Addis Ababa University

School of Graduate Studies

Department of Biology

Evaluation of powder and essential oils of some botanical plants for their efficacy against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) on haricot bean (*Phaseolus vulgaris* L.) under laboratory condition in Ethiopia

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Biology (Insect Science)

August, 2007

ACKOWLEDGEMENTS

I would like to express my genuine appreciation to my advisor Dr. Emana Getu of Addis Ababa University for his excellent guidance, follow up and regular supervision during the study. I am also very grateful to Dr. Mekuria Tadesse of Essential Oil Research Centre for his constructive comments in the methodologies and the overall script of the text, and in plant material collections and extraction. I am also indebted to Dr. Bekele Jembere for his kind help in giving me some technical advices regarding the methodologies I have followed.

I wish also to extend my deepest thanks to the Essential Oil research Center staffs, especially to Dr. Tesfaye Bekele for allowing me to use the laboratory equipments in the center, Miss Tadelech Atew and Mrs. Fosia Ali for their full collaboration in extracting the essential oils of the test plants. My special thanks as well go to Mr. Merid Negash for his technical, moral and statistical advice. Many thanks to all my colleagues and friends in particular Biniam Wondale, Alemnesh Hailemariam, Ibrahim Mama, Essayas Akililu, Mesfin Wondafrash Tamirat Adnayehu and Solomon Yared.

I sincerely also like to thank Addis Ababa University, Biology Department for providing me financial support to conduct the study and to the Ministry of Education for the sponsorship it provided to me to pursue the post- graduate program.

Last, but not least to the Almighty God and to all my lovely parents for their moral support and encouragements throughout the study.

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Abstract

Experiments were conducted to evaluate the efficacy of botanical powders of *Jatropha curcas* (L.), *Datura stramonium* (L.), *Chenopodium ambrosioides* (L.), *Phytoloca dodecondra* (L'Herit), *Azadrachta indica* (A. Juss) and *Parthenium hysterophorus* (L.) and essential oil extracts of *C. ambrosioides* (L.), *Rosmarinus officinalis* (L.), *Eucalyptus globulus* (Labill), *Trachyspermum ammi* (Sprague) and *Cymbopogon citratus* (Stapf) against *Zabrotes subfasciatus* (Boh.) under laboratory conditions (27±3 ^oC, 50-70% RH). The test insect was reared feeding in glass jar (1L volume) and tested on whole haricot bean grains. For comparison Primiphos-methyl (0.125 g/150 g of grain) and untreated bean seed were included. The experiments were arranged in a completely randomized design with three replications.

Hundred percent mortality of *Z .subfasciatus* was obtained with *C. ambrosioides* leaf powder at all levels of concentrations (5, 10 and 15 g/150 g of grain) after 24 hour exposure time. More than 90% mortality of the adult *Z. subfasciatus* was also observed for bean seeds treated with *J. curcas*, *D. stramonium* and *P. dodecondra* within 96 hour after treatment. Similarly, essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* admixed to the bean at the rate of 750 mg/ 150 g showed 100% mortality of *Z. subfasciatus* in 24 hours after treatment. Haricot bean seeds treated with powder and essential oil extracts of the plant materials also reduced progeny production by the pest. All the five essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* applied at the highest dose (750 mg) caused over 97% reduction in progeny production by the bruchid. Likewise, using powder treatments of *C. ambrosioides* and *A. indica* at all tested levels gave more than 97% inhibition in F1 progeny production by *Z. subfasciatus*.

Essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* at the highest concentration (750 mg) diluted with 10 ml acetone and applied at 1, 2 and 3 ml per filter paper was significantly ($P \leq 0.05$) toxic, causing 100% mortality in 24 hour. All the tested essential oil extracts had also a significant ($P \leq 0.05$) level of toxicity in the fumigation bioassay against *Z. subfasciatus* in impregnated filter paper adjusted to 24 hour exposure period. Powder and essential oil extracts of the plant materials provided effective protection of haricot bean against attack by *Z. subfasciatus*. Additionally, plant materials admixed to the grain did not show any significant ($P > 0.05$) effects on the germination potential of the treated bean seeds. In general, these results indicate that the use of powder and essential oils of the tested plants can give effective control of the most important pest of stored bean, *Z. subfasciatus*.

1. INTRODUCTION

Grain legumes play an important role in the world food and nutrition requirements, especially in the dietary pattern of low-income group of people in developing countries. They are considered as "poor man's meat" and are important inexpensive sources of protein, dietary fiber and starch (Perla *et al*., 2003). They contain almost 2 or 3 times more protein than cereals (Deshpande *et al*., 2000). Because of their high protein and lysine content, they also represent good sources of supplementary protein when added to cereal grains and root crops, which are low in essential amino acids. In addition to their food value, pulses also play an important role in cropping systems because of their ability to fix nitrogen and thereby enrich the soil fertility.

The common bean (*Phaseolus vulgaris* L.) is one of the principal food and cash crop legume grown in the tropical world and most of the production takes place in developing countries, particularly in Africa, India, Latin America and Mexico (Abate and Ampofo, 1996; Songa and Rono, 1998; Schmale *et al*., 2002; Shimelis, 2005). Africa is considered to be a secondary center for bean genetic diversity. Beans are a major staple in Eastern, Southern and Great Lakes of Africa, where they are the second most important source of dietary protein after maize and the third most important source of calories after cassava and maize (Abate and Ampofo, 1996; Hillocks *et al*., 2006). The major bean producing countries of Africa are Kenya, Tanzania, Malawi, Uganda, Zambia and Ethiopia (Abate and Ampofo, 1996; Firdissa *et al.*, 2000; Nchimbi-Msolla and Misangu, 2002; Shimelis, 2005; Hillocks *et al*., 2006).

Around 30 species of grain legumes are grown both in the lowland and medium altitude areas of Ethiopia ranging from 700-2000 m above sea level (Ohlander, 1980). Among these, haricot bean is the most important food legume either as source of protein for local consumption or as an export crop for generating foreign currency (Belay *et al*., 1998; Mebeasilassie, 2004; Dawit, 2005; Tadele, 2006). This crop was introduced to the northern parts of the country around the 16^{th} century (Shimelis, 2005). Haricot bean has a wide range of adaptation and its production is very heterogeneous interms of ecology, cropping system and agronomic performance. Under Ethiopian situation, the primary producers of haricot bean in Ethiopia lie between altitudinal ranges of 1400 to 2000 m for rain fed agriculture and 700 m and beyond for irrigated agriculture (Ohlander, 1980; Belay *et al*., 1998). It also requires the minimum and maximum temperature of 10^0C and 32 ^{0}C, respectively, and an annual rainfall distribution of 200 to 600 mm and relative humidity of 15% (IAR, 1995; Belay *et al*., 1998; EARO, 2000).

Post-harvest system, encompassing primary processing, storage, secondary processing, handling and movement of natural products, is a crucial task in meeting the overall goals of food security which is a major prominent problem for the third world as it rapidly deteriorates. Haines (1982) stated that storage of durable grain produce has been practiced in various forms since man started growing his own food and other resources rather than being dependent on hunting and gathering. It has been estimated that between 60 and 80 % of all grain produced in the tropics is stored at the farm level (Boxall, 1998). A good quality of grain legumes in general, and haricot bean in particular is their ability to be stored for long period for latter consumption or to look for good price. However, most of the grain legumes including haricot bean suffer major economic loss caused by grain infesting insects because of cummulative effects of feeding, breeding, transmission of toxic and saprophytic fungi and associated changes in the micro-ecological conditions in the grain bulk, which hasten the deterioration process in the grain (Allotey, 1991). De Lima (1987) demonstrated that between 25 % and 40 % of stored agricultural produce is lost annually in the tropics because of the activity of storage pests.

Generally, a wide range of insect pests, the commonest among them being beetles and moths in the orders of Coleoptera and Lepidoptera (Emana and Assefa, 1998; Allotey, 1991; Bekele *et al*., 1997; Ferdu *et al*., 2001) attack all stored products. Every year, large quantities of stored beans are destroyed or contaminated because of the presence of insect pests especially beetles, which are the most important group of arthropods attacking these products. Among the beetles the predominant damaging pests of stored grain legumes are bruchids: *Callosobruchus maculatus* (Fab.), *C. chinensis* (L.), *Caryedon serratus* (Oliver), *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) (Nahdy and Agona, 1995; Sing, 1997; Nchimbi-Msolla and Misangu, 2002; Schmale *et al*., 2002; Emana *et al*., 2003; Harberd, 2004). By nature, these insect pests are cosmopolitan, prolific and rapid in their breeding and thus, they can quickly render a serious reduction in the quantitative and nutritive value of stored seeds (Mebeasilassie, 2004).

Control methods available for use in storage systems are numerous and diverse as many of their environmental factors are easily adjusted, mainly because of the nature of the post-harvest system. However, stored-product insect pests have been prevented principally by synthetic pesticides for many years worldwide. This continued and wide spread use of synthetic pesticides and fumigants has given rise to the development of resistance, pest resurgence, lethal effects on non-target organisms, toxic residues, worker safety, increasing cost of application, applying inappropriate doses by farmers, and stricter regulations for their use (Bekele *et al*., 1997; Mohale, 2004; Guedes *et al*., 2005). As a result, under such circumstances, alternative methods for reducing stored-product loses by insect pests are attractive and preferable over conventional control measures to be considered as stored-grain pest management tools.

The application of traditional materials like botanical insecticides and plant-derived pesticides to protect stored products against insect infestation is a prevalent and an age-old practice of farmers in small-scale stores (Gwinner *et al*., 1990; Mekuria, 1995; Bekele *et al*., 1997; Shaaya and Kostyukovysky, 2006). The wide-scale commercial use of plant extracts as insecticides began in the 1850s with introduction of nicotine from *Nicotiana tabacum*, rotenone from *Lonchocarpus sp*., derris dust from *Derris elliptica*, pyrethrum from the flower heads of *Chrysanthemum cinerariaefolium*, *Tagetes* sp, *Capsicum* sp, *Lanthana* sp, and so on. Some plant families may accumulate a restricted number of anti-insect chemicals, so called secondary metabolites, whilst others possess a wide variety of different structural compounds. The synthetic pesticide approach had its beginning in the use of botanical materials. Generally, botanical pesticides can be used as crude extracts, development of prototypes of known active ingredients, and sources of known active ingredients (Mekuria, 2003).

Currently, in developing countries including Ethiopia, there is an increasing interest and experience in the use of different types of plant products for the control of stored product insect pests because of drawbacks of conventional control measures (Bekele *et al*., 1995;

Mekuria, 1995; Emana, 1999; Bekele *et al.*, 1997; Dawit, 2005; Shaaya and Kostyukovysky, 2006). In spite of this increased demand and experience in the use of these eco-friendly natural products, which could supplement synthetic chemicals, it is often observed that the existence of difficulities around resource poor farmers of developing countries in terms of the use and the application aspects of these potent plants. Hence, the current investigation was intiated with the following objectives:

General objective:

To investigate the potential of botanical extracts for the management of Mexican bean weevil, *Z. subfasciatus*.

Specific objectives

- ✓ To study the effects of botanical plant powders and essential oils against *Z. subfasciatus*.
- ✓ To investigate antifeedant effects of different botanical products against Mexican bean weevil
- ✓ To determine the contact and fumigation potential of essential oils of botanicals against *Z. subfasciatus.*
- ✓ To establish effective dose for test materials against *Z. subfasciatus.*

2. LITERATURE REVIEW

2.1 Mexican bean weevil *Zabrotes subfasciatus* (Boheman)

Bruchids are the major problem affecting bean (*P. vulgaris*) seed both in the field and storage. Two species of bruchids are major pests of stored beans, the Mexican bean weevil *Z. subfasciatus* and the common bean weevil *A. obtectus*. These insect pests are endangering stored legumes throughout the world, particularly in the tropical belt (Abate and Ampofo, 1996; Schmale *et al*., 2002; Nchimbi-Msolla and Misangu, 2002). According to Ferede and Tsedeke (1995), these two species are the major pests of stored legumes in Ethiopia. Mexican bean weevil is of recent introduction to Ethiopia (Emana *et al*., 2003). *Z. subfasciatus* was recorded for the first time in 1989 in Ethiopia from haricot bean with which maize seeds were sparsely mixed in the Bako Research Center farm store (Abraham Tadesse, 1996). It was observed that when the populations of bruchids left unchecked, they grow exponentially and can completely destroy the crops within few months (Schmale *et al*., 2001).

Bruchids of economic importance in Ethiopia include the bean bruchids, the Mexican bean weevil or spotted bean weevil and the cowpea bruchids (Emana *et al*., 2003). The study conducted in Bako showed that about 14% of grain loss by Mexican bean weevil in haricot bean stored for 12 months (Adane and Abraham Tadesse, 1996). Moreover, Mc Farlane (1988) showed that infestation by Mexican bean weevil could cause a decrease of 12% of the available protein content.

2.1.1 Descriptions and Identification

Mexican bean weevil, *Z. subfasciatus* belongs to the order Coleoptera, family Bruchidae and subfamily Amblycerinae, commonly known as bruchids. It was first described and named by Boheman as *Z. subfasciatus* in 1833 (CPC, 2004). This species probably evolved in South and Central America and used as original hosts of the wild ancestors of the modern cultivated forms of the Lima bean *Phaseolus lunatus* (L.), and the common bean, *P. vulgaris* (Fabaceae) (Sing, 1997; Rodríguez *et al*., 2002). The adult *Z. subfasciatus* is about 1.8 to 2.8 mm in body length, which makes it the smallest of the bruchids commonly infesting stored legume seeds (Dendy and Credland, 1991). The adults exhibit strong sexual dimorphism. The elytra are short, relatively broad and together are somewhat square in shape. The elytra of the female are strongly marked with a pattern of white and pale grey setae on a dark (almost black) background while that of the male has rather uniform light grey-brown pubescence (sometimes mottled with darker brown) over a dark-grey cuticle. On the apex of the tibia of each hind leg there are two movable spurs, called calcaria, which are reddish in color and equal in length (CPC, 2004; Graham, 2006). On the other hand, the more closely related bruchid *A. obtectus* possess no teeth on the hind femur (FAO, 1992; CPC, 2004). Moreover, it has long, filiform antennae that are black with basal segments reddish yellow.

2.1.2 Biology and Behavior

The species *Z. subfasciatus* usually exhibits a common mode of oviposition and larval emergence in the storage habitat (Sing, 1997). The adult female commonly lays and distributes some of its eggs uniformly on the seed available on the grain surface in groups of two or more eggs (Utida, 1967; Pimbert and Pouzat, 1988). The egg, which is more ellipsoid than the usual flattened ovoid, is protected by a covering excuded at the time of oviposition that fixes the egg firmly to the substrate (Southgate, 1979). In contrast to many other bruchids, adult females of *Z. subfasciatus* most frequently do not glue their eggs on the pod (Pimbert, 1987 cited in Sperandio and Zucoloto, 2004) rather on the seed coats to stimulate ovarian production and to induce oviposition activity (Pimbert and Pierre, 1983). The egg stage usually lasts 5-6 days. Though there are variable figures with regard to the total number of eggs laid by a single female, many researchers reported to be 22 to 55 eggs/ female (Utida, 1967; Golob and Kilminster, 1982; Dendy and Credland, 1991; Abate and Ampofo, 1996). Dendy and Credland (1991) also reported that fecundity of female is often correlated with the weight of the females at emergence.

The newly hatched larvae bore via the eggshell and immediately penetrate the legume testa in the cotyledons. The larvae continue to feed inside the cell and tunnel, forming a cell and usually molting four times before pupation. The late instar larvae concentrate feeding on a small region, directly below the testa forming a distinctive circular “windows” that commonly become visible externally as insect development progresses (Abate and Ampofo, 1996; Teixeira and Zucoloto, 2003). The larval and pupal stage lasts about 23 and 6-7 days, respectively. The newly formed adults may remain in the cell for several days before pushing out the window in order to mediate adult emergence. The emerging adults usually escape the host seed coat by pushing with their legs and head (Sing, 1997). Adults are relatively short lived in which mating occurs soon after emergence, and egg laying immediately follows. The adult longevity is about 13 days under optimum conditions. The adults are not known to feed any appreciable degree on stored legume host (Abate and Ampofo, 1996; Teixeira and Zucoloto, 2003), which makes the choice of oviposition sites even more important to the biological success of the descendants.

The duration of the life cycle varies with temperature, relative humidity, egg load and diet (Carvalho and Rosseto, 1968 cited in Teixeira and Zucoloto, 2003; Dendy and Credland, 1991; Sperandio and Zucoloto, 2004). In general, the life cycle of the insect at 27°C and 70% relative humidity takes around 34 days (Dendy and Credland, 1991).

The female will remain productive for short period of its lifetime, in which the rate of oviposition falls off as the female ages (Pimbert and Pierre, 1983; Dendy and Credland, 1991; Sperandio and Zucoloto, 2004). The sex ratio of emerging adults is about 1:1, but sometimes male biased (Dendy and Credland, 1991). Behavioural experiments showed that females of *Z. subfasciatus* produce a sex pheromone, which is perceived only by male (Pimbert and Pouzat, 1988). The production and/or release of this pheromone vary with the age and reproductive status of the females as shown by the olfactometer tests. Older females release a chemical cue that is more attractive for the males, and the emission of the sex-pheromone is temporarily inhibited following mating (Pimbert and Pouzat, 1988).

2.2 Post-harvest loss of beans

The term loss is often expressed in various ways and it is not clear whether it refers to the amount of damage or total amount of grain lost. Similarly, the word loss has been interchangeably used with the term damage. Nevertheless, in the context of stored food legumes, post-harvest loss is usually expressed as loss of commodity weight in the period between harvest and consumption; loss of nutrients in stored legumes; qualitative deterioration caused by contaminants or biochemical changes rendering legumes unfit for human consumption; loss of seed viability; and loss as a result of physical damage (Salunkhe *et al*., 1985).

It has been estimated that about 60 to 80 % of all grain produced in the tropical countries is stored at farm level (Boxall, 1998). Crop products must be stored in the way that the quality does not deteriorate during the storage period; the quantity is not unintentionally reduced; it is secure against pests, disease and physical loss; and is accessible at the time and quantity required.

Crop losses and deteriorations of produce during storage are likely to occur unless adequate precautions are taken. Frequently, reported that worldwide a minimum of 10 % of cereals and legumes are lost after harvest (Boxall *et al*., 2002). Like wise, in Ethiopia loss of stored produce reached up to 20-30 % (Abraham, 1996; Emana, 1999). However, it is widely agreed that food losses after harvest can be substantial and are important, especially in the developing world in terms of quantity, quality, nutritional and economic value (Golob *et al.*, 2002).

Harris and Libland (1978) estimated appreciable quantities of stored legumes are lost to bruchids every year. A study conducted in Honduras evaluated and demonstrated that post-harvest weight losses associated with bruchid infestations in dry beans stored by subsistence farmers ranges 5.5% to 8.5% (Espinal, 1993). FAO (1985a) estimated losses on pulses in the range of 25- 50 % due to infestations from bruchids, weevils and other insects in Africa. In Tanzania, for example, bean losses of up to 40% due to bean bruchids have been reported (Nchimbi-Msolla and Miswangu, 2002). Abate and Ampofo (1996) also showed that in Ethiopia stored bean damage by *A. obtectus* and *Z. subfasciatus* reaches up to 38 % and bean weight loss to reach 3.2 %.

Z. subfasciatus and *A. obtectus* are very closely related to common bean. Many legumes including common bean consist of chemical substances that make them resistant to invasion by most insects, but bruchid species have developed tolerance to many of plant defense chemicals (Golob *et al*., 2002). Given this fact, together with evolution of different behavioral strategies has provided different species of Bruchidae with potential of feeding on specific legume varieties (Roger and Hamraoui, 1995).

Apart from direct feeding, damage by bruchids can reduce weight, quality, nutritional and viability of bean seeds. The damaged grains can be infested by surface microorganisms including fusarium (mycotoxins, aflatoxins), penicilium, apergillus and zearalenone (Tesfaye and Dawit, 2000).

Weight loss of bean to bruchids infestation usually related to quantitative loss of produce that is measured as a reduction in weight or volume, and hence can be valued and measured more efficiently. However, a reduction in weight may not necessarily because of feeding of insects, but also reduction in moisture content of the produce. Actual weight loss usually happens from feeding by storage pests. Study conducted in 1993 in southeastern Honduran communities indicated that post-harvest weight losses associated with bruchid infestations in dry beans stored by subsistence farmers ranges from 5.5% to 8.5% (Espinal, 1993). In a related report, weight loss of pulses ranges from 4-29 % in Eritrea (Adugna, 2006) and 3.2 % in Ethiopia (Abate and Ampofo, 1996).

The degree of loss due to bruchid damage is quite variable and depends on the storage period, storage conditions, and storage container and varieties (Nchimbi-Msolla and Miswangu, 2002; Mebeasilassie, 2004). In Ethiopia, the structural condition of the traditional granaries used for storage of beans varies from one farm to another. It includes “Koffo”, “Debignet”, “Gotera”, tin, clay pot and polypropylene bags. The main problems associated with these storage structures are lack of repair or replacement of the old structures, poor hygiene store, and the distance at which they are located. Granaries that are not repaired permit easy access to rodents, insects and flooding. Moreover, poor store hygiene and storage structures located nearby the farm land may cause the development, carry over and cross-infestation of insect pests from previous season harvest. As a result, they attribute to different types of stored bean loss and damage by bruchids. For instance, in Rift valley zones of bean producing areas percent weight loss of seeds varied with the type of seed storage structures. According to Mebeasilassie (2004), the highest percentage weight loss was seen on seeds kept in tins and clay pot, while the lowest was recorded on polypropylene bags and gotera. The losses were 0.04 and 3.47% for tins and polypropylene bags, respectively.

In addition to direct weight loss, bruchids also render qualitative loss, which is more frequently based up on subjective judgments and is perhaps identified via comparison with locally accepted quality standards. It may include the presence of contaminants, such as uric acid and other nitrogenous wastes; the presence of adult bruchids inside the seeds, exit holes, glued eggs to the seed, casted larval skin, and pieces of insect chitin; and changes in appearance, texture and taste, making it unfit for human consumption (Hill, 1990; Espinal, 1993; Nchimbi-Msolla and Miswangu, 2002). Commercial grain buyers usually reject or refuse to accept delivery of insect contaminated grain or may pay very less price to it. According to FAO (1985b) importers of beans and peas in the UK, object to accept products infested with most bruchid species. This situation will lead to economic losses to the producer and quantitative losses to the consumer (Nchimbi-Msolla and Miswangu, 2002) In addition to, their probable negative impacts on the quality of products, these foreign particles have been reported to have serious abrasive effects on human alimentary canal (Southgate, 1978).

Nutritional loss is represented by a reduction in the food value of the grain because of decreasing in its hydrocarbon, protein and vitamin content (Sing 1997). It is also the product of both the quantitative and qualitative losses. Mc Farlance *et al*. (1994) could demonstrate that a reduction in the protein content of pulses due to the feeding activity of bruchids on the cotyledons. In beans in particular, loss of protein is very important where there is infestation, as up to 25% of the dry matter may be crude protein (FAO, 1992). Besides, contamination from uric acid by bruchids is correlated with negative changes in nutritive composition of pulses causing an increased fat acidity and decreased levels of various vitamins in particular thiamin and essential amino acids (Salunkhe *et al*., 1985). Finally, the damage of beans by insect can provoke loss in qualitative and quantitative grain production enhancing more instability and poverty.

2.3 Management practices of *Z. subfasciatus*

Insects within stored feed and food cause numerous quality and health issues, which prompt the promoting and developing of possible methods to come up with these problems. According to Savidan (2002), control of stored pests will vary with the type of facility, the pest species and the type of food supporting the infestation, and the legal and economic methods of control available at a given time. Most often, however, effective management efforts always begin with a thorough inspection of the site to determine the source, type, and importance of the infestation (Gwinner *et al.,* 1990). Overall, chemical, biotechnical, biological, cultural and chemical methods are crucial tasks for successful control of the most dominant stored produce pests in general and *Z. subfasciatus* in particular.

2.3.1 Cultural Control

The principle involved in the cultural control of insect pests is purposeful manipulation of the environment to make it less favorable, thereby exerting economic control of the pests or at least reducing their rates of increase and damage. The development of cultural method requires a thorough knowledge of the life history and habits of the insect, and the plant host (Songa and Rono, 1998). The most vulnerable stage or stages of the insect pest's life cycle must be determined and storage practices must be altered to prevent attack, kill the pest, or slow down its rate of reproduction. Proper modification of storage practices has controlled many species of insect pests in the storage structures.

Sanitation or store hygiene is the leading preventive task in insect pest control in stored grain. Stores, silos, cribs and others and their nearby surroundings must be kept as clean as possible. Sanitation imparts its crucial role in preventing or reducing insect infestation in stored grains or foodstuffs. This method can be applied through removal of old grains, mechanically damaged grains, which attract secondary pests and residue of organic matter present in storage structure including sub- floor spaces, bins and old bags. Emana *et al*. (2003) also suggested that bean stores must be free from bruchids and only adequately dried clean seeds be stored. Manson and Obermeyer (2004) stated that a newly harvested product should never be stored with remainders of previous harvest as well as in used bags with out cleaning. Sanitation practices can bring satisfactory output if it is coupled with appropriate and adequate drying technologies (FAO, 1985b; Harberd, 2004). Thus, perfect storage hygiene is the basic prerequisite for successful storage and for the effectiveness of all on- going measures, like the use of insecticides or fumigants (Gwinner *et al*., 1990).

Farmers have practiced different types of traditional methods of control for many decades to prevent insect infestation. These methods will certainly keep on playing a role in small farm storage in the future (Gwinner *et al*., 1990; Golob, 1997). The most common treatments to limit insect activity are mixing inert materials and organic materials with stored grain. According to Golob (1997), the protection success depends up on the effect of the preservation on the grain, the rapidity of its action, the period of storage and proper mixing.

Inert dusts are finding increasing use as storage protectants in the grain industry. These dust particles primarily exert their effects on insects as a result of desiccation: water loss is a consequence of the destruction of the cuticle (Gwinner *et al*., 1990; Salunkhe *et al.*, 1985; Golob, 1997; Golob *et al*., 2002). They also fill in the spaces between the grains, usually causing restricted locomotion towards partners whereby they cause reduced progeny emergence (Gwinner *et al*., 1990).

Inert dusts commonly used in storage structure against storage insect pests include: non-silica dusts as rock phosphate, lime and lime stone; sand, wood ash, tobacco and saw dust, and clays; diatomaceous earths; and silica aerogels. For example, a laboratory experiment in Zimbabwe (Golob, 1997) showed that Drycide at 0.2 % (w/w) caused more than 90 % mortality of *A. obtectus* and *Z. subfasciatus,* within 2 days of exposure and completely prevented the emergence of F_1 progenies. Similarly, hydrated amorphous silica has been found to control *Z. subfasciatus, A. obtectus* and *C. maculatus* (Golob *et al*., 2002). Grahn & Schmutterer (1995) cited in Golob (1997) also showed that hydrophobic amorphous silica dusts resulted in efficient control of *C. chinensis*, as no beetles survived after 48 hours at a concentration of 0.1%. A similar effect can also be achieved via treatment with wood ash, clay, sand and tobacco and sawdust. Emana and Assefa (1998) also showed that tobacco dust result in efficient control of *Sitotroga* *cereallela* (Oliver). Myers *et al*. (2001) also pointed out that the use of wood ash is an effective practice to reduce bruchid damages. Hakbijl (2002) also reported the use of ash from burnt cow dung as an insecticide against *T. castaneum*, *S. granaries* and *Cryptolestes ferrugineus* (Stephens) larvae.

Other cultural control methods most predominantly experienced by small-scale farmers include storing unthreshed bean where the dry pod provides a physical barrier against oviposition, and enrobing the seed with mud or cow dung (Salunkhe *et al.*, 1985; Abate and Ampofo, 1996). A few farmers store unthreshed bean to protect against *Z. subfiscaitus*, which infests only harvested beans (Abate and Ampofo, 1996). Moreover, sun drying at monthly intervals; crushing eggs or perturbing oviposition by bean sieving or bean tumbling; restaking grains and mixing small sized grains (teff); and use of different storage methods like Wooden bin, Earthen pot-pile, Mud house and Bamboo bin are some of the feasible cultural practices (Salunkhe *et al.*, 1985; Abate and Ampofo, 1996; Agona and Nahdy, 1998; Songa and Rono, 1998; Kiruba *et al*., 2006). For instance, Lale (1998) reported decreased oviposition and increased adult mortality of *C. maculatus* in grains stored after exposure to sun.Research on tumbling containers and repeated sieving as control methods has produced encouraging results, but the applicability of these techniques to large-scale production remains to be proven (Myers *et al*., 2001).

2.3.2 Biological control

Nontoxic means of control are of particular significance for on-farm storage in developing countries (Schmale *et al*., 2001). Biological control can be a viable tool, as beneficial insects may occur naturally or can be released where needed by farmers. Additionally, this method is of interest as a safe alternative to fumigation and traditional chemical insecticides since it is nontoxic and does not damage human health or the environment (Adane *et al*., 1998; Flinn *et al.*, 2006). Though many species of natural enemies that attack stored-product insects have been reported (Adane *et al*., 1998; Savidan, 2002; Flinn *et al.*, 2006), literature on the natural enemies and their impact as biological control agent against bruchids is scanty (Schmale *et al*., 2001).

The hymenopteran parasitoids have been shown to be effective in suppressing a limited number of pest species both in bulk grain storages and in food processing facilities and warehouses, promising that they can be used as potential biological control agents (Imamura *et al*., 2004; Flinn *et al*., 2006). Southgate (1979) has also reviewed that parasitoids belonging to 10 families of Hymenoptera and one Diptera attack bruchids at different developmental stages. Nonetheless, the egg stages with high degree of vulnerability because they are laid on the surface of the ripening pod. The major natural enemies of *Z. subfasciatus* and *A. obtectus* are the hymenopteran parasitoids such as *Stenocorse bruchivora* (Crawford) (Brachonidae), *Dinarmus basalis* (Rondani), (Pteromalidae) and *Horisemenus* sp. (Eulophidae)

S. bruchivora is a specialist, solitary ectoparasitoid of bruchids. Rios (1997) cited by Campan (2002) stated that ectoparasitoids of female *S. bruchivora* were effective in paralyzing totally and definitely the larva inside the bean before laying a single egg on bean surface. Campan (2002) also indicated that *S. bruchivora* parasitizes the 3rd and 4th instar larvae and occasionally prepupae of bruchids. In the experiment, the parasitoid caused significant reduction in the population density of *Z. subfasciatus.*

Like wise, *D. basalis* is a solitary ectoparasitoid that feed on larvae and pupae of several bruchid species developing in Leguminosae seeds. Compared to *S. bruchivora*, *D. basalis* is most often considered a generalist since it is able to parasitize a wide range of hosts, in a wide variety of legumes (Rasplus, 1988; Campan and Benrey, 2004). It has been proved a very good biological control agent against bruchids. Sanon *et al*. (1998) demonstrated that the introduction of *D. basalis* to the populations of *Z. subfasciatus* caused a total suppression of bruchid members. These parasitoid most often prefer to oviposit on fourth instar larvae and prepupae of *Z. subfasciatus* and *A. obtectus* (Schmale *et al*., 2001; Campan, 2002). Schmale *et al*. (2001) conducted an experiment on the control potential of three hymenopteran parasitoid species against the bean weevil in stored beans and found that the pteromalid parasitoid *D. basalis* appears to be a promising candidate for biological control of *A. obtectus* in stored beans.

Additionally, other polyphagous parasitoids of internally developing coleopteran and lepidopteran stored product pests include *Anisopteromalus calandrae* and *Pteromalus calandrae* (Sing, 1997). *A. calandrae* has been associated with the bruchid host species *C. maculatus*, *C. chinensis* and *Z. subfasciatus* infesting various stored grain legumes. In the same way, *P. calandrae* has also been seen parasitizing *Z. subfasciatus* and other bruchids. Rasplus (1988) reported that these two polyphagous larval parasitoids caused up to 90 % reduction in number of emerging bruchid progeny.

Currently, studies in the biological control of bruchids have also focused on the use of egg parasitoids *Uscana* spp. Sing (1997) indicated that around nine trichogrammatid *Uscana* species have been known to attack bruchid eggs. Study by Sood *et al*. (2002) has also revealed that trichogrammatid *Uscana mukerji* could cause 10-15 % egg parasitism, which result in additional mortality of the egg stages of *Z. subfasciatus* apart from natural mortality.

Some predators play a key role in regulating the population density of *Z. subfasciatus* and other associated primary stored legume pests, especially *A. obtectus* in stored bean ecosystem. *Xylocoris flavipes* (Reuter) is a generalist predator of stored product insects, largely to bruchids. Sing (1997) showed that the warehouse pirate bug could cause up to 75% populations suppression against *Z. subfasciatus*. The population suppression by the predator was likely being because of either predation on the parental bruchid or indirectly by disruption of mating and oviposition. Moreover, entomopathogenic fungi have been shown to be effective biological control agents against several insect pests of both field and stored grain. For instance, fungi such as *Beauveria bassiana* (Vuill.), *B. brongniartii* (Sacc.), *Metarhizium anisopliae* (Mots.) and *Paecilomyces fumosoroseus* have been found to control a number of stored product pests including *S. zeamais* and other bruchids as *C. maculatus* (Adane *et al*., 1998).

2.3.3 Host Plant Resistance

The use of varietal resistance is a prominent component of integrated pest management, particularly for small holding farmers where economic status does not permit them to apply synthetic insecticides. Plant resistance is an indirect pest control method, which involves the manipulation of the genetic make-up of the host so that it is resistant to pest attack. The principle behind the use of varietal resistance is mostly based on one of the mechanisms of antibiosis, non-preference or tolerance, where biophysical or biochemical factors are involved (Savidan, 2002).

Over the years, there have been numerous successes in breeding for resistance to a variety of pests and currently many crops are being selected for this purpose. Schoonhoven and Cardona (1982) tested more than 4000 of dry bean from the germ plasm bank and observed low levels of resistance to *Z. subfasciatus*. Schoonhoven *et al*. (1983) observed high levels of resistance against Mexican bean weevil in a number of wild haricot bean genotype accessions than cultivated genotype accessions. Fory *et al*. (1996) cited by Mebeasilassie (2004) reported resistance to *A. obtectus* and *Z. subfasciatus* in a wild line of *P. vulgaris*, which contained a soluble carbohydrate component that was strongly antimetabolic to larvae of *A. obtectus* and *Z. subfasciatus*. Lara (1997) also reported that the wild genotypes Arc. 5S and Arc. 1S and the near isogenic lines Arc. 1 and Arc. 2 presented high resistance of the antibiosis type to *Z. subfasciatus.*

In Ethiopia, research on host plant resistance of bruchids has identified cultivars against *Z. subfasciatus*. Firdissa *et al*. (2000) evaluated that 56, 32, and 65 indeginous and exotic haricot bean genotypes for their resistance against Mexican bean weevil. All of the the 25 CIAT accessions were resistant to *Z. subfasciatus*. However, from commercial bean varieties only Roba-1 exhibited resistance to the pest. Emana *et al*. (2003) also reviewed that out of 100 CIAT accessions tested in the laboratory, the genotypes “RAZ1”, “RAZ7”, “RAZ8”, and“RAZ11” have shown high levels of resistance to this pest.

Some of the mechanisms that attribute resistance to storage pests of legumes include morphological characters as seed size, seed coat, texture and color (Lara, 1997; Goossens *et al*., 2000; Mazzoneto and Vendramim, 2002). Researches targeted on searching for factors associated with resistance to bruchids clearly indicated that antibiosis is responsible for resistance to the common pests of grain legumes (Cardona *et al*., 1990; Goossens *et al*., 2000). They have also reported that a novel seed storage protein, “arcelin”, is an abundant, lectin-like seed storage protein that is present in wild *P. vulgaris* accessions resistant to *Z. subfasciatus*, but not cultivated haricot bean. So far, seven allelic arcelin variants have been identified (Acosta- Gallegos *et al*., 1998), designated arcelin1 to arcelin 7, of which variants 1 and 5 seem to be the most promising candidates to provide bruchid resistance in leguminous crops. According to Cardona *et al.* (1990) and Goossens *et al*. (2000) wild common bean accessions containing one of these two variants showed the highest resistance levels to the *Z. subfasciatus*. Moreover, when arcelin-containing accessions are used in a breeding program, high resistance levels will only be maintained in lines generated from crosses with arcelin-1 or arcelin- 5 parents (Kornegary *et al*., 1993). It is also shown that as the dose of arcelin increases its antibiotic effect is also greater against Mexican bean weevil.

Quite apart from a novel protein arcelin, another a novel inhibitor alpha- amylase variant found in some resistant bean varieties has been identified to have larval inhibition effect on *Z. subfasciatus* (Ishimoto and Chrispeels, 1996 cited in Goossens *et al*., 2000). In all together, resistance against *Z. subfasciatus* as a result of arcelin is shown by: no oviposition; disrupted embryo development; failure of larvae to penetrate the testa; death of larvae within the cotyledon; failure of pupation or adult emergence; reduced fitness of adults; and reducing male and female weight (Simmonds *et al*., 1989; Firdissa *et al*., 2000; Mazzoneto and Vendramim, 2002).

2.3.4 Use of pheromones

Insects use pheromones to communicate (Linn *et al*., 1987) in many insect orders, females produce sex pheromone and release into the air to attract conspecific males. Sex pheromones have been identified from most economically important insects and pheromones are used to monitor and control insects (Carde and Minks, 1997). Sex pheromone based monitoring methods are nowadays used against most insect pests.

The use of pheromones is one of the most promising techniques aimed at the control of stored-product insects (Burkholder and Ma, 1985; Trematerra, 1997). The use of these substances may lead to a drastic reduction of chemical treatments, thus determining remarkable economic advantages and improvement of product quality (Trematerra, 1997). In recent years, considerable progress has been made in monitoring and control of stored-product insects by using mass trapping, mating disruption and attracticide (lure and kill) methods.

Mass trapping of pest insects is an alternative to mass killing by using insecticides. The intention is to catch a pest species selectively and thereby suppresing the population to a level below the threshold of damage. This method is especially appreciable: where control by conventional insecticide is inapplicable and resistance has developed to conventional insecticides. For instance, successful control of Mediteranean flour moth *E. kuehniella* in stored-product was achieved using mass trapping method (Trematerra, 1997).

2.3.5 Botanical Control

Currently, the measures to control pest infestation in grain and dry foodstuffs rely heavily upon the use of gaseous, liquid and dust formulated insecticides, which pose possible health hazards and a risk of environmental contamination. Dusting and fumigation is still one of the most effective methods for the protection of stored, feedstuffs and other agricultural commodities from insect infestation. Some years onward, however, the number of conventional pesticides for the management of insect pests has decreased drastically. Hence, there is an immediate need to develop cost effective, cheap, safe alternatives that have the potential to replace the toxic synthetic chemical controls, yet which are simple and convenient to use by local farmers in small- scale farm storages.

The use of botanical insecticides and plant-derived pesticides for the control of insect damage to stored foodstuffs is a very common and an age- old practice of farmers in small- scale stores in tropics (Mekuria, 1995; Rajapakse and Emden, 1997; Bekele *et al*., 1997; Bekele, 2002; Tapondjou *et al*., 2002; Shaaya and Kostyukovysky, 2006). However, the use of insecticidal plants has more likely declined since the advent of synthetic chemicals usually due to farmers' intense rely upon commercial products, overlooking natural products as grain protectants (Golob *et al*., 2002).

Botanical insecticides frequently work in the same fashion as commercial products do. Nonetheless, plant derived pesticides exhibit several modes of action. Accordingly, toxicity against insect may be expressed by direct killing a particular life stage of insect, reproductive inhibition, acting as repellent, interference in host finding and selection of the insect in a manner that prohibits infestation, antifeedant, growth inhibitor and sterilant (Abate and Ampofo, 1996; Shaaya and Kostyukovysky, 2006).

Botanical products for the control of storage by insect pests can be collected either from the whole plant or from specific parts like seed, leaf, and bark by extraction. The most predominant way of using plants in post- harvest protection is the admixture of powders, oils and more purified insecticides including use of essential oils and organic solvent extract of plant parts as fumigants and repellents (Sing, 1997; Tapondjou *et al*., 2002; Harberd, 2004; Shaaya and Kostyukovysky, 2006).

Recently, there has been a renewed interest in the use of plant products as crop protectants for the control of both field and stored produce insect pests. Plant materials with insecticidal properties provide small-scale farmers with locally available, biodegradable and inexpensive method of pest control for storage. The plants of original insecticides have therefore, drawn attention for extensive research, which are now highly encouraged in order to meet the demands of IPM and environmental safety (Mulungu *et al*., 2007).

The insecticidal activity of many plant derivatives against several storage pests has been demonstrated. Rajapakse and Emden (1997) tested ten botanical powders for the protection of cowpea seeds against *C. maculatus*, *C. chinesis* and *C. rhodesianus*. The results showed that all the treatments were significantly superior to the untreated seeds.

The biological activity of several edible and non- edible oils against stored-product insect pests was reported by Messina and Renwick (1983), Shaaya *et al*. (1997), Rajapakse and Emden (1997), Khattak *et al*. (2001). Their findings also showed that the plant oil treatments were effective in causing 80 to 100% mortality of adults, reduced progeny emergence, and high percentage protection with no adverse effect on the viability of the seed. According to Hill and Schoonhoven (1981), crude palm and cotton oils were effective for the control of *Z. subfasciatus*. Hall (1998) also reported that vegetable, mineral and soybean oils tested at the rate of 5 mg/ kg bean against *Z. subfasciatus* caused reduced beetle oviposition and decreased embryo and larval survival greatly compared to the untreated seeds. Similarly, experiments by Lemma (1994) cited by Emana *et al*. (2003) showed that vegetable oils could also offer effective control of bruchids in stored beans.

Delobel and Malonga (1987) evaluated *C. ambrosioides* leaf powder for its toxicity and oviposition deterrent effect against *C. serratus*. They reported more than 90% mortality and a complete reduction in fecundity. Su (1991) also evaluated chenopodium oil for its toxicity and repellency to *C. maculatus, S. oryzea, Lasioderma serricorne* (F.) and *T. confusum*. He reported 100%, 92.5% and 52.5% mortality for *C. maculatus, S. oryzea* and *Lasioderma serricorne,* respectively. In Ethiopia, Mekuria (1995) also demonstrated that *C. ambrosioides* powder at the rates of 2 and 4% w/w performed nicely and resulted in high percent adult mortality, reduced progeny emergence and low percent grain damage.

Bekele *et al*. (1995) evaluated the bioactivity of materials from the leaves of *Ocimum kilimandscharicum* against *S. zeamais*, *R. dominica* and *Sitotroga cerealella* (Olivier). They reported 100% mortality after 48 hr exposure of adults of the three insect to dried leaves and essential oil extract of the plant. Bekele (2002) also evaluated the toxicity of different plant parts of *Milletia ferruginea* (Hochst.) against *S. zeamais* in maze seeds. Seed powder applied at 10%w/w to maize seeds was toxic to the weevil and caused significant reduction in reproduction. Moreover, Mebeasilassie (2004) screened the efficacy of *M. ferruginea* seed powder against *Z. subfasciatus* and found that 100% mortality at the dose of 15 g/ 250 of grain within 24-hour exposure time. Dawit (2005) also demonstrated that orange peel oil applied at the rate of 0.3% w/w caused 100 % mortality of *Z. subfasciatus*.

Adane and Abraham (1996), Emana (1999) and Asmare (2002) evaluated the efficacy of local plant materials including datura, chenopodium, endod, neem, croton, and castor against maize weevil. They reported that the treatments resulted in high percentage of adult mortality, reduced progeny emergence and low percent grain damage. Sharma *et al*. (2003) also studied the repellent activity of some plant extracts viz., *Acacia arabica* (L.), *R. cummunis* and *P. hysterophorus* against pulse beetle on chickpeas under laboratory conditions. *P. hysterophorus* extract showed maximum repellency against *C. maculatus*. Mulungu *et al*. (2007) evaluated insecticidal effect of some locally available plant products, namely garlic, pyrethrum and nyongwe for controlling *Z. subfasciatus.* The result showed that the locally available plant products were effective in reducing number of holes, percentage damage and weight loss of bean grains treated. Similarly, Bamaiyi *et al*. (2007) also evaluated the bioactivity of *Khaya senegalensis* (Desv.) seed oil and powder against *C. maculatus* on stored cowpea. The result indicated that adult mortality of *C. maculatus* was highest within 24-hour post treatment. Furthermore, the seed powder and oil treatments significantly reduced the F_1 and F_2 progeny emergence of the insect, without significant phytotoxicity to the host plant.

Generally, effective control of bean bruchids was also achieved by powdered leaf, seed and oil extracts of plant products such as *D. stramonium*, *Piper guineese*, *Capsicum annum*, *Ocimmum canum*, *O. bacilicum*, *E. globulus*, *C. citratus*, *R. officinalis*, neem, *Nerium oleander*, rhizome, turmeric, coconut, sesame and mustard (Abate and Ampofo, 1996; Gakuru and Faua, 1996; El-Atta and Ahmed, 2002; Saljoqi *et al*., 2006; Shaheen, 2006).

2.3.6 Chemical Control

Despite the technical and financial constraints, environmental effects and enormous other associated problems, curative rather than preventative measures are more frequently employed in both large and small-scale grain legume storages to cause bruchids below damaging levels. Commercially available chemicals most commonly applied to control bruchid infestation in stored grain legumes include organophosphates, carbamates and synthetic pyrethroids. These groups of insecticides have been used for over five decades to control insect pests both at the field and storage conditions. Many researchers have reported that the effective utilization of synthetic insecticides including fumigants, dusts or admixture of seeds and sprays for the control of bruchids in general and *Z.*

subfasciatus in particular (Schoonhoven and Dam, 1982; Gwinner *et al*., 1990; Golob, 1997; Harberd, 2004).

Dust formulations of insecticides, which are sold ready for use usually contain 0.1- 5 % active ingredient (Gwinner *et al*., 1990). These formulations often contain additives, which increase the adhesive power of the active ingredients to the stored grain. Dust formulations can be applied mixed with grains by shovel, on floors, flat surfaces and around the bottom of storage containers. Dusts should be mixed thoroughly and distributed allover the produce in order to achieve effective control of bruchids.

Most of synthetic pesticides in use for liable emergency action against bruchids when their population approaches or exceeds economic threshold level include: malathion, lindane, pirimiphos-methyl, permethrin, deltamethrin, metacrifos, fenitrothin, iodofenphos, chloropicrin, and cocktail of malathion and permethrin, primophos-methyl and permethrin ("Actellic super"), fenitrothion, iodofenphos, chloropicrin, deltamethrin and permethrin (Evans, 1985; Salunkhe *et al*., 1985; Gwinner *et al*., 1990; Hill, 1990). For instance Emana *et al*. (2003) reviewed that pirimiphos-methyl at the rate of 4-5 ppm provided effective control of bruchids in Ethiopia and is widely used at present. Moreover, Schoonhoven and Dam (1982) illustrated that fungicide Arasan at the recommended dosage of 85 g/kg provided complete protection from *Z. subfasciatus* attack for the 600-day test period. It is also seen that other fungicides like thiram and methoxychlor are effective in causing significant mortality of *Z. subfasciatus*.

Chemical fumigation of stored grains is another approach to insecticidal control. Fumigants are low molecular weight chemicals, highly toxic and volatile and are hence self- dispersing and non- persistent. It is noted that fumigation is one of the technique that most widely practiced allover the world in the control of bruchid, especially in large scale storage. Correctly applied, the tiny gas molecules of fumigants easily penetrate large stacks of grain right in to the individual grains, reaching and killing all stages of development of the pests. At least 16 chemicals have been registered as fumigants, but because of concern for human safety, methyl bromide, phoshine, methyl iodide, Carbon disulfide and aluminium phosphide are the primary fumigants currently being used commercially for stored products (Lee *et al*., 2003; Faruki *et al*., 2004). El-Nahal *et al*. (1984) cited by Mebeasilassie (2004) reported that methyl bromide on exposed larvae of *C. maculatus* reduced fecundity, number of adult progeny and there was a tendency of progeny treated females to last longer in their development stage. However, its main disadvantages are that the treatment confers no residual protection against reinfestation, once the commodity is again exposed, and the fact that the most effective fumigants are all highly toxic to humans and other non-target organisms.

Generally, the introduction of chemical grain protectants a number of years ago greatly assisted the achievement of the goal, but their role in pest management systems of the future is now open to question, due to the increasing incidence of insecticide resistance and the increasing health hazard, risk of environmental contamination and the increasing tolerance of residues in foodstuffs (Mohale, 2004).

2.3.7 Integrated Pest Management

Integrated pest management (IPM) provides feed manufacturers an effective alternative strategy to sole reliance on chemical control of stored product pests. IPM relies on managing insect populations through physical and biological control techniques and, as necessary, chemical insecticides. The adoption of an IPM strategy requires an understanding of stored product insects including their identification; biology and ecology, sampling to monitor insect populations, physical, biological and chemical control (Rees, 1995).

Current interest in IPM results from food safety concerns related to the use of residual insecticides and the development of resistance in populations of insects to insecticides (Herrman, 1998). In addition, IPM is an approach to pest control that uses cost benefit analysis in decision-making. Thus, in some cases, IPM offers a more economical means of controlling stored product insects than traditional chemical control techniques that relied on pre-determined (calendar based) application intervals.

3. MATERIALS AND METHODS

3.1 Insect Rearing

Adults of *Z. subfascitus* were obtained from Melkasa Agricultural Research Center and reared in the Insectory of Addis Ababa University, Biology Department. The rearing procedure was similar to that of Schoohoven and Cardona (1982). The experiment was conducted at 27± 3^0C and 50-70% RH. Haricot bean seeds used for the rearing were bought from local farmers in Melkasa and were kept in an oven at 40^0C for 4 h to disinfest the seeds against internal infestation and allowed to cool for 2 h before use (Bekele, 2002). Fifty pairs of unsexed adult *Z. subfascitus* were placed in 1-litre volume glass jars containing 250 gm of haricot bean seeds. The jars were then covered with nylon mesh to allow ventilation and held in place with rubber bands to prevent the escape of bruchids after introduction. The parent bruchids were removed by sieving after 13 days of oviposition period and seeds were kept under laboratory condition until emergence of F_1 progeny.

3.2 Description of the test plants

Jatropha curcas (L.) commonly called as physic nut and "Ayderke" in Amharic.

The physic nut is a drought-resistant species, which is widely cultivated in the tropics as a living fence. It belongs to the family of Euphorbiaceous. It reaches a height of up to 8m and is mainly cultivated for the production of seeds with oil content of 55-60 % seed yields (Adebowale, and Adedire, 2006).The seed, which is black and oval in shape, is rich in fixed oil (Shukla *et al*, 1996 cited in Adebowale, and Adedire, 2006).

Many parts of the plant are used in traditional medicine (Heller, 1996). The kernels of the fruit from the plant are pressed to give oil, which is used as a biodiesel, lubricant, dibble oil, soap production and medicinal uses. It is also reported that the plant is used as potential insecticide against a number of insect pests (Asmare, 2002; Ohazurike *et al*., 2003; Adebowale, and Adedire, 2006). The leaves used for fumigating houses against bedbugs. Additionally, the ether extracts of the leaves show antibiotic properties against *Staphylococcus aurous* and *Escherichia coli* (Adedire *et al.*, 2003).

Chenopodium ambrosioides (L.) commonly called as “Worm seed” or “Mexican Tea” and “Gime” in Amharic.

C. ambrosioides belongs to the family Chenopodiaceas. It is a strongly aromatic, hairy, annual or perennial herb, 40 cm to 1 m (1.3 to 3.3 ft) tall that grows throughout the world as weed, though its original home is in Central America (Rendle, 1983). The leaves are oval (up to 4cm long and 1cm wide) and toothed. The plant has been reported to have a wide variety of both medicinal and insecticidal properties against a number of helments (internal parasites) and insect pests for many years (Dobel and Malonga, 1987; Mekuria, 1995; Tapondjou *et al*., 2002). There are many compounds in Chenopodium. The compound considered the active ingredient is ascaridole, a monoterpene. The major components of oil of chenopodium are ascaridole (60-80%), isoascaridole, p-cymene, limonene, and x-terpinene (Chiasson *et al*., 2004; Quarles, 2006).

Phytolacca dodecandra L’Herit commonly called as “Endod”

Endod belongs to the family Phytolaccaceae. It is a native herb to Ethiopia and grows as a weed in many parts of the country. It is a perennial, climbing plant with hanging branches, growing up to 10 m and usually fruits twice a year. Endod has small berries, which, when dried, powdered, and mixed with water, yield a foaming detergent solution that has been traditionally used in Ethiopia for washing clothes. Furthermore, Kloos and McCullough (1985) reported molluscidal properties of endod fruits. Assefa and Ferdu (1999) noted that fruits of extracts endod significantly reduced the levels of leaf infestation and dead heart injury due to larvae of the maize stalk borer. Likewise, powdered endod berry applied on maize and sorghum grain has resulted in high percent adult mortality, reduced progeny emergence, and low percent grain damage against the maize weevil (Firdissa and Abraham, 1999; Asmare, 2002).

Datura stramonium (L**.)** commonly called as thorn-apple and Jimsonweed, and "Atsefaris" in Amharic.

Thorn-apple belongs to the family Solanaceae. It is an annual herb native to Asia and now grows all over the world as weed along roadsides, old fields, pastures and waste places. All parts, mainly seeds and leaves posses tropane alkaloids that attribute to its poisonous nature (Cooper and Johnson, 1984). In ingesting, any of its parts by human beings may have a varied type of physiological effects. It was also reported as it possesses insecticidal properties against storage insect pests including *C. chinensis* and *S. zeamais* under laboratory conditions indifferent parts of the world (Adane and Abraham, 1996; Firdissa and Abraham, 1999; Asmare, 2002; Shaheen, 2006).

Azadirachta indica A. Juss

The neem tree (*Azadirachta indica* A. Juss), from the Meliaceae (mahogany) family, known as margosa or Indian lilac, has long been recognized for its properties both against insects and in improving human health. The neem tree is an attractive broad-leaved evergreen, which can grow up to 30m tall with spreading branches covering some 10 m across.The seed consist of a shell and 1-3 kernels that contain azadirachtin and its homologues. Both the bark and leaves also contain biologically active molecules but not high levels of azadirachtin, which is found mainly in the seed Kernels (Mordue and Nisbet, 2000).

Azadirachtin, a complex tetranortriterpenoid limonoid from the neem seeds, is the main component responsible for both antifeedant and toxic effects in insects. Other limonoid and sulphur-containing compound with repellent, antiseptic, contraceptive, antipyretic and antiparasitic properties are found elsewhere in the tree, e.g. leaves, flowers, bark, roots (Buss and Park-Brown, 2002). Moreover, insects from different Orders differ markedly in their behavior responses to azadirachtin with Lepidoptera being the most susceptible one followed by Coleoptera, Hemiptera and Homoptera (Martinez and Emden, 1999).

Parthenium hysterophorus (L.) popularly known as Congress weeds, Carrot weed

Parthenium is a herbaceous annual or ephemeral member of the Asteraceae, reaching a height of 2 m. in good soil and flowering within 4-6 weeks of germination. Chemical analysis has indicated that all the plant parts including trichomes and pollen contain toxins called sesquiterpene lactones (Datta and Saxena, 2001). The major component of these toxins being parthenin and other phenolic acids of caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid and p-anisic acid, which are lethal to humans and animals (Oudhia, 2001). Despite the fact that *Parthenium* is considered a toxic plant, industrial uses are reported in the literatures (Sastri and Kavathekar, 1990). Parthenium is reported as promising remedy against hepatic amoebiasis and also roots to cure amoebic dysentery (Sharma and Bhutani, 1988 cited in Oudhia, 2001).

Moreover, parthenin derived from parthenium plant was shown to act as a feeding deterrent to the adults of *Dysdercus koenigii* F, *T. castaneum, Phthorimaea operculella* (Zell), *C. chinensis* (Sharma and Joshi, 1997) and sixth-instar larvae of *Spodoptera litura* (F) (Datta and Saxena, 2001). Shaheen (2006) as well studied the repellent and toxicant property of *P. hysterophorus* extracts against *C. chinensis*

Cymbopogon citratus [DC] Stapf commonly called as Lemon Grass; Lomi sar (Amharic).

Lemon Grass is a tall tropical grass belonging to the family Poaceae. It is an aromatic tropical plant with long, slender blades that can grow to a height of 5 ft (1.5 m). It is grown throughout the world (Saljoqi *et al*., 2006). The plant is believed to have a wide range of therapeutic effects including antibacterial, antifungal, and fever-reducing effects and also antimutagenic properties (Wilson *et al*., 2002). It contains 75 to 80% citral, an essential oil constituent that attributes to its insecticidal, fungicidal and bactericidal activities of the plant (Paranagama *et al*., 2003). Moreover, powder, solvent extracts and the essential oil of *C. citratus* have been known to have insecticidal activities against *C. maculatus*, *C. chinensis*, *C. rhodesianus*, *Sitophilus zeamais* (Motschulsky), *S. oryzae* (L) (Rajapakse and Emden, 1997; Saljoqi *et al*., 2006).

Rosmarinus officinalis (L.) commonly called rosemary and “Siga metbesha” in Amharic

Rosemary is a member of the mint family Lamiaceae, which also includes many other herbs. Native to the Mediterranean area, rosemary is now cultivated widely in different parts of the world, although it thrives in a warm and relatively dry climate (Al-sereiti *et al*., 1999).The plant takes its name from rosmarinus, a Latin term meaning "sea dew." Rosemary is an attractive evergreen shrub with pine needle-like leaves.

Traditionally, rosemary has been used by herbalists to improve memory, relieve muscle pain and spasm; stimulate hair growth, and support the circulatory and nervous systems; and recently in the prevention of cancer and its antibacterial properties (Al-sereiti *et al*., 1999).

Similarly, *R. officinalis* is also well-known for its substantial insecticidal activities against a number of insect pests in storage structures. It was reported that rosemary essential oil have contact and fumigant toxicity towards *O. surinamensis, R. dominica, S. oryzae* and *T. castaneum* (Shaaya *et al*., 1997). Papachristos and Stamopoulos (2002) also studied the contact and fumigation potential of rosemary against immature stages of *A. obtectus*. Tunc *et al*. (2000) also indicated ovicidal activity of *R. officinalis* against two stored-product insects, the confused flour beetle, *T. confusum* and the Mediterranean flour moth, *Ephestia kuehniella* Zeller.

Trachyspermum ammi (Sprague) popularly called as Bishop's weed, Carum, Ajowan and also "Nech Azmud" in Amharic.

Bishop's weed is a smooth or slightly hairy-branched perennial shrub belonging to the family Apiaceae, reaching a height of 90 cm. It is an aromatic spice closely resembling thyme in flavour and is native to Egypt and is now distributed in Mediterranean region, Africa and South-West Asia (Srivastava and Satyanarayana, 2006). Bishop’s weed oil is a colourless liquid possessing a characteristic odour of thymol and sharp burning taste (Ravindran and Balachandran, 2004). The major active components of this oil are thymol and carvacrol as the major constituent (35% to 60%). Other minor components are paracymene (15.6 percent), ã- terpinene (11.9 percent), â- pinene (4-5 percent), dipentene (4-6 percent), camphene and Myrcene (FAO, 1999).

Mintesnot and Mogessie (1999) indicated that crude extracts of bishop's weed contain antibacterial effect against food-borne pathogens. The methanolic extract of the fruits of this plant yielded a monoterpene with phenolic properties, which when tested in vitro against adult worms of *Setaria digitata*, the filarial parasite of cattle, resulted in more than 80% immobilization of the worms at an incubation period of 2hours (Srivastava and Satyanarayana, 2006).

Eucalyptus globulus (Labill) "Nech bahirzaf" in Amharic

Eucalyptus globulus is one of the most widely cultivated of Australia's native trees, belonging the family Myrtaceae. It is a medium to very tall forest tree, which may reach 70 metres in ideal conditions but is more commonly 15-25 metres in height. Leaves are mostly curved, acuminate at tip, and thick and leathery entire surface with fine straight veins and vein inside marlin, shiny dark green on both surfaces.

It was reported to have a number of medicinal values against diseases of abscess, arthritis, asthma, boils, bronchitis, burns, malaria, cold, cough, croup, and the like (Jones, 1998). Moreover, leaf powder, essential oil and solvent extracts of the plant part have been noted to have the potential of using as insect pest control against *S. oryzae* (L.), *Stegobium paniceum* (L.), *T. castaneum, C. chinensis* , *C. maculates* and *A. obtectes* (Tunc, 2000; Papachristos and Stamopoulos, 2002; Lee *et al*., 2003; Papachristos *et al*., 2004). For instance, the oil of *E. globulus* was reported to have contact toxicity and a seed protection effect against the pulse beetle (Srivastava *et al*., 1988). Negahban and Moharramipour (2007) evaluated fumigant toxicity of different *Eucaluptus* species.

3.3 Plant material collection and extraction

Plant materials (i.e. leaf and seed) used for the study were collected from natural habitats in Addis Ababa, Wondo Genet, the garden of Essential Oil Research Center in Addis Ababa and Melka Werer (Afar) (Table1). The identity of the plant materials collected was confirmed at the National Herbarium, Biology Department, Addis Ababa University.

3.3.1 Dried and ground materials

Fresh plant materials (whole leaves and seeds) of a known weight were kept in a well-ventilated room under shade for 2-3 weeks depending on weather conditions. Dried materials were ground to fine powder using mortar and pestle following the procedure of Habte (1999). Dried ground powders were applied at the rates of 5 g (3.3 %), 10 g (6.6 %) and 15 g (10 %)/150 g of grains.

3.3.2 Distillation of Essential oils

The essential oils of the test plants (fresh leaves, inflorescences, succulent stems and seeds) parts were extracted by hydro-distillation using Clevenger apparatus by the staff of the Essential Oil Research Center of Ethiopian Institute of Agricultural Research, Addis Ababa. Some amount of these fresh plant materials were cut into small pieces and were placed in a distillation flask with approximately 5 times water in volume and 10 glass beads. The distillation flask was placed in heating mantle (> 100^{0}C) for 3 hours and allowed to boil the sample until the distillation is completed. The distillate was collected in a separating funnel in which the aqueous portion is separated from the volatile oil. The water (lower) layer was slowly drowned off until only the oil layer remains. The oil was collected in a container and srored at about 4^{o}C in refrigerator until use.

Table 1: List of botanicals tested against *Z. subfasciatus* on haricot bean.

Treatments	Local name	Common name	Parts used
Jatropha curcas	Ayderke	Physic nut	seed powder
Datura stramonium	Atsefaris	Thorn-apple	leaf powder
Phytoloca dodecondra	Endod	-	seed powder
Chenopodium ambrosioides	Gime	Mexican tea	leaf powder
Azadirachta indica	Kinin	Neem	seed powder
Parthenium hysterophorus	Faramisesa	Congress weeds	Seed powder
Parthenium hysterophorus	Faramisesa	Congress weeds	leaf powder
Chenopodium ambrosioides	Gime	Mexican tea	fresh leaves and inflorescences
Eucalyptus globulus	Nech baharzaf	Eucalyptus; blue gum tree	leaves
Rosmarinus officinalis	Siga Metbesha	Rosemary	fresh leaves and inflorescences
Cymbopogon citratus	Lomi sar	Lemmon grass	leaves
Trachyspermum ammi	Nech Azimud	Bishop's weed	seed

3.4 Admixture toxicity assessment bioassay with botanical dusts and essential oils

For the dried and ground powder, 150 g of healthy disinfected haricot bean seeds were placed in 1-litre volume glass jars and were treated with 5, 10 and 15 g of dried and ground leaf, and seed powder of each test plant. Primiphos methyl at the rate of 0.125 g/ 150 g grain dust was also applied as a standard check. In addition, untreated grains were included as a control. After treatment, 20, 3-5 days-old *Z. subfascitus* of unsexed was introduced to the treated and untreated seeds in the glass jar. The jars were covered with nylon mesh and held in place with rubber bands. The number of dead insects in each jar was sieved and counted after 24, 48, 72 and 96 hr after treatment application. The experiment was designed in a completely randomized design (CRD) in three replications.

Likewise, the oils were applied to the grain at the rate of 30, 150 and 750 mg/ 150 gm of grain dissolved in 10 ml of acetone. The jar contents were shaken thoroughly for five minutes to ensure uniform distribution of the solution over grain surface. One set of the jars was treated with acetone alone and used as a control. Then, the treated grains were kept for 24 h to allow complete evaporation before the conduct of bioassays. Mortality observation was conducted at 24, 48, 72 and 96 h after treatment application and the percentage insect mortality was calculated using Abbott formula (Abbott, 1925 cited by Tapondjou *et al*., 2002) as follows:

$$\textbf{Corrected \% mortality} = \left(1 - \frac{\textbf{n in T after treatment}}{\textbf{n in Co after treatment}}\right) \times 100$$

Where : n = Insect number , T = treated , Co = control

3.5 F_1 Progeny Assessment bioassay

The treated jars were kept for additional 10 days of oviposition time after mortality assessment. All live and dead insects were sieved and discarded after 13 days of introduction. The treated and control grains were then kept until emergence of F_1 progeny. Then, the number of F1 progeny produced by the *Z. subfascitus* was counted. Counting was stopped after 45 days from the days of introduction to avoid overlapping of generation. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated using Tapondjou *et al*. (2002) method as follows:

$$\% \text{ IR} = (C_n - T_n) \times 100 / Cn$$

Where Cn is the number of newly emerged insects in the untreated (control) jar and Tn is the number of insects in the treated jar.

3.6 Damage Assessment Assay

Damage assessment was carried out on treated and untreated grains. Samples of 100 grains were taken randomly from each jar and the number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Percentage weight loss was calculated following the method cited in FAO (1985b) as:

$$\% \text{ Weight loss} = \frac{[U_{aN} - (U+D)] * 100}{UaN}$$

Where U= weight of undamaged fraction in n^{th} sample,

N= total number of grains in a sample,

U_a= average weight of one undamaged kernel

D= weight of damaged fraction in n^{th} sample. The assessment was repeated five times for each treatment.

3.7 Contact toxicity test of essential oils on a filter paper against parent bruchids

The contact toxicity effect of essential oils against *Z. subfascitus* was evaluated on filter paper discs by treating Whatman No.9 filter paper with the oils diluted in acetone. The filter paper (9-cm diameter) was placed in a 10-cm Petri dish (78.5 cm^2). Different levels of essential oil extracts (30 mg, 150 mg and 750 mg) were diluted in 10 ml acetone and applied uniformly to the filter paper discs at the rate of 1, 2 and 3 ml per filter paper. The acetone was allowed to evaporate for 20 min., prior to the introduction of the insects. Then, 1ml distilled water was added to the whole surface of filter paper of each treated paper discs, as the carrier of the active materials to the insect body, after evaporation of the solvent (Bekele, 2002). Other filter papers were also treated with three levels (1, 2 and 3 ml) of acetone as a control. After treatment, 5 pairs of 3-5 day- old adults of mixed sex of *Z. subfasciatus* were introduced in to the treated and control filter papers in the Petri dishes. The treatments were replicated three times. Insect mortalities were recorded up to 4 days for every 24 h. When no leg or antennal movements were observed, insects were considered dead as suggested by Negahban and Moharramipour (2007).

3.8 Essential oils as Fumigants

The fumigation toxicity of the essential oil extracts was tested following the method by Dawit (2005) with some modifications. Wide mouth bottles of 1-liter capacity with lids were used as exposure chamber. Filter papers of 9 cm diameter were treated with 1, 2 and 3 ml of essential oil at the rate of 12 mg, 60 mg, and 300 mg/100 g grain dissolved in 10 ml of acetone. On the other hand, the same amount of acetone alone was applied as a control. The solvent was allowed to evaporate for 20 minutes and then the filter paper was placed at the bottom of a 1-liter bottle. Twenty insects in small nylon mesh bag with 100 g food substrate were hung at the center of the glass bottle above the filter paper (Annex 11). The bottles were then closed tightly with a lid. Each treatment with respective control was replicated three times. Mortality was recorded after 24 h.

3.9 Germination test Assay

For seed germination test, 100-seed samples were taken at random from each replication of all the treatments and primiphos-methyl treated seeds. The seeds were placed in Petri dishes containing moistened filter paper (Whatman No. 1) and arranged in a CRD in five replications. Healthy untreated seeds were used as a control. The number of emerged seedlings from each Petri dish was counted and recorded after 7 days. The percent germination was computed according to Ogendo *et al*. (2004) as follows:

Viability index (%) = (NG*100)/TG

Where NG = number of seeds germinated and TG = total number of seeds tested in each Petri dish.

3.10 Data Analysis

Data entry and analysis were done using Microsoft Excel and SPSS Ins., Version 13, 2004, respectively. Data were transformed using Arcsine transformation when necessary. To observe the effects of the treatment on % mortality, % number of F1 progeny reduction, % weight loss and effect of treatments on germination of bean seeds one-way analysis of variance (ANOVA) was run. In cases where significant results were obtained, means separation were conducted using Tukey's studentized (HSD) test at 5% level of significance.

4. RESULTS

4.1 The effect of powders and essential oil treatments of different botanicals on mortality of parent bruchids

Results on the rate of mortality of adult *Z. subfasciatus* starting from 24 hr up to 96 hr after introduction to the seed mixture with different powders of botanical and essential oil extracts are shown in Tables 2 and 3.

One hundred percent mortality of *Z. subfasciatus* was recorded on seeds treated with primiphos-methyl and all doses of *C. ambrosioides* 24 hr after treatment application. Significantly, lower mortality was recorded with seeds treated with *J. curcas, D. stramonium*, *P. dodecondra, P. hysterophorus* and *A. indica* at all rates of treatments in 24 hr after treatment application. However, these same treatments caused significantly ($P \leq 0.05$) higher mortality against *Z. subfasciatus* at 15 g/150 g of grain 96 hr after treatment application (Table 2).

Different types of essential oils applied at different rates resulted in significantly higher ($P \leq 0.05$) mortality of *Z. subfasciatus* at 24 hr after treatment application (Annex 2). Essential oil extracts of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* applied at the rate of 0.75 g / 150 g of haricot bean induced 100 % mortality against *Z. subfasciatus* at 24 hr after treatment application (Table 3). However, essential oils from *R. officinalis*, *E. globulus* and *C. citratus* essential oils at lower dose (0.03g) caused less significant mortality against *Z. subfasciatus* at 24 hr after treatment application. Generally, results of these experiment showed that both powder and essential oil extracts of the plant materials were effective in controlling *Z. subfasciatus* comparable to primiphos-methyl.

Table 2: Cummulative mean % mortality±SE of adult *Z. subfasciatus* due to different rates of plant powder treatments, after different exposure time.

Treatments	Dose (g/ 150g grain)	Mean % adult mortality, h after treatment application			
		24	48	72	96
J. curcus	5	33.33±3.33cde	70.00±5.77abc	83.33±0.81abc	93.33±6.67ab
	10	56.67±5.15bc	73.33±6.18ab	86.67±2.71ab	93.33±3.33ab
	15	63.33±2.01b	93.33±3.33a	100.00±00a	100.00±00a
D. stramonium	5	10.00±00ef	33.33±0.88de	50.00±3.31fgh	66.67±2.01bcdef
	10	20.00±.6.93def	33.33±2.01de	63.33±00abcd	80.00±00abcd
	15	33.33±2.00cde	63.33±3.93abcd	80.00±00bcdefg	93.33±6.67ab
C. ambrosioides	5	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	10	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	15	100.00±00a	100.00±00a	100.00±00a	100.00±00a
P. dodecondra	5	16.67±3.33def	46.67±3.33bcd	56.67±3.93defg	66.67±4.22bcdef
	10	30.00±.00de	46.67±1.92bcd	66.67±2.0bcdef	76.67±6.05abcde
	15	36.67±2.01cd	50.00±3.31bcd	76.67±6.05abcde	90.00±00abc
A. indica	5	10.00±5.77ef	33.33±0.88de	40.00±00gh	46.67±3.82f
	10	13.33±0.88def	40.00±3.40bcde	43.33±1.92fgh	51.67±0.96ef
	15	16.67±2.71def	40.00±00bcde	56.67±1.92defg	66.67±5.39bcdef
P. hysterophorus (Seed)	5	13.33±0.33def	36.67±8.82cde	50.00±3.33fgh	56.67±3.93def
	10	20.00±4.27def	36.67±2.01cde	50.00±00fgh	63.33±2.01cdef
	15	26.67±6.18de	46.67±5.17bcd	60.00±5.85cdefg	76.67±4.92abcde
P. hysterophorus (Leaf)	5	10.00±00ef	20.00±2.89e	36.67±6.15h	53.33±3.82def
	10	23.33±2.22def	43.33±1.92bcde	46.67±1.92fgh	60.00±1.92def
	15	33.33±2.01cde	43.33±1.92bcde	53.33±1.92efgh	70.00±3.66bcdef
Primiphos- methyl	0.125	100±00a	100±00a	100±00a	100±00a
Control(untreated)	0	0±00f	0±00f	0±00i	0±00g

Means within same column followed by the same letters are not significantly different, P> 0.05%, Tukey student test (HSD)

Table 3: Cummulative mean % mortality±SE of adult *Z. subfasciatus* due to essential oils treated to seeds after different exposure time.

Treatments	Dosage (g / 150 grain)	Mean % adult mortality , h after treatment application			
		24	48	72	96
C. ambrosioides	0.03	83.33±8.82a	96.67±3.33a	100.00±00a	
	0.15	96.67±3.33a	100.00±00a	100.00±00a	100.00±00a
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
R. officinalis	0.03	33.33±5.39bc	66.67±5.39b	83.33±3.33ab	90.00±5.77ab
	0.15	53.33±0.33b	70.00±3.65b	88.33±0.60ab	93.33±0.29ab
	0.75	90.00±5.73a	96.67±3.33a	96.67±3.33ab	100.00±00a
E. globulus	0.03	43.33±1.92bc	63.33±3.93b	80.00±6.93b	83.33±4.92b
	0.15	43.33±3.33bc	73.33±6.17b	83.33±3.33ab	93.33±0.29ab
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
T. ammi	0.03	93.33±6.67a	100.00±00a	100.00±00a	100.00±00a
	0.15	96.67±3.33a	100.00±00a	100.00±00a	100.00±00a
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
C. citratus	0.03	23.33±6.05cd	35.00±4.46c	43.33±3.47c	45.00±2.88c
	0.15	96.67±5.77a	98.33±1.67a	98.33±1.67ab	98.33±0.19a
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
Primiphos-methyl	0.125	100.00±00a	100.00±00a	100.00±00a	100.00±00a
Control(untreated)	0	0±00d	0±00d	0±00d	0±00d

Means within same column followed by the same letters are not significantly different, P> 0.05%, Tukey student test (HSD).

4.2 Effect of powders of different plant materials on the F_1 progeny production of *Z. subfasciatus*, percent inhibition and percent grain weight loss.

The number of F_1 progeny produced by *Z. subfasciatus* in untreated and treated grain seeds with different powder plant materials is presented in Table 4. The number of F_1 progeny produced in most of treatments by using botanical powder materials was significantly ($P \leq 0.05$) low compared to the untreated check. Powder treatments prepared from *C. ambrosioides*, *A. indica*, *J. curcas* and *D. stramonium* at all tested concentrations caused more than 90% reduction in F_1 progeny production by *Z. subfasciatus* when compared to the untreared check. Nevertheless, more number of F_1 progeny and comparatively less percent in inhibition of reproduction by *Z. subfasciatus* were recorded in grains treated with leaf and seed powder treatments of *P. hysterophorus*

Moreover, results on assessment of percent weight loss caused by infestation of *Z. subfasciatus* to treated and untreated seeds are indicated in Table 4. Most of the powder plant materials admixture significantly ($P \leq 0.05$) reduced weight loss of the bean seed compared to the untreated check after 45 days infestation by *Z. subfasciatus*. However, *P. hysterophorus* seed powder at the lower doses (5 and 10 g) was markedly less effective in reducing the damage caused by *Z. subfasciatus*. There was no weight loss recorded on grains treated with primiphos-methyl.

Table 4: Mean Number of F_1 progeny, percent inhibition and percent grain weight loss caused by *Z. subfasciatus* on seeds treated with powders of different plant materials at different rates.

Treatments	Dosage (g / 150 grain)	Mean number of F1 progeny± SE	% inhibition	% weight loss
J. curcus	5	3.00±0.58abcd	90.42	0.04±0.03a
	10	2.33±1.85abcd	92.56	0.00±00a
	15	2.33±0.33abcd	92.56	0.00±00a
D. stramonium	5	1.00±0.57abc	96.81	0.03±0.03a
	10	0.00±0.00a	100.00	0.00±00a
	15	0.00±0.00a	100.00	0.00±00a
C. ambrosioides	5	0.67±0.67ab	97.86	0.00±00a
	10	0.00±0.00a	100.00	0.00±00a
	15	0.00±0.00a	100.00	0.00±00a
P. dodecondra	5	1.97±0.48abcde	93.71	0.05±0.02a
	10	1.38±0.21abcd	95.60	0.03±0.01a
	15	1.38±0.21abcd	95.60	0.00±00a
A. indica	5	0.33±0.33ab	98.95	0.00±00a
	10	0.00±0.00a	100.00	0.00±00a
	15	0.00±0.00a	100.00	0.00±00a
P.hysterophorus (seed)	5	10.00±2.31def	68.08	0.16±0.08ab
	10	9.00±5.13cdef	71.21	0.16±0.08ab
	15	4.33±0.88bcde	86.18	0.04±0.03a
P. hysterophorus (leaf)	5	20.33±5.17fg	35.11	0.13±0.1a
	10	0.00±0.00a	100.00	0.00±00a
	15	14.33±1.45efg	54.26	0.10±0.05a
Primiphos- methyl	0.125	0.00±0.00a	100.00	0.00±00a
Control (untreated)	0	31.33±4.81g	0.00	0.35±0.07b

Means within a column followed by different letters are significantly different, P≤0.05%, Tukey student test (HSD).

4.3 Effect of essential oil extracts of different plant materials on F_1 progeny production of *Z. subfasciatus*, percent inhibition and percent grain weight loss.

Results on F1 progeny production, percent inhibition in reproduction and percent weight loss caused by *Z. subfasciatus* are presented in Table 5.

Essential oil extracts of *C. ambrosioides, R. officinalis, E. globulus, T. ammi* and *C. citratus* applied at the highest rate (750 mg) (0.5 % w/w) caused significant ($P \leq 0.05$) reduction in the number of progeny produced. Progeny production was completely inhibited by bean seeds treated with primiphos-methyl dust and *T. ammi* essential oil extract at all levels of application. Alternatively, grain beans treated with lower dose (0.03 g) (0.02% w/ w) of *C. citraus* essential oil extract were significantly less effective in inhibition of oviposition and subsequent progeny emergence of *Z. subfasciatus* (Table 5).

No weight loss of stored bean seeds was recorded in seeds treated with primiphos-methyl dust. Similarly, essential oil extracts applied at the highest dose (750 mg) significantly ($P \leq 0.05$) protected the grain against the attack by the insect pest, without noticeable feeding damages (Annex 6). Significantly, lower percent protection of bean seed was recorded with essential oil extracts of *C. ambrosioides*, *E. globulus* and *C. citratus* at the lower concentrations.

Table 5: Mean Number of F_1 progeny, percent inhibition and percent grain weight loss caused by *Z. subfasciatus* on seeds treated with different essential oil extracts at different rates of application.

Treatments	Dosage (g / 150 grain)	Mean number of F1 progeny±SE	% Inhibition	% weight loss
C. ambrosioides				
	0.03	12.33±0.67ab	67.26	0.13±0.09ab
	0.15	0.67±0.05a	98.22	0.00±00a
	0.75	0.00±0.00a	100.00	0.00±00a
R. officinalis				
	0.03	7.67±1.13ab	79.64	0.02±0.01a
	0.15	5.00±0.54ab	86.73	0.01±0.00a
	0.75	1.00±00a	97.35	0.00±00a
E. globulus				
	0.03	10.67±1.50ab	71.68	0.08±0.05ab
	0.15	8.33±1.26ab	77.89	0.02±0.01a
	0.75	0.00±00a	100.00	0.00±00a
T. ammi				
	0.03	0.00±00a	100.00	0.00±00a
	0.15	0.00±00a	100.00	0.00±00a
	0.75	0.00±00a	100.00	0.00±00a
C. citratus				
	0.03	26.33±1.23bc	30.10	0.24±0.07bc
	0.15	2.67±0.94a	92.91	0.00±0.00a
	0.75	0.00±00a	100.00	0.00±00a
Primiphos-methyl	0.125	0.00±0.00a	100.00	0.00±00a
Control(acetone treared)	0	37.66±2.03c	0.00	0.36±0.04c

Means within a column followed by different letters are significantly different, P≤0.05%, Tukey student test (HSD).

4.4 The effect of filter paper contact toxicity of essential oils on adult mortality of *Z. subfasciatus*

Percent mortality of *Z. subfasciatus* on filter papers treated with different dosages of essential oil extracts is shown in Table 6. All the essential oil extracts caused significant ($P \leq 0.05$) mortality of adult *Z. subfasciatus* than the control and enhanced insecticidal effects were recoreded starting from 24 to 96 hours of post exposure periods after introduction of the bruchids on oil impregnated filter papers with essential oil.

Essential oils from *C. ambrosioides*, *R. officinalis, E. globulus, T. ammi* and *C. citratus* essential oil extracts provided 100% mortality at the dose of 750 mg / 10 ml of acetone and application rates of 1, 2 and 3 ml per filter paper in 24-hr exposure time (Table 6). For essential oil extracts of *C. citratus, R. officinalis, E. globulus, C. ambrosioides* and *T. ammi,* mortality of *Z. subfasciatus* ranged between 10- 100% at the dose of 0.15 g/ 10 ml of acetone per filter paper starting with 24h up to 96 h after treatment (Table 6). The lowest mortality was observed in *R. officinalis, E. globulus* and *C. citratus* oils at the rate of 0.03 g / 10ml of acetone and applied at 1, 2 and 3 ml / filter paper adjusted to 96 hour, which was not significant with the control (Table 6). However, application of essential oil extracts of *C. ambrosioides* and *T. ammi* caused 100% mortality of *Z. subfasciatus* at the dose of 0.03 g applied at 1, 2 and 3 ml / filter paper adjusted to 24 hr treatment application (Table 6).

However, in the bioassay the acetone treatment used as control did not cause any significant mortality of *Z. subfasciatus* at all levels of application, where they were introduced to treated filter papers from, which all the solvents had been evaporated (Table 6).

Table 6: Cummulative mean % mortality±SE of *Z. subfasciatus* due to essential oils from *C. ambrosioides, R. officinalis, E. globulus, T. ammi* and *C. citratus* applied at the dose of 30, 150 and 750 mg/ 10 ml and rate of 1, 2 and 3 ml after different times of exposure.

Treatments in mg	Dose (ml/ filter paper)	Mean % adult mortality, h after exposure			
		24	48	72	96
C. ambrosioides					
0.03	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
0.15	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
0.75	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
R. officinalis					
0.03	1 ml	6.67±3.33b	10.00±00cde	10.00±00ef	10.00±00fgh
	2 ml	3.33±0.36b	3.33±0.36de	10.00±5.77ef	13.33±3.33fgh
	3 ml	13.33±3.33b	26.67±0.20bc	33.33±5.39de	46.67±8.82d
0.15	1 ml	13.33±2.71b	20.00±5.77bcde	20.0±5.77de	20.00±4.27fg
	2 ml	16.67±3.33b	36.67±0.84b	53.33±1.67c	66.67±1.67c
	3 ml	90.00±3.33a	93.33±6.67a	96.67±5.27a	100.00±00a
0.75	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
E. globulus					
0.03	1 ml	0.00±00b	0.00±00e	0.00±00f	0.00±00h
	2 ml	6.67±0.42b	6.67±0.42cde	6.67±0.42ef	6.67±0.42gh
	3 ml	6.67±0.42b	6.67±0.42cde	13.33±6.67ef	13.33±6.67fgh
0.15	1 ml	10.00±5.77b	23.33±8.82bcd	33.33±8.82de	40.00±2.87de
	2 ml	80.00±5.77a	86.67±3.33a	86.67±3.33ab	93.33±3.33ab
	3 ml	80.00±5.77a	90.00±5.77a	93.33±3.33ab	100.00±00a
0.75	1 ml	100.00±00a	100.00±00a	100.00±00	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00	100.00±00a
T. ammi					
0.03	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
0.15	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
0.75	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a

Continued

C. citratus					
0.03	1 ml	0.00±00b	3.33±3.33de	6.67±6.67ef	6.67±6.67gh
	2 ml	3.33±0.36b	10.00 ±5.77cde	16.67±1.50def	23.33±8.81efg
	3 ml	6.67±3.33b	13.33±3.33cde	16.67±3.33def	26.67±3.33ef
0.15	1 ml	80.00±11.55a	80.00±11.55a	80.00±11.55b	80.00±11.55bc
	2 ml	96.67±3.33a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
0.75	1 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	2 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
	3 ml	100.00±00a	100.00±00a	100.00±00a	100.00±00a
Control (acetone)	1 ml	00±00b	00±00e	00±00f	00±00h
	2 ml	00±00b	00±00e	00±00f	00±00h
	3 ml	00±00b	00±00e	00±00f	00±00h

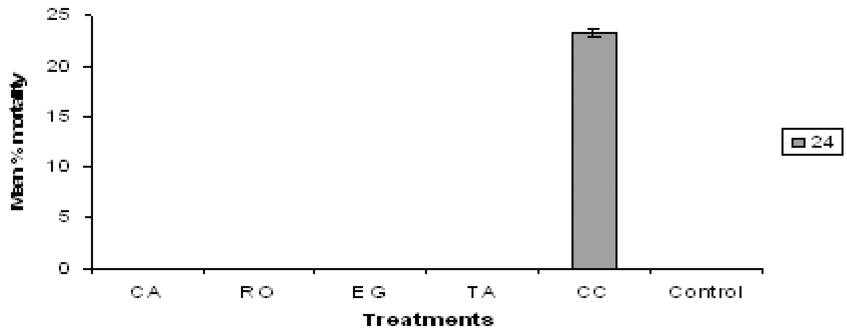
Means within a column followed by different letters are significantly different, $P \leq 0.05\%$, Tukey student test (HSD).

4.5 Fumigation potentials of essential oils on *Z. subfasciatus*

Bioassays conducted to determine fumigation potential of the essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* against *Z. subfasciatus* is shown in figures 1 to 3. Essential oils applied at the highest doses of 0.06 and 0.3 g/ 100 g grain caused significant ($P \leq 0.05$) mortality of adult *Z.subfasciatus* over 24 hr exposure time (Annex 8). Particularly, essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus* and *T. ammi* at the dose of 0.3 g/100 g of grain applied at 2 and 3 ml application rates gave 100% mortality of *Z. subfasciatus* 24 h after treatment application (Fig. 3), while *C. citratus* essential oil extract resulted in 93.3% mortality. Significantly, lower mortality of adult bruchids was observed on bean seeds fumigated with lower dose of essential oil extracts. No death of *Z. subfasciatus* was observed at the lower dose of 0.012 g/ 10 ml acetone applied at the rate of 1, 2 and 3 ml / filter paper, except that for *C. ambrosioides* and *C. citratus* (Fig. 1).

Acetone did not cause any significant mortality of the *Z. subfasciatus* at 1, 2 and 3 ml level of applications (Fig. 1 to 3).

a



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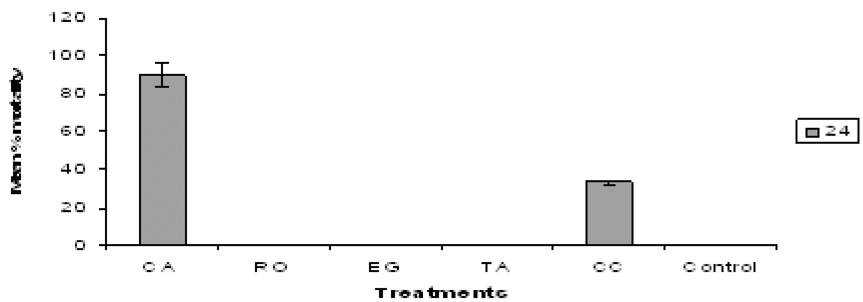

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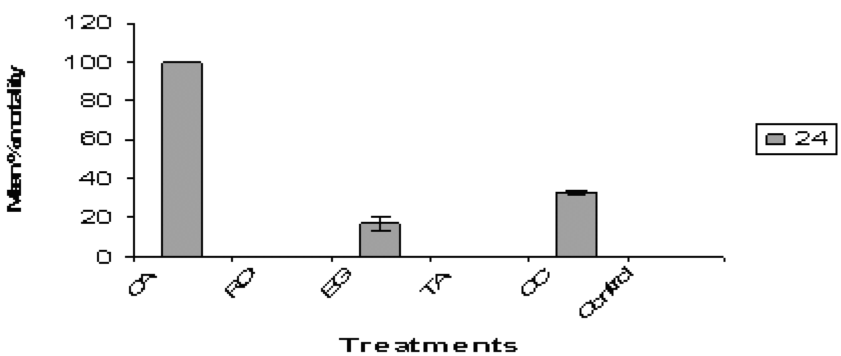
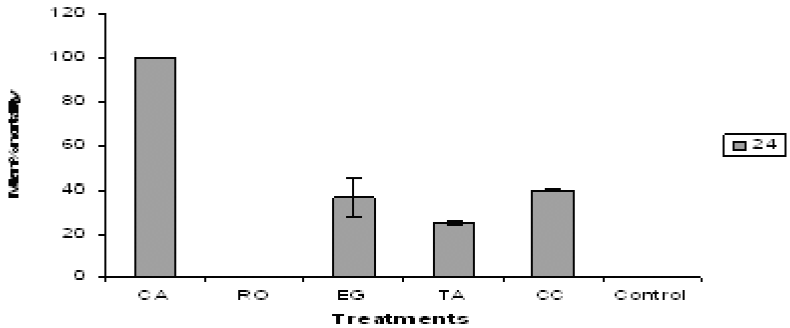


Figure1: Fumigant toxicity of essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* treated bean seeds to adult *Z. subfasciatus* applied at the dose of 0.012g / 10 ml and rate of 1ml (a), 2 ml (b) and 3 ml (c) at 24-hour exposure time.

Key: **CA =** ***C. ambrosioides*****; RO=** ***R. officinalis*****; EG=** ***E. globulus*****; TA=** ***T. ammi*****; CC= C.** ***citratus.***

a



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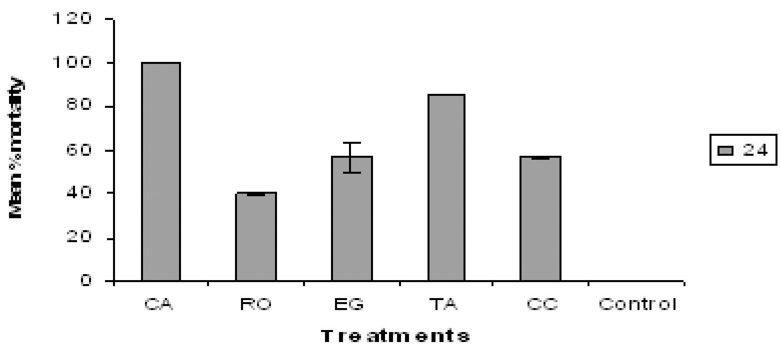

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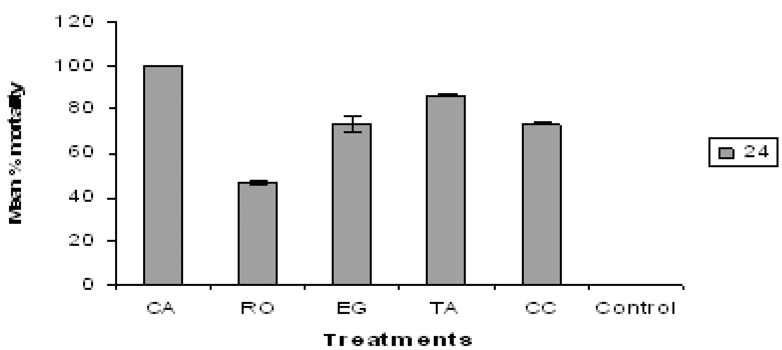
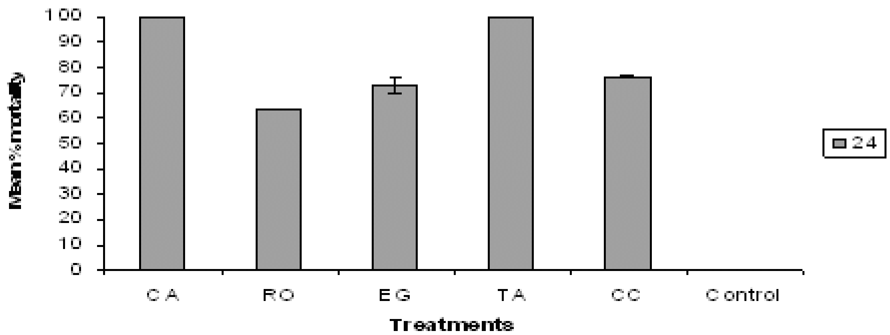


Figure 2: Fumigant toxicity of essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* treated bean seeds to adult *Z. subfasciatus* applied at the dose of 0.06 g/ 10 ml and rate of 1ml (a), 2 ml (b) and 3 ml (c) at 24 hour exposure time.

Key: CA = *C. ambrosioides*; RO= *R. officinalis*; EG= *E. globulus*; TA= *T. ammi*; CC=*C.citratus*

a



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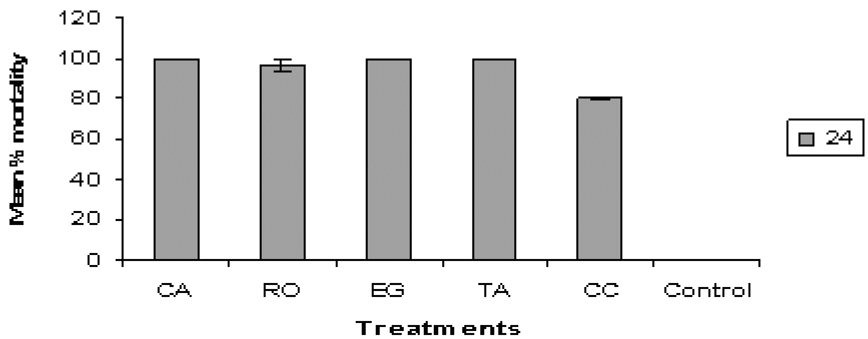

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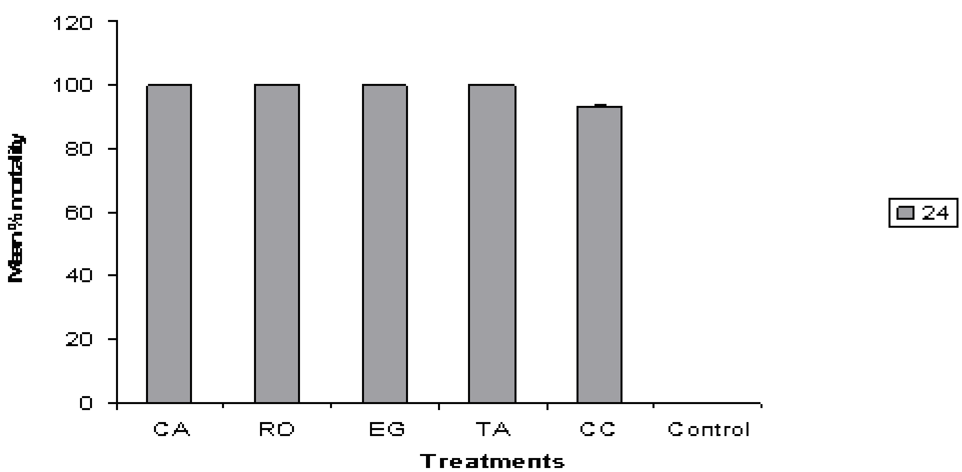

Figure 3: Fumigant toxicity of essential oils from *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* treated bean seeds to adult *Z. subfasciatus* applied at the dose of 0.3 g/ 10 ml and rate of 1ml (a), 2 ml (b) and 3 ml (c) at 24 hour exposure time.

Key: CA = *C. ambrosioides*; RO= *R. officinalis*; EG= *E. globulus*; TA= *T. ammi*; CC= *C. citratus*

4.6 The effect of powder and essential oil treatment of different plant materials on germination of haricot bean seeds

Percent germination of haricot bean seeds treated with different type of powder and essential oil extracts of plant materials are presented in Tables 7 and 8. There was no significant (P > 0.05) difference in germination capacity of haricot bean seeds treated with different powders and the untreated control (Table 7). The highest germination (95%) was recorded on bean seeds treared with *D. stramonium* leaf powder at the rate of 15 g/ 150 g of grain and the least on *A. indica*. Similarly, essential oil extracts and control did not differ significantly (P> 0.05) in their effects on the germination of haricot bean during this investigation (Table 8). The highest and the lowest germination of bean seeds treated with essential oils were 89.67% and 93%, respectively. Bean seeds treated with primiphos-methyl dust exhibited good germination capacity.

Table 7: Effect of powder plant materials on percent germination of haricot bean seeds (ns= non-significant)

Treatments	Dosage (g / 150 grain)	% germination of seeds±SE
J. curcus	5	92.33±0.33
	10	91.00±0.58
	15	93.00±2.52
D. stramonium	5	91.67±1.45
	10	92.33±1.20
	15	95.00±0.58
C. ambrosioides	5	91.33±0.33
	10	91.67±0.88
	15	93.67±0.88
P. dodecondra	5	90.67±0.88
	10	92.67±0.33
	15	92.33±0.88
A. indica	5	90.00±1.15
	10	90.33±1.76
	15	90.67±1.45
P. hysterophorus (seed)	5	91.00±1.15
	10	90.33±1.20
	15	92.00±1.00
P. hysterophorus (leaf)	5	90.00±1.15
	10	92.33±0.88
	15	91.33±0.88
Primiphos-methyl	0.125	90.67±1.76
Control (Untreated)	0	95.67±0.88

Table 8: Effect of different essential oil extracts on percent germination of haricot bean seeds (ns= non-significant)

Treatments	Dosage (g / 150 grain)	% germination of seeds±SE
C. ambrosioides		
	0.03	91.33±1.20
	0.15	92.00±1.53
	0.75	92.00±0.58
R. officinalis		
	0.03	90.00±1.53
	0.15	90.67±0.67
	0.75	90.67±0.88
E. globulus		
	0.03	89.67±1.76
	0.15	91.33±1.45
	0.75	90.00±2.00
T. ammi		
	0.03	90.67±1.53
	0.15	90.33±0.57
	0.75	93.00±1.00
C. citratus		
	0.03	90.00±1.00
	0.15	90.00±1.00
	0.75	92.00±1.00
Primiphos-methyl	0.125	90.67±1.76
Control (Untreated	0	95.67±0.88

5. DISCUSSION

Results of the present laboratory study indicated that most of the plant materials tested gave a comparable control of *Z. subfasciatus* with that of the standard insecticide, primiphos methyl.

Among the powder plant materials, *C. ambrosioides* leaf powder applied at all concentrations caused 100% mortality of *Z. subfasciatus* within 24 hours after treatment application. Such biocidal effect of *C. ambrosioides* have had adequate efficacy, like that of primiphos-methyl, which could induce100% mortality of the tested insect within 24 hr of treatment application.

Dried ground leaves of *C. ambrosioides* were shown to have different insecticidal activities against different storage insect pests. Delobel and Malonga (1987) found that a dosage of 1.4% w/w of *C. ambrosioides* dry ground leaf was enough to cause more than 90% mortality of *C. serratus* adults. Mekuria (1995) also reported that *C. ambrosioides* leaf and inflorescent parts applied at the rate of 4% w/w induced 100% mortality against maize weevil. Similarly, Tapondjou *et al*. (2002) also indicated that cowpea treated with dry ground leaf at the dosage of 0.4% killed 100% of *C. chinensis* and 80 % of *C. maculatus* within 48 hour. Hence, the current findings are in agreement with the previous works, suggesting that the efficacy of botanical dust from *C. ambrosioides* could serve as alternative bean bruchid controlling material.The major active constituent in Mexican tea responsible for its insecticidal property might be associated with its high content of essential oil (Tapondjou *et al*., 2002).

All powder plant materials admixed with haricot bean seeds caused significant mortality of adult *Z. subfasciatus*. Mortality of 100%, 93.33%, 90%, 66.67%, 76.67% and 70% of *Z. subfasciatus* were recorded on seeds treated with *J. curcas, D. stramonium*, *P. dodecondra*, *A. indica* and *P. hysterophorus* seed and leaf powders within 96 hrs of treatment application. Mortality of adult bruchids due to treatment of plant powders was directly related to application dosages and the time of exposure of the pest to the tested material. It indicated that higher dosage and longer exposure periods are needed to achieve appreciable management of *Z. subfasciatus*.

The present laboratory trails showed that locally available *J. curcas, D. stramonium*, *P. dodecondra*, *A. indica* and *P. hysterophorus* plant materials possess insecticidal activities that may be used in the control of *Z. subfasciatus*. Many researchers have also reported the insecticidal activities of these plant derivatives against different types of storage insect pests. Leaf powder of *J. curcas* was found to have insecticidal effect against *S. zeamais* (Asmare, 2002). The works of Adebowale and Adedire (2006) noted that jatropha seed oil extract inflicted a significantly high mortality of *C. maculatus, C. chinensis* and *S. zeamais.* Amaugo and Emosairue (2003) have also illustrated that aqueous and acetone extracts of seed kernel were superior to the other plant extracts in controlling stem borers and influencing yields of the crop.

Dry ground leaf and seed powders of datura were also evaluated for their bioactivies against storage insect pests. Adane and Abraham (1996), Emana (1999) and Asmare (2002) stated that dried leaf powder of *D. stramonium* has shown 60- 85% effectiveness in the control of *S. zeamias*. Similarly, Shaheen (2006) also reported that dry leaf powder and oil of datura were efficient, in inducing 100% mortality of *C. maculatus* within 5 and 7 days of treatment, respectively. Maize grain treated with seed powder of *P. dodecondra* induced 68- 100% mortality of *S. zeamais* (Adane and Abraham, 1996; Asmare, 2002).

Interestingly, leaf and seed powder of *P. hysterophorus* also exhibited toxicity to the tested bean bruchid. Datta and Saxena (2001) reported that parthenin isolated from parthenium possess both antifeedant and toxicant properties against *C. maculatus* and *Spodoptera litura* (Fab.). Sharma *et al*. (2003) also indicated the maximum repellent activity of *P. hysterophorus* extract against *C. maculatus* on chickpeas under laboratory conditions. In the present study, there was a difference in the efficacy of parthenium plant parts, where the seed part with higher effectiveness compared to the leaf part. This difference probably indicates that the seed contains higher content of the active components responsible for the insecticidal properties.

On the other hand, neem seed powder at all levels of concentrations and exposure periods was less effective compared to other plant powders against *Z. subfasciatus*. Ogunwolu and Idowu (1994) noted that 2.5% w/w powder seed of *A. indica* was highly toxic.

Shaheen (2006) also reported the neem seed powder applied at the rate of 1% w/w caused 100% mortality against pulse beetle within four days. Results in the present study on neem seed powder against *Z. subfasciatus* were in disagreement. This reduced in efficacy of the neem powder might be because of several factors including: the harvesting time of the seed, other ecological factors (temperature, rainfall and soil type), the concentrations used, and variations in insect behavior and species susceptibility. Schmutterer (1990) stated that neem products vary in their efficacy against insect pests for a number of factors.

In general, the insecticidal activities of the botanical powders could be attributed to the presence of some major and minor active constituents. Adebowale and Adedire (2006) indicated that the toxicity of *J. curcas* seed is mainly because of the oil that possesses sterols and terpene alcohols. The toxicity of *P. hysterophorus* may also be attributed to the sesquiterpene lactone parthenin and other phenolic acids such as caffeic acid, vanillic and parahydroxy benzoic acid, which are secondary metabolites (Datta and Saxena, 2001). Sharma and Joshi (1977) evaluated antifeedant and insecticidal activities of parthenin against *T. castaneum* and *C. chinensis*. Additionally, the toxicity of *D. stramonium* could be owing to the presence of flavanoids and withanolides (Cooper and Johnson, 1984; Shaheen, 2006). The major active ingredients of botanical powders exert their bioactivities against insect pests in various modes of actions. The insecticidal effect of plant powders may attribute to one or more of the following properties including fumigation, repellency, stomach poisoning effect where insects feed on admixed grains and pick up lethal doses of treatment particles, and physical barrier effect that is blocking the spiracles to impair respiration (Law-Ogbomo and Enobakhare, 2007; Mulungu *et al*., 2007).

All essential oil extracts at the highest concentration (750 mg) applied at all rates 1, 2 and 3 ml exhibited 100% mortality of *Z. subfasciatus* within 24 hours after treatment application. Essential oils from *T. ammi* and *C. ambrosioides* produced similar levels of effectiveness even at the lowest concentration (30 mg) levels of application. Conversely, essential oils from *R. officinalis*, *E. globulus* and *C. citratus* applied at lower concentrations depicted significantly less toxicity to *Z. subfasciatus*. Like those tested botanical powders, activity of essential oils against adult bruchids appeared to be directly related to level of application and exposure periods. In general, these results indicate that it seems possible an effective control of *Z. subfasciatus* using essential oil extracts though there appeared variation of efficacy due to concentration and longer exposure period.

Recently, in elsewhere the use of different plant extracts and plant derived oils for the control of stored-product insect pests has been reported in progress. Large number of plant extracts and essential oils are known to have insecticidal activities against various storage insect pests on filter paper and admixture bioassays (Bekele *et al*., 1996; Bekele, 2002). Similarly, Hill and Schoonhoven (1981) found that crude palm and cotton oils were more effective in the control of *Z. subfasciatus*. Effectiveness of orange peel oil against adult *Z. subfasciatus* was reported (Dawit, 2005) and hundred percent mortality was recorded on filter paper bioassay in 24 hours.

The current investigation showed that essential oil of *C. ambrosioides* with higher insecticidal property against *Z. subfasciatus* both in filter paper and grain admixture bioassays. Likewise, the work of Tapondjou *et al*. (2002) demonstrated that a dosage of 0.2µl/ cm^2 of filter paper was enough to induce more than 80 % mortality against *C. maculatus*, *A. obtectus* and *P. trunctus* within 24 hour. Quarles (2006) also reviewed that chenopodium essential oil extract is toxic to at least four species of stored-product insects. Topically added oil caused 100% mortality (40µg/insect) in cowpea weevil, and about 92% mortality (50µg/insect) in cigarette beetle. Such higher toxicity of *C. ambrosioides* may be attributed to ascaridole and other active compounds (Tapondjou *et al*., 2002; Quarles, 2006). Quarles (2006) indicated that the symptoms of ascaridole poisoning include nausea, dizziness and weakness in extremities in humans.

Essential oil extracts of rosemary, eucalyptus, bishop’s weed and lemon grass also desplayed highly effective control of *Z. subfasciatus* in admixture and filter paper trials. Usually many of the essential oil extracts from different plant families are known to have chemical active compounds that show different biological activities against various insect pests. Waliwiyita *et al*. (2005) indicated that rosemary oil have both contact and fumigant toxicity. The same author reported that concentration of 15.9µg/ cm^2 caused significantly high mortality of the late instar larvae of *Agriotes obscurus*. Recent investigation of Miresmailli and Isman (2006) demonstrated that rosemary oil resulted in complete mortality of spider mite at concentrations that are not phytotoxic to the host plant.

Likewise, the present study in the toxicity of *E. globulus* oil against *Z. subfasciatus* is in line with previous investigations. The essential oils of *E. globulus* were reported to have contact toxicity and a seed protection effect against pulse beetles (Srivastava *et al*., 1988). El-Atta and Ahmed (2002) as well revealed that eucalyptus oil distilled from *E. camaldulensi* caused significantly high mortality against *C. serratus*. Rahman and Talukder (2006) also reported *E. globulus* essential oil extracted by acetone was highly effective against *C. maculatus*.

Solvent extracts of different lemon grass parts were reported to have toxicant, repellent and fumigant activities against storage pests. Chungsamarnyart and Jiwajinda (1992) cited by Saljoqi *et al*. (2006) found that fresh *C. citratus* essential exhibited high (85-100% mortality) acricidal activity. Similarly, Odeyemi (1993) found that *C. citratus* essential oil applied at 0.7 ml /50g of maize increased the mortality of maize weevil compared to the control. Saljoqi *et al*. (2006) also reported the insecticidal activity of ethanol extract of lemon grass roots against rice weevil.

Insecticidal activity of the essential oils evaluated might be attributed to the presence of some major and minor active chemical constituents in the extract. Based on Tecle and Abegaz (1983) the major active ingredients of bishop's weed oil are thymol and carvacrol. Thymol is well known for its fumigant and contact toxicity to storage insects. Roger and Hamraoui (1995) evaluated the toxicity of thymol against *A. obtectus* and found that LC_{50} value of less than 1 mg/ L air for 24 hour. A monoterpenoid citral is mainly responsible for the biological activity of *C. citratus* essential oil extracts (Paranagama *et al*., 2003). The works of Lee *et al*. (2003) demonstrated that toxicity assay of citral caused 80-90% mortality of housefly and sawtoothed grain beetle in 14 h, respectively. The rosemary oil also contains 1, 8-cineole, camphor, bornyl acetate and high amount of monoterpene hydrocarbons (Chalchat *et al*., 1993). These chemical substances may be responsible for the toxicant nature of rosemary essential oil against *Z.*

subfasciatus in the present study. The toxicity of essential oil of *E. globulus* contains 1,8-cineole as its major constituent (Gupta *et al*., 1990; Dagne *et al*., 2000). Arannilewa *et al*. (2006) reported that essential oil extracts of plant origin are highly lipophilic and therefore have the ability to penetrate the cuticle of insects. This might be among the suggested the mechanisms how current essential oils impregnated on filter paper caused mortality of *Z. subfasciatus.*

All the botanical powders and essential oil treatments induced significant reduction in F_1 adult emergence of Z. *subfasciatus* compared to the untreated check. The plant materials added to the bean seed vary among themselves interms of the extent to which they affected the survival of the subsequent progeny. During progeny count, only live newly emerged bruchid adults were considered. Powder treatments of *C. ambrosioides*, *A. indica*, *J. curcas*, *D. stramonium* and *P. dodecondra* powder treatments were superior to the untreated check and other powder treatments in reducing F_1 progeny production. The study showed that the botanical powders were even better than the primiphos-methyl making 100% reduction of F_1 emergence at the lower doses. Likewise, it was reported that chenopodium leaf powder mixed to maize and sorghum grains at the rates of 2 and 4% w/w caused complete reduction in F_1 progeny production by maize weevil (Mekuria, 1995; Asmare, 2002). Tapondjou *et al*. (2002) noted that all concentrations of dry ground leaves of *C. ambrosioides* resulted in complete (100%) inhibition of oviposition and subsequent progeny production by *C. chinesis*, *C. maculatus* and *A. obtectus*. El-Atta and Ahmed (2002) further reported that neem seed kernel admixed to the groundnuts at 5% rate gave comparable effect as BHC in reducing the emergence of adult *C. serratus.*

Similar works also indicated that leaf and seed powder treatments of *D. stramonium*, *J. curcas*, *P. dodecondra* and *P. hysterophurus* were effective in reducing F_1 progeny production by storage insect pests. The effectiveness of dry ground leaf of *D. stramonium* in reducing the emergence of F_1 progeny of *S. zeamais* and *C. maculates* was reported (Emana, 1999; Asmare, 2002; Shaheen, 2006). Similarly, treatment of maize grain with dry seed and leaf powder of endod caused a lower level of progeny emergence of maize weevil (Ferdu *et al*., 2001). Interestingly, in this study seed and leaf powder of *J. curcas* was observed to have progeny emergence inhibition effect against grain weevils. Adane and Abraham (1996) and Asmare (2002) reported that maize grain treated with leaf and seed powder of jatropha produced low level of F_1 progeny. Cowpea seeds treated with oil of jatropha also significantly reduced oviposition by *C. maculatus* and there was no adult emergency in all levels of oil application (Adebowale and Adedire, 2006).

The reduction in F_1 progeny emergence in the treated grains could be resulted from increased adult mortality, ovicidal and larvicidal properties of the tested leaf and seed powders. Ofuya (1990) suggested that weakening of adults by botanical powders may make them lay fewer eggs than the normal. Tapondjou *et al*. (2002) also reported that powder plant materials may kill the larvae hatching from eggs laid on grains, preventing feeding and damage.

Moreover, essential oils of plant materials admixed to bean seeds exhibited ovicidal properties that suppressed the emergence of adult *Z. subfasciatus.* Bean grains treated with essential oil extracts of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* oils at the highest dose of 750 mg/ 150g of haricot bean caused significant reduction in F_1 progeny production by *Z. subfasciatus.* The treatments manifested up to 96-100% reduction in adult emergence. A similar result, 100% reduction in the emergence of adult bruchid, was recorded for bishop's weed oil at the lowest application dose. Conversely, lower dose of lemon grass essential oil extract markedly enhanced oviposition and subsequent progeny production by *Z. subfasciatus*.

Practically, plant oils coating can be effective in reducing progeny production by storage insect pests. Srivastava *et al*. (1988) stated that eucalyptus oil prevented oviposition of pulse beetles. Rahman and Talukder (2006) also reported that *E. globulus* essential oil extract completely inhibited F_1 progeny emergence of *C. maculatus*. Similarly, ovicidal and reduction in adult emergence activity of rosemary essential oil against *A. obtectus* was documented (Tunc *et al*., 2000; Papachristos and Stamopoulus, 2002; Papachristos *et al*., 2004). Paranagama *et al*. (2003) also indicated that *C. citratus* and *C. nardus* showed deleterious effects on oviposition and F_1 adult emergence of cowpea bruchid, *C. maculatus* compared to the control during no-choice tests. Essential oils of spices such as nutmeg, cardamom, turmeric and caraway were also seen to have ovicidal and larvicidal properties (Triphathi *et al*., 2002). Hence, the current findings regarding the use of essential oil extracts of aromatic and spice plants in reducing the emergence of F_1 progeny by *Z. subfasciatus* is in line with the earlier findings.

Generally, the possible cause for reduction of F_1 progeny production by *Z. subfasciatus* in treated grains was likely that immature stages of the insect were being killed physically by oil coating and impairing respiration through blockage of spiracles thereby resulting in inhibiting immature stages survival or reduced longevity of adult females. Bamaiyi (2007) reported that application of oils occlude seed funnels leading to the death of the developing insect by asphyxia.

A significant protection of haricot bean seeds against attack by *Z. subfasciatus* was provided by powder and essential oil extracts of different plant materials. This suggests their protectant potential for further investigation. All essential oil extracts at their higher doses of application appeared to be effective compared to the control. Among the treatments, *T. ammi* was highly significant in reducing bean seed damage at all concentrations. It appears therefore that the essential oil extracts screened have pesticidal properties which account for much higher levels of their effectiveness in reducing the feeding damage of *Z. subfasciatus*. Additionally, most of botanical powders admixed to the haricot bean seed provided significant reduction of grain weight losses compared to the untreated check, suggesting that the presence of chemical factors that can interfere with the feeding habit of the bruchid. A number of powder and essential oil extract of plant materials were reported to have the potential of protecting cereals and pulses against attack by insects. Mekuria (1995), Bekele *et al*. (1996), Firdissa and Abraham (1999), Dawit (2005) and Mulungu *et al*. (2007) reported that botanical powders added to grains (i.e. cereals and pulses) gave effective protection against attack by insect pests.

Like wise, different types of oil extracts of plant materials were also identified with a potential of reducing the loss in weight against attack by stored produce insect pests. Bekele *et al*. (1995) indicated that essential oil extracts of *Ocimum kilimandscharicum* provided greatest protection of maize and sorghum against attack by *S. zeamais, R. dominica* and *S. cerealella*. Shaheen (2006) also reported that datura and neem oil extracts considerably reduced percent weight loss of cowpea by *C. maculatus* compared to the control. *C. citratus* oil treatment significantly reduced percent seed damage of rice compared to the control (Paranagama *et al*., 2003). Dawit (2005) also reported that haricot bean seeds treated with orange peel oil extract highly protected against feeding damage by *Z. subfasciatus*. Malik *et al*. (1984) cited by Quarles (2006) also indicated that *C. ambrosioides* essential oil extracts are antifeedants against *R. dominica*, and average effectiveness over a four period was not significantly different from that of azadiractin isolated form neem seed.

Bioassays determining the toxicity data showed that the essential oil vapours in a confined airtight bottle exhibit a strong fumigant toxic action against *Z. subfasciatus.* Of the five essential oils, *C. ambrosioides*, *R. officinalis*, *E. globulus* and *T. ammi* exhibited the highest fumigant activity against *Z. subfasciatus*. Exposure of adults of bruchid to higher dosages of essential oil extracts caused significantly higher mortality rates. These oils caused 100% mortality of *Z. subfasciatus* at 0.3 g/ 100 g of haricot bean applied at 2 and 3 ml adjusted to 24 hour exposure. On the other hand, lemon grass oil caused 93.3% mortality at the highest dose. The fumigant activity of essential oils decreased with decreasing concentration, where most of essential oil vapours caused markedly less toxicity to *Z. subfasciatus* at the lowest tested dose.

The higher fumigant activity of a number of essential oil extracts and their major constituents have been investigated against insect pests (Shaaya *et al*., 1991; 1997). The fumigant toxicity of 16 essential oils against *A. obtectus* was studied (Shaaya *et al*., 1991). Among those, rosemary and caraway oils were most effective causing 100% mortality after 3 hours at 31.4µl/l air. Another study by Shaaya *et al*. (1997) also revealed fumigation toxicity of essential oils from lavender, rosemary, basil, peppermint and oregano on adults of *O. surinamensis*, *S. oryzae*, *R. dominica* and *T. castaneum*. A concentration of 4.54µl/l air or less was enough to obtain 90% lethal effects of all the test insects within 24 hour in space tests.

Moreover, essential oils from chenopodium, eucalyptus and lemon grass were observed to have potential fumigant toxicity to a number of stored produce insect pests. For instance, the oil of *E. globulus* has been reported to have fumigant and knocking-down effect on adult bean bruchids of *C. serratus*, *C. maculatus* and *A. obtectus* (Papachristos and Stamopoulus, 2002; Papachristos *et al*., 2004). The broad fumigation activity of eucalyptus is most likely attributes to the presence of a wide variety of volatile compounds, especially 1, 8, cineol, aldehydes, terpenes, sequiterpenes, alcohols and phenols (Gupta *et al*., 1990; Negahban and Moharramipour, 2007).

Similarly, essential oil of *C. citratus* contains high fumigant activity against *S. oryzae* (Saljoqi *et al*., 2006). Lee *et al*. (2003) also indicated that citral derived form essential oil of *C. citratus* showed strong fumigation toxicity against housefly and sawtoothed grain beetle in14-hour exposure time at 50µg/ ml of air, respectively. Consequently, the present study depicted that essential oils of aromatic and spice plants have strong fumigant toxicity potential against *Z. subfasciatus*. Generally, Keita *et al*. (2001) suggested that the mode of action of fumigant toxicity of essential oil extracts against insect pests might be reversible competitive inhibition of acetylcholinestrase by occupation of the hydrophobic site of the enzymes active center.

Using essential oil vapours as a fumigant for stored grain legumes and cereals could be particularly appropriate, as the number of synthetic fumigants has decreased lately because of their adverse effects to the environment (WMO, 1995) and the development of pesticide resistance. Weaver and Subramanyam (2000) cited by Lee *et al*. (2004) suggested that fumigant activity in botanicals could have a great potential than grain protectants in future on the basis of their efficacy, economic value and use in large-scale storages. However, information on the distribution characteristics of the essential oils after application to a commodity, e.g. absorption and desorption is scarce (Tunc *et al*., 2000). In addition, there is only very limited evidence demonstrating their ability to penetrate through grain bulks which must occur if plant extracts are to follow fumigant gases.

During the germination test, it was detected that plant materials screened against *Z. subfasciatus* did not show any visible adverse effects on germination capacity of seeds. Some of the treatments were affected by moulds as a result a reduced germination percentage ranging 90-96% was recorded. Asmare (2002) revealed that powders of jatropha, chenopodium, neem, and endod used in the control of maize weevil did not show any significant effect on the germination capacity of sorghum. Keita *et al*. (2001) and Rahman and Talukder (2006) also reported that plant oil extract treatments used for the control of bruchid pest on cowpea did not exhibit significant or adverse effect on the seed germination rate.

However, Mital (1971) cited in Songa and Rono (1998) found that a complete loss of viability of cowpea seed treated with ground nut oil. Another study conducted by Paranagama *et al*. (2003) pointed out that *C. citratus* oil treatment reduced the germination capacity of rice paddy as compared to the control. Fortunately, the present study showed that lemon grass and other plant material treatments did not cause any noticable adverse effect on the germination capacity of the bean seed. This indicates that most of the treatments do not interfere with viability of seeds and can be applied for the protection of stored grain for food and seed purposes.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

According to the results of the present investigation, both powders and essential oil extracts of botanicals possessed insecticidal properties, feeding and ovipositional deterrent effect against the most economically important haricot bean storage pest, *Z. subfasciatus*.

The protection of haricot beans against *Z. subfasciatus* damage provided by plant powders varied in terms of plant materials, their application rates and exposure time. This suggests that longer exposures with higher applications provide significantly higher mortality of *Z. subfasciatus*.

The application of *C. ambrosioides, R. officinalis, E. globulus, T. ammi* and *C. citratus* essential oil extracts at the highest rate significantly reduced F_1 progeny production and associated weight loss of bean seed by *Z. subfasciatus*. Additionally, essential oil extracts from *C. ambrosioides*, *R. officinalis, E. globulus, T. ammi* and *C. citratus* possessed contact and fumigant toxicity against *Z. subfasciatus*. Mortality of *Z. subfasciatus* due to fumigation and contact toxicity effect of essential oil extracts were largely dependent on the type the essential oil extracts, doses and application rates and exposure period. Consequently, longer exposure and higher application rates of the essential oils are required to achieve effective control of *Z. subfasciatus*.

Over-all, the plant materials screened against *Z. subfasciatus* did not have adverse effects on the germination capacity of *P. vulgaris*.

6.2. Recommendations

- The present laboratory evaluation on the efficacy of some local plant powders and essential oil extracts illustrated their potential as grain protectant. Nevertheless, further evaluation of these plant materials under real storage conditions is needed.

- Farmers living in developing countries including Ethiopia should use locally available plant materials, possessing insecticidal activities for the management of bean bruchids owing to their easy and local availability, economic and safe use.

- Much work needs to be done around plant based botanicals to develop effective formulations, which can be commercialized for insect control. Thus, this study provide a gate way for the researchers/ bio-chemists to find out active materials/ fractions of these plants for developing economic and commercial formulations, which will definitely lead to the development and establishment of biopesticide industry in Ethiopia.

- The use of these essential oil extracts to stored grain legumes as fumigants for the control of *Z. subfasciatus* is a possible supplement to synthetic fumigants, as most of conventional fumigants are out of market because of development of resistance to storage insect pests and their adverse effects on ozone layer and human toxicity.

- The blend effect of essential oils as grain protectant should be evaluated in the future.

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APPENDICES

Annex 1: Summary table for analysis of variance (ANOVA) for mean % mortality of *Z. subfasciatus* due to powder of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *A. indica*, *P.dodecondra* and *P. hysterophorus* over different period of time on treated haricot bean seeds.

Source of error		Sum of Squares	df	Mean Squares	F	P-value
Mortality after 24hrs	Between Groups	70228.986	22	3192.227	48.947	0.000*
	Within Groups	3000.000	46	65.217		
	Total	73228.986	68			
Mortality after 48hrs	Between Groups	52428.986	22	2383.136	25.693	0.000*
	Within Groups	4266.667	46	92.754		
	Total	56695.652	68			
Mortality after 72hrs	Between Groups	45457.971	22	2066.271	29.096	0.000*
	Within Groups	3266.667	46	71.014		
	Total	48724.638	68			
Mortality after 96hrs	Between Groups	38231.159	22	1737.780	23.396	0.000*
	Within Groups	3416.667	46	74.275		
	Total	41647.826	68			

* = Significant at $P \leq 0.05$

Annex 2: Summary table for analysis of variance (ANOVA) for mean % mortality of *Z. subfasciatus* due to essential oil of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* over different period of time on treated haricot bean seeds.

Source of error		Sum Squares	df	Mean Square	F	P-value
Mortality after 24hrs	Between Groups	52925.490	16	3307.843	49.618	0.000*
	Within Groups	2266.667	34	66.667		
	Total	55192.157	50			
Mortality after 48rs	Between Groups	39385.294	16	2461.581	45.651	0.000*
	Within Groups	1833.333	34	53.922		
	Total	41218.627	50			
Mortality after 72hrs	Between Groups	33883.333	16	2117.708	57.602	0.000*
	Within Groups	1250.000	34	36.765		
	Total	35133.333	50			
Mortality after 96hrs	Between Groups	33657.843	16	2103.615	93.291	0.000*
	Within Groups	766.667	34	22.549		
	Total	34424.510	50			

* = Significant at $P \leq 0.05$

Annex 3: Summary table for analysis of variance (ANOVA) for mean F_1 progeny emergence of *Z. subfasciatus* on seeds treated with different powder materials of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *A. indica*, *P. dodecondra* and *P. hysterophorus*.

Source of error	Sum of Squares	df	Mean Square	F	P-value.
Between Groups	164.177	22	7.463	18.239	0.000*
Within Groups	18.821	46	0.409		
Total	182.998	68			

* = Significant at $P \leq 0.05$

Annex 4: Summary table for analysis of variance (ANOVA) for mean F_1 progeny emergence of *Z. subfasciatus* on seeds treated with different essential oil extract treatments of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus*.

Source of error	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5392.824	16	337.051	7.051	0.000*
Within Groups	1625.333	34	47.804		
Total	7018.157	50			

* = Significant at P≤ 0.05

Annex 5: Summary table for analysis of variance (ANOVA) for weight loss produced by *Z. subfasciatus* on seeds treated with different powder materials of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *A. indica*, *P. dodecondra* and *P. hysterophorus*.

Source of error	Sum of Squares	df	Mean Square	F	P-value
Between Groups	0.455	22	0.021	4.809	0.000*
Within Groups	0.198	46	0.004		
Total	0.653	68			

* = Significant at P≤0.05

Annex 6: Summary table for analysis of variance (ANOVA) for weight loss produced by *Z. subfasciatus* on seeds treated with different essential oil extract treatments of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus*.

Source of error	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.505	16	0.032	9.942	0.000*
Within Groups	0.108	34	0.003		
Total	0.613	50			

* = Significant at P≤ 0.05

Annex 7: Summary table for analysis of variance (ANOVA) for mean percent mortality of *Z. subfasciatus* due to different essential oil extract treatments of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* at 1, 2 and 3 ml concentrations, respectively.

Source of error		Sum Squares	df	Mean Square	F	P-value
Mortality after 24hrs	Between Groups	236765.217	45	5261.449	132.015	0.000*
	Within Groups	3666.667	92	39.855		
	Total	240431.884	137			
Mortality after 48rs	Between Groups	236765.217	45	5261.449	132.015	0.000*
	Within Groups	3666.667	92	39.855		
	Total	240431.884	137			
Mortality after72hrs	Between Groups	195924.638	45	4353.881	124.526	0.000*
	Within Groups	3216.667	92	34.964		
	Total	199141.304	137			
Mortality after 96hrs	Between Groups	186240.580	45	4138.680	135.985	0.000*
	Within Groups	2800.000	92	30.435		
	Total	189040.580	137			

* = Significant at $P \leq 0.05$

Annex 8: Summary table for analysis of variance (ANOVA) for mean percent mortality of *Z. subfasciatus* due to fumigation toxicity of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* at 1, 2 and 3 ml concentrations after 24 hours.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	223925.540	45	4976.123	53.193	0.000*
Within Groups	8700.000	93	93.548		
Total	232625.540	138			

* = Significant at P≤ 0.05

Annex 9: Summary table for analysis of variance (ANOVA) for mean germination test of haricot bean treated by different powder materials of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *A. indica*, *P. dodecondra* and *P. hysterophorus*.

Source of error	Sum of Squares	df	Mean Square	F	P-value
Between Groups	151.159	22	6.871	1.681	0.069
Within Groups	188.000	46	4.087		
Total	339.159	68			

Annex 10: Summary table for analysis of variance (ANOVA) for mean germination test of haricot bean treated by different essential oil extracts of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus*.

Source of error	Sum of Squares	df	Mean Square	F	P-value
Between Groups	105.412	16	6.588	1.672	0.102
Within Groups	134.000	34	3.941		
Total	239.412	50			

Annex 11: The fumigation Set up for the fumigation bioassay of essential oils against *Z. subfasciatus*.