THE EFFICACY OF SOME BOTANICAL INSECTICIDES AGAINST COMMON BEAN BRUCHIDS (*Zabrotes subfasciatus* Boh and *Acanthoscalides obtectus* Say)

BY

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ABSTRACT

Laboratory studies were carried out to investigate the effectiveness of nine botanical extracts in three formulations for the control of common bean bruchids, *Zabrotes sabfasciatus* Boh. and *Acanthoscalides obtectus* Say. on common beans and to compare the performance of dust, water and oil extract formulations. Powder formulation of *Ocimum canum, Tagetes minuta, Eucalyptus globus, Ricinus communis* leaves and *Neuteraunenia mitis* tuber were tested under laboratory conditions. The effectiveness of the extracts was compared with actellic super dust a synthetic chemical (pirimiphos methyl + permethrin) commonly used for control of bean bruchids, *T.minuta* was the least effective botanical followed by *R. communis, O. canum* and *E. globus* while *N. mitis* was the most effective, the mortality of weevils after 14 days was 100% as that of Actellic Super Dust. The results indicate that all tested botanicals have potential for protecting bean seeds against infestation by bean weevils, but *N. mitis* is the most effective. Water extracts formulation of *O. canum, T. minuta, E. globus and R. communis* had lower efficacy compared to *N. mitis* and Actellic Super Dust which had performed better. Oil extracts of botanicals have shown higher efficacy on bean weevils as Actellic Super Dust, and therefore have potential for protecting stored beans against infestations. The result from this study showed that *N. mitis* extracts and all oil extracts have higher efficacy as that of Actellic Super Dust on protecting common beans against common bean bruchids. Hence, can be used efficiently as the recommended synthetic insecticides.

DECLARATION

I, Bernadeta Celestine Fivawo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has not been submitted for any degree in any other University.

Signature ----------Date 15/08/08

Fivawo Bernadeta Celestine
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The above declaration is confirmed

Signature ---------- Date 15/8/08

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(Supervisor)

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DEDICATION

To my mother Mhafiwa Maria-lusia Kabaga and my children Irene, Innocent and Anna.

DEDICATION

To my mother Mhafiwa Maria-lusia Kabaga and my children Irene, Innocent and Anna.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance.
CRD	Complete Randomized Design
DMRT	Duncan's Multiple Range Test
EMS	Error Mean Square
Fig.	Figure.
g	grammes.
IPM	Integrated Pest Managemen
Kgs	Kilogrammes
LD_{50}	Lethal Dose that kills 50% of tests insects.
LSD	Least Significant Different.
Mls	Milliliters
NS	Not Significant different
P	Probability
SAS	Statistical Analysis System.
SUA	Sokoine University of Agriculture.
UV	Utral-Violet

CHAPTER ONE

INTRODUCTION

1.1. General introduction

Botanical insecticides are naturally occurring chemicals extracted from plants. Plants have developed protection mechanisms such as repellence and even insecticidal effects for defense against insect attack, (Gonzalo, 2004). The oldest known pest control method is the use of dusts and plant extracts. For instance, there are reports that in 400 B.C in Persia, the delousing procedure for children was with a powder obtained from dry flowers of the pyrethrum plant (*Chrysanthemum cinerariofolium* Compositae). The first botanical insecticide used as such dates back to the 17th century, when nicotine obtained from tobacco leaves was used to kill plum beetles. Around 1850, a new plant insecticide known as rotenone was obtained from the roots of plants called timb, but was for catching fish. Later on, plants with irritating properties like incense and sabadila were used as decongestants; the plants that do not kill insects directly but scares them off. Massive use of these insecticides has had a long and difficult road because the earliest information was based on superstition, which when tested by scientific methods was not shown to be effective (Addor, 1995). After the Second World War, synthetic insecticides replaced few plants and plant extracts that had shown promising effects and were of widespread use (Anard and Jagadiswari, 1996).

When synthetic insecticides appeared in the 1940s, problems of environmental contamination, residues in food and pest resistance and resurgence rose with time. There is no doubt botanical insecticides are an interesting alternative to insect pest

control. However, only few have been properly evaluated for this purpose (Heal *et al.*, 1950). Plants are like natural laboratories where a great number of chemicals are biosynthesized and infact they may be considered the most important source of chemical compounds which have protective action against insects: they include alkaloids, non-proteic amino acids, steroids, phenols, flavonoids, glycosides, grucosinolates quinines, tannins and terpenoids, which are known as secondary compounds that have no known function in photosynthesis, growth or other aspects of plant physiology (Bell *et al.*, 1990). More recently a number of botanical insecticides are being marketed which are extracted from Neem (*Azadirachta indica*), Tobacco (*Nicotiana tabacum*), *Derris spp* and *Conchocarpus spp.* (Rotenone) Pyrethrum (*Chrysanthemum cinerariaefolium*), S*choenocaulon officinale* (Sabadila), *Ryania speciolsa* and *Tephrosia spp* (Arnason *et al.*, 1989). Other researchers point out that some products are important chemical signals in ecosystem because most of these compounds are found in flowers, leaves, roots, stems and seeds.

Information and scientific support on botanicals is generally inadequate which makes it difficult to recommend particular plant materials as a replacement for chemical insecticides. Efficacy levels of botanicals can vary among pests. Since the cost of testing and registration is prohibitive and no commercial company is likely to feel that the market is large enough to justify such an outlay, it would appear that the best chance of using plant materials in developing countries is of the whole unchanged material, the use of which would not require registration (Anard and Jagadiswari., 1996).

Many researchers are attempting to validate the efficacy of traditional storage protectants whilst others are seeking effective plant species, which would be readily available for farmer's use at village level (Weaver *et al.*, 1991a). Some researchers are seeking plant extracts, which can become the focus of local pesticide industries (Kis-Tomas, 1990). Decision on ultimate intended usage need to be made because botanicals have got several advantages that outlay their disadvantages. It is believed that botanical insecticides will minimize the undesirable side effects of synthetic insecticides and help to preserve the environment for future generations (Anard and Jagadiswari, 1996).

1.2 Objectives of the study

(a) General objective

To establish suitable botanical insecticide as plant extract in form of oil, powder or solution in water with different concentrations in comparison with Actellic Super Dust, a recommended chemical for control of common bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus)*.

(b) Specific objectives

(i) To assess the efficacy of nine botanical extracts against bruchid beetles *Acanthoscelides obtectus* and *Zabrotes subfasciatus* (Bruchidae) in common beans and compare with Actellic Super Dust.

(ii) To evaluate the efficacy of different formulations against *Acanthoscelides obtectus* and *Zabrotes subfasciatus*

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of insect pests of common beans (*Phaseolus vulgaris* L.)

Two species of bruchids, the common bean weevil (*Acanthoscelides obtectus* Say) and Mexican bean weevil (*Zabrotes subfasciatus* Boh) are major pests of stored beans (Nchimbi-Msolla and Misangu, 2002). *Acanthoscelides obtectus* start to infest beans in the field and continue to infest them in storage, while *Z. subfasciatus* infest beans only in storage (Kiula and Karel, 1985). Bruchid beetles can consume up to 50% by weight of the seed (Reddy and Reddy, 1987). The degree of loss due to bruchid damage is quite variable depending on the storage period and storage conditions. In Tanzania for example, bean losses could be as high as 40% due to bean bruchids (Kiula and Karel, 1985). It has been reported also that the damage increases as storage period is prolonged (Singh, 1990). Bruchid damage reduces the weight, quality and viability of the bean seed (Reuben *et al.*, 2006). The presence of even a small portion of holed seeds is likely to lead to down grading and a consequent reduction in value (FAO, 1999).

2.1.1 Details of common bean weevils (*Zabrotes subfasciatus* Boh. and *Acanthoscelides obtectus* Say.)

Z. subfasciatus predominate more in warmer areas and *A. obtectus* at higher altitudes in the tropics and throughout temperate climates in general (Biedmont and Bonet, 1981; Slim, and Agona 1996; Masolwa and Nchimbi, 1991). In Africa, however, this differentiation is less marked. *Z. subfasciatus* does not attack beans in the field. Fresh eggs are attached to the testa of bean seed. The adults exhibit strong sexual

dimorphism. Female are large and have four characteristic cream-colored spots on the elytra while the male is entirely brown. *A. obtectus* scatters its eggs among stored seed and oviposits in maturing bean pods in the field. It is difficult to distinguish between male and female as size and coloring is the same (Jones, 1998).

2.1.2 Life history

In storage, the life histories of *Z. subfasciatus* and *A. obtectus* are similar, larvae of both species moult four times before pupating. During the last larval instars, the feeding and pupation cell become externally visible as a circular window in the seed where the larvae feed on the lower testa surface. After pupation, the adult may remain in the cell for several days before pushing or biting out the window with its mandibles. Adults are short-lived, mate and oviposit soon after emergence (Jones, 1998) For the Mexican bean weevil, the egg stage lasts 5-6 days, different larval instars last 14 days, pupal stage 6-7 days; adults live for 10-13 days; and females lay an average of 36 eggs. For the bean weevil, the egg stage lasts 6-7 days, combined larval and pupal stage 23 days; adults live for 14 days; female lay about 45 eggs.

2. 2 Methods of controlling bruchid beetle of common beans

Various techniques have been used to control bruchid pests of beans (*Phaseolus vulgaris* L.) in storage. The techniques range from physical, tumbling (Quentin *et al.*, 1991), sunning and sieving regimes (Silim and Agona, (1996), contact insecticides and fumigants (Schoonhoven and Cardona, 1986, NRI, 1991), botanicals (Silim and Agona, 1996) and vegetable oils (Silim and Agona, 1996).

2.2.1 Physical control

The use of chemically inert materials such as ashes, sand or other minerals, powder of crop residue or seeds in large quantities to fill up the interstitial space in grain bulks and provide a barrier to insect movement is quite widespread. For example, farmers living on the Lilongwe plain and in the Dedza Hills of the central region in Malawi, commonly use ash from the cooking fire to protect beans stored at homes for consumption and seed, while in India, sand is mixed with both cereals and pulses for storage (Golob and Webley, 1980)

2.2.1.1 Ashes

To treat the stored grain with ash is a common method of protection against insect pests (Golob and Webley, 1980). The ash is either mixed thoroughly with the grain or added to stored products in various layers. In Zimbabwe, ashes from unnamed sources and ashes from maize cobs and Mopane tree (*Colophospermum mopane*) are predominant in Mashonaland Provinces, (Giga and Mvumi, 1993). Ashes from cattle and goat droppings and from the Lead wood (*Combretum imberbe*) are preferred in Matabeleland Provinces (Giga and Mvumi, 1993). Lead wood is very heavy and burns down to almost floury ash. It is also used by people in Botswana and was shown to be fairly effective against bruchids in pulses. Wood ash, as well as sand, tobacco dusts and dolomite provided a good protection of maize stored for six months in Malawi (Golob and Webley, 1980).

The protective mechanisms of ash are unclear; desiccation and suffocation are two possible ways of how insects are affected. There may be a variation in how effective

different ash products are owing to differences in texture and chemical composition. Although the choice of ash products is likely to be dependent on what is available for the people in the particular area, it could be of interest to carry out comparative experiments of several ash products against the most common storage pests. The optimal mix of ash is 20 percentage of the weight of bean seed being treated (FAO, 1999).

2.2.1.2 Trampling

In an interesting experiment in the USA, half-filled kidney beans containers were rolled or tumbled every 8 hours or 2-3 times per day for 2 weeks, which resulted in disturbing the alignment between stable bracing sites of *A. obtectus* and target beans, and prevented the larvae from completing entrance holes. Populations of *A. obtectus* in all rolled or tumbled containers were reduced by about 97% compared with stationary controls (Spencer *et al.*, 1991). Some control of *Z. Subfasciatus* is achieved by storing beans in their pods, as *Zabrotes* prefer laying their eggs on shelled beans. These methods are suitable for small amounts of produce.

2.2.1.3 Timely harvesting

The time of harvesting determines the level of field infestation by *A. obtectus*, but longer term storage can be achieved by adding dust or by drying or smoking the harvested beans. It has been reported that each week of delayed harvest resulted in a seven percent increase in infestation by *A. obtectus* (Sindi and Hendry, 2004).

2.2.1.4 Good storage hygiene

Cleaning the store and the containers before storing the beans, disinfecting the store if necessary, reduces infestation of beans. Avoiding mixing newly harvested beans with stored ones reduce the infestation of beans with weevils from the field. Also storage of beans in air tight containers, e.g. Polythene bags, drums; calabash and clay pots make unfavorable conditions for weevils to reproduce (Sindi and Hendry, 2004).

2.2.1.5 Control by sunlight

Exposure to sunlight followed by sieving of the grains is a well known technique among farmers in sub-Saharan Africa, especially against the different pests of beans (Chinwada and Giga, 1996). In this method, the grains are spread on the ground or black polythene sheet and are left exposed to sunlight for at least seven hours. After sunning, it is sieved by using a 5 mm sieve. The process may be repeated every three to four weeks depending on the size of production and availability of labor. The method proved to be quite effective in reducing bruchid infestation with no or minimal effect on grain quality or germination (Chinwada and Giga, 1996).

2.2.1.6 Control by drying

Proper drying of beans before storage to maximum moisture content of 12% in beans can keep the produce for four months without bruchid infestation (Sindi and Hendry, 2004). Farmers may use bush dryers, solar dryer or light fire underneath the crop to reduce the water content and to deter or kill the different insect stages.

2.2.2. Chemical control

Due to the significant increase in human population and the consequent increase in the amount of food and grains produced, many small scale farmers adopted the use of pesticides as a means of pest control. Dusting and fumigation of grains are the most commonly used chemical methods among small-scale farmers (Gwinner and Muck, 1996). Dusting in particular is an easily applied method and can be implemented with very cheap tools such as small perforated metal cans or jute bags. For small amounts of grain, dust can be mixed thoroughly and distributed evenly all over the produce. The most commonly used chemicals in storage beans are pirimiphos methyl (Actellic 2%) at the rate of 200-500g per bag and pirimiphos methyl + permethrin (Actellic Super Dust) at the rate of 100g per bag (Hand Book of Field Pests, 2002) and have for long been effective and the most important in grain storage. However there are several disadvantages accompanied with their use. Most of the synthetic pesticides in use have persistent residues, which can hardly be removed during grain processing (Kabungo *et al.*, 1998) and are neither user nor environment friendly; can result in harmful residues in foodstuff and development of resistance in the target insect populations (Reddy and Reddy, 1987, Zettler and Cuperus, 1990). Farmers treat their produce with synthetic insecticides during storage of which majority of them still lack the knowledge on proper, safe and effective use (Baier and Webster, 1992). These synthetic chemicals have become expensive and therefore, resource poor farmers fail to utilize them at the recommended dose rendering them ineffective and making pest control difficult (Bhaduri *et al.*, 1985; Talukder and Howse, 1994). Synthetic pesticides also may cause serious health hazards, insect pest resistance,

resurgence, environmental pollution, ecological imbalance and residues in market produce (Bhaduri *et al.*, 1985; Addor, 1995).

2.2.3 Use of plant materials

Studies on the use of plant materials, extracts and oils for the control of storage product pests show that over the past 21 years, a large number of plant species from a wide range of families have been evaluated. Jacobson (1989) suggested that the most promising botanicals were to be found in the families of Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae. In the current review, references to trials using plant species from over 50 families have been found. The most numerous being in the families of Compositae, Fabaceae, Labiatae, Leguminoceae, Solanaceae and Umbelliferae (Golob and Webley, 1980). The presence of secondary compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts the anti-insect activity. Secondary compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals. They can affect insects in several different ways; they can disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants and antifeedants or modify oviposition. They may retard or accelerate development or interfere with the life cycle of the insect in other ways (Bell *et al.*, 1990). Scientists are now experimenting and working to protect insect infestation by indigenous plant materials. The use of such plant extracts to control pests is not a new innovation, as it has been widely used by small-scale subsistence farmers. The use of locally available plants in the control of pests is an ancient technology in many parts of the world (Roy *et al.*, 2005)

The plant species that have been investigated are frequently those used locally within individual countries as culinary species or in traditional medicine. Some researchers infer that the materials are therefore safe to use as insecticides because most of them are known by the farmer at a particular locality and are easy to process and use. The rapid degradation of the active product by Ultra Violet (UV) light reduces the residue risks on food and tends to be safe for higher animals and environment, also posing less risk to non target organisms. Resistance to botanical materials not developed as quickly as with synthetic insecticides (Gonzalo Silva-Aguayo; 2002, 2004). Beans treated with different plant materials were significantly less infested by the Mexican bean weevil (*Z. subfasciatus*) than untreated seeds after 90 days. Seed treatments comprised of Coconut oil, powder of *Eucalyptus camaldulensis* and Neem, ashes and a synthetic pesticide (Busungu and Mushobozy, 1991).

From the evidence available to date, there are many plant materials that can be used as grain protectants which include: *Ocimum canum* Sims (Asteraceae), *Tagetes minuta* (Asteraceae), *Ricinus comunis* L (Euphorbiaceae), *Eucalyptus globus* Labill (Myrtaceae) and *Neurautanenia mitis* (Leguminaceae).

2.2.3.1 *Ocimum canum* (Sims) Hoary or American basil, (Lamiaceae: Labiatae)

2.2.3.1.1 Description and habitat of the plant

Ocimum canum is a bushy, semi-woody herb that can grow up to 40 cm high (Plate 1), (Olive-Baver, 1986). It is widely spread in Tropical Africa and the old world.

2.2.3.1.2 Uses of *Ocimum canum*

The leaves are used as a traditional medicine in West Africa for the treatment of fever, dysentery and to relieve toothache. It is also used for flavoring and as an insect repellent (Oliver- Baver, 1986). In Rwanda, it is used for protection against post-harvest insect damage. Dried milled leaves (1%w/w) admixed with Pinto beans caused 100% mortality in adult *Z. subfasciatus* after 48 hours (Weaver *et al.*, 1994). LD 50 values for Linalool applied to paper after 24 hours exposure were 412 $\mu g/cm^2$ for *A. otectus*, 429 $\mu g/cm^2$ for *Z. subfasciatus*. A dose of 750 µg/cm2 caused 100% mortality in all species within 24 hours (Weaver *et al.*, 1994).

2.2.3.1.3 Constituents

Ocimum canum consists of Linalool, a terpenoid which is the major component of the oil, forming 60-90% of the total volatiles collected (Weaver *et al.*, 1991a). Composition of the essential oil varies according to its origin. In East Africa, it contains 16-25% camphor, while in West Africa it may contain 75% Methylchaviol (estrago) (Oliver-Baver, 1986).

2.2.3.1.4 Mode of action

Linalool is an oxygenated monoterpenoid which acts as a reversible competitive inhibitor of acetyl cholinesterase (Weaver *et al.*, 1991b).

2.2.3.2 *Tagetes minuta* (Mexican marigold; Asteraceae).

2.2.3.2.1 Description and habitat

T. minuta is native to the temperate grass-lands and montane regions of South America (Soule, 1993). *T. minuta* is often found growing in disturbed areas during

early seasonal stages. *T. minuta* is an erect annual herb reaching 1-2m high, leaves are slightly glossy green and pinnately dissected into 4-6 pairs of pinnae, leaf margins are finely serrate (Plate 2). The under surface of the leaves bear a number of small punctuate, multicellular glands, orangish in colour, which exude a liconce like aroma when ruptured. Glands may also be found on the stems and involucres bracts, four or five fused involucre bracts surround each head. There are typically 3-5 yellow, orange ray florets and 10-15 yellow orange disk florets per capitula, the heads are small, 10-15 mm long and including ray florets, 10-20 mm in diameter. The heads are borne in a chistered panicle of 20-80 capitula, the dark brown achenes are 10-12 mm long with a pappus of 1-4 tiny scales and 0-2 retresely serrulate awns which are 1-3 mm long.

2.2.3.2.2. Use of *Tagetes minuta*

The new world people have been using *T. minuta* as a flavourful, beverage, a medicinal and condiment for a long time. *T. minuta* is referred to as a weed (Cubrera, 1971) and is commercially grown and harvested for its essential oils *which* are used in flavour and perfumes. There is evidence that the secondary compounds in *Tagetes* are effective deterrents of numerous organisms, including fungi which are pathenogenic on humans (Chan *et al.*, 1985; Camm *et al.*, 1975), bacteria (Grover and Rao, 1978), round worms in general and nematodes (Graham *et al.*, 1980; Grainge and Ahmed, 1988) and numerous insect pests through several different mechanisms (Jacobson, 1990).

2.2.3.2.3 Constituents of *T. minuta*

T. minuta is rich in many secondary compounds including acyclic, monocyclic and bicyclic monoterpenes sesquiterpenes, flavonoids and aromatics (Redrigues and Mabry, 1977)

2.2.3.3 *Eucalyptus globus* Labill (Myrtaceae) (Tasmanian blue gum tree, Australian fever tree)

2.2.3.3.1 Description and habitat

E. globus is a wood, pale, strong and durable tree, leaves are tough, leathery, greyish green, scimitar shaped 10-15 cm long and about 2.5-4 cm wide, shortly stalked and rounded at base (Plate 3), with numerous transparent oil dots (Rehm and Espig, 1991). Mainly cultivated in Brazil, ex-USSR, Spain, Ecuador, Portugal and India. .

2.2.3.3.2 Uses of *Eucalyptus globus*

The LD50 for exposure to leaf powder for seven days was 4.1.g/100g rice and 4.86g/100g rice for adult *Sitophilus oryzae* and *Sitophilus granarius*, respectively. The leaves showed repellent activity against *Sitophilos* (Sharaby, 1989). Oil (0.4% v/w) admixed with red gram prevented emergence of F1 adults in *Callosobruchus chinensis* after an exposure period of 90 days (Strivastava *et al.*, 1988).

2.2.3.3.3. Constituents of *E. globus*

The oils contain over 70% cineole (Rehm and Espig, 1991). Major constituents are monoterpenoid esters (66.12%) i.e. 1.8 cineole (66.1%) and the monoterpenes (21.15

%) i.e. α-pinene (14.7%). Steam- volatile constituents include cineole-β-phell, (86 %), α-penene 3.8%) and para pcymene 2.4%) (Rehm and Espig, 1991).

2.2.3.4 *Ricinus communis* L (Castor bean; Euphorbiaceae)

2.2.3.4.1 Description and habitat

The Castor bean plant is a native of tropical Africa, it is a shrub which can grow up to 2.4-3.6 m high, which is widely distributed, occurs either wild or in a state of semi-cultivation in tropical and non-tropical countries found on wasteland and field borders (Rehm and Espig, 1991). It is cultivated in several varieties for the oil found in its seeds and for its bold foliage. The stalked leaves consist of usually eight radiating pointed leaflets with slightly serrated edges and prominent control veins. Many varieties are green, but some are reddish brown (Plate 4). The flowers are green and inconspicuous, but pink or red in the pigmented varieties (Rehm and Espig, 1991).

2.2.3.4.2 Uses of Castor bean plant

Castor oil extracted from seeds is used mainly in industry as a lubricant and additive in rubber and as a plasticizer in plastic industry and traditional medicines (Rehm and Espig, 1991). Dried ground leaves (16g/kg) admixed with cowpea caused 100% mortality in adult *C. maculatus* within seven days and reduced F1 emergence (Okonkwo and Okoye, 1992). 10 ml/kg admixed with pigeon pea caused 100% mortality in adult *A. obtectus* and *C. maculatus* within 18 hours (Salas and Hernandez, 1985)

2.2.3.4.3 Constituents

The seeds contain 2.8-3.0% toxic substances. The principal toxin is an albumin called ricin and also contains undecyclenic acid and ricinine (Grainge and Ahmed, 1988). The leaf consists of alkaloid ricinine, cyanogenic glycoside, flavonoids, steroidal sapogenin, garlic acid and potassium nitrate. The oil consists of ricinoleic, stearic, dihydroxystearic, oleic and linoleic acids (Ayensu, 1981). The castor beans are pressed to extract castor oil that is used for medicinal purposes. Ricin does not partition into the oil because it is water soluble, therefore castor oil does not contain ricin, provided that no cross contamination occurred during its production (Grainge and Ahmed, 1988).

2.2.3.4.4 Toxicity

Poisoning of livestock and humans by castor beans has been reported. Ingestion of two to four seeds may cause serious poisoning and human fatalities (Grainge and Ahmed, 1988).

2.2.3.5 *Neuteraunenia mitis* (Leguminoseae)

2.2.3.5.1 Description and habitat

N. mitis is a leguminous creeper plant, shrub like, 1-1.5 m high, with trifoliate leaves and hairy big pods, the seeds are round with big eye, the flowers are pinkish or white in color the stems originate from a huge root tuber (Plate 5). Normally these plants are widely distributed in the Southern highlands of Tanzania, especially in miombo woodlands, open woodlands and open grasslands. Collected information about plants that can be used in crop protection that was done with villagers in Handeni, Tanga

region documented the leguminous plant creeper, *N. mitis* (Kajias *et al.*, 1994). Farmers cut the huge root of the plant creeper into pieces and soak them in water; the extract is then sprayed for protection against maize stalk borers. *N. mitis* showed a great potential in the control of maize weevils and possibly other storage pests and can protect grains up to six months with a minimal damage (Kabungo *et al.*, 1998).

Plate 1: *Ocimum canum* plant.

Plate 2: *Tagetes minuta* plant.

Plate 3: *Eucalyptus globus* plant.

Plate 4: *Ricinus communis* plant.

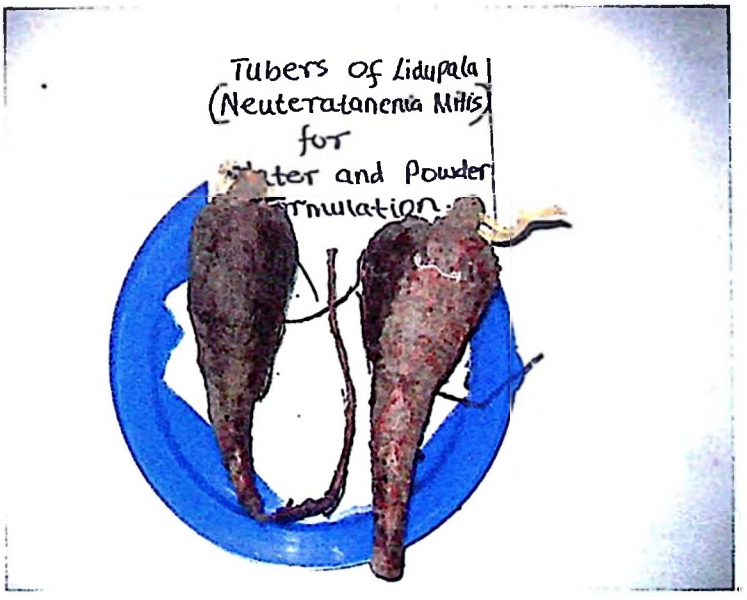

Plate 5: *Neuteraunenia mitis* tuber

2.2.4 Use of vegetable oils

The application of oils of botanical origin (vegetable oils) to beans is a method of protection against bruchid beetle attack, which has been confirmed as effective by many workers. In addition to action against adult insects, vegetable oils are generally reported to exert ovicidal action (Don-Pedro, 1989). The protection of stored products such as rice, wheat, maize and beans with vegetable oils is another simple, convenient and cost-effective method. The oils from peanut groundnut, coconut, sunflower, mustard, castor bean, cottonseed, soybean and maize were tested and found effective. Sunflower seed oil has not proved very effective (Ketker, 1989; Ramzan, 1994; Shaaya *et al.*, 1997; Ahmed *et al.*, 1999 and Yalamanchilli and Punukollu, 2000). According to Lienard *et al.* (2004) the effect of vegetable oil on insect pests of stored products involves four different and complementary mechanisms. Toxicity to the eggs and first instar larvae is the consequence of the occulation of a short funnel at the posterior end of the egg. The oils coat leads to a reduction of egg adherence on the treated seed which prevents the first instars larva from penetrating the seeds. Some oil constituents have a direct toxic effect. As time passes after a treatment, two factors change; adult females increasingly avoid laying eggs on seeds which have been treated with oil more than 7 days earlier. Very few eggs indeed are laid on seeds which were treated 60 days before being newly infested with beetles (Gabriele and Stoll, 2003). Also it was suggested that egg mortality in bruchids is caused by the physical properties of the oil coating, blocking respiration rather than by a specific chemical effect, because the larvae which hatch from the eggs must penetrate the seed to survive and are unable to do this unless the egg is firmly attached to the seed surface. Eggs on the oil treated seeds were found to be

less firmly attached than on the controls, suggesting that the oil may inhibit successful larval penetration into the seeds (FAO, 1999). Credland (1992) examined the structure of bruchid eggs and suggested that the funnel structure at the posterior pole of *Callosobruchus* eggs may be the major route for gaseous exchange hence application of oil to bruchid eggs might occlude the funnel and thus lead to the death of the developing insect by asphyxiation.

2.2.4.1 Sunflower seed oil (*Helianthus annus* L, Compositae; Asteraceae)

Sunflower is an annual herbaceous plant. Seeds are opaque/white in colour, obovate, wedged- shaped, broader and truncate at the apex, convex, compressed on two sides which form sharp margin (Plate 6). A black variety has black glossy surface and is larger and thinner than a white variety which often has black, longitudinal stripes. Sunflower is widely cultivated (Ahmed *et al.*, 1999).

2.2.4.1.1. Uses of sunflower oil

Culinary oil is produced from the seeds; it is also used for animal fodder (Doharey *et al.*, 1990). 2.5 ml/kg applied to *Phaseolus vulgaris* protected the beans against attack by A. obtectus for six months (Rheenen *et al.*, 1989).

2.2.4.1.2 Constituents

Sunflower seed oils consists of linoleic acid (58-67 %) (Rheenen *et al.*, 1989).

2.2.4.2 Castor bean oil (*Ricinus communis* L) Euphorbiaceae.

The castor oil is extracted from the seeds of castor beans (Plate 7) and is mainly used in industry as a lubricant, an additive in rubber and as a plasticizer in the plastics

industry and in traditional medicines (Rehm and Espig, 1991). 10 ml/kg of oil admixed with pigeon pea caused 100% mortality in adult *A. obtectus* within 18 hours (Salas and Hernandez, 1985). The oils of castor beans consists ricinoleic, steraric, dihydroxystearic, oleic and linoleic acid (Ayensu, 1981).

2.2.4.3 Soybean oils

Soybean oil is extracted from the seeds (Plate 8) of soybean plant (*Glycine max* L.) (Leguminosae or Fabaceae) and is refined by several methods but the most common is by solvent extraction. The oil is available in crude, degummed and refined forms but degummed form is mostly used because it is more uniform than crude oil and has been described as stable in storage than refined oils (Singal and Singh, 1990). Soybean oil sprays are relatively safe to humans and are effective on insects, mites and insects unlikely to develop resistance to oil sprays (Singal and Singh, 1990). 5 ml/kg of highly saturated soybean oil applied on common beans (*P. vulgaris)* reduced oviposition and larvae development by *Z. subfasciatus* after an exposure period of 7 and 28 days, respectively (Hall and Harman, 1991). 10 ml/kg applied to pigeon pea seed caused 97% mortality in *A. obtectus* within 1 hour and also prevented egg laying (Salas and Hernandez, 1985).

2.2.4.4 Coconut oils

The oil is extracted from the nut of the coconut seed (*Cocos nucifera* L.) (Plate 9). 10 ml/kg admixed with pigeon pea caused 97.5% mortality in adult *A. obtectus* and 100% mortality in *C. maculatus* within 1 hour. It has also prevented reproduction and F1 emergence (Salas and Hernandez, 1985).

2.2.4.4.1 Constituents of coconut oils

Coconut oil contains high proportions of saturated fatty acids, especially lauric acid, Trimyristin, Triaurin, Tripalmitin, Tristearin and other glycerides, (Messina and Renwick, 1983; Jouhar and Poucher, 1991).

2.2.4.5 Groundnut oils

Groundnut oil is extracted from the seeds of groundnuts (Plate 10) by several methods, either by boiling, solvent extraction or by squeezing the seeds. The oil is used for cooking but also used as storage protectant (Rehm and Epsig, 1991). 10ml/kg admixed with cowpea reduced damage by *C. maculatus* over 24 week storage (Cockfield, 1992).

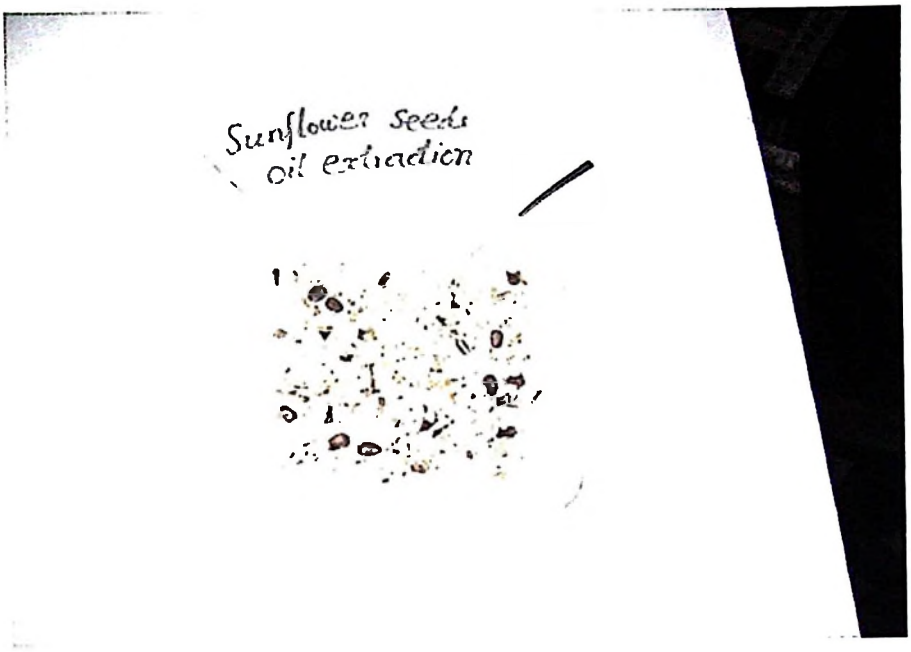

Plate 6: Sunflower seeds

Plate 7: Castor bean seeds

Plate 8: Soybean seeds

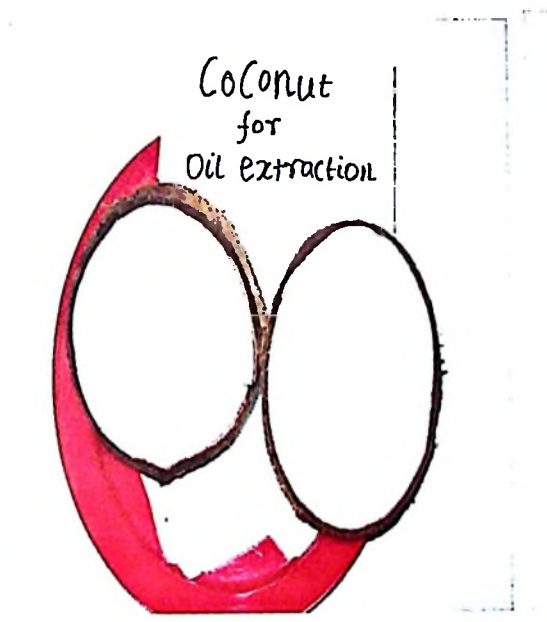

Plate 9: Coconut seed

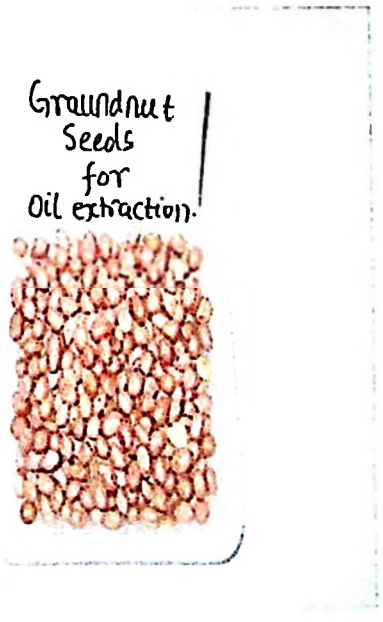

Plate 10: Groundnut seeds

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study location

Laboratory experiments were conducted at the Pest Management Center, Sokoine University of Agriculture, in Morogoro, from November 2006 to April 2007.

3.2 Bean samples

The bean samples used for the experiment were collected from the Bean Improvement Project at SUA. Five (5) kgs of beans were kept in the freezer for 48 hours to destroy insect infestations in the seeds. 20kgs of Kablanketi variety bean seeds were used during the experiment.

3.3. Bean weevils

The bean bruchids (*A. obtectus* and *Z. subfasciatus*) were collected from farmer's stores and introduced into five culture bottles, each with 1 kg of clean bean seeds for 60 days at room temperature.

3.4 Plant materials

3.4.1 Botanicals

The plant materials and vegetable oils with insecticidal properties used for the experiments were *Ocimum canum* (Hoary or American basil, Camphor basil), *Tagetes minuta* (Mexican marigold), *Eucalyptus globus* Labill (Tasmanian blue gum tree or Australian fever tree), and *Ricinus communis* L (Castor bean), *Neuteraunenia*

mitis (Lidupala or Nyongwe), sunflower, groundnuts, coconut, soybean and castor bean oils. The plant materials were collected from Iringa and Morogoro Regions

3.4.2 Preparation of materials

3.4.2.1 Powder formulation

The leaves of *O. canum, T. minuta; R. communis* and *E. globus* were dried at ambient temperature under diffused light for seven days to avoid degradation of active ingredients. Tubers of *N. mitis* were dug then chopped into pieces and dried. Both dried leaves and pieces of tuber from all five plants were ground into a very fine powder and kept in tightly closed bottles.

3.4.2.2 Water extracts formulation

The fresh leaves of *E. globus, R. communis, O. canum* and pieces of *N. mitis* tubers were pounded in a mortar to form a paste and soaked in water for twelve hours to allow the active ingredient to dissolve. Clean piece of cloth was used to filter the extracts. Five grams of detergent soap were added to the extracts to enable the active ingredient to spread uniformly on the bean seeds.

3.4.2.3 Oil extracts formulations

The oils from the seeds of sunflower, castor bean, groundnuts, coconut and soybeans were obtained by pounding the seeds, paste was mixed with 1 liter of water and 500mls of organic solvent (acetone) and stirred for 10 minutes to make a thorough mixture of paste and the solvent, the mixture was left undisturbed for 12 hours to

allow the solvent and extracts to separate. The oils were collected and kept in glass bottles.

3.5 Control (Actellic Super Dust)

A synthetic insecticide (Actellic Super Dust) was used as a control. 1g of Actellic Super Dust was mixed thoroughly with 1kg of bean seeds. Twenty five treated seeds were placed in glass vials and 10 pairs of newly emerged bruchids were introduced in each glass vial.

3.6 Dust formulations

The experiment involved five botanicals at two different concentrations. The first concentration was 16g of powder mixed with 1kg of bean seeds for each particular botanical and the second concentration was 8g/kg of *O. canum*, 10g/kg *T. minuta*, 12g/kg *E. globus*, 14g/kg *R. communis* and 18g/kg *N. mitis* powder mixed with clean bean seeds. 25 treated seeds were selected from each botanical and placed into glass vials and 10 adult weevils were introduced per glass vial for both bruchid species (*A. obtectus* and *Z. subfasciatus*).

3.7 Water extracts formulations

This experiment involved five botanical water extracts. Each extract was applied in two different concentrations. The first concentration was 50 mls/kg for all five botanical extracts and the second involved different concentrations for each extract. These were 10ml/kg, 20ml/kg, 30ml/kg, 40ml/kg and 60mls/kg for *O. canum*, *T.*

minuta, E. globus, R. communis and *N. mitis* extracts, respectively. For each of the two treatment levels, two vials were used. Each vial contained 25 treated seeds and 10 pairs of either one of the two species of adult weevils were introduced.

3.8 Oil extracts formulations

Five different types of vegetable oil extracts in two different concentrations were used. The first concentration was 2.5ml/kg and the second was varied for each oil extract. The concentrations applied were 1ml/kg, 2ml/kg, 3ml/kg, 4ml/kg and 5ml/kg for sunflower, castor bean coconut soybean and groundnuts, respectively. 25 treated bean seeds were placed in a glass vial and 10 pairs of each of the two species of adult weevils were introduced in separate vials.

3.9 Actellic Super Dust treatment

Actellic Super Dust at1g/kg was used as a standard to compare with the efficacy of the botanicals against the two species of bruchids.

3.10 Experimental layout

The layout of the three experiments was a split-plot arrangement in a Complete Randomized Design (CRD) in which two species of bruchids were the main plots and extracts were subplots.

3.11 Data collection

Mortality was assessed at 24, 48 hours, and one week after treatment and after there at intervals of 2 weeks for duration of three months, the number of live and dead

insects, damaged, undamaged seeds and number of holes in damaged seed were assessed.

3.12 Data analysis

The collected data were subjected to Analysis of Variance (ANOVA) procedure (SAS, 1997) with the following model:

$Y_{ijk} = \mu + R_i + P_j + (RP)_{ij} + Tk + (PT)_{ij} = (RPT)_{ijk}$.

Where as:

Y_{ijk} = Response of variables investigated

μ = General mean.

$R_i = i^{th}$ effect of replication.

$P_j = j^{th}$ effect of pest species.

$(R, P)_{ij}$ = Main plot error (a).

$T_k = k^{th}$ effect of treatments.

$(P, T)_{jk}$ = Interaction of pest species and treatment effects.

$(R, P, T)_{ijk}$ = Experimental error (b).

The variations in mean values were tested using Least Significant Differences (LSD) method.

CHAPTER FOUR

RESULTS

4.1 Powder formulations

4.1.1. The efficacy of *O. canum, T. minuta, E. globus, R, communis* and *N. mitis* in dust formulations against bean bruchids (*A. obtectus* and *Z. subfasciatus*)

4.1.1.1 Efficacy of *Ocimum canum*

The results show that there were no significant differences between the two concentrations of *O. canum* (16g/kg and 8g/kg) and control (Actellic Super Dust) for the mortality of adult bean weevils. The mean percent mortality of weevils was 85% for both concentrations of *O. canum* and 100 % for Actellic Super Dust at 14^{th} day after treatment. Significant differences between treatments and control in the mortality of bean weevils were observed on the 1^{st} and 2^{nd} day after treatment. During this period higher mortality was observed in weevils treated with Actellic Super Dust than in the two concentrations of *O. canum* (Fig.1).

4.1.1.2 Efficacy of *Tagetes minuta*

There were significant differences ($P < 0.05$) in mortality of bean weevils between treatments with *T. minuta* at 16g/kg and 10 g/kg and the control (Actellic Super Dust. The mean percent mortality of adult bean weevils treated with 16g/kg and 10g/kg of *T. minuta*. Higher mortality occurred in the control than in the two treatments with *T. minuta* (Fig. 2).

4.1.1.3 Efficacy of *Eucalyptus globus*

There were significant differences in mortality of bean weevils between treatments with 16g/kg and 12g/kg of *E. globus* and the control. Higher percent mortality was observed in the control. All introduced adult weevils were dead after two days of treatment while in bean seeds treated with *E. globus* there were few live adult weevils (Fig. 3).

4.1.1.4 Efficacy of *Ricinus communis*

The results show significant differences ($P < 0.05$) between the treatment of bean seeds with two different concentrations of *R. communis* (16g/kg and 14g/kg) and the Actellic Super Dust (1g/kg). Mortality of weevils in bean seeds treated with Actellic Super Dust was 65% on the 1st day after treatment and 100% on the 2nd day (Fig. 4)

4.1.1.5 Efficacy of *Neuteraunenia mitis*

There were no significant differences ($P > 0.05$) in mortality of adult bean weevils between treatments with the two different concentrations of *N. mitis* (16g/kg and 18g/kg) and on the 14th day after treatment where the mortality were 100% in the three treatments on the 14th day after treatment, (Fig. 5).

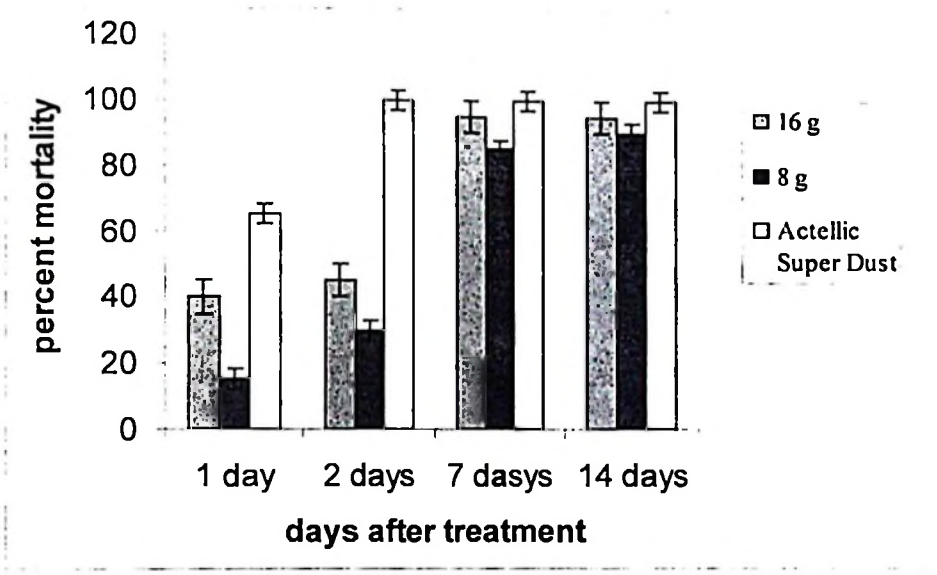

Figure 1. Mortality of bean weevils after treatment with two different concentrations of O. canum and Actellic Super Dust.

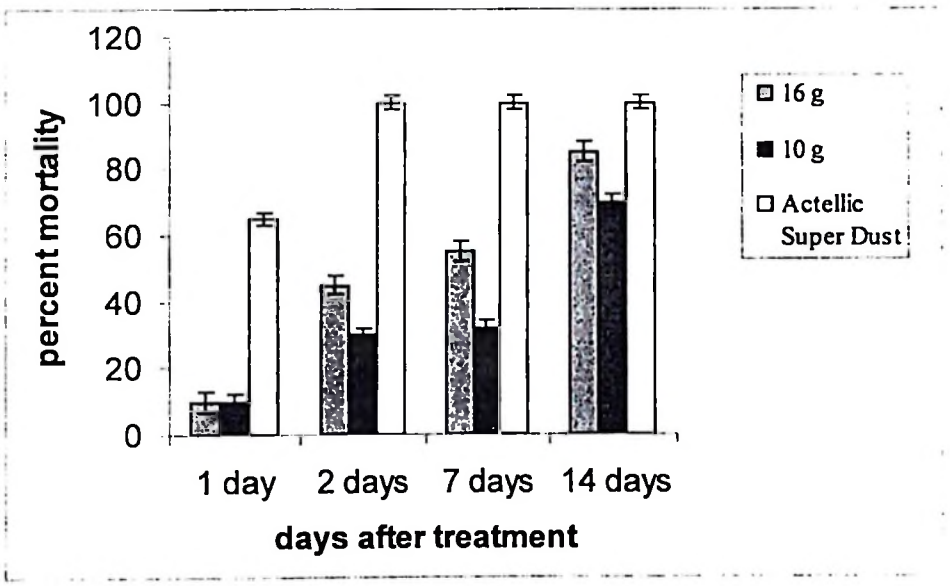

Figure 2. Mortality of bean weevils after treatment with two different concentrations of *T. minuta* and Actellic Super Dust.

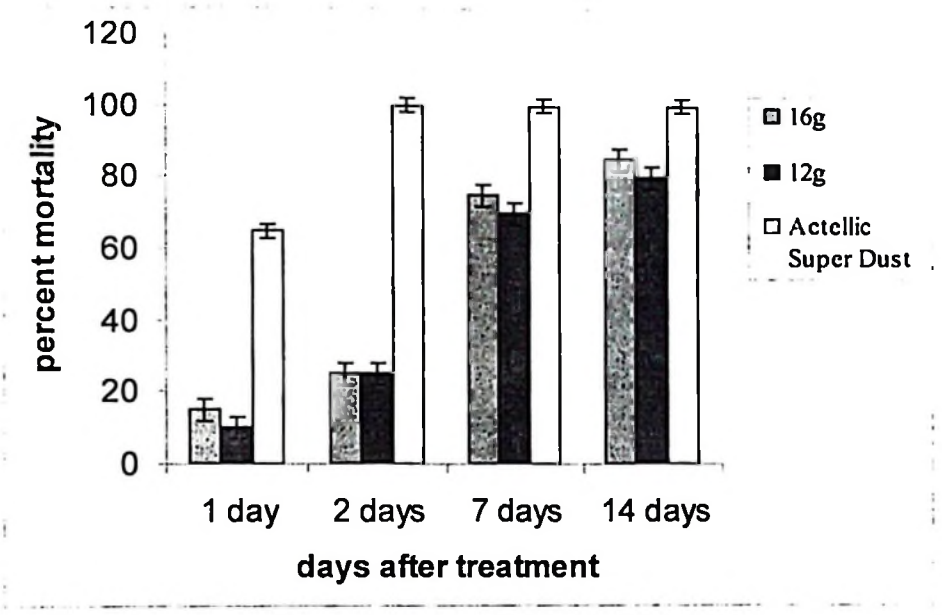

Figure 3. Mortality of bean weevils after treatment with two different concentrations of *E. globus* and Actellic Super Dust.

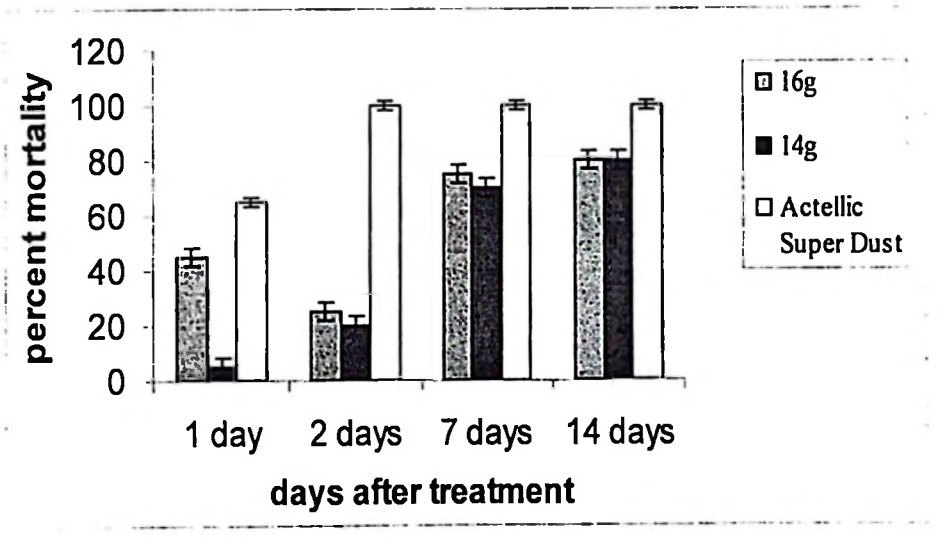

Figure 4. Mortality of bean weevils after treatment with two different concentrations of *R. communis* and Actellic Super Dust.

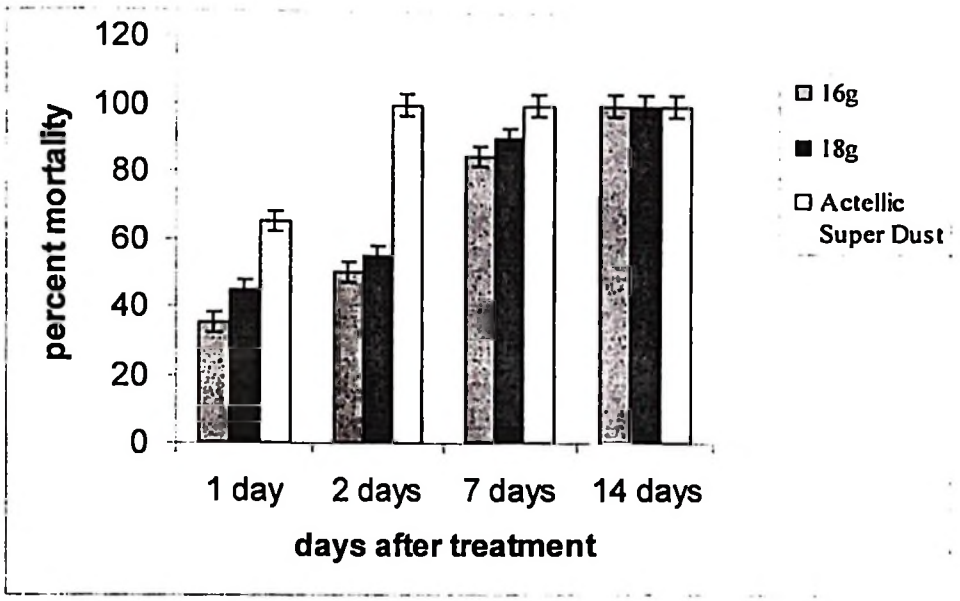

Figure 5. Mortality of bean weevils after treatment with two different concentrations of *Neuteraunenia mitis* and Actellic Super Dust.

4.1.2 Bean seeds damage after treatment with two different concentrations of botanicals in powder formulation

The number of damaged bean seeds treated with *O. canum* (16g/kg and 8g/kg), *T. minuta* (16g/kg and 10g/kg,), *E. globus* (16g/kg and 12g/kg) and *R. communis* (16g/kg) were significantly different ($P < 0.05$) from the control.There were no significant differences between damaged bean seeds treated with *R .communis* (14g/kg) and *N. mitis (*16g/kg and 18g/kg) and the control (Actellic Super Dust*)*. Higher numbers of damaged seeds were observed in treatment with *O. canum, T. minuta, E. globus* at all concentrations and *R. communis* at 14g/kg than bean seeds treated with *R. communis* at 16g/kg, *N. mitis* at both concentrations and control (Table 1).

Table 1 Mean numbers of damaged, undamaged and holed bean seeds treated with two different concentrations of botanicals in powder formulation and Actellic Super Dust after three months

Botanicals	Concentration g/kg	Total Treated seeds.	Damaged seeds	Undamaged seeds	Number of holes
O canum	16	25	4.67^{abc}	20.33^{cde}	11.94^{bcd}
O. canum	8	25	5.83^{abc}	19.17^{bcde}	33.83^{abc}
T. minuta	16	25	7.33^{a}	16.56^{e}	53.20^{a}
T. minuta	10	25	6.89^{ab}	18.11^{cde}	37.11^{ab}
E. globus	16	25	5.67^{abc}	19.33^{bcde}	23.06^{abcd}
E. globus	12	25	3.89^{abcd}	21.11^{abcd}	14.61^{bcd}
R. communis	16	25	7.67^{a}	17.33^{de}	38.94^{ab}
R. communis	14	25	6.44^{a}	18.56^{cde}	27.44^{abcd}
N.mitis	16	25	2.94^{bcd}	22.06^{abc}	11.61^{bcd}
N. mitis	18	25	1.89^{cd}	23.11^{ab}	3.67^{cd}
Actellic	1	25	0.00^{d}	25^{a}	0.00^{d}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05), DMRT.

4.2 Oil extract formulations

4.2.1 Efficacy of oil extracts of Sunflower, Castor bean, Soybeans, Coconut and Groundnuts against bean weevils (*A. obtectus* and *Z. subfasciatus*).

4.2.1.1 Efficacy of Sunflower oils

There were no significant differences (P > 0.05) in mortality of bean weevils for the two concentrations of sunflower oil and control (Actellic Super Dust). The mean percent mortality of weevils in seeds treated with sunflower oil (2.5mls/kg and 1ml/kg) on the 7th day after treatment was 100% for both concentrations and the control (Fig. 6).

4.2.1.2 Efficacy of Castor bean oils

The results show significant differences in mortality of bean weevils for the two concentrations of castor bean oil (2.5 mls/kg and 2mls/kg) and the control on the 1st and 2nd day after treatment. High mortality of bean weevil on treated bean seeds was

observed after 7days (90% and 70%). After 14 days mortality of bean weevils was 100% for all treatments (Fig. 7).

4.2.1.3 Efficacy of Soybean oils

There were significant differences ($P < 0.05$) in mortality of bean bruchids between the two concentrations of soybean oil (2.5 mls/kg, 3mls/kg) and the control (1g/kg). The results show that the mean percent mortality of bruchids increased with time of storage. After 14 days of treatment percent mortality of bean was 100% for all treatments (Fig. 8).

4.2.1.4 Efficacy of coconut oils

There were no significant differences ($P > 0.05$) in mortality of bean bruchids between the two concentrations of coconut oil and the control. The results show that the mortality of bean seeds treated with coconut oil (2.5mls/kg and 4mls/kg) after 1 day was 15% and 25%, respectively while bean seeds treated with Actellic Super Dust was 65% and after 14 days mortality of bean bruchids was100% for all treatments (Fig. 9).

4.2.1.5 Efficacy of groundnut oils

The results show significant differences ($P < 0.05$) in mortality of bean bruchids between the two concentration of groundnut oil and control on the 1st and the 2nd day after treatment. Percent mortality of bean bruchids in bean seeds treated with groundnut oil (2.5mls/kg, and 5mls/kg) was 100% after 14 days (Fig. 10).

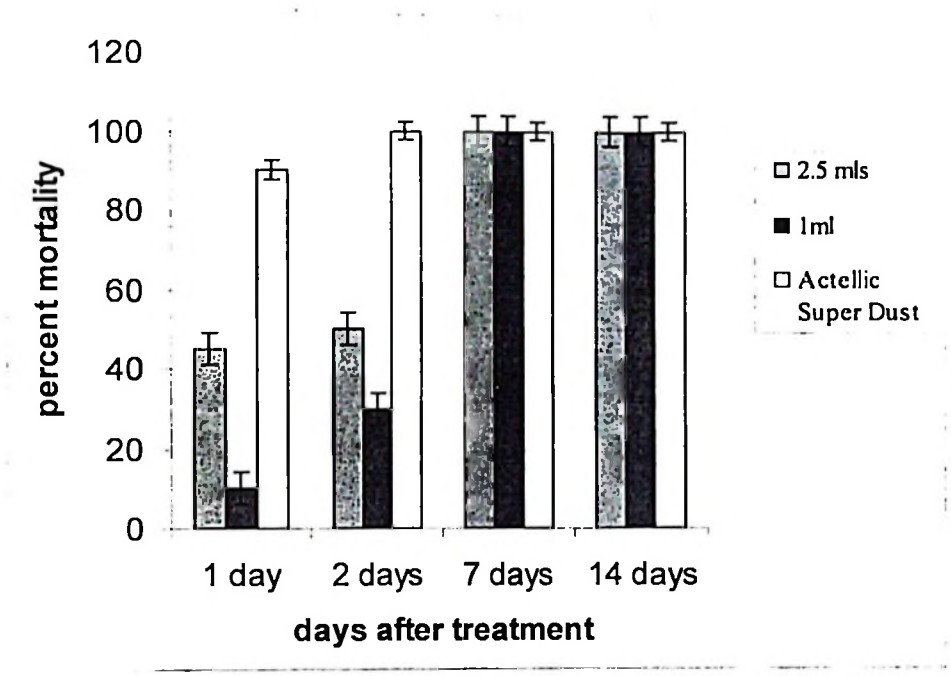

Figure 6. Mortality of bean weevils after treatment with two different concentrations of sunflower oil and Actellic Super Dust.

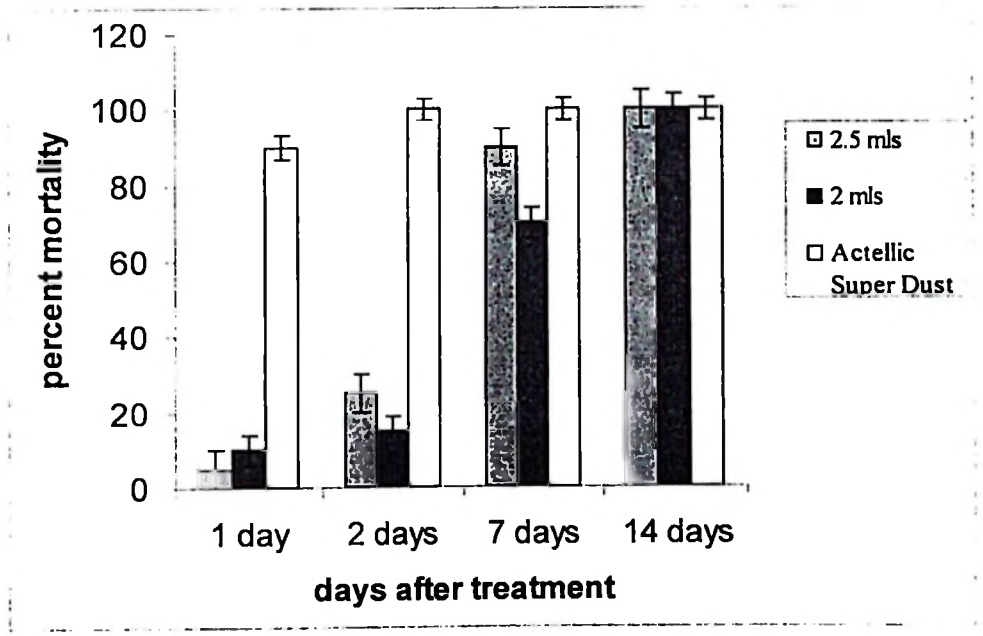

Figure 7. Mortality of bean weevils after treatment with two different concentrations of castor oil and Actellic Super Dust

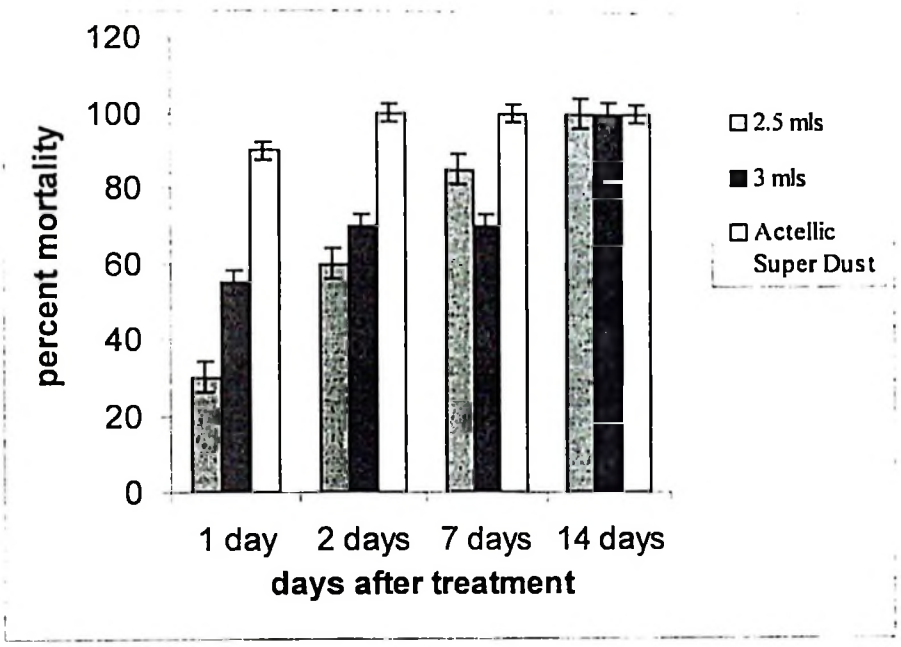

Figure 8. Mortality of bean weevil after treatment with two different concentrations of soy bean oil and Actellic Super Dust

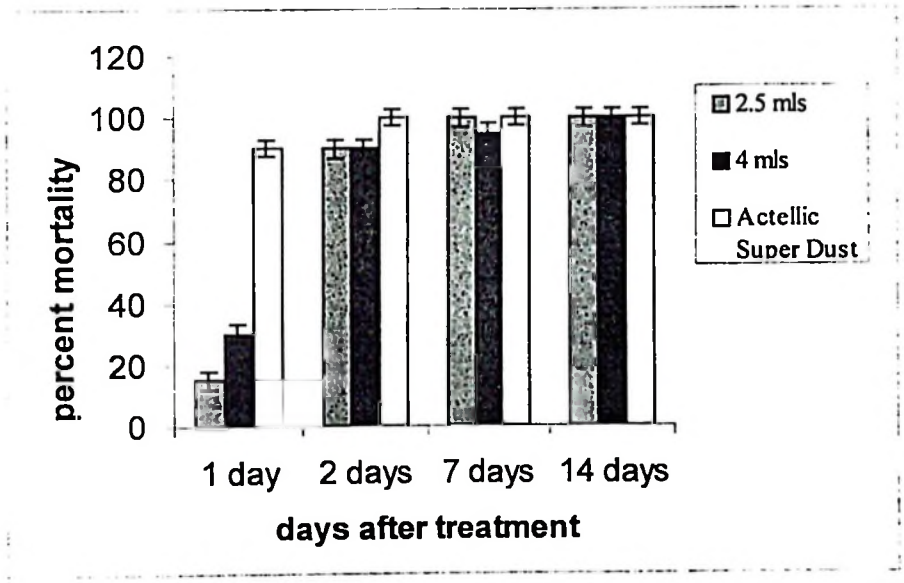

Figure 9. Mortality of bean weevils after treatment with two different concentrations of coconut oil and Actellic Super Dust.

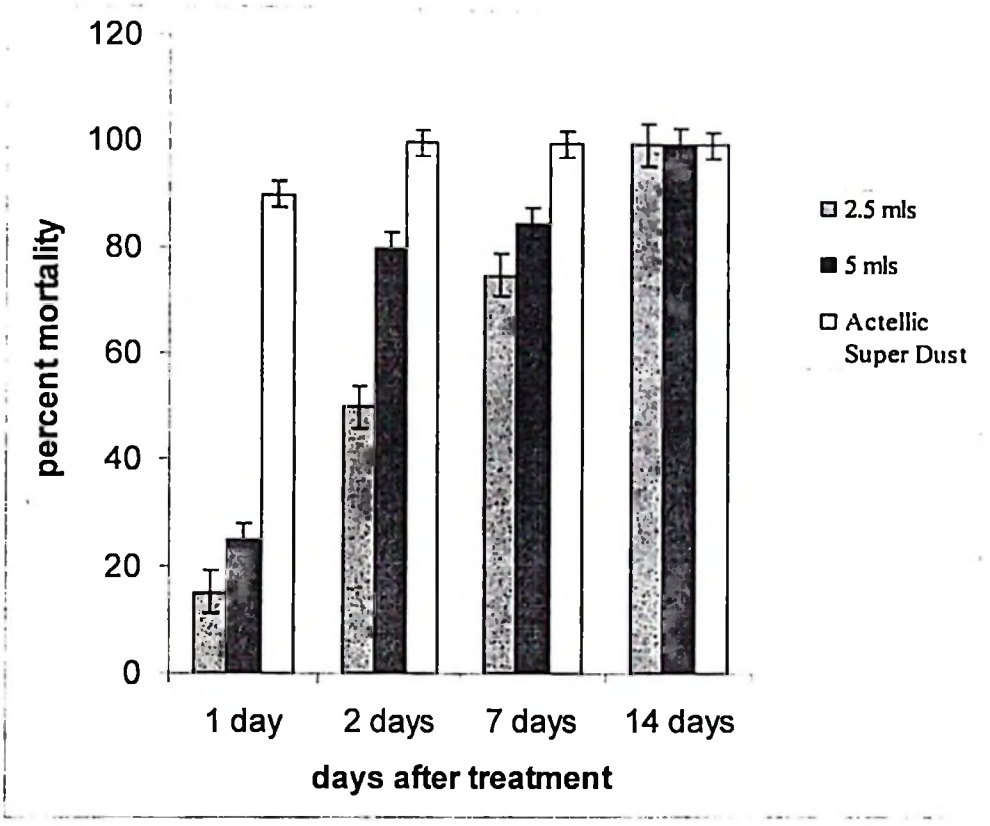

Figure 10. Mortality of bean weevils after treatment with two different concentrations of groundnut oil and Actellic Super Dust

4.2.2. Bean seeds damage in oil extract treatments

The results show that there were no significant differences ($P < 0.05$) in damaged/undamaged seeds and number of holes in seeds treated with sunflower, castor bean, coconut, groundnuts and soybean oil extract and control. There was a higher number of undamaged than damaged seeds in all treatments.

Table 2: Mean number of damaged, undamaged seeds and number of holes on bean seeds treated with two different concentrations of different botanicals in oil extract formulation after three months

Botanicals	Concentration mls/kg	Total treated seeds	damaged seeds	Undamaged seeds	Number of holes
Sunflower	2.5	25	0.34^{a}	24.66^{b}	0.22^{ab}
Sunflower	1	25	0.25^{ab}	24.75^{ab}	0.22^{ab}
Castor bean	2.5	25	0.00^{b}	25^{a}	0.00^{b}
Castor bean	2	25	0.00^{b}	25^{a}	0.00^{b}
Coconut	2.5	25	0.00^{b}	25^{a}	0.00^{b}
Coconut	3	25	0.25^{ab}	24.75^{ab}	0.44^{ab}
Groundnut	2.5	25	0.00^{b}	25^{a}	0.00^{b}
Groundnut	4	25	0.16^{ab}	24.84^{ab}	0.11^{b}
Soy bean	2.5	25	0.16^{ab}	24.84^{ab}	0.22^{ab}
Soy bean	5	25	0.34^{a}	24.66^{b}	0.61^{a}
Actellic	1	25	0.00^{b}	25.00^{a}	0.00^{b}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05) DMRT.

4.3 Water extract formulations

4.3.1 Efficacy of water extracts of *Ocimum canum, Tagetes minuta, Eucalyptus globus, Ricinus communis* and *Neuteraunenia mitis* against bean weevils (*A. obtectus* and *Z. subfasciatus*).

4.3.1.1 Efficacy of *Ocimum canum*

The results show significant differences ($P < 0.05$) in mortality of bean weevils between the two concentrations of *O. canum* and control. The percent mortality of weevils in bean seeds treated with *O. canum* (50mls/kg and 10mls/kg) was 20% and 5%, respectively while for the control (Actellic Super Dust) it was 95% in the 1st day (Fig. 11).

4.3.1.2 Efficacy of *Tagetes minuta*

There were significant differences ($P < 0.05$) in mortality of bean weevils between the two concentrations of *T. minuta* extract and the control. The results show that treatment with *T. minuta* (50mls/kg and 20mls/kg) gave mortality of 20% on the 1st day, 50 and 40% on the 2nd day, 70% and 75% on the 7th day and 95% and 85% on the 14 day after treatment while in control (Actellic Super Dust) mortality was 100% from the 2nd day after treatment (Fig.12)

4.3.1.3. Efficacy of *Eucalyptus globus*

The results show significant differences ($P < 0.05$) in percent mortality of adult weevils between the two concentrations of *E. globus* and control. There was significant lower mortality of weevils in bean seeds treated with *E. globus* (50mls/kg and 30mls/kg) after 14 days than the in control were observed (Fig. 13). .

4.3.1.4 Efficacy of *Ricinus communis*

There were significant differences ($P < 0.05$) in percent mortality of bean weevils between the two concentrations of *R. communis* (50mls/kg and 40mls/kg) and the control (Actellic Super Dust 1g/kg). The percent mortality of weevils was low in bean seeds treated with *R. communis* than in the control (Fig. 14).

4.3.1.5 Efficacy of *Neuratanenia mitis*

The results show no significant differences ($P > 0.05$) in percent mortality of bean weevils between the two concentrations of *N. mitis* (50 mls/kg and 60mls/kg) and the

control (1g/kg). Mortality of bean weevils in bean seeds treated with *N. mitis* after 14 days was 100%, which was similar to the control (Fig. 15).

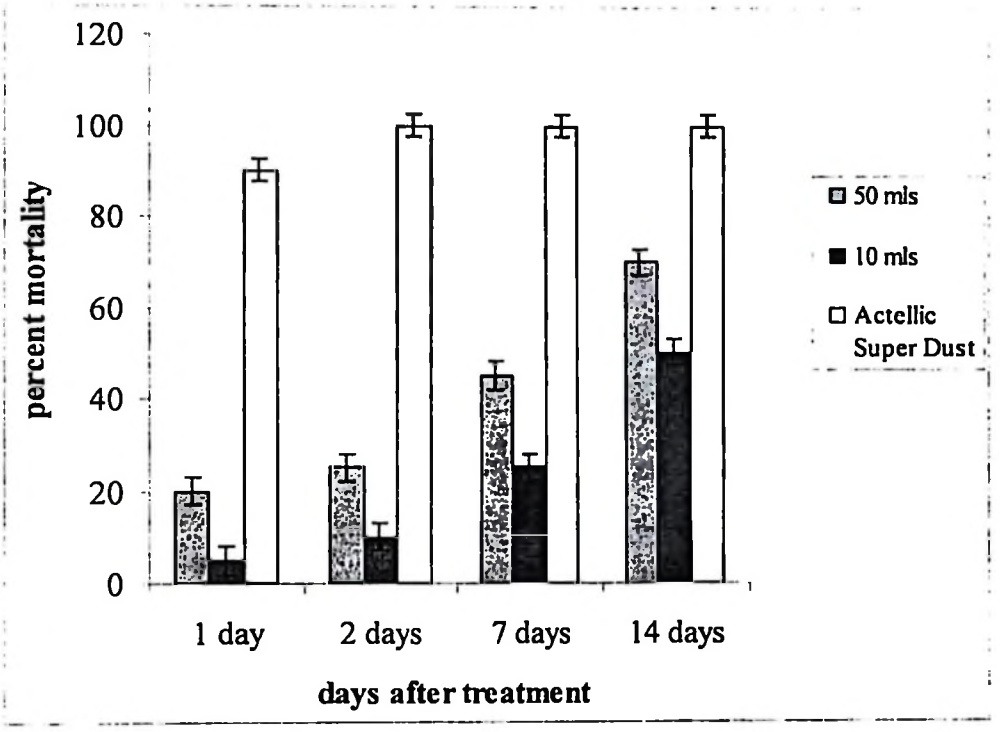

Figure 11. Mortality of weevils after treatment with two different concentrations of *O. canum* water extracts and Actellic Super Dust.

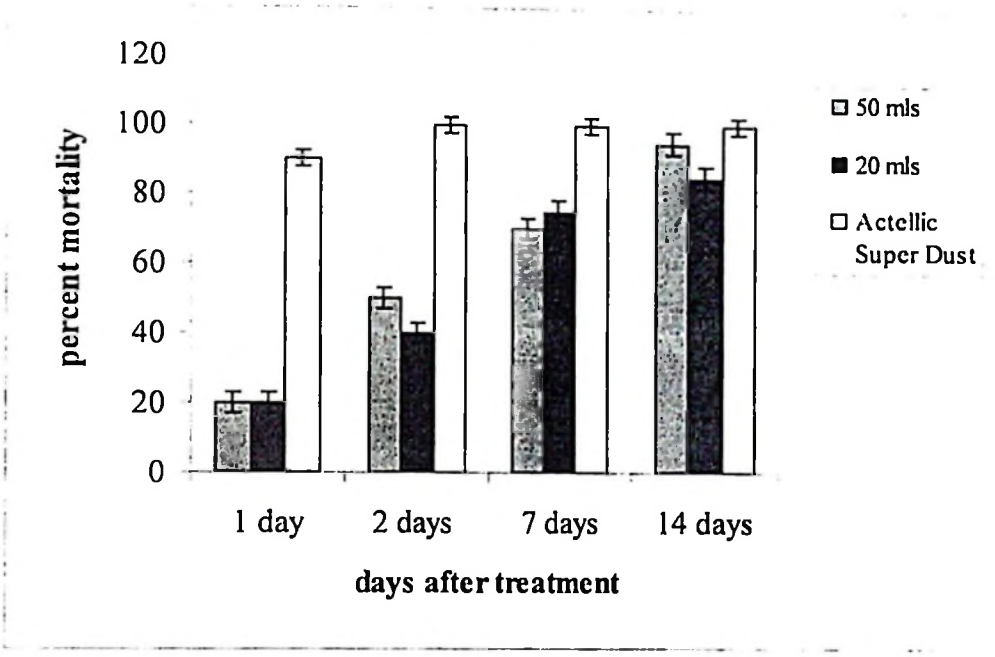

Figure 12. Mortality of bean weevils after treatment with two different concentrations of *T. minuta* water extract and Actellic Super Dust.

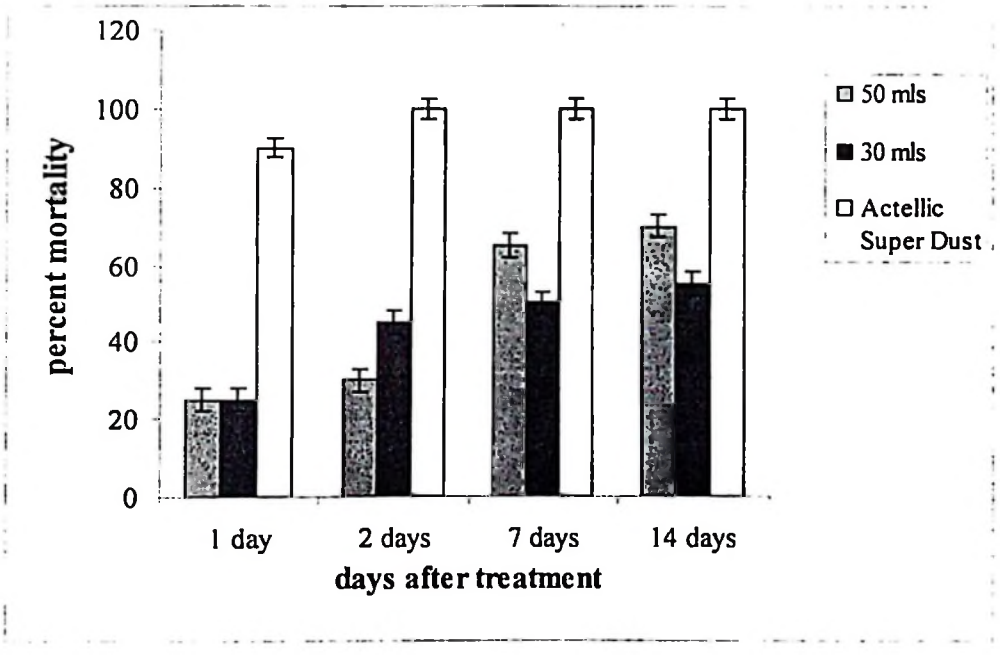

Figure 13. Percent mortality of weevils after treatment with two different concentrations of *E.globus* water extract and Actellic Super Dust.

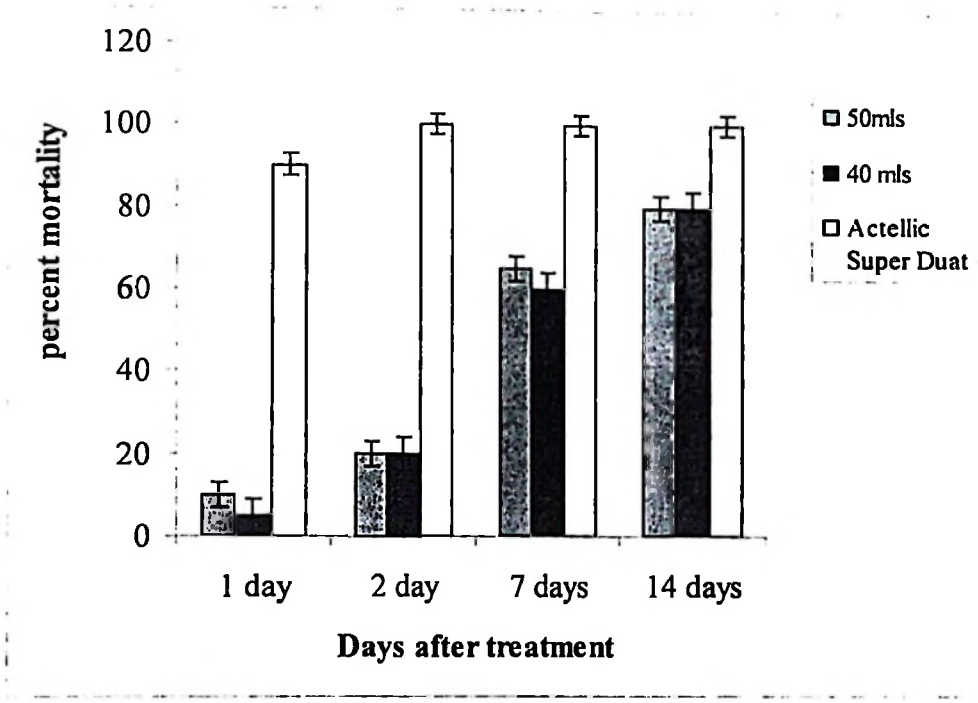

Figure 14. Percent mortality of weevils after treatment with two different concentrations of *R. communis* water extract and Actellic Super Dust.

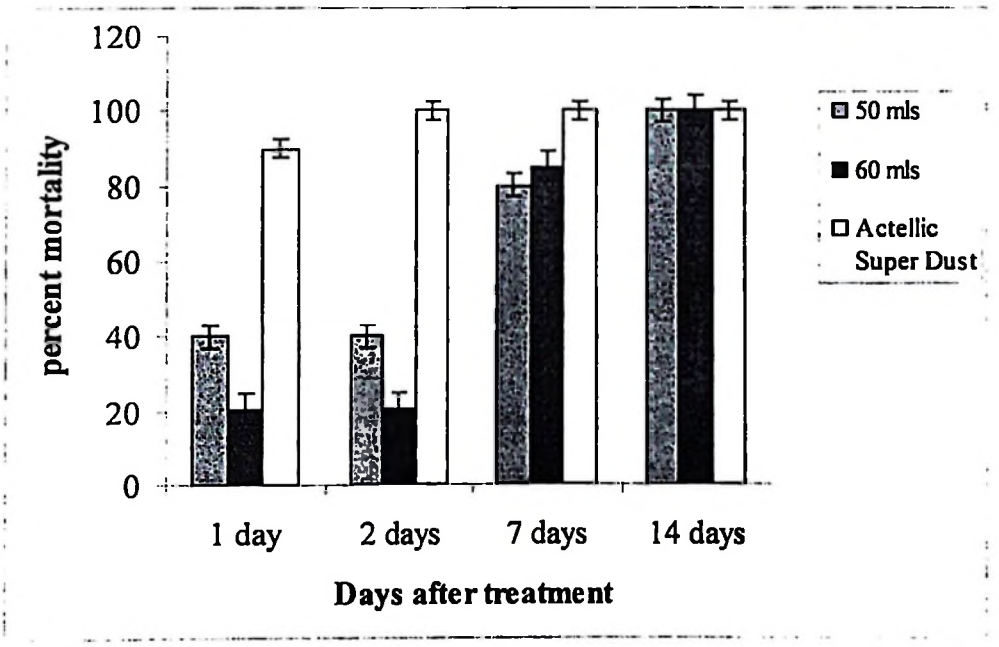

Figure 15. Percent mortality of weevils after treatment with two different concentrations *N. mitis* water extracts and Actellic Super Dust.

4.3.2 Bean seed damage in water extracts treatments against bean weevils (*A. obtectus* and *Z. subfasciatus*)

The results show significant differences ($P < 0.05$) in damaged, undamaged seeds and number of holed seeds treated with *O. canum, T. minuta E. globus* and *R. communis* in all concentrations and control except bean seeds treated with *N. mitis* after three months, (Table 3).

Table 3 Mean numbers of damaged, undamaged and number of holed bean seeds treated with two concentrations of botanicals in water extract formulation after three months.

Botanicals	Concentration mls/ kg	Total treated seeds	Damaged seeds	Undamaged sees	Number of holes
O. canum	50	25	12.42^{a}	12.58^{c}	50.33^{a}
O. canum	10	25	10.92^{a}	14.08^{c}	52.67^{a}
T. minuta	50	25	12.42^{a}	12.58^{c}	52.06a
T. minuta	20	25	12.72^{a}	12.25^{c}	52.06^{a}
E. globus	50	25	13.50^{a}	11.64^{c}	57.61^{a}
E. globus	30	25	14.25^{a}	10.75^{c}	48.11^{a}
R.communis	50	25	15.16^{a}	9.91^{c}	$52,17^{a}$
R.communis	40	25	14.08^{a}	10.82^{c}	57.50^{a}
N. mitis	50	25	5.83^{b}	19.17^{b}	11.50^{b}
N. mitis	60	25	1.17^{bc}	24.75^{ab}	2.11^{b}
Actellic	1	25	0.00^{c}	25.00^{a}	0.00^{b}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05) DMRT.

4.4 Comparison of performance of dust, oil and water formulations against common bean weevils (*A. obtectus* and *Z. subfasciatus*)

The results show significant differences ($P < 0.05$) in percent mortality of bean weevils between the three formulations. Bean seeds treated with oil extract formulation resulted to higher mortality of adult bean weevils followed by dust formulation. Water extract formulations gave the lowest percent mortality at 14 days after treatment (Fig. 16).

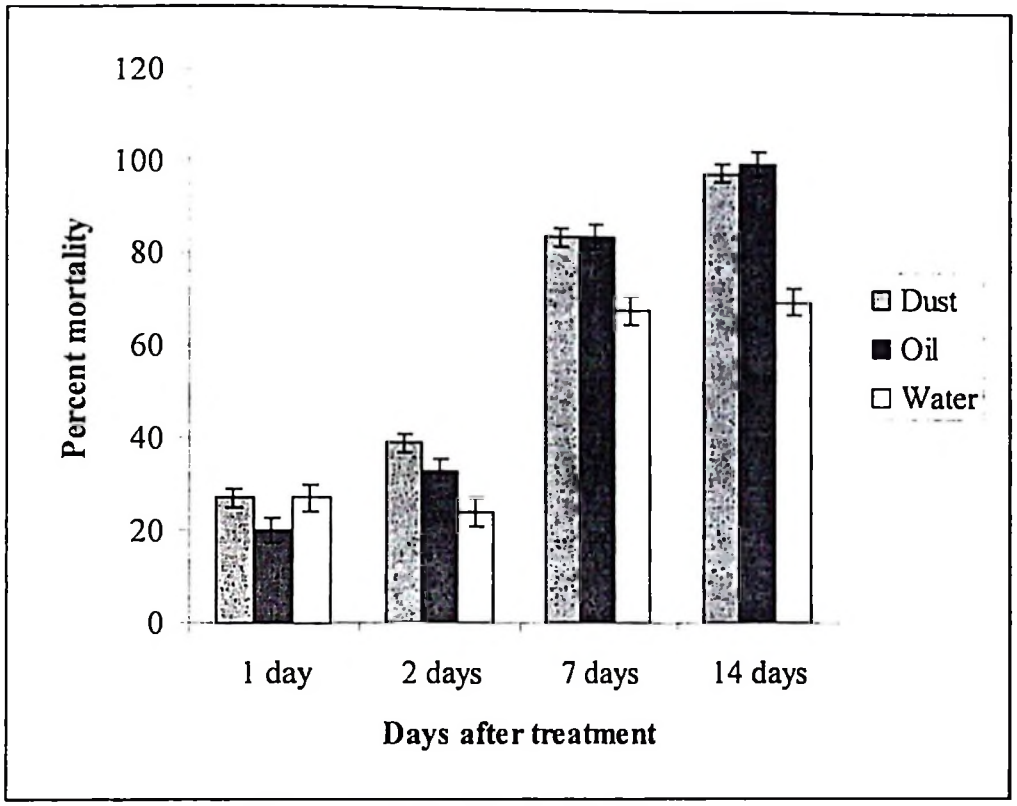

Figure. 16 **Percent mortality of weevils in bean seeds treated with dust, oil and water extract formulations.**

4.4.1 Mean number of damaged seeds in dust, water and oil extract treatment against common bean weevils

Table 4 shows significant differences ($P < 0.05$) in number of damaged seeds between the three different formulations. Higher numbers of damaged seeds were observed in bean seeds treated with water extract formulations followed by dust formulations. Bean seeds treated with oil extract formulations showed very low number of damaged seeds which was not significantly different from the control (Actellic Super Dust) (Table 4).

Table 4: Mean number of damaged bean seeds treated with dust, water and oil extract formulations against bean weevils

Formulations	botanicals	Treated seeds	Damaged seeds
Water	*O. canum*	25	15.50^{abc}
Water	*T. minuta*	25	16.78^{abc}
Water	*E. globus*	25	18.21^{abc}
Water	*R. communis*	25	20.05^{a}
Water	*N. mitis*	25	4.78^{ef}
Dust	*O. canum*	25	10.50^{d}
Dust	*T. minuta*	25	14.22^{cd}
Dust	*E.globus*	25	9.56^{de}
Dust	*R. communis*	25	14.11^{cd}
Dust	*N. mitis*	25	3.83^{ef}
Oil	Sunflower	25	0.39^{f}
Oil	Groundnut	25	0.33^{f}
Oil	Coconut	25	0.11^{f}
Oil	Castor bean	25	0.00^{f}
Oil	Soy bean	25	0.17^{f}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05) DMRT

CHAPTER FIVE

DISCUSSION

5.1 Performance of *O. canum, T. minuta, Globus, R. communis* and *N. mitis* powder formulation against bean weevils (*A. obtectus* and *Z. subfasciatus*)

The results of the study have shown that all botanicals in powder formulation have some effects on common bean weevils. However, the mortality of weevils was slow and the effectiveness of botanicals varied considerably depending on their source and concentrations applied. This study has shown that higher concentrations, all the botanicals gave high mortality of bean weevils in comparison to application of low concentrations. Low mortality of weevils and higher number of damaged seeds treated with *T. minuta* and *R. communis* indicates low efficacy of these botanicals. The low mean number of damaged bean seeds in treatments with *O. canum* and *E. globus* shows higher efficacy of these botanicals. However *N. mitis* had highest efficacy compared to other botanicals used as dust formulations. Shetto *et al.* (1995) reported that *N. mitis* has shown a great potential in the control of maize weevils and possibly other storage pests. Other results (Mulungu *et al.* 2007) have indicated that *N. mitis* plant possess insecticidal properties that can be used in control of insect pest on stored beans. Sharaby (1989) reported that the leaves of *E. globus* powder showed repellent activity against bruchids. Secondary compound in *T. minuta* are effective deterrence of numerous insects through several different mechanisms (Jacobson, 1989). However appropriate harvesting of *N. mitis* need to be studied because the organ used is roots which is an important part of the plant

5.2 Performance of Sunflower, Castor bean, Soybean, Coconut and Groundnut oil extract formulations against common bean weevils (*A. obtectus* and *Z. subfasciatus*).

The results from this study have shown that all vegetable oil extracts have high efficacy against common bean weevils. Although their action was slow, many seeds were free from damage, which implies that mortality of weevils was initially high. A report by Don-Pedro (1989) suggests that vegetable oils exert ovicidal action on bean seeds. Also Gabriele and Stoll (2003) found that adult females increasingly avoid laying eggs on seeds which were treated with oil more than 7 days previously and very few eggs indeed era laid on seeds which were treated 60 days before being newly infested with beetles. Credland (1992) and FAO (1999) suggest that oil may inhibit successful larval penetration into the seeds. Also the oils coat leads to a reduction of egg adherence on the treated seeds and some oil constituents have a direct toxic effect resulting to high mortality as was observed in the current study. Oil may become rancid after a period of storage thus making the treated produce impalatable when cooked.

5.3 Performance of *O. canum, T. minuta, E. globus, R. communis* and *N. mitis* water extract formulation against common bean bruchids (*A. obtectus* and *Z. subfasciatus*).

The results show low efficacy of the above botanicals in water extract formulations. The low mortality of bean weevils in botanical treatments than in the control indicates that botanical extracts in water formulation have low efficacy against bruchids. Reason could be that water is not a good solvent for extraction of the active

components in the botanicals. In a tight containers moisture may encourage fungal growth and may be absorbed in bean seeds and affect viability.

5.4 Performance of dust, water and oil extract formulations against common bean weevils

The results show a better performance of oil extract formulation followed by dust formulation compared to water extract formulation, which had the poorest performance. This indicates that extracts from vegetable oils are the most effective compared to dust and water extract formulations, which is in agreement with Don-Pedro (1989) that application of oils of botanical origin to stored beans is a method of protection against bruchid beetle attack which has been confirmed to be effective by many farmers. Credland (1992) examined the structure of bruchid eggs and suggested that the funnel structure at the posterior pole of *Callosobruchus* egg is the major route for gaseous exchange hence application of oil to bruchid eggs might occlude the funnel and thus lead to death of developing insect by asphyxiation. Also, Lienard *et al.*, (2004) reported that the oil coat leads to a reduction of egg adherence on treated seeds which prevent the first instars larva from penetrating the seeds.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

All the tested botanical extracts have shown potential for management of common bean weevils (*Acanthscalides obtectus* and *Zabrotes subfasciatus)* although the efficacy varied depending on the plant species and formulations used. In this study the oil extract formulations have shown higher efficacy than water and dust formulation against bean weevils due to the mode of action and compound properties. The performance of water and dust formulations can be improved by regular applications. Since all extracts of sunflower, castor bean, coconut, groundnut and soybean as well as dust and water extracts of *N. mitis* have shown higher efficacy as post harvest protectants against bean weevils, they are complementary to Actellic Super Dust. It is recommended that the knowledge about their use as post-harvest protectants should be spread to farmers so that they can apply them appropriately and thus improves their storage techniques. The use of plant material as an insecticide, especially in protecting beans can minimize the synthetic insecticides drawbacks. Because botanicals can grow in a wide range of environment many farmers obtain and use it by using their local methods. Currently there is a lack of systematic approach to use botanicals for crop protection. It is recommended that more investigations on the constituents of botanicals and mode of application be enhanced especial in developing countries. Information on their potency should be disseminated to farmers. A strong research-farmer linkage is needed to advocate the use of botanicals for improved crop protection, minimizing food insecurity and enhancing preservation of the natural environment to increase standards of living and reduce poverty. Other recommendations are to check the culinary factors, taste, smell

and cookerbility, to increase the production of botanicals, to check the shelf life of the extracts, dust water and oils after formulation. Also for sustainability there is need to domesticate the botanicals so that they are not completely lost due to environmental degradation leading to loss of native vegetation and to check whether they fit for exported produce. Since the way of obtaining and preparing botanical materials is prohibitive, I recommend farmers to obtain and prepare these botanical materials by using their local available methods they know or by using as raw for immediate use.

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APPENDECES

Appendix 1. ANOVA table for variables investigated in dust formulations

Source of variation	Degree of freedom	Dead insects	Live insects	Damaged seeds	Undamaged seeds	Number of holes
Time	8	7935.26***	6771.08***	608.05***	637.92***	21767.5***
Species	1	2081.64	3152.02*	11.64	3.96	19066.91***
Time*species	8	1214.25	3817.29***	39.92	37.47	7574.10***
Treatment	10	962.93	1536.29*	106.76**	118.76***	4917.11*
Treat*time	80	664.32	1126.80*	48.56	50.63	2827.45
Species*treat	10	1023.79	1219.68	47.11	39.88	4407.55*
Exp. Error	80	661.98	704.74	38.39	36.14	2273.86
Total	197					

Mean separation test for variables investigated on time, treatment and species in powder formulation.

Time	Dead insects	Live insects	Damaged seeds	Undamaged seeds	Number of holes
24 hours	2.81^{d}	7.82^{bc}	0.00^{c}	25.00^{a}	0.00^{b}
48 hours	4.09^{d}	5.82^{c}	0.00^{c}	25.00^{a}	0.00^{b}
1 week	8.36^{cd}	1.64^{c}	0.00^{c}	25.00^{a}	0.00^{b}
2 weeks	9.96^{cd}	0.05^{c}	0.09^{c}	24.91^{a}	0.09^{b}
4 weeks	7.91^{cd}	4.86^{c}	4.09^{b}	20.91^{b}	6.77^{b}
6 weeks	12.32^{cd}	6.59^{c}	5.82^{b}	19.18^{b}	12.00^{b}
8 weeks	20.77^{cd}	56.36^{a}	9.96^{a}	15.05^{c}	53.59^{a}
10 weeks	32.36^{b}	22.59^{b}	13.18^{a}	11.82^{c}	54.50^{a}
12 weeks	62.23^{a}	13.73^{bc}	10.41^{a}	13.68c	82.09^{a}
Treatments					
O.canum 16g	11.50^{b}	9.39^{bcd}	4.67^{abc}	20.33^{bcde}	11.94^{bcd}
O.canum 8g	24.33^{ab}	19.06^{abc}	5.83^{abc}	19.17^{bcde}	33.83^{abc}
T.minuta 16g	28.83^{a}	30.11^{a}	7.33^{a}	16.57^{c}	53.28^{a}
T.minuta 10g	21.94^{ab}	25.33^{ab}	$6.89^{ab.}$	18.11^{cde}	37.11^{ab}
E.globus 16g	15.67^{ab}	7.00^{cd}	5.67^{abc}	19.33^{bcde}	23.06^{abcd}
E.globus 12g	13.89^{ab}	9.89^{bcd}	3.89^{abcd}	21.11^{abcd}	14.61^{bcd}
*R.communis*16g	30.17^{a}	13.61^{abcd}	7.67^{a}	17.33^{de}	38.94^{ab}
*R.communis*14g	16.61^{ab}	19.33^{abc}	6.44^{ab}	18.56^{cde}	27.44^{abcd}
N. mitis 16g	13.61^{ab}	6.78^{cd}	2.94^{bcd}	22.06^{abc}	11.61^{bcd}
N. mitis 18g	10.39^{b}	4.28^{cd}	1.89^{cd}	23.11^{ab}	3.67^{cd}
Actellic 1g	9.61^{b}	0.39^{d}	0.00^{d}	25.00^{a}	0.00^{d}
Species					
Z.subfasciatus	21.11^{a}	17.19^{a}	5.08^{a}	11.59^{a}	33.04^{a}
A. obtectus	14.63^{a}	9.21^{b}	4.59^{a}	11.87^{a}	13.41^{b}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05), by DMRT.

Appendix 2. ANOVA table for variables investigated in oil extracts formulations

Source of variation	Degree of freedom	Dead insects	Live insects	Damaged seeds	Undamaged seeds	Number of holes
Time	8	198.19***	201.25***	0.33***	3398.25***	1.02*
Species	1	0.05	0.00	0.01	0.01	0.01
Time*species	8	2.99	2.16	0.08	0.08	0.85
Treatments	10	11.50***	11.50***	0.15**	0.15**	0.77
Species*treat	10	0.09	1.13	0.22	0.22	0.67
Exp. Error	160	2.86	2.81	0.09	0.09	0.15
Total	197					

Mean separation test for variables investigated on time, treatment and species in oil extracts formulations.

Time	Dead insects	Live insect	Damaged seeds	Undamaged seeds	Number of holes
24 hours	2.72^{c}	7.41^{a}	0.00^{b}	25.00^{a}	0.00^{b}
48 hours	3.32^{c}	6.73^{a}	0.00^{b}	25.00^{a}	0.00^{b}
1 week	8.36^{b}	1.64^{b}	0.00^{b}	25.00^{a}	0.00^{b}
2 weeks	10.00^{a}	0.00^{c}	0.00^{b}	25.00^{a}	0.00^{b}
4 weeks	9.73^{a}	0.27^{c}	0.27^{a}	24.73^{b}	0.59^{a}
6 weeks	9.91^{a}	0.09^{c}	0.23^{a}	24.77^{b}	0.23^{ab}
8 weeks	10.18^{a}	0.05^{c}	0.27^{a}	24.73^{b}	0.23^{ab}
10 weeks	9.95^{a}	0.05^{c}	0.05^{b}	24.94^{a}	0.41^{ab}
12 weeks	9.86^{a}	0.14^{c}	0.05^{b}	24.95^{a}	0.05^{b}
Treatments					
Sunflower 2.5ml	8.89^{abc}	1.22^{cd}	0.34^{a}	24.66^{b}	0.22^{ab}
Sunflower 1ml	8.61^{bcd}	1.56^{bcd}	0.25^{ab}	24.74^{ab}	0.22^{ab}
Castorbean 2.5m	7.78^{cde}	2.22^{abc}	0.00^{b}	25.00^{a}	0.00^{b}
Castorbean 2ml	7.83^{cdc}	2.17^{abc}	0.9^{ab}	25.00^{a}	0.00^{b}
Soybean 2.5ml	7.78^{cde}	2.22^{abc}	0.00^{b}	25.00^{a}	0.00^{b}
Soybean 3mls	7.22^{e}	2.78^{a}	0.25^{ab}	24.75^{ab}	0.44^{ab}
Coconut 2.5mls	9.11^{ab}	0.94^{de}	0.00^{b}	25.00^{a}	0.00^{b}
Coconut 4mls	8.00^{bcdc}	2.17^{abc}	0.16^{ab}	24.84^{ab}	0.11^{b}
Groundnut 2.5m	9.50^{ab}	2.50^{ab}	0.16^{ab}	24.84^{ab}	0.22^{ab}
Groundnut 5ml	7.89^{cde}	2.11^{abc}	0.34^{a}	24.66^{b}	0.61^{a}
Actellic 1g	9.89^{a}	0.11^{e}	0.00^{b}	25.00^{a}	0.00^{b}
Species					
Z. subfasciatus	8.21^{a}	1.82^{a}	0.15^{a}	24.85^{a}	0.17^{a}
A. obtectus	8.24^{a}	1.82^{a}	0.13^{a}	24.87^{a}	0.16^{a}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05), by DMRT.

Appendix 3. ANOVA table for variables investigated in water extracts formulations

Source of variation	Degree of freedom	Dead insects	Live insects	Damaged seeds	Undamaged seeds	Number of seeds
Time	8	21606.47***	9624.57***	990.51***	1600.88***	52098.2***
Species	1	36139.52***	5213.41***	1418.69***	1424.05***	95304.73***
Time*species	8	9829.71***	3267.60***	150.88***	151.76***	20099.68***
Treatments	10	2446.92**	1294.14***	222.58***	222.53***	9151.95***
Time*treat	80	893.94	753.94**	45.52***	45.54***	2557.44***
Species*treat	10	2061.01*	277.69	81.03***	81.35***	4128.25***
Exp. Error	80	707.93	457.71	22.62	22.61	1273.49***
Total	197					

Mean separation test for variables investigated on time, treatment and species in water extracts formulation.

Time	Dead insects	Live insects	Damaged seeds	Undamaged seeds	Number of holes
24 hours	3.50^{c}	6.73^{cd}	0.00^{c}	25.00^{a}	0.00^{c}
48 hours	3.23^{c}	6.96^{cd}	0.00^{c}	25.00^{a}	0.00^{c}
1 week	7.14^{c}	2.86^{c}	0.00^{c}	25.00^{a}	0.00^{c}
2 weeks	8.41^{c}	1.82^{c}	0.00^{c}	25.00^{a}	0.00^{c}
4 weeks	9.14^{c}	13.4^{bc}	9.23^{b}	15.77^{b}	14.05^{bc}
6 weeks	30.91^{b}	7.82^{bc}	10.41^{b}	14.50^{bc}	29.86^{b}
8 weeks	39.32^{b}	69.64^{a}	14.46^{a}	10.55^{d}	106.41^{a}
10 weeks	72.32^{a}	17.73^{b}	13.27^{a}	11.73^{cd}	109.32^{a}
12 weeks	86.10^{a}	13.50^{bc}	14.27^{a}	10.77^{d}	90.00^{a}
Treatments					
O.canum 50mls	27.28^{abc}	19.50^{ab}	12.42^{a}	12.58^{c}	50.33^{a}
O.canum 10mls	38.22^{a}	9.17^{bcd}	10.92^{a}	14.08^{c}	44.67^{a}
T.minuta 50mls	36.22^{a}	19.44^{ab}	12.42^{a}	12.58^{c}	52.06^{a}
T.minuta 20mls	34.22^{a}	16.72^{abc}	12.75^{a}	12.25^{c}	52.06^{a}
E.globus 50mls	31.39^{ab}	26.11^{a}	13.50^{a}	11.64^{c}	57.61^{a}
E.globus 30mls	34.69^{ab}	18.06^{abc}	14.25^{a}	10.75^{c}	48.11^{a}
*R.communis*50ml	34.17^{ab}	22.22^{ab}	15.16^{a}	9.91^{c}	52.17^{a}
*R.communis*40ml	44.61^{a}	25.28^{a}	14.08^{a}	10.83^{c}	57.50^{a}
N.mitis 50 mls	18.22^{bcd}	10.78^{bcd}	5.83^{b}	19.17^{b}	11.50^{b}
N.mitis 60 mls	8.83^{d}	4.39^{cd}	1.75^{bc}	24.75^{ab}	2.11^{b}
Actellic 1g	10.00^{cd}	0.00^{d}	0.00^{c}	25.00^{a}	0.00^{b}
Species					
Z. subfasiatus	42.40^{a}	20.74^{a}	9.53^{a}	7.13^{b}	60.86^{a}
A. obtectus	15.38^{b}	10.48^{b}	4.17^{b}	12.49^{a}	16.98^{b}

Means within columns followed by the same letter(s) are not significantly different (P = 0.05), by DMRT.