analysis of Kafur Extract showed that LC_{50} were 157.177, 109.526 and 94.425 after 24, 48, and 72 hours respectively. Probit Analysis of Usher Extract showed that LC_{50} were 22.842, 18.187 and 11.926 after 24, 48, and 72 hours respectively.

The present results are in agreement with Bakavathiappan. *et al.*, (2012) who found that chloroform extract of *C. procera* showed maximum 67% larvicidal activity at 5% concentration whereas, LC50 and LC90 values are presented was 2.854 and 17.940. Irrespective of the concentrations and the solvents used for extraction the larvicidal activity has been varied.

Conclusion and Recommendations

- Based on the above mentioned results, leaves powder aqueous extract of *Calotropis procera* and *Eucalyptus sp* can be considered as promising to be used as a control agent for *Rhyzopertha dominica.*
- Cafur extract was gave high percentage of mortality was 63.3% by concentration 30% after 72 hours.
- The best result obtained by Uher extract at concentration 30% was 80% adult mortality after 72 hours.
- Further comparative studies should be conducted to evaluate the effects of these leaves with other organic solvents and also in other insect pests.
- A comprehensive study should be conducted to specify the active ingredients.

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Appendices

Appedix 1: Number of mortality (actual data) (out of 10 adults) after 24 hour. Effects of leaves powder aqueous extract of Kafor (*Eucalyptus sp*) and Usher plant (*Calotropis sp*) against the adults stage of *Rhyzopertha* dominica under laboratory conditions

Conc.	Mortality			
	R1	R2	R3	Mean
USHER 10%	50	20	10	26.6
USHER 20%	50	30	10	33.3
USHER 30%	60	30	20	36.6
K 10%	10	50	10	23.3
K 20%	40	50	30	40
K 30%	80	50	60	63.6
CONTROL	0	0	0	0.7

Appedix 2: Number of mortality (actual data) (out of 10 adults) after 48 hour. Effects of leaves powder aqueous extract of Kafor (*Eucalyptus sp*) and Usher plant (*Calotropis sp*) against the adults stage of *Rhyzopertha* dominica under laboratory conditions

Conc.	**Mortality**			
	R1	**R2**	**R3**	**Mean**
USHER 10%	**50**	**40**	**10**	**33.3**
USHER 20%	**60**	**30**	**20**	**36.6**
USHER 30%	**60**	**30**	**30**	**40**
K 10%	**30**	**60**	**20**	**36.6**
K 20%	**40**	**50**	**50**	**46.6**
K 30%	**80**	**50**	**70**	**66.6**
CONTROL	**0**	**0**	**0**	**0.7**

Appedix 3: Number of mortality (actual data) (out of 10 adults) after 72 hour. Effects of leaves powder aqueous extract of Kafor (*Eucalyptus sp*) and Usher plant (*Calotropis sp*) against the adults stage of *Rhyzopertha* dominica under laboratory conditions

Conc.	**Mortality**			
	R1	**R2**	**R3**	**Mean**
USHER 10%	**50**	**50**	**10**	**36.6**
USHER 20%	**70**	**30**	**20**	**40**
USHER 30%	**60**	**30**	**40**	**43.3**
K 10%	**30**	**70**	**40**	**46.6**
K 20%	**50**	**60**	**70**	**60**
K 30%	**90**	**80**	**70**	**80**
CONTROL	**0**	**0**	**0**	**0.7**

Appedix 4: Transformed data after 24 hour. Effects of leaves powder aqueous extract of Kafor (*Eucalyptus sp*) and Usher plant (*Calotropis sp*) against the adults stage of *Rhyzopertha dominica* under laboratory conditions

Conc.	**Mortality**			
	R1	**R2**	**R3**	**Mean**
K 10%	**45**	**26.5**	**18.4**	**29.6**
K 20%	**45**	**33.2**	**26.5**	**34.9**
K 30%	**50.7**	**33.2**	**26.5**	**36.8**
USHER 10%	**18.4**	**45**	**18.4**	**27.2**
USHER 20%	**39.2**	**45**	**33.2**	**39.1**
USHER 30%	**63.4**	**45**	**50.7**	**53**
CONTROL	**0**	**0**	**0**	**0.7**

Appedix 5: Transformed data after 48 hour. Effects of leaves powder aqueous extract of Kafor (*Eucalyptus sp*) and Usher plant (*Calotropis sp*) against the adults stage of *Rhyzopertha dominica* under laboratory conditions

Conc.	Mortality			
	R1	R2	R3	Mean
K 10%	45(6.7)	39.2(6.3)	18.4(4.3)	34
K 20%	50.7(7.1)	33.2(5.8)	26.5(5.1)	36.8
K 30%	50.7(7.1)	33.2(5.8)	33.2(5.8)	39
USHER 10%	33.2(5.8)	50.7(7.1)	26.5(5.1)	36.8
USHER 20%	39.2(6.3)	45(6.7)	50.7(7.1)	44.9
USHER 30%	63.4(7.9)	45(6.7)	55.7(7.5)	55.0
CONTROL	0(0.7)	0(0.7)	0(0.7)	0.7

Appedix 6: Transformed data after 72 hour. Effects of leaves powder aqueous extract of Kafor (*Eucalyptus sp*) and Usher plant (*Calotropis sp*) against the adults stage of *Rhyzopertha dominica* under laboratory conditions

Conc.	**Mortality**			
	R1	**R2**	**R3**	**Mean**
K 10%	**45(6.7)**	**45(6.7)**	**18.4(4.3)**	**36.1**
K 20%	**56.7(7.5)**	**33.2(5.8)**	**26.5(5.1)**	**38.8**
K 30%	**50.7(7.1)**	**33.2(5.8)**	**39.2(6.3)**	**41.03**
USHER 10%	**33.2(5.8)**	**56.7(7.5)**	**39.2(6.3)**	**43.03**
USHER 20%	**45(6.7)**	**50.7(7.1)**	**56.7(7.5)**	**50.8**
USHER 30%	**71.5(8.4)**	**63.4(7.9)**	**56.7(7.5)**	**63.8**
CONTROL	**0(0.7)**	**0(0.7)**	**0(0.7)**	**0.7**