Garlic and orange plant materials evaluation for the control of *Sitophilus spp.* (Coleoptera: Curculionidae) and *Zabrotes subfasciatus* (Coleoptera: Bruchidae) in Ethiopia.

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ABSTRACT

At present much emphasis is being placed in the use of botanical pesticide in the protection of storage insect pests, which have gradually been ignored and also to avoid problems with insecticide resistance. Experiments were conducted to determine the efficacy of *Allium sativum* L. and *Citrus sinesis* L. against *Sitophilus spp.* and *Zabrotes subfasciatus* under laboratory conditions. For comparison pirimiphos-methyl and untreated check were included and the experiments arranged in a completely randomized design with three replications. One hundred percent mortality of *Sitophilus spp.* was obtained with water and acetone extracts at the rate of 30g/100ml applied at 3ml/ filter paper after 48 and 96 hour of exposure, respectively. Similar result was obtained for *Z. subfasciatus* at 20g/100ml rate of extraction. Essential oil from *C. sinesis* at the highest rate of 750mg/10 ml of acetone applied at 3ml per filter paper gave 100% mortality for both *Z. subfasciatus* and *Sitophilus spp.*

Allium sativum applied as powder and extracts to the haricot bean were toxic to *Z. subfasciatus* which caused significant percent mortality of weevils. The highest concentration of acetone extract of *A. sativum* applied at the rate 15ml/250g of grain caused 82 percent mortality after 96 hours. Grains treated with 15g of sun dried powder of orange peel and 750mg of essential oil killed 65 and 67 percent of *Z. subfasciatus* after 96 hours respectively. Similarly, *A. sativum* power had low effect on progeny production of *Z. subfasciatus,* but grains treated with ethanol and acetone extracts of *A. sativum*, orange peel powder and essential oil of orange peel reduced progeny production of *Z. subfasciatus* by more than 92 percent. *Citrus sinesis* peel oil reduced 100 percent progeny

production of *Z. subfasciatus* at all dosage levels used. All the treatments were repellent to *Z. subfasciatus*, with the highest dosage of ethanol extracts of *A. sativum* and fresh chopped garlic evoking the highest repellent action. The treatments, however, showed lower repellency effect against *Sitophilus spp*. except fresh chopped garlic with 72 percent repellency. The essential oil of orange peel had a high level of toxicity in the fumigation bioassay against *Z. subfasiatus* and *Sitophilus* species in impregnated filter paper. The effect of these promising botanicals on weevil progeny survival as well as on seed germination and seed weight loss are discussed.

1. INTRODUCTION

Food security is a major problem for the world today, particularly the third world, where food security is rapidly deteriorating. In third world much produce is kept on farm stores in small quantities under quite primitive storage system. These countries are experiencing not only producing low yield, but also having traditional storage system which aggravate the problem. Efforts at improving agricultural production have always been concentrated on increasing production through the breeding of high-yielding varieties with little emphasis on conservation of what is produced (Boxall, 1998). Such efforts can not fulfill the goals of meeting food and nutritional levels with out matching care to reduce, if not completely eliminate, post-harvest losses (quantitative and qualitative) caused by various physical, biological and mechanical factors.

Increasing the production of food alone can not be a solution rather it has to be supplemented with appropriate scientific storage system. Since all that is produced can not be consumed immediately, there is always a need for adequate and efficient storage facilities to save the excess that is produced from deterioration and waste. Therefore, post-harvest system is crucial in meeting the overall goals of food security, poverty alleviation and sustainable agriculture. Reduced wastage during storage reduces food and income losses for farmers. Indeed, in view of the hunger in the world today loss of food and food products in storage is tragic. To bring economic benefit to farmers productivity of grain should be complemented with sound and reliable post harvest control measures. More effective control of storage pests could mean an immediate increase in the world's edible grain and food with out any change in agricultural productivity. This is significant when the money and use of energy necessary to increase food production are considered.

Stored product pests are very important in Ethiopian agriculture. Considerable amounts of stored products, both in terms of quantity and quality are lost in this country. The main agents causing deterioration of stored product are microorganisms, rodent, birds, insets and mites. Among these insects are the principal pests responsible for losses to food grain. The most economically important stored product insects belong to two insects orders: Coleoptera and Lepidoptera (Olkowski *et al.,* 1991; Abrham 1997). Every year, large quantities of stored products are destroyed or contaminated because of the presence of beetles which are the most

important group of arthropods attacking these products (Emana and Assefa, 1998; Firdissa and Abrham, 1999).

The major insect pests related problem in stored grains and grain products in the tropics is the attack by the maize weevil, *Sitophilus zeamaiz* (Mots.) (Coleoptera, Curculionidae), the lesser grain moth, *Rhyzoptera dominica* (Fab.) (Coleoptera., Bostrichidae), angoumois grain borer, *Sitotroga cerealella* (Oliv.) (Lepidoptera, Gelechidae). The major primary pests of legumes are Bruchids: *Callosobruchus maculates* (Fabicius), *Caryedon serratus* (oliver), *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) (FAO, 1985; Abraham *et al.*, 1993; Nahdy and Agona, 1995; Tsedeke, 1995). Stored product insects damage grains directly by feeding and there by reduce dry weight, germination, nutritional value or the grade of harvested grain and indirectly by increasing susceptibility to secondary pests. The Food and Agricultural Organization of the United Nation (FAO) estimates that 5-10% of harvested grain is lost in storage with losses being higher in developing countries (Hall, 1970). Pimentel and David (1984) also indicate 10-20% of the crops are destroyed after harvest by pests.

Stored product losses have been prevented predominantly by synthetic insecticides. However, considerable problems may arise from the continued application of these insecticide including the development of resistance by insects, pollution of the environment and hazards from handling toxic compounds (Champ and Dyte, 1976; Sharaby, 1988a; White and Bell, 1988; Zettler and Cuperus, 1990; Guedes *et al.*, 1996; Fragoso, 2003). In addition, in small scale storages, which are prominent in developing countries, the use of synthetic chemicals becomes uneconomical and dangerous. In Ethiopia, about 95% of food production comes from peasant sector, where production technologies and storage system are primarily traditional (Tsedeke, 1998). The farmers have little access to modern crop protection technologies. More over, insecticides are too expensive and are unavailable to the bulk of subsistence farmers in Ethiopia in particular and in Africa in general. Post-harvest improvement that target farmer storage can therefore achieve impact in reducing losses, increasing farm incomes and improving rural household food security. Hence, priority for research should be to find alternatives that preserve the natural control agents of existing and potential pests.

The use of traditional materials to protect stored products against damage by insect infestation is widespread and undoubtedly and some are quite effective. Currently, in developing countries including Ethiopia laboratory investigations into the use of some of the locally available materials are being undertaken (Jilani *et al*., 1988; Cobbinhah and Appiah-Kwarteng, 1989; Jembere *et al*., 1995; Mekuria, 1995; Adane and Abrham, 1996; Bayeh and Tadesse, 1996; Firdissa and Abrham, 1998; Emana, 1999; Glob *et al.*, 1999; Amhara Regional State Agriculture Bureau, 2000).

Despite of the cosmopolitan use of plants as a grain protectant by traditional farmers in Ethiopia (Amhara Regional State Agriculture Bureau, 2001), knowledge of methods and efficacy of these plants is scanty. Therefore, the present study was undertaken with the following objectives:

General objective

i. to determine the efficacy of orange peel, *Citrus sinensis* (Rutales: Rutaceae) and garlic, *Allium stavium* (Liliales: Liliaceae) plant materials for the control of *Sitophilus sp.* and *Z. subfascitus.*

Specific objective

ii. to investigate the potential of garlic and orange peel extracts as *Sitophilus sp.* and *Z. subfascitus* repellent;

iii. to investigate the toxicity of the plants extracts against *Sitophilus sp.* and *Zabrotes subfascitus* and to establish best and effective dose;

iv. to evaluate antifeedant effect of the two botanical extracts against the two insect pests; and

v. to identify best extraction method (solvent) of the active component of the two botanicals.

2. LITERATURE REVIEW

2.1 Production of Beans and Maize

The common bean, *Phaseolus vulgaris* L, is the most important food legume for direct consumption in the world. It is a major food crop and one of the main sources of protein for inhabitants of tropical and subtropical regions of the world. Beans form an important food and cash crop in Africa, particularly in the eastern, southern, and great lakes of the continent (Jaetzold and Schmidst, 1983; IAR 1990; Silim, 1993). In Ethiopia, beans are important food crops particularly in Sidama, western Ethiopia, highlands of Harergae and Riftvally coverings 35, 95, 25 and 64 thousands of hectares respectively (Wartman *et al.*, 1998).

Haricot bean has a wide range of adaptation and noted for its diversity and exhibits considerable variation in growth habit and seed type. It is normally grown from sea level to about 2800masl. Under Ethiopia condition, it is well adapted to altitude ranges between 1400 to 2000masl under irrigated and rain fall conditions. The minimum and maximum temperature requirements are 10^{o}c and 32^{o}c respectively, with rainfall distribution of 350 to 500mm and relative humidity below 15% (IAR, 1995; EARO, 2000).

In Ethiopia, common beans are produced by small scale farmers for cash and subsistence. The Central Statistical Authority (CSA, 2003) indicates the total land holding area covered by pulse 1,016,790 hectares and total production was estimated at 10,212,150 quintal in 2001/02. Average yield of common bean in Ethiopia is low ranging between 5 & 8 qt/ha. Ethiopia is second in east Africa in production next to Burundi (FAO, 1997). Beans are nearly 'perfect' food, nutritionally rich; they are also a good source of protein, folic acid, dietary fiber and complex carbohydrates. Further, when beans are part of the normal diet, the amino acids are complementary and increase the cereal proteins. Beans are also one of the best non-meat source of iron, providing 23-30% of daily recommended levels from a single serving. For the poor of the world, they are a means of reducing malnutrition. Apart from its nutritional value the high quality dry beans have been exported by Ethiopia and have considerable foreign exchange value (FAO, 1997).

Maize (*Zea mays* L.) is an annual plant which belongs to Maideas tribe and the grass family of Germinea. Maize is the most widely grown cereal crop. In the global production of cereal crops, maize ranks first. Like wise, in countries with developing economics, such as Latin America and Africa the maize ranks first (FAO, 2003). Maize production and consumption vary greatly through out Africa. In large parts of eastern and southern Africa, maize is the principal staple food produced and consumed by most farming households. Maize is produced in Africa on over 20 million hectare, which is 16% of the total world area under maize production. The total annual production is about 25 million tones and this represent 6% of the total world production. The eastern and southern African countries produce 64% (16.2 million tones) of all Africa maize. Ethiopia is the second most important producer next to Kenya in east Africa (Ristanovic, 2001). The crop is widely produced in western, southern, south western, north western and eastern part of the country. Maize is extremely an important crop grown in Ethiopia. At present, it is the first in total production and yield per hectare among cereals. According to the Central Statistical Authority (CSA, 2003), about 17% of the crop land is covered by maize and contributing about one quarter of cereal crop production with an average yield of 21 quintal per hectare, which is the highest yield of cereal crops in the country.

The crop is one of the most productive species of food plants in Ethiopia and 90% of the maize produced is used as food (Ferdu *et al.,* 2001). The crop posses great genetic diversity and grown across varied agro-ecological zones. The optimum temperatures and soil moisture for growth and development of the maize plant are 25 to 30^{o}C and 60-70%, respectively (Ristanovic, 2001). Under Ethiopian condition it is well adopted to altitude ranges of 1400m and 2000m in irrigated and rainfall condition (IAR, 1995). Maize is of great economic significance world wide as human food, as animal feed and as a source for a large number of industrial products. In fact, maize accounts for at least 15% of the total calories daily intake in 28 developing countries, almost all of them in Africa including Ethiopia (FAO, 2003). It can supply the minimum daily caloric requirements for a person.

2.2 Mexican Bean Weevil, *Zabrotes subfasciatus* Boheman

The two most universal and important pests in storage are the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) and the common bean weevil *Acanthoscelides obtectus* (Say) in Africa (Slim, 1993). Ferede and Tsedeke (1995) reported that these two species are the major pests of stored beans in Ethiopia. The study conducted in Bako recorded 14% of total loss by *Z. Subfasciatus* in haricot been stored for 12 month (Adane and Abrham, 1996). Infestation by *Z. subfasciatus* can cause a loss of about 12% of the available protein (Mc Farlance, 1988).

2.2.1 Recognition and Identification

Adult *Z. subfasciatus* is oval shaped beetles that have two movable spurs attached to the apex of the tibia of each hind leg. There are no teeth on the hind femur. The elytra of the female are strongly marked with white pottering on a dark back ground. The male has uniform light brown pubescence over a dark cuticle. The elytra are short and relatively broad, and almost square in shape (Dobie *et al.*, 1984).

2.2.2 Biology and Behavior

Zabrotes subfaciatus is a major primary pest of common bean (*Phaseolus vulgaris*), cow pea (*Vigna unguiculata*) and lima bean (*Phaseolus lunatium*). It sometimes attacks the seeds of other legumes. The adult beetles, which do not feed on stored products usually, live up to 13 days under optimum conditions. During this time the female lays up to 100 eggs. The optimum conditions for development are a temperature of between 27^{o}C and 31^{o}C and humidity of 70% (Hill, 1990).

Infestation occurs in the store. The eggs laid in the pods, after they have been perforated on piles of grain. When newly laid they are small gray and inconspicuous. The larva, which hatches out bores tunnels in the grain on which it feeds and the same grain may accommodate

several individuals. The larvae moult four times before pupation. After pupation the adult may remain in the cell for several days before pushing the 'window' prepared by the last instar larvae to facilitate adult emergence. The life cycle takes 24-25 days under optimum conditions. Sex ratio is usually 1:1 (Hill, 1990).

2.3 *Sitophilus Spp.*: Storage Pests of Maize

Weevils (*Sitophilus* spp.) (Coleopteran; Curculionidae) are among the most destructive and wide spread pests of stored cereals (Hill, 1990). The maize weevil *Sitophilus zeamais* Motsch. is cosmopolitan and especially abundant as pests in worm temperature to tropical regions, like the prevailing condition in Ethiopia, where it is the main pest of stored maize (Nansen *et al*., 2004). Infestation by the maize weevil frequently starts in the field before harvesting. Its strong flying ability and destructive power account for a great part of the 20% loss in total weight in stored grain at the farm level in Ethiopia (Beyene *et al.*, 1996).

2.3.1 Recognition and Identification:-

Although *Sitophilus sp.* is among the commonest and most destructive storage pests in the world the taxonomy of the *Sitophilus* group has been confused till recent time. *S. zeamais* and *S. oryzea* are almost indistinguishable from each other externally. These species can be distinguished from each other based on the shape of the male aedeigus and sclerite in the female genitalia (Dobie *et al.*, 1984). Despite of the difference both have a characteristic sortie elbowed antenna and four reddish orange circular markings on the elytra.

2.3.2 Biology and Behavior

Weevils are a common species in hot regions. The eggs are laid only on grains which are sufficiently large to enable the larvae to develop. Usually the insect starts to lay eggs in the field before harvest. The female chews holes in the grain and deposits an egg in each of them before sealing them with a hard secretion. The female lay eggs through out most of the adult life, although 50% may be laid with in the first 4-5 weeks. Upon hatching from the egg, the white leg less larva begins to feed inside the grain, excreting a tunnel as it develops. The larva moults three

times at 25ºC & 70% relative humidity in about 25 days. Fully grown larvae measure about 4mm (Dobie *et al*., 1984; Hill, 1990).

Pupation takes place with in the grain and the newly developed adult chews its way out of the grain leaving a characteristic circular emergence hole. The adult is an elongated dark brown little weevil, 2.4 – 4.5mm long with four reddish brown patches on the elytra. It is an active insect, diurnal and usually flies readily and crops in the field at about one kilometer distance from grain stores are at risk (Hill, 1990). Under optimum conditions (temperature = 30ºC; humidity = 70%), a female lays some 300 eggs over several weeks and the egg-to-adult cycle lasts 26 days. The species can develop only at a temperature of between 13º and 34ºC, provided that humidity is in excess of 60% (Appert, 1987).

2.4 Storage Loss

The term loss has been defined in different ways and it was not clear whether it refers to the amount of damage or total amount of grain lost. Loss has been used synonymously with the term damage. In the context of storage losses, "loss" means a measurable decrease of the food staff, which may be qualitative or quantitative. While damage refers to the superficial evidence of deterioration.

In the developing world, durable commodities, notably the grains and legumes, have been the most important food stuffs in terms of quantities produced. In Ethiopia, the cereals (maize, wheat, sorghum, teff, barley) and pulses (beans, chick pea,) are common products stored in compliance with local traditions. Crop products must be stored in the way that the quality does not deteriorate during the storage period; the quantity in storage is not unintentionally reduced; it is secure against pests, disease and physical loss; and it is accessible at the time and quantity required. The efficiency of these storage systems may well determine the security and survival of the local community.

Loss and deterioration of products during storage is likely to occur unless adequate precautions are taken. Globally a minimum of 10% of cereals and legumes are lost after harvest (Boxall *et al.*, 2002). Information on post-harvest losses of food grain in Ethiopia is really scarce to estimate the loss trough out the country. Several studies of storage losses were undertaken, but comparison of results is very difficult for a number of reasons including insufficient information about the methodology used; results being aggregated for several different crops and different storage systems; and the studies being conducted over different storage periods and in different agro-ecological zones. Nevertheless, it is widely agreed that food losses after harvest are substantial and important in Ethiopia, in terms not only of quantity but also quality and nutritional and economic value (Teshome *et al.,* 1998).

Crop losses with an average of 16% was reported by Abrham (1997) on stored maize in Bako area. This figure is similar to the report by Adhanom (1990) who found weight loss of 16.48% on farm-stored maize in Awassa. Both authors indicate the maize weevil, *Sitophilus zeamais* was most common and distractive pest inflicting heavy damage and losses on stored maize. A survey conducted in 1996/97 by Firdissa and Abrham (1999), to assess the species of insects and the associated damage level in farm-stored sorghum indicate weevils of *Sitophlius* species followed by the angumois grain moth were found to be the major insect pests causing average damage of 38.7% and weight loss of 14.5% respectively on stored sorghum . Storage loss of bean due to *Acanthocelides obtectus* and *Zabrotes subfaciatus* in east Africa have been estimated to be between 30% and 73% (Nahdy and Agona, 1995). Ferede and Tsedeke (1995) also indicate bruchid damage to reach 38% and seed weight loss to reach 32%.

Most survey appears to have concentrated on the cereal grains, maize and sorghum in particular. However, the more recent studies conducted during 1997/98 in three region of Ethiopia (Amhara, Oromia and Southern regions of Ethiopia) includes storage loss of pulses in addition to maize, sorghum, wheat and barley and estimate overall loss of 9% in peasant farm-storage (Boxall, 1998).

All the study emphasize insect damage in farm-storage is a general problem through out the country, particularly in the lower altitude. And it is widely accepted that the main cause of storage loss at farm-level in Ethiopia is insect infestation though loss caused by mould is

important in underground storage (Tsedeke, 1985; Abrham 2003). Losses in farm-storage in Ethiopia can be expected to increase with the wide adoption of new, improved or high-yielding varieties of grain. Hence these new varieties are more susceptible to pest attack after harvest (Boxall, 1998; Abrham, 1997, 2003; FAO, 2003).

Direct feeding damage by insects reduces grain weight, nutritional value and germination of stored grain. Infestation also cause contamination, odor, mould and heat-damage problems that reduce the quality of the grain and may make it unfit for processing into food for humans or animals. Commercial grain buyers may refuse to accept delivery of insect contaminated grain or may pay a reduced price. There are many causes of loss in storage and they may be categorized in a number of ways. One method identifies primary and secondary causes of loss (Golob *et al*., 2002).

According Boxall *et al*. (2002) primary causes of loss are those that directly affect the stored commodity and include: biological, microbial, chemical, mechanical and physical
The second causes of loss are those that lead to conditions that encourage primary causes of loss and are usually a result of inadequate handling equipment, technology and control. They include: post harvest facilities, lack of equipment and lack of skill; lack of adequate containers and package for transport and handling; inadequate transport to move products quickly from field; inadequate drying facilities or equipment; inadequate storage facilities; traditional processing methods giving rise to breakage in grain or excessive removal of nutrients; and inadequate management. Generally loss can be considered in terms of quantity or quality.

Quantitative loss is a physical loss of produce that can be measured as a reduction in weight or volume and, therefore can be measured and valued most readily. But a reduction in weight may not necessarily indicate a loss of food material. Hence reduction in weight may occur due to reduction of moisture content of the grain. True weight loss may result from feeding by insects, rodents and birds or growth of micro organisms. In assessing loss it is important, therefore, to take account of changes of moisture content.

Qualitative loss is more frequently based up on subjective judgments and is perhaps identified through comparison with locally accepted quality standards. It may include the

presence of contaminants and changes in appearance, taste and texture that may cause the product to be rejected as an aspect of quality loss (Hill, 1990).

Nutritional loss represent a reduction of the food value of the grain as a result of a lowering of its protein, hydrocarbon and vitamin content (Appert, 1987). Selective feeding of pests largely lead to nutritional loss beside of weight loss. Weevils feed mainly on the endosperm and reduce the carbohydrate content (NRI, 1996). Bruchids feeds on the cotyledons of pulses and reduce the protein content (Mc Farlance *et al.*, 1994). High moisture content and the associated growth of micro-organisms also lead to changes in vitamin content of grain (FAO, 1983).

2.5 Management of Storage Insect Pests

Food grains are liable to suffer heavy losses during storage as a result of infestation by insects. So as to benefit from the harvest, attempts will have to be made to control insect pests. The control methods may be hygienic, cultural, host resistance, chemical or biological. These measures directed towards control of storage pests involve the manipulation of the storage environment to make it less favourable to the insects and ensure adequate protection of the stored commodities (Allotey, 1991).

2.5.1 Cultural Control

Store hygiene is the fore most preventive factor in pest control in stored grain. Stores, silos, cribs etc. and their immediate surroundings must be kept as clean as possible. Removal of old grains and residue of organic matter present in storage structure including sub-floor spaces, bins and old bags is important in the preparation of insect-free environments and prevention of carry over of pests to new grain. A new harvested should never be stored with remainders of previous harvest as well as in used bags with out washing (Manson and Obermeyer, 2004). However, it should be pointed out to small-sclae farmers that practical hygienic control can bring satisfactory result if it is combined with good and adequate drying (FAO, 1985).

Farmers use a range of traditional methods of control or devise strategies to cope with insect infestation, which are selected from one generation to the next. The most common traditional treatments to limit insect activity are mixing inert materials and organic materials with

stored grain. The success of protection depends upon the effect of the preservative on the grain, the rapidity of its action, the period of storage and proper mixing (Gahukar, 1994).

Friction of dust particles with insect's cuticle leads to desiccation and hampers the development of the pest (Golob, 2002). Emana and Assefa (1998) showed that tobacco dust resulted in efficient control of *Sitotroga cereallela* applied at the rate of 30 percent. A similar effect can also be achieved through treatment with wood ash, collected from burnt tree wood or a farmers stove. Some farmers may also add fine sand to hinder the pest activity, in which the high proportion of the quartz damage to sensitive cuticle of the newly hatched insect (Girma *et al.,* 2000). In an experiment in Bako Research Center, heating maize grain at 70-80^{o}C in an oven for 1 hour, maize plus dockage, and exposure to the sun showed comparable results to the standard insecticide pirimiphos-methyl at 10ppm in protecting maize grain from the maize weevil (Demissew *et al.*,2002).

Exposure to sunlight or exposure followed by sieving of the grains usually at monthly interval is also well known technique among farmers and create an unfavourable environment for weevils (Girma *et al.*, 2000). Other traditional methods include winnowing, shaking, restaking grains and mixing grains with small sized grains (teff) reduce insect activity.

2.5.2 Botanical Control

The use of plants as traditional protectants of stored products is an old practice use all over the world particularly (Golob and Wbley, 1980). However, the use of insecticidal plants has more than likely declined since the advent of synthetic chemicals. Associated ethno botanical information on the uses of plants may also be under threat as farmers increasingly rely upon commercial products, leading to a breakdown in the passing on of local knowledge between generations (Golob *et al.*, 2002, Ivbijaro, 1990).

Botanicals are typically pesticides, working in the same fashion as commercial synthesis. However, botanical have several modes of action. Toxicity against insect may be expressed by direct killing particular life stages of the insect, interfering with mating or suppressing reproduction, acting a repellent or affecting host finding and selection in a way that prevents infestation , reducing or preventing feeding (Sharma and Sexana, 1974, Tripathi *et al*., 2000). The

other characteristics shared by most botanicals are their persistence in the environmental is less; they act very quickly to stop feeding by plant insects that is they cause immediate mammalian toxicity and not phytotoxic (Weinzierl and Henn, 1992).

Plant products can be obtained either from the whole plant or from a specific part by extraction. The most common 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 extracts (Weinzierl and Henn, 1992).

Use of plant products or insecticides is one of the important approaches of insect pest management and it has many advantages over synthetic insecticides (Weinzierl and Henn, 1992). Plant materials with insecticidal properties provide small-scale farmers with locally available, biodegradable and inexpensive method of pest control for storage. Farmers, in Ethioipia, use local herbs by mixing with grain to reduce infestation in stored grains (Yemane and Yilma, 1998). Firdissa and Abrham (1999) reported *Chenopodium sp.* performed very well and resulted in high percentage of adult mortality, reduced progeny emergence and low percent grain damage. Bayeh and Tadesse (1996) found that neem (*Azadirachta indica*), birbira (*Milletia sp.*) oil and pyrethrum flower powder to be toxic against *Callosobruchus chinensis*. Other botanicals that gave good control include *Croton macrostachyus, Ricinus communis, Datura stramonium, Cpsicum frutescens, Azadirchata indica, Ocimum sp.* and *Eucalypyus sp*. (Jembere *et al*., 1995; Emana, 1997; EARO, 1999; El Altta and Ahmed, 2002).

2.5.2.1 Garlic (*Allium sativum* L.)

Garlic is a perennial plant with a compound bulb composed of several partial bulbs (cloves) enclosed in a common membrane. It is widely cultivated plant used as flavouring in cooking. They are also used in traditional medicine (Dawit *et al.*, 2003). The fresh bulb contains alliin, alicin and volatile oils. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via the enzyme allinase. Allicin gives garlic its characteristic pungent smell (Williamson, 2003). This pungent odure enables garlic to have antifeedant and repellency effect against storage insect pests (Amhara Regional State Agriculture Bureau, 2001). Garlic extracts were also found to prevent damage caused by *Callosobruchus chinensis* (Glob *et al.*, 1999). Two percent (w/w) admixed with wheat caused a reduction in percentage of damage caused by

Tribolium garnarium larvae (Jood, *et al*., 1993). Garlic oil exhibits antibacterial, antifungal, amebicidal and insecticidal qualities (Owens, 2002). The constituents of the essential oil responsible for these characteristics are allicine and sulphide (Buss and Park-Brown, 2002).

2.5.2.2 Orange (*Citrus sinensis* L.)

Orange is widely cultivated fruit tree in sub-tropics including Ethiopia. The peel powder and oil were found to have insecticidal effect. The peel powder mixed with cow pea produced LD_{50} of 4% (w/w) for *Callosobruchus maculates* (F.) (Don Pedro, 1985). The peel oil has been reported to have toxicity, feeding deterrent and development effects on lesser grain borer, *Rhyzoperta domonica* (F.), rice weevils, *Sitophilus oryzae* (L.) and red floor beetle, *Tribolum castaneum* (Herbst) (Tripathi, *et al.,* 2003). The peel oil was also reported to have toxicity toward *Culex pipiens* (Mwaiko and Savaeli, 1992); and cow pea weevils, *Callosobruchus maculates* (F.) (El-sayed and Abdel-Razik, 1991). Further more, the peel oil has fumigant action against fleas (Weinzierl and Henn, 1992) and house hold insects *Blatella germanica* (L.) and *Musca domestica* (L.) and stored product *Sitophilus oryzae* (Karr and Coats, 1988).

2.5.3 Host Resistance

Plant resistance is an indirect pest control method which involves breeding in the field. This method, though long and tedious, is most profitable as resistance or tolerance to both field and storage pests can be combined. The use of varietal resistance for insect control is based on one of the mechanisms of antibiosis, non-preference or tolerance, in which biophysical or biochemical factors are involved. In small-scale farmers' stores the use of resistant varieties may extend the period during which the produce can be safely stored with out the use of pesticides. In cereals, hard grains, a flinty corneous endosperm, the presence of glumes and the compact arrangement of pericarp layers are important grain characters (Wargo, 1990).

Firdissa *et al.*, (2000) evaluated several haricot bean and maize genotypes for their resistance against Mexican bean weevil and maize weevil, respectively. Though host plant resistance is a promising strategy in pest control, insect population are able to develop biotypes that can attack formerly resistant varieties and there is evidence that improved varieties tend to

perform poorly under low input conditions. However, this strategy may result, along with other control methods, in a significant degree of pests' population regulation (Norris *et al.,* 2003).

2.5.4 Biological Control

Biological control may provide a useful safe alternative for the control of crop pests. Various instances are well documented with regard to the possibilities of using natural enemies to suppress the stored grain insects (Gahukar, 1994). However, the use of biological control against stored product is still limited though recently gaining emphasis due to environmental and health concern. The study done by Adane *et al.*, (1998) showed *Beauveria bassina* as a potentially valuable mycopathogen for microbial control of storage pests.

2.5.5 Chemical Control

Despite the obvious environmental effect, conventional synthetic products will continue to be popular until alternatives are shown to be effective in curtailing pest problems. Many small-scale farmers adopted the use of insecticides for limiting the population of storage insect pests. Chemicals that are used to treat grain stores and grain must posses the correct blend of biological activity and either low mammalian toxicity or short residual life. Dusting and fumigation of grains are the most commonly used chemical methods (Gwinner *et al*., 1996).

Dusting is an easy applied method, can be implemented with very cheap tools such as small containers or cloth bags with punctured holes in the lid. For small amounts of grains, dust can be mixed with grains using a shovel. Dust should be mixed throughlly and distributed evenly all over the produce. Dusts can also be applied on floors, flat surfaces and around the bottom of storage containers. The most commonly used insecticide dusts among farmers are malathion, deltamethrin, permethrin and primiphos-methyl (Hill, 1990).

Fumigants are low molecular weight chemicals, highly toxic and volatile and are, therefore self-dispersing and non-persistant. Fumigation is a widely used method all over the world particularly for large scale storage. Fumigants have an ability to kill all insect stages residing in the grains, but do not protect grain from new attacks. Fumigants must be used in air tight containers. The most widely spread fumigants in use are Phosphine (PH_3) and Methyl bromide (CH_3Br) (Manson and Obermeyer, 2004).

3. MATERIAL AND METHODS

3.1 Mass rearing of test insects

Adults of *Z. subfasciatus* (Boheman) collected from Melkassa Agricultural Research Center were cultured in Addis Ababa University, Department of Biology Insect Science insectory at 27 ± 3ºC and 55-70% RH (Schoonven, 1978). Whole haricot bean seeds bought from local farmers from Melkassa were kept in an oven at 60ºC for 6 hrs to disinfest the seeds if there is any prior infestation before using it as a substrate for mass rearing (Jembere, 2002). Fifty pairs of the adult of *Z. subfasciatus* were placed in 1-litre glass jars containing 250g seeds. The jars were then covered with nylon mesh and held in place with rubber bands. The parent bruchids were sieved out after 13 days of oviposition time and seeds were kept under laboratory condition until F1 progeny emergence. When the F1 progeny started to emerge after 30 days they were sieved out and used for test.

Adult *Sitophilus spp*. used for the study was obtained from grain store house of Melkassa Agricultural Research Center. This area has worm and humid climate that makes favourable environment for the insect reproduction. All the experiments were carried out at 27 ± 3ºC and 55-65%RH.

3.2. Plant material collection and extraction

3.2.1 Solvent extract of plant materials

The bulbs of garlic, *Allium sativum* were bought from local farmers around Addis Ababa and kept in refrigerator before use. The bulbs were removed from compound bulb and chopped using mortal and pistle and soaked in distilled water, petroleumether, ethanol and acetone at the rate of 10, 20 and 30g/100ml of each solvent for extraction (Jembere, 2002). After 24 hours the mixtures were filtered with cheath cloth and filter paper (Watman No 9). Then the filtrates were ready to be used for the different treatments.

Fresh orange fruit (Valencia variety) bought from Eatfruit Ethiopia were peeled with a knife; this left the albedo (the white spongy portion) on the fruit. The fresh orange peel were then chopped with a knife and prepared for treatment following the above mentioned procedure.

3.2.2 Dried and ground materials

The fresh orange peel of *Citrus sinensis* and some bulbs of garlic were dried under shade. Ground materials were obtained by grinding dry plant materials to a fine powder using mortal and pistle. The rates used were 5g (2%), 10g (4%) and 15g (6%)/250g of grains.

3.2.3 Isolation of essential oil extract

Essential oil of *C. sinesis* was isolated by hydrodistillation of fresh orange peel using a Clevenger type apparatus. An average yield of 7.4 ml oil was collected from 1kg of orange peel. The oil was kept in refrigerator before use. The oil was weighed in three doses of 30mg, 150mg and 750mg and dissolved in 10ml of acetone before use.

3.3 Toxicity assessment

Different levels of the extracts and essential oil were applied to a filter paper of 9 mm diameter at rate of 1, 2 and 3ml per filter and placed in a Petri dish of 10cm diameter. In the case of organic solvent extracts, the treated filter was exposed to the open air to allow the organic solvent to evaporate. Then, 1ml distilled water was added to the whole surface of filter paper of each treated filter papers, as a carrier of the extracts. Variable exposure times were used based on the nature of the solvent. In case of acetone and petroleum ether the exposure times were 30 minutes, while this was 60 minutes for ethanol. Other filter papers were also treated with three levels of different solvents as control. After treatment, 5pairs of 3-7 day-old adults of mixed sex of *Zabrotes subfasciatus* and 6-12 day-old adults of unsexed *Sitophilus spp*. were introduced into the treated and control filter papers in the Petri dishes. The treatments were replicated three times. Mortality of the adult insects were counted after 24, 48, 72 and 96hrs starting with 24h after treatment.

For admixture treatment, 250g disinfested common dry bean (*Phaseolus vulgaris L.*) seeds (variety Awash 1) were placed in 1-litre glass jars and treated with the different extracts of garlic at the rate of 5,10 and15 ml being dissolved in 10ml of acetone. After application the seeds were shacked vigorously to coat the extracts with the seeds. These treatments were kept aside for 24hrs, while this was 36 hrs for ethanol extract before introduction of the test insects to the

treated seeds. For comparison a standard insecticide pirimiphos-methyl and solvent treated and untreated seeds were included.

For powder treatment, 250g of disinfected haricot bean seeds in 1-litre glass jars were treated with three levels (5, 10 and 15g) of the garlic bulb and orange peel powder. Untreated grains were included as control. After treatment, 20, 3-7 day-old *Z. subfsciatus* of mixed sex were introduced to the treated and untreated seeds in the glass jars. The jars were covered with nylon mesh and held with rubber bands. The number of dead insects in each jar was sieved and counted after 24, 48, 72 and 96 hrs.

3.4 Progeny assessment

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 F1 progeny. Then the number of F1 progeny produced by *Z. subfasciatus* was counted. Counting was stopped after 45 days from the day of introduction to avoid overlapping of generation.

3.5 Damage assessment

Damage assessment was carried out on treated and untreated grains. Samples of 100 grains were taken from treated and untreated grains and the number of damaged (grains with characteristic holes) and undamaged grains were counted and weighed. Percent weight loss was calculated by count and weight method cited in FAO (1985) as:

$$\% \text{ Weight loss} = \frac{(UaN - (U+D) \times 100}{UaN}$$

Where U = weight of undamaged fraction in n the sample, N.

N = total number of grains in sample.

Ua = average weight of one undamaged kernel.

D = weight of damaged fraction in n the sample

The assessement was done five times for each treatment as replication.

3.6 Repellency bioassay

Repellency of plant materials, mentioned above were assessed against *Z. subfasciatus* and *Sitophilus spp*. in a choice and with no choice bioassay system in a 'Y' olfactometer. In choice test hundred grams of untreated disinfected maize for *Sitophilus spp.* and haricot bean for *Z. subfasiatus* were put into one of the gas washing bottle, while differently treated seeds were put in the other gas washing bottle. For no choice test one of the gas washing bottle was left empty. Then air was pumped with regulated air pump at a rate of 1.2 l/minute into a gas washing bottle containing activated charcoal for filtration through rubber tubing. The filtrated air then passes to the two washing gas bottles containing untreated and treated seeds or with no seeds. Finally the air reachs the two arms of the "Y" tube glass which is attached to the stem where the insects are released. After this set up following the method of Jembere *et al*. (1995), twenty five adults of each *Sitophilus spp.* and *Z. subfasiatus* of mixed sex and age were released into the "Y" olfactometer glass. After 30minutes, the numbers of insects which moved into the untreated (Nc) and treated bottle (Nt) were counted. After each test the "Y" glass tube and the gas washing bottles were washed and dried at 80^{o}C for 1h. Each treatment was replicated four times and percentage repellency (PR) values were computed using the methods of Jilani *et al*. (1988) as:

$$PR\ (\%) = \frac{Nc - Nt}{N} \times 100$$

Where Nc is the individuals in the control bottle (untreated seeds).

Nt is the individuals on the treated bottle.

N is the total number of insects tested.

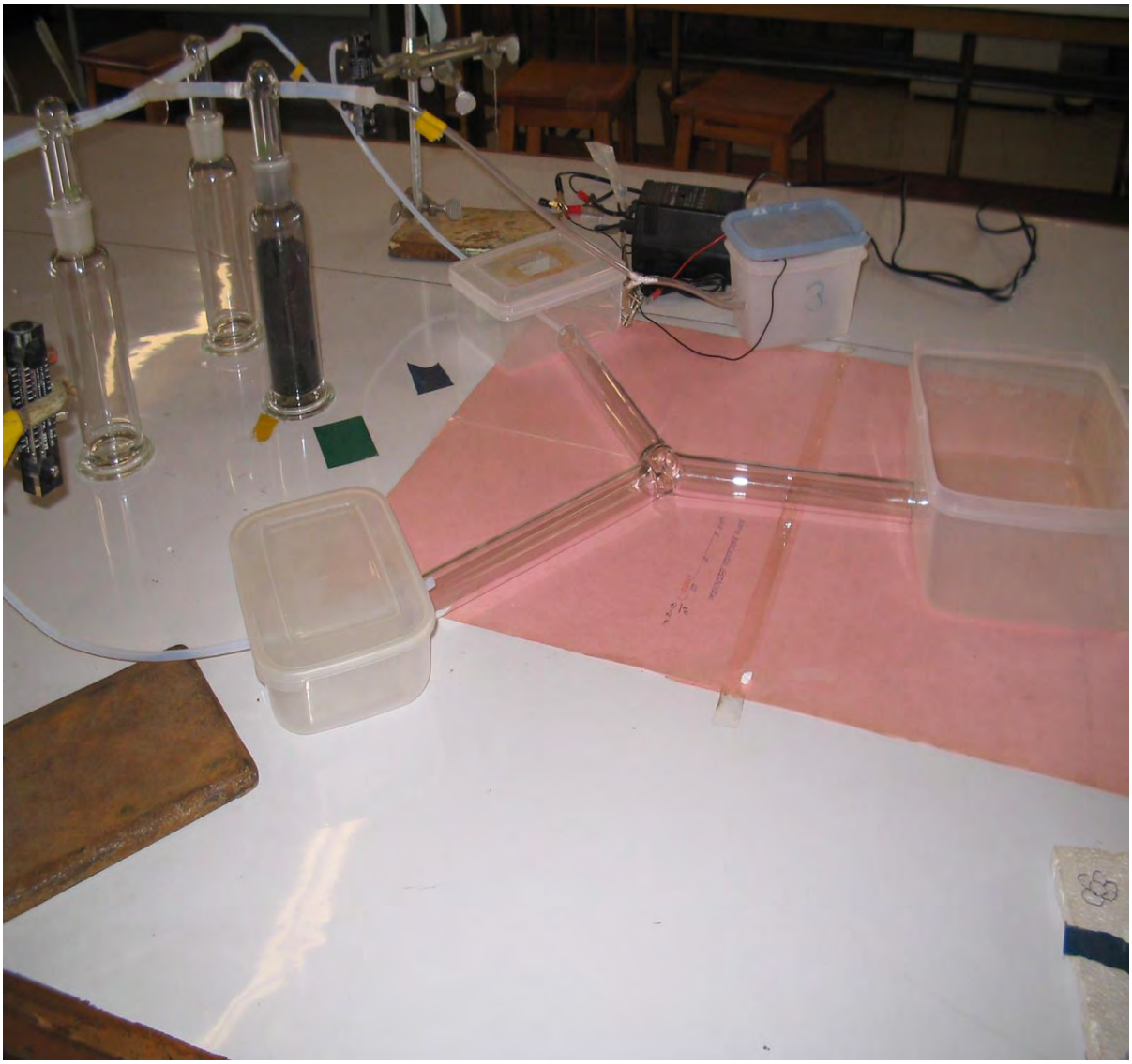

Figure 3.1 Repellency bioassay set up

3.7 Fumigation toxicity

The fumigation toxicity of the essential oil was tested following the method of Wang *et al.* (2001) with some modification. Wide mouth bottles of 1-liter capacity with lids were used as exposure chamber. Filter papers of 9mm diameter were treated with 1, 2 and 3ml of essential oil at the rate of 12mg, 60mg, 300mg dissolved in 10ml of acetone; the same amount of acetone alone was applied as control. The solvent was allowed to evaporate for 20minutes and then the filter paper was placed at the bottom of a 1-liter glass bottle. Twenty insects in small nylon mesh bag with 100g food substrate were hung at the center of the glass bottle (7cm high) above the filter paper (figure3.2). The bottles were then closed tightly with a lid. Each treatment with respective control was replicated five times. Mortality was checked after 24hrs.

Figure 3.2 Fumigation set up

3.8 Germination test

For seed germination test, 100-seed samples were taken at random from each replication of all treatments and pirimiphos-methyl treated seeds. The seeds were placed in petri dishes containing moistened filter paper. Healthy untreated seeds were also tested as control. After six days the percentage of seeds that germinated were recorded.

3.8 Data analysis

All the experiments were laid in a completely randomized design (CRD). Data entry and analysis were done using Microsoft Excel and SPSS Version 10, respectively. Data were transformed by Arcsine transformation when necessary. To find out the effects of the treatment on %mortality, %number of F_1 progeny and % weight loss one-way analysis of variance was used (Gomez and Gomez, 1984). In cases where significant results were obtained, mean comparisons were conducted using Tukey's Studentized range test at 5% level of significance.

4. RESULT

4.1 Toxicity of botanical to adult bruchids and weevils.

4.1.1 Filter paper bioassay

Figure 4.1- 4.8 shows the percent mortality of the *Z. subfasciatus* and *Sitophilus spp.* on the filter paper treated with different dosages of plant materials. Extracts of polar solvents of garlic applied at 1, 2 and 3ml/filter paper showed significant toxicity effect, while acetone, petroleum ether, water and ethanol extracts of orange peel and petrolumether extracts of *A. sativum* did not show any significant effect at all levels of applications tested (Appendix 1).

One hundred percent mortality of *Sitophilus spp*. was obtained with water and acetone extracts of *A. sativum* at the rate of 30g/100ml and applied at 3ml/filter paper after 48 and 96 hour of exposure time, respectively (Figure 4.1). While the extracts of *A. sativum* at lower rate was less effective to S*itophius spp*. (Figures 4.2 and 4.3). Essential oil of *C. sinensis* at the highest rate (750mg/10ml of acetone) applied at 3ml gave 100% mortality of both *Sitophilus spp* and *Z. subfasciatus* after 24 hour exposure time (Figures 4.4 and 4.8). However the lower dosages were not toxic to *Sitophilus spp.*

Acetone, ethanol and water extracts of garlic at all rates of application showed significant toxicity ($P<0.05$). As indicated in figures 4.5 and 4.6 garlic bulbs extracted with water and acetone at the rate of 20gm/100ml and ethanol at 30gm/100ml applied at the dose of 2, 3 and 2 ml/filter paper, respectively induced 100% mortality of *Z. subfasciatus* after 24 hr of exposure time. Even at the lower rate of extraction 10/100ml polar extracts of *A. sativum* showed significant toxicity towards *Z. subfasciatus* (Figure 4.7).

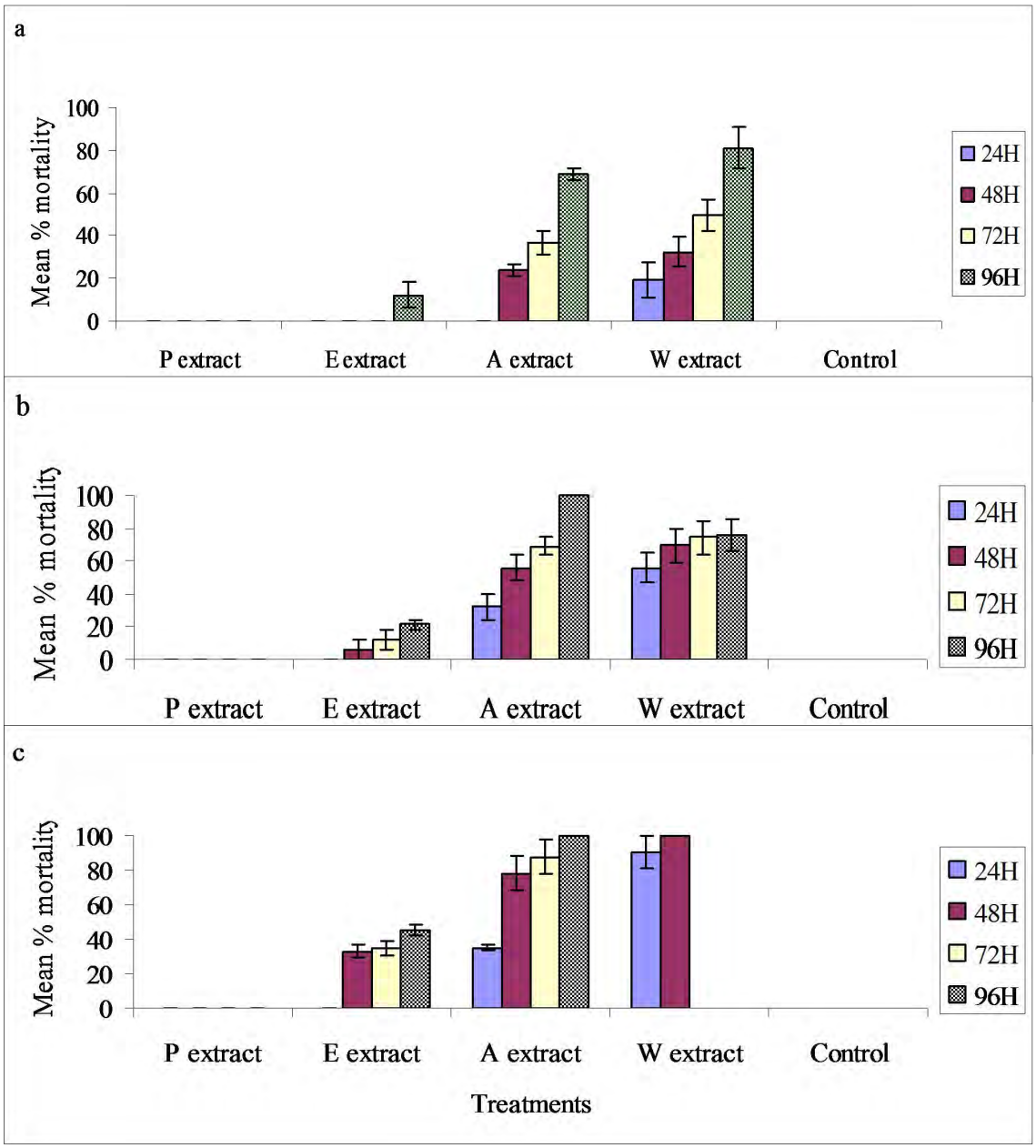

Figure 4.1 Mean % mortality of *Sitophilus spp*. due to solvent extracts of *A. sativum* extracted at the rate of 30g/100ml and applied at the dose of 1ml (a), 2ml (b) and 3ml (c) after different times of exposure.

* P = Petrolum ether extract; E = Ethanol extract;A = Acetone extract; W = Water extract

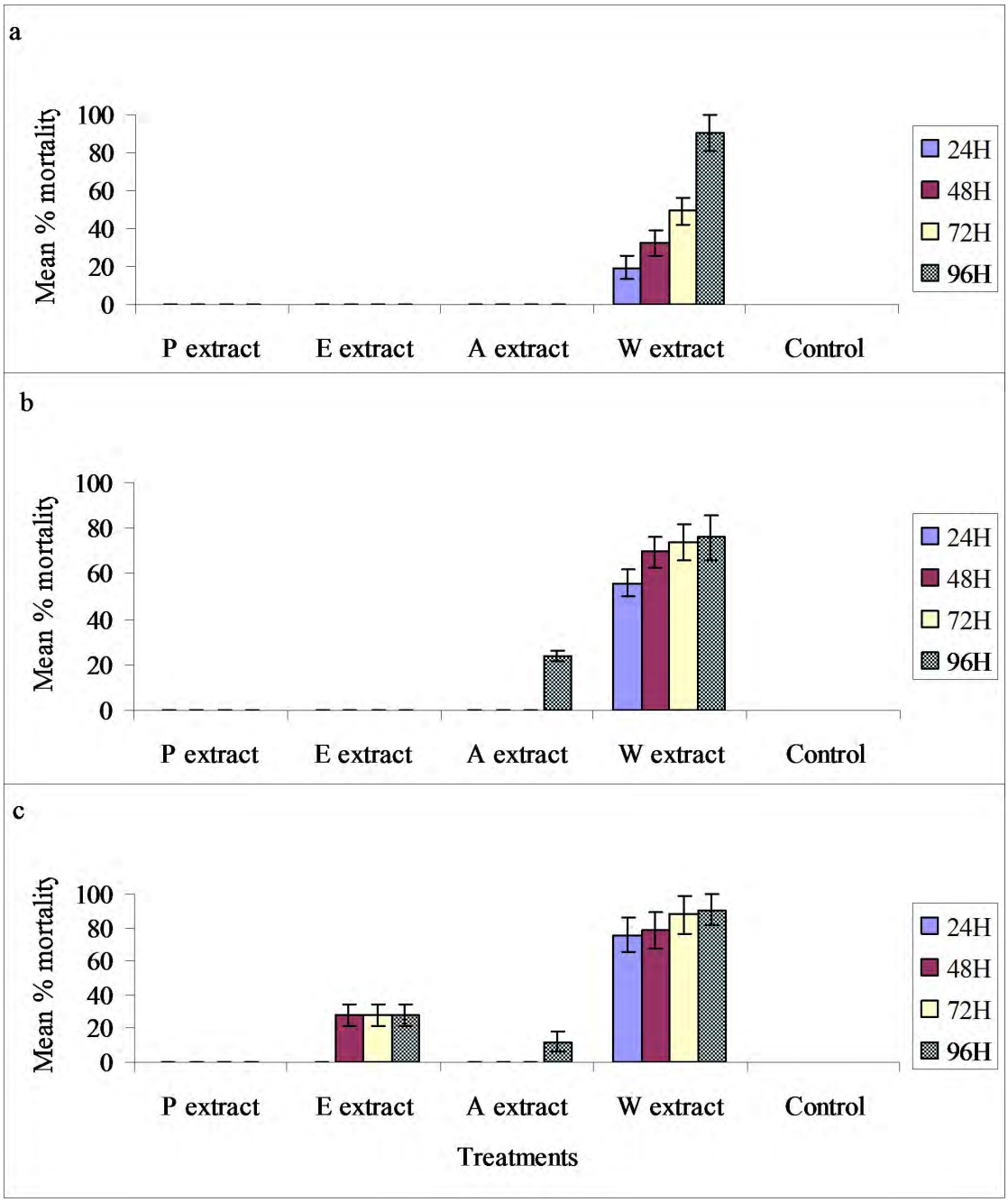

Figure 4.2 Mean % mortality of *Sitophilus spp.* due to solvent extracts of *A. sativum* extracted at the rate of 20g/100ml and applied at the dose of 1ml (a), 2ml (b) and 3ml (c) after different times of exposure.

* P = Petrolum ether extract; E =Ethanol extract; A = Acetone extract; W = Water extract

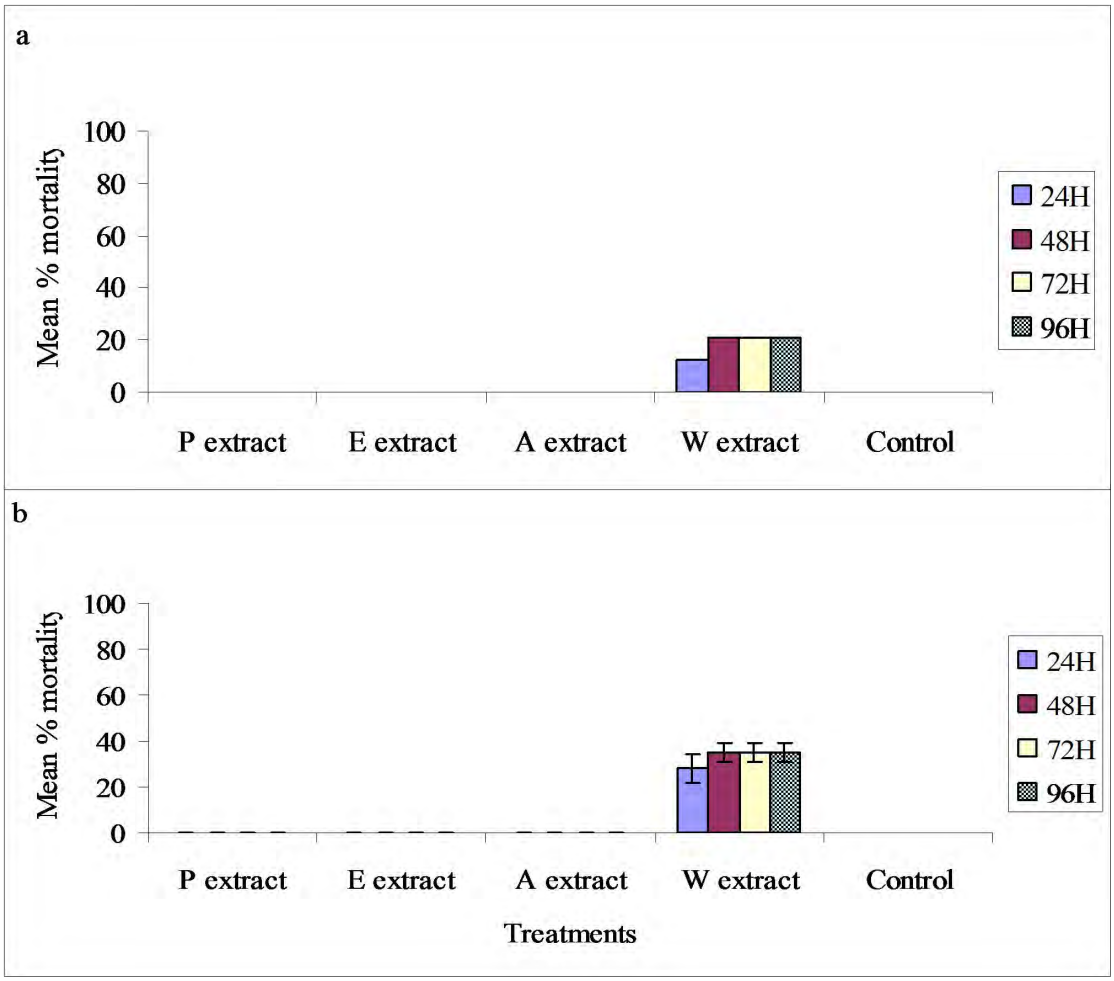

Figure 4.3 Mean % mortality of *Sitophilus spp.* due to solvent extracts of *A. sativum* extracted at the rate of 10g/100ml and applied at the dose of 2ml (a) and 3ml (b) after different times of exposure.

* P = Petrolum ether extract; E =Ethanol extract; A = Acetone extract; W = Water extract

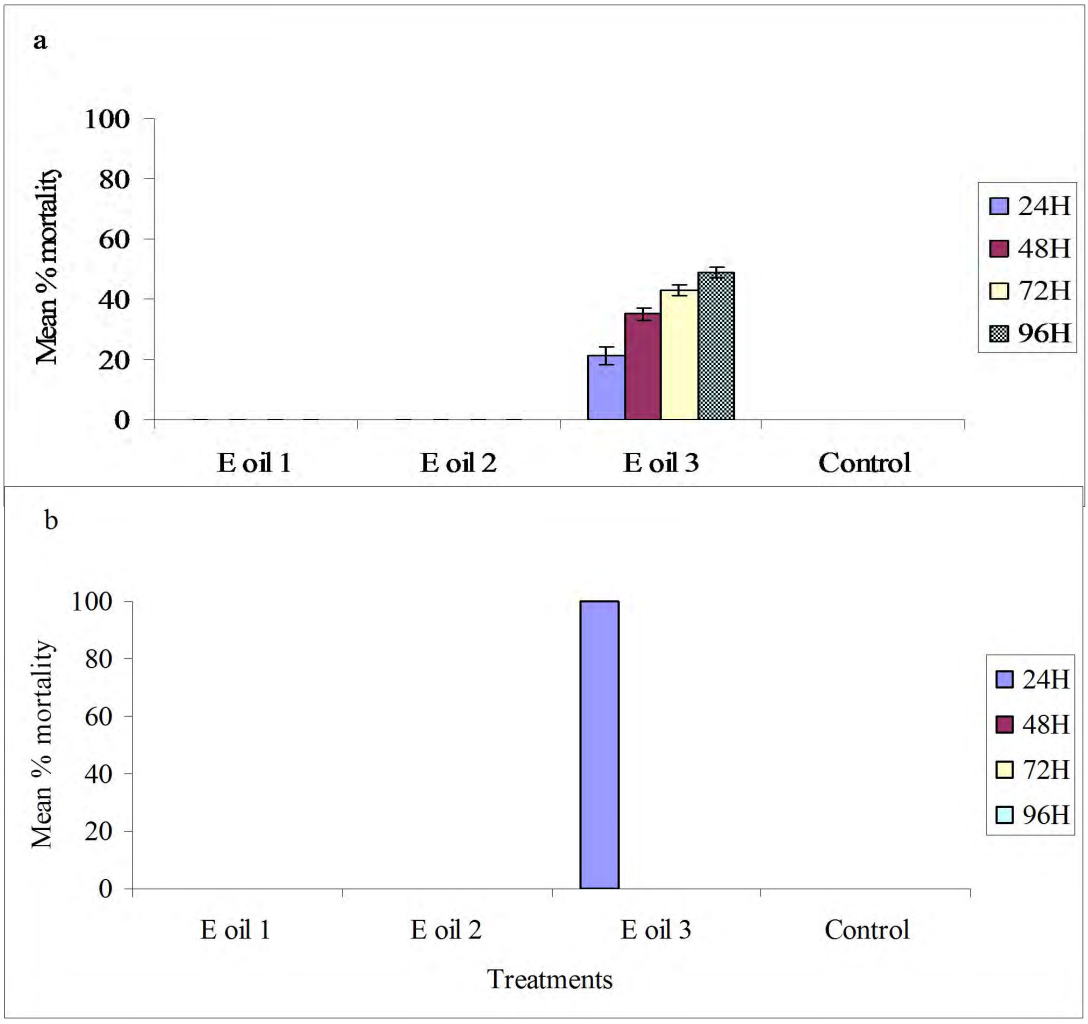

Figure 4.4 Mean % mortality of *Sitophilus spp.* due to essential oil of *C. sinesis* applied at the rate of 2ml (a) and 3ml (b) after different times of exposure.

E oil1 = Essential oil at 0.03g

E oil 2 = Essential oil at 0.15g

E oil 3 = Essential oil at 0.75g

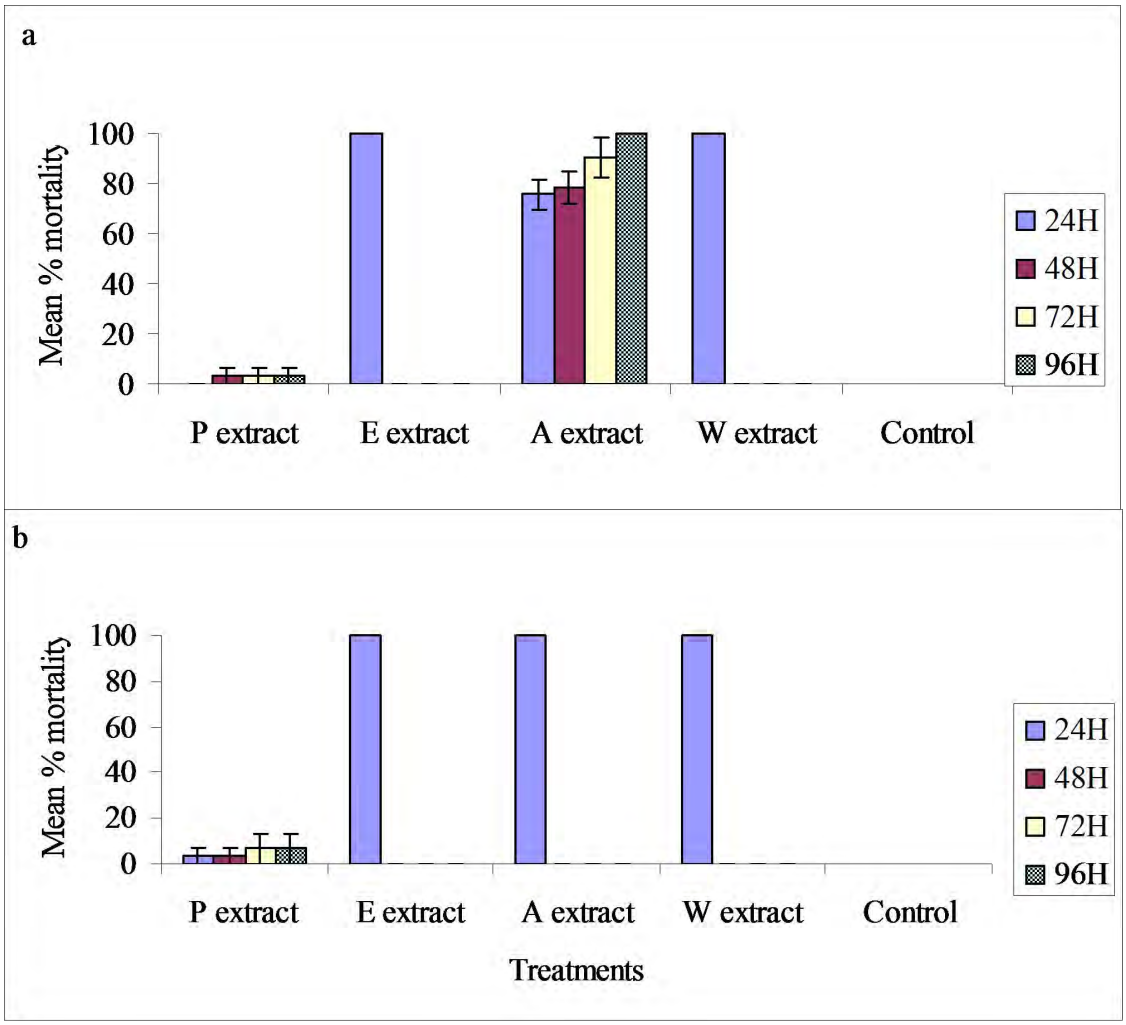

Figure 4.5 Mean % mortality of *Z. subfasciatus* due to solvent extracts of *A. sativum* extracted at the rate of 30g/100ml and applied at the dose of 1ml (a) and 2ml (b) after different times of exposure.

* P = Petrolum ether extract; E =Ethanol extract; A = Acetone extract; W = Water extract

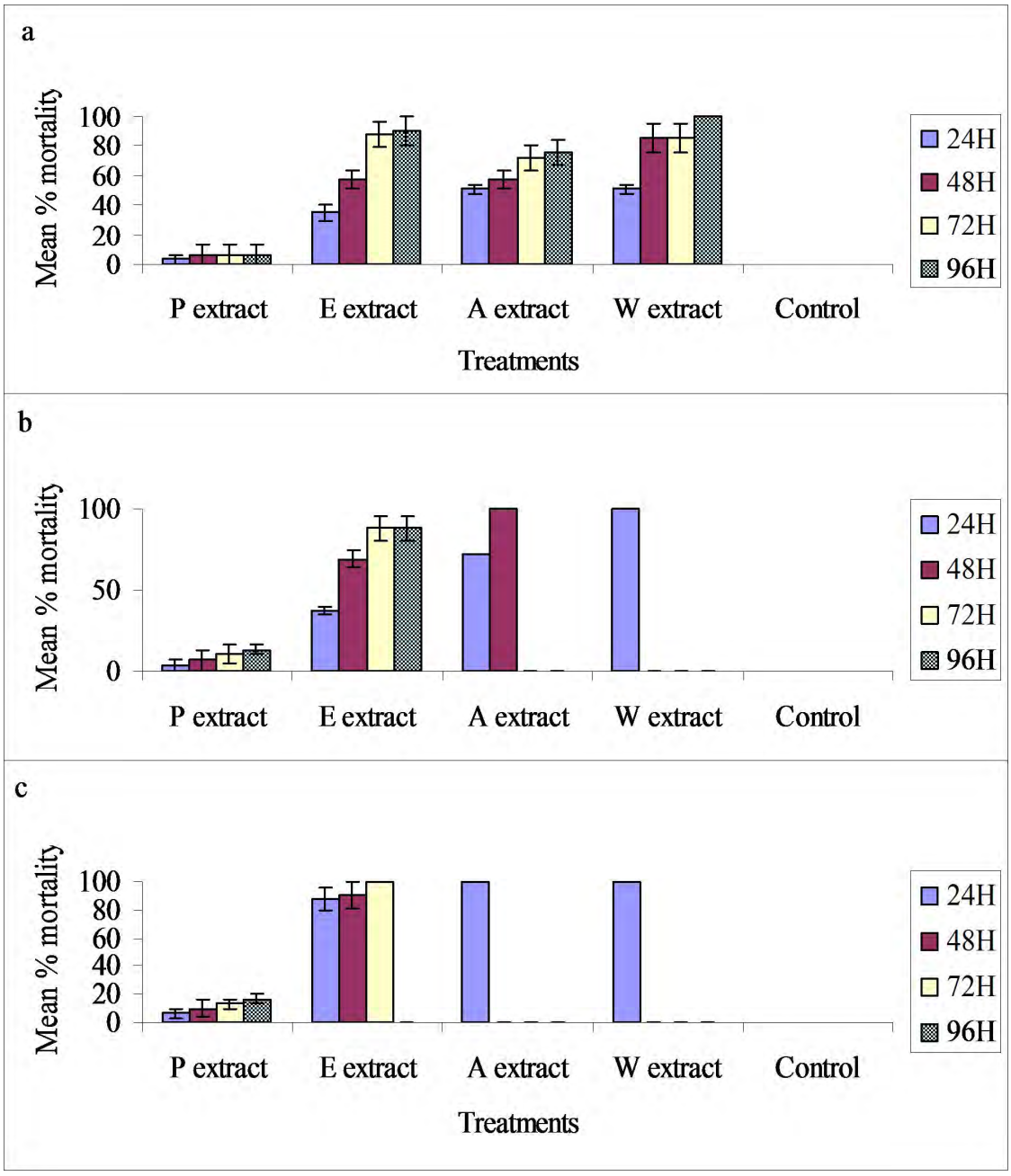

Figure 4.6: Mean % mortality of *Z. subfasciatus* due to solvent extracts of *A. sativum* extracted at the rate of 20g/100ml and applied at the dose of 1ml (a), 2ml (b) and 3ml (c) after different times of exposure.

* P = Petrolum ether extract; E =Ethanol extract; A = Acetone extract; W = Water extract

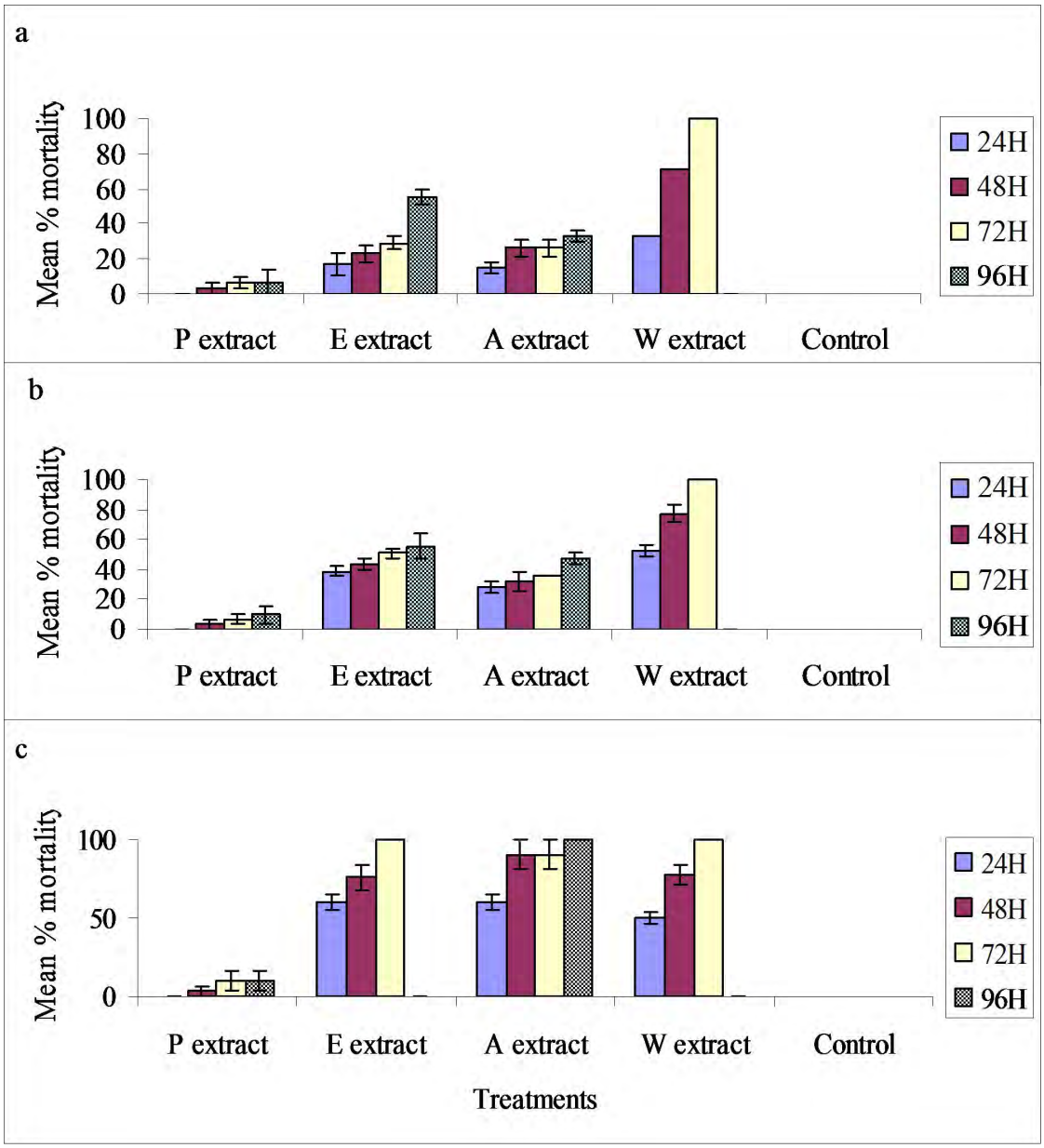

Figure 4.7: Mean % mortality of *Z. Subfasciatus* due to solvent extracts of *A. sativum* extracted at the rate of 10g/100ml and applied at the dose of 1ml (a), 2ml (b) and 3ml (c) after different times of exposure.

* E extract = Ethanol extract; A extract = Acetone extract; W extract = Water extract

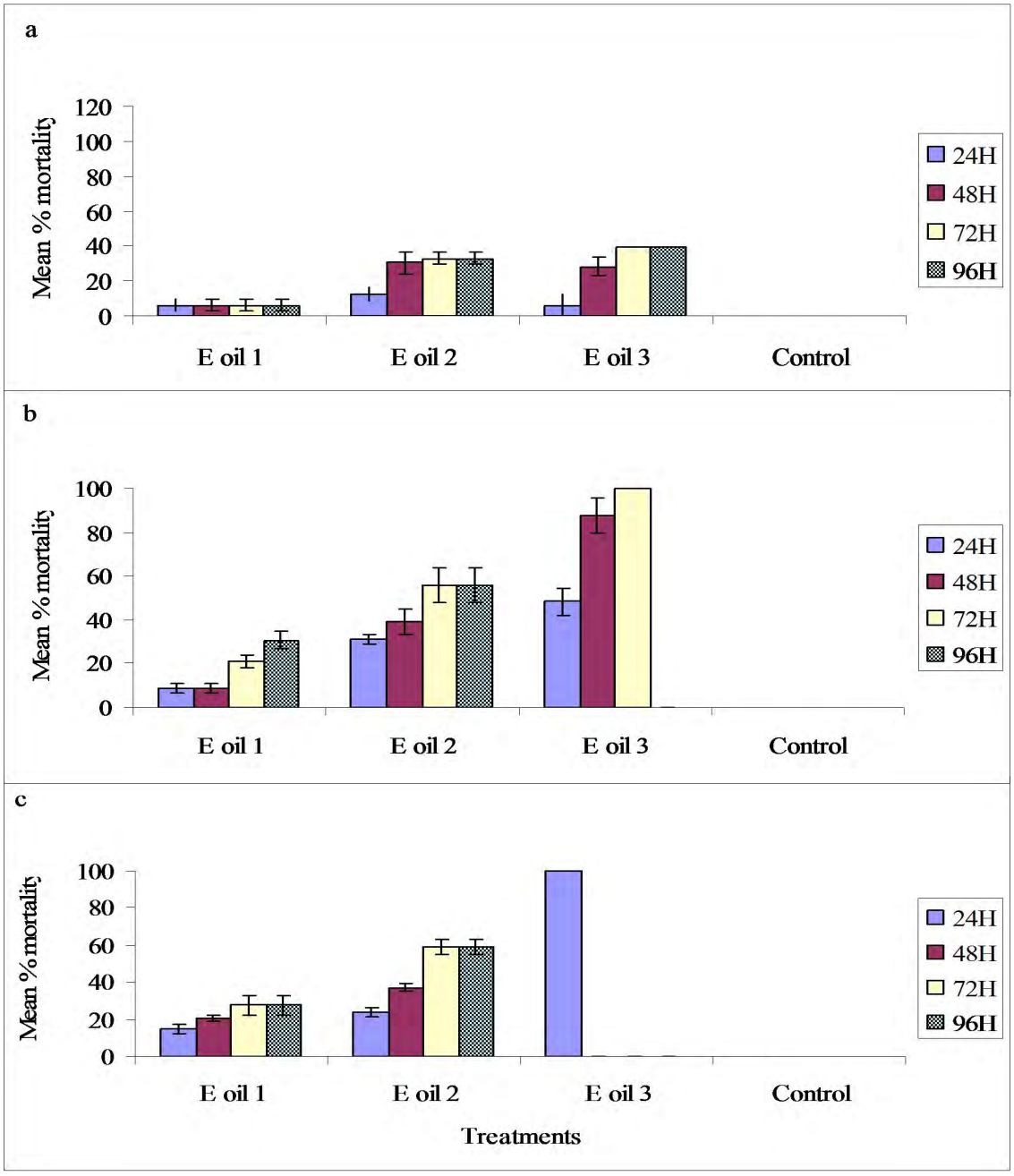

Figure 4.8 Mean % mortality of *Z. Subfasciatus* due to essential oil of *C. sinesis* applied at rate of 1ml (a), 2ml (b) and 3ml (c) after different times of exposure.

E oil1 = Essential oil at 0.03g

E oil 2 = Essential oil at 0.15g

E oil 3 = Essential oil at 0.75g

4.2 Admixture toxicity

Extracts that showed significant toxicity effect in the filter paper bioassay were applied onto seeds. Percent mortality of *Z. subfasciatus* in haricot bean treated with different dosages of plant is indicated in tables, 4.1 and 4.2.

Mortality was significantly high ($P<0.05$) on seeds treated with pirimiphos-methyl at the rate of 0.125g/205g of grain. The highest doses of acetone extracts of *A. sativum* at 15ml/250g of seeds and essential oil of *C. sinensis* at the highest dose of 0.75g/250g of haricot bean gave 82.87 and 67.40% mortality after 96 hours of exposure, respectively (Table 4.1). *Allium sativum* and *C. sinesis* applied as powder were also toxic at higher dosage of 15g/250g of grain causing 63.55 and 65.95% mortality after 96 hour of exposure (Table 4.2). Compared to other treatments ethanol, powder of garlic bulb and orange peel powder at lower dose were less toxic. The result of this experiment showed that all treatments of the essential oil of orange peel and acetone extract of garlic were relatively toxic to *Z. subfasciatus*.

Table 4.1: Mean % mortality adult *Z. subfasciatus* due to toxicity of beans treated with different extracts of *A. sativum*.

Treatments	Level of extraction	Dose (ml/250g)	Hours after treatment			
			24	48	72	96
Ethanol extract	10g	5	0.00 ± 0.00a	16.35 ± 6.58ab	33.16±1.81abc	45.00 ± 1.65b
		10	8.87 ± 4.79ab	25.30 ± 1.25bcd	27.52±3.04b	45.95 ± 0.95b
		15	10.29±0.24ab	27.70 ±b-e 1.14	36.23±1.73abc	47.88 ± 1.66bc
	20g	5	13.74± 0.98ab	22.42 ± 9.23abc	43.08±0.95cde	53.86 ± 3.50bcd
		10	17.80± 6.96ab	38.16 ± 3.53c-f	43.05±4.21cde	52.88 ± 3.65bcd
		15	15.09± 5.66ab	34.14 ± 2.70cde	45.03±4.42cde	56.84 ± 1.81bcd
	30g	5	17.80± 6.96ab	34.18 ± 2.09cde	45.00±3.33cde	50.85 ± 3.40bcd
		10	28.54± 4.01bc	40.11 ± 3.54def	49.88±3.54def	57.00 ± 3.65bcd
		15	11.07± 1.07ab	44.04 ± 0.95ef	50.85±3.40ef	63.85 ± 3.85cd
Acetone extract	10g	5	0.00 ± 0.00a	23.85 ± 2.70bcd	36.23±1.73abc	50.79 ± 1.69bcd
		10	0.00 ± 0.00a	28.66 ± 3.08b-e	39.15±2.97b-e	59.05 ± 2.84bcd
		15	0.00 ± 0.00a	29.92 ± 1.92b-e	44.04±1.91cde	55.98 ± 3.82bcd
	20g	5	2.70 ± 1.35a	26.45 ± 2.08bcd	40.16±2.56b-e	54.88 ± 3.62bcd
		10	19.30 ± 3.96ab	28.77 ± 2.21b-e	42.09±4.42cde	60.31 ± 3.89bcd
		15	15.00 ± 5.59b	31.07 ± 1.07b-e	46.92±2.53c-f	65.95 ± 1.25de
	30g	5	2.70 ± 1.35a	34.23 ± 1.02cde	45.00±1.65cde	58.93 ± 1.07bcd
		10	22.59 ± 2.34ab	40.19 ± 0.96def	49.80±0.96def	67.40 ± 2.34de
		15	50.77 ± 0.00c	54.75 ± 1.02f	60.07±1.92f	82.88 ±8.70ef
Ethanol/ Acetone		15	0.00 ± 0.00a	0.00 ± 0.00a	0.00±0.00a	0.00 ± 0.00a
Pirimiphos-methyl		0.125g	100.00 ± 0.00d	Nob	Nob	Nob
Control		0g	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey studentized range test (HSD).

Table 4.2: Mean % mortality of *Z. subfasciatus* due to *A. sativum* powder, *C. sinensis* powder and essential oil of treated seeds.

Treatment	Dosage (g/250g)	Mean % adult mortality, h after exposure			
		24	48	72	96
Garlic powder					
5g	5	0.00 ± 0.00a	15.09±5.66b	32.14±1.07b	45.00±1.65b
10g	10	0.00 ± 0.00a	29.92±1.92c	41.07±7.86bc	53.76±1.73bc
15g	15	0.00 ± 0.00a	39.21±1.69cd	48.93±4.26bcd	63.55±2.08cde
Orange powder					
5g	5	21.34±1.45b	26.45±2.08bc	36.23±1.73b	45.00±3.33b
10g	10	27.70±1.14bc	34.18±2.09cd	44.04±3.46bc	54.83±2.72bc
15g	15	35.25±1.02c	47.91±3.34de	57.98±2.86cd	65.95± 1.25de
Orange essential oil					
0.03g	0.03	21.14±2.70b	39.15±2.97cd	53.81±2.97cd	56.79±0.00cd
0.15g	0.15	26.07±4.27bc	45.96±2.54de	61.33±3.08d	61.33±3.08cde
0.75g	0.75	47.89±2.88d	57.00±3.65e	63.55±2.08d	67.40±2.34e
Pirimiphos-methyl					
0.125g	0.125	100±0.00e	Nob	Nob	Nob
Control 0.0g	0	0.00 ± 0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey studentized range test (HSD).

Nob = No observation

4.3. Effect of the plant extracts, powder and essential oil on F_1 progeny of *Z. subfasciatus*.

The number of F_1 adult progeny produced by *Z. subfasciatus* in untreated and different plant extracts treated haricot bean is shown in tables 4.3 and 4.4. The number of F_1 progeny produced in each treatment were significantly low due to treatments compared to the number of progeny produced in control. Haricot bean treated with ethanol and acetone extracts of *A. sativum* at higher extraction level (30g/100ml) applied at 15ml/250g of grain reduced the emergence of *Z. subfascitus* by 98.0 and 96.2%, respectively (Table 4.3). All levels of the powder of *A. sativum* and peel powder of *C. sinensis* caused significant reduction in progeny emergence of *Z. subfasciatus* ($P< 0.05$). However, the percent reduction in adult emergency by *A. sativum* powder is less compared to *C. sinesis* peel (Table 4.4).

No emergence of adult progeny was observed from pirimiphos-methyl treated haricot beans. A similar result was obtained with haricot beans treated with 750mg (0.3%) of essential oil, but there was no significant difference ($P>0.05$) between the mean number of F_1 adult progeny emerged in beans treated with 30, 150 and 750mg (Table 4.4). The result of this experiment showed that all treatments of essential oil were effective as the standard insecticide, pirimiphos-methyl, by significantly reducing F_1 progeny emergence.

4.4 Effect of the plant extracts, powder and essential oil on weight loss of haricot bean due to feeding by *Z. subfasciatus*.

Weight loss assessments result of treated and untreated grains are shown in tables 4.3 and 4.4. All the treatments significantly reduced weight loss compared to the untreated check 45 days after introduction of *Z. subfasciatus* into treated and untreated beans. No weight loss of stored haricot beans was observed in seeds that were treated with pirimiphos-methyl at a recommended rate. The highest dosage of ethanol and acetone extracts of *A. stavium* and peel powder of *C. sinesis* protected the haricot beans against feeding by *Z. subfasciatus* with no noticeable feeding damage on seeds, but this result was achieved at a minimum dose (30mg) for essential oil of *C. sinesis*. The damage caused by *Z. subfasciatus* was significantly higher in grains treated with lower dosage of powder of *A. sativum,* but higher doses reduced weight loss (Table 4.4).

The present finding showed that essential oil of orange peel effectively reduced the weight loss even at lower dose than the standard check pirimiphos-methyl at recommended rate. Apart from the essential oil of orange peel all the solvent extracts show similar result at the highest dosage.

Table 4.3: Mean number of F_1 progeny produced and weight loss caused by *Z. subfasciatus* on seeds treated with different extracts of *A. stavium.*

Treatments	Level of extraction	Dose (ml/250g)	Mean number of F_1 progeny	Mean % weight loss
Ethanol extraction	10	5	49.00 ± 3.05fg	0.61 ± 0.05de
		10	50.66 ± 4.97fg	0.63 ± 0.06e
		15	40.66 ± 7.31efg	0.50 ± 0.07de
	20	5	47.66 ± 4.66fg	0.45 ± 0.04cde
		10	36.66 ± 2.84def	0.414 ± 0.06cde
		15	23.66 ± 1.76b-e	0.37 ± 0.07b-e
	30	5	19.66 ± 3.52a-d	0.27 ± 0.07a-d
		10	7.33 ± 2.33ab	0.00 ± 0.00a
		15	1.66 ± 1.66a	0.00 ± 0.00a
Acetone extract	10	5	57.00 ± 2.08g	0.72 ± 0.14e
		10	51.00 ± 2.88fg	0.47 ± 0.05de
		15	36.00 ± 3.60c-f	0.38 ± 0.07b-e
	20	5	47.33 ± 3.17fg	0.46 ± 0.05cde
		10	37.66 ± 3.17def	0.60 ± 0.11de
		15	6.33 ± 3.17ab	0.08 ± 0.03ab
	30	5	27.66 ± 1.66cde	0.28 ± 0.06a-d
		10	18.00 ± 4.04abc	0.11 ± 0.02abc
		15	3.33 ± 2.02a	0.00 ± 0.00a
Ethanol	0	15	89.00 ± 2.30h	1.97 ± 0.05f
Acetone	0	15	91.666 ± 3.48h	1.99 ± 0.11f
Control (untreated)	0	0	123.33 ± 6.88^{i}	2.33 ± 0.09g

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey studentized range test (HSD).

Table 4.4: Mean number of F1 progeny produced and weight loss caused by *Z. subfasciatus* on seeds treated with *A. stavium* and *C. sinesis* powder and essential oil.

Treatments	Dosage (g/250g)	Mean number of F1 progeny	Mean %weight loss
Orange powder	5	31.66 ± 5.81^{b}	0.53 ± 0.09^{b}
	10	17.66 ± 0.88^{ab}	0.38 ± 0.08^{ab}
	15	13.66 ± 1.45^{ab}	0.00 ± 0.00^{a}
Garlic powder	5	110.33 ± 3.17^{de}	1.86 ± 0.15^{d}
	10	89.33 ± 5.20^{d}	1.28 ± 0.14^{c}
	15	64.00 ± 9.84^{c}	0.95 ± 0.11^{c}
Orange essential oil	0.03	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}
	0.15	0.66 ± 0.66^{a}	0.00 ± 0.00^{a}
	0.75	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Pirimiphos-methyl	0.125	$0.00 \pm .00^{a}$	0.00 ± 0.00^{a}
Control (untreated)	0	123.33 ± 6.88^{e}	2.33 ± 0.09^{e}

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey studentized range test (HSD).

4.5 Fumigation toxicity of *C. sinesis* essential oil

The result on fumigant toxicity of essential oil of *C. sinensis* towards *Z. subfasciates* and *Sitophilus spp.* are presented in table 5. No dead insects were observed at lower dose of 0.012 mg/10ml of acetone applied at all levels 1, 2 & 3ml/filter paper in both insects. The highest dose of essential oil showed significantly higher ($P< 0.05$) mortality of both species of storage insects. Particularly essential oil of 0.3g applied at the rate of 2 & 3ml per filter paper induced 100 and 61.25% mortality of *Z. subfasciatus* and *Sitophilus spp.* respectively (Table 4.5). *Zabrotes subfascitus* was most susceptible to the essential oil of *C. sinesis* compared to *Sitophilus spp*. Acetone treated controls did not cause any significant mortality of the bruchids at 1, 2 and 3ml level of application.

4.6 Effect of the plant extracts, powder and essential oil on viability of treated bean seeds.

The percent germination of haricot bean seeds treated with different treatments and untreated check are shown in figure 4.9. There was a no significant difference in germination of haricot bean except with seeds treated with ethanol extracts. The result showed the other extracts of both *A. stavium* and *C. sinesis* did not have any effect on seed germination.

Table 4.5 Fumigant toxicity of *C. sinensis* essential oil treated seeds to *Z. subfasciates* and *Sitophilus* spp.

Treatments (g/100g of seed)	Dose (ml/filter)	% mortality after 24h exposure	
		Zabrotes subfasciatus	*Sitophilus spp*
0.06 gram	1ml	0.25 ±0.25a	0±0.00a
	2ml	0±0.00a	0±0.00a
	3ml	18.75±2.39b	0±0.00a
0.3gram	1ml	26.25±1.25c	0±0.00a
	2ml	100.00±0.00d	15.00±0.00b
	3ml	100.00±0.00d	61.25±3.75c
Control (acetone treated)		0.00±0.00a	0±0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey studentized range test (HSD).

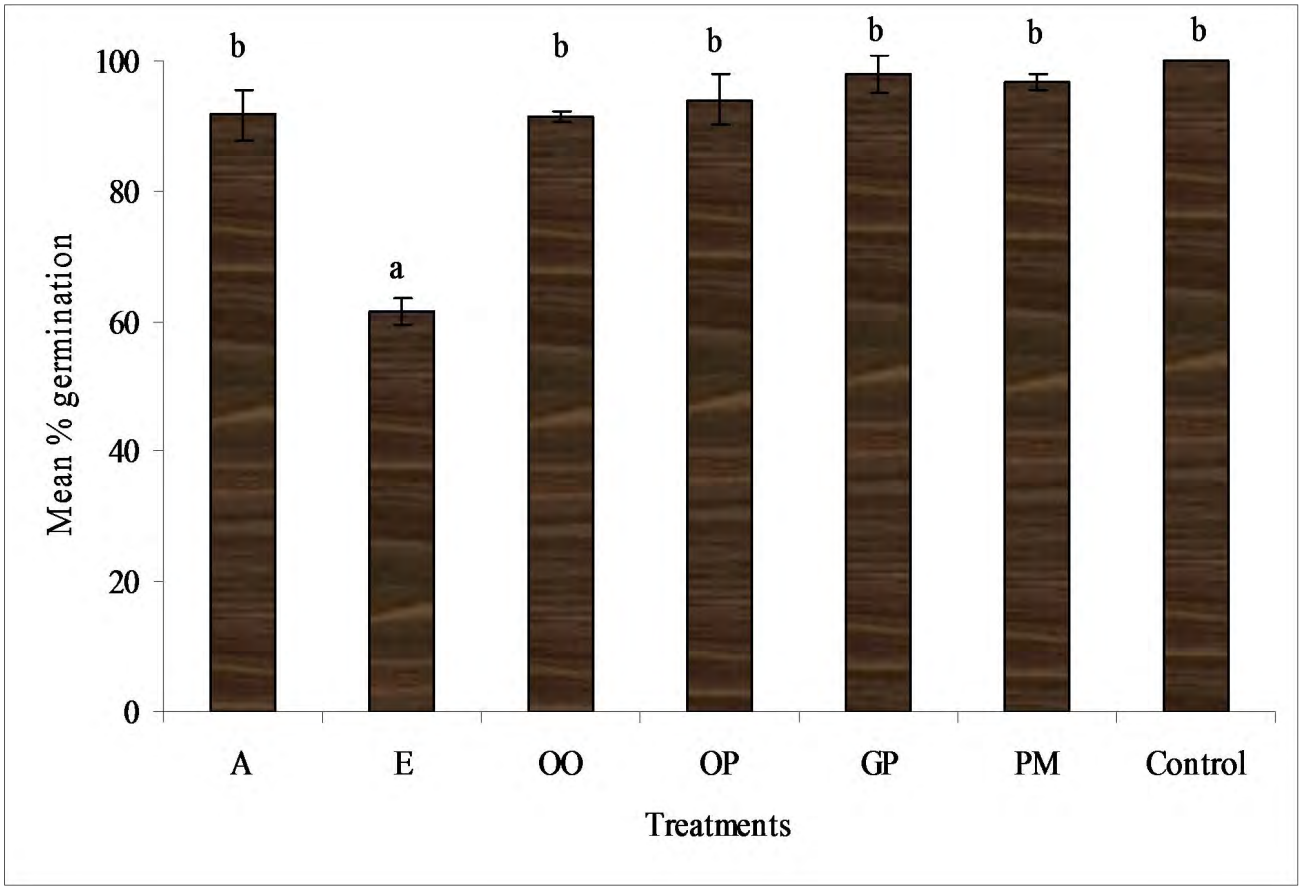

Figure 4.9: Effect of plant extracts, powder and essential oil on bean seed germination.

Key: A = Acetone extract of *A. sativum* at level of 30g/100ml applied at 15ml.

E = Ethanol extract of *A. sativum* at level of 30g/100ml applied at 15ml.

OO= Orange oil at 0.75g.

OP= orange powder applied at 15g.

GP= Garlic powder applied at 15g.

PM= Pirimiphos-methyl applied at 0.125g.

4.7 Repellency of the plant extracts powder and essential oil to *Z. subfasciatus* and *Sitophilus spp.*adults.

Tables 4.6 and 4.7 shows the mean repellency values for each of the test materials at different doses for each insect species. Analysis of variance indicated significant differences ($P< 0.05$) between the responses of the two insects to the plant materials. The percent repellency obtained with out choice was less than with choice test. There was a considerable variation in the repellency of the materials against *Z. subfasciatus* and *Sitophilus spp.* All the treatments showed significant repellent effect against *Z. subfasciatus* except acetone extracts at lower level of extraction (10g/100ml). Ethanol extracts of *A. sativum* extracted at levels of 20 & 30g/100ml and applied at all levels were most repellent to *Z. subfasciatus,* but did not show any repellent effect against *Sitophilus spp*. The percent repellency of *Sitophilus spp*. due to fresh chopped garlic was significantly high ($P< 0.05$) followed by garlic powder (Table 4.6).

Table 4.6: Mean percentage repellency of different plant materials to *Z. subfasiatus* and *Sitophilus spp*.

Treatments (g/100gm seeds)	Mean % repellency (PR)			
	Zabrotes subfasciatus		*Sitophilus spp*	
	No choice	With choice	No choice	With choice
Fresh chopped garlic				
2g	2.00±1.15ab	16.53±1.44cd	6.25±1.31ab	54.57±2.75g
4g	4.00±0.00ab	24.57±1.29d	33.15±2.44ef	72.25±2.39h
6g	20.47±0.65d	56.87±3.82g	55.07±2.17h	68.62±1.72h
Garlic powder				
2g	17.33±0.88cd	13.44±0.79bc	19.50±1.80de	41.61±1.32fg
4g	10.75±1.03bc	5.50±0.64ab	18.25±1.88cd	37.00±1.83fg
6g	4.25±1.84ab	2.00±1.15a	21.25±1.25de	45.22±2.09g
Orange powder				
2g	25.00±4.04de	25.00±1.73de	5.00±2.04ab	10.50±.50bc
4g	33.25±.85e	33.80±1.97f	14.05±1.21bc	18.32±1.47cd
6g	32.50±1.44e	38.45±1.65f	20.75±1.88cd	27.75±1.65ef
Orange oil				
0.012g	25.75±2.56de	33.27±2.03ef	3.90±2.30a	10.75±1.01bc
0.06g	33.50±2.39e	37.60±0.90f	8.80±0.69b	21.00±0.70cd
0.3g	37.87±0.96f	53.75±1.37f	25.70±3.34de	35.95±2.69f
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey studentized range test (HSD).

Table 4.7: Mean percentage repellency of ethanol and acetone extracts of *A. sativum* to *Z. subfasiatus* and *Sitophilus spp*.

Treatments	Level of extraction (g/100ml)	Dose	Mean % repellency (PR)			
			Zabrotes subfasciatus		*Sitophilus spp*	
			No choice	With choice	No choice	With choice
Ethanol extracts	(30g)	6ml	61.65±2.32e	67.65±3.28f	35.10±3.37c	17.43±1.49c
		4ml	60.82±2.46e	62.00±2.58def	0.00±0.00a	9.87±0.87b
		2ml	41.77±1.89bc	64.50±1.25ef	0.00±0.00a	0.00±0.00a
	(20g)	6ml	57.00±1.91de	57.00±1.91cde	0.00±0.00a	0.00±0.00a
		4ml	50.00±3.16cde	48.00±4.32c	0.00±0.00a	0.00±0.00a
		2ml	47.00±3.41cd	48.00±2.16c	0.00±0.00a	0.00±0.00a
	(30g)	6ml	55.00±6.60cde	54.00±2.70cd	0.00±0.00a	0.00±0.00a
		4ml	3.00±1.00a	10.33±1.41a	0.00±0.00a	0.00±0.00a
		2ml	1.00±1.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Acetone extracts	(30g)	6ml	54.00±1.15cde	51.00±2.51c	15.44±1.26c	23.50±1.84b
		4ml	47.00±2.79cd	50.50±1.70c	0.00±0.00a	6.25±2.13b
		2ml	29.00±3.62b	30.50±1.19b	0.00±0.00a	0.00±0.00a
	(20g)	6ml	5.50±0.64a	3.00±1.00a	0.00±0.00a	0.00±0.00a
		4ml	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
		2ml	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Control	0g	0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Means with in a column followed by different letters are significantly different from each other (P< 0.05%), Tukey studentized range test (HSD).

5. DISCUSSION

Among the treatments, extracts of the polar solvents (acetone, ethanol and water) caused very high mortality of both Sitophilus weevils and Mexican bean bruchid. Water extracts of *A. sativum* was significantly toxic to *sitophilus spp.* than other extracts and control at all levels of application in filter paper bioassay followed by acetone and ethanol extracts, while all these extracts were highly toxic against Mexican bean weevil even at lower doses. The result demonstrates the active ingredient of *A. sativum* was highly soluble both in water and acetone.

Orange peel oil at high concentration (750mg) applied at 3ml showed 100% mortality for both insects. At lower dose the orange peel oil also showed higher mortality against *Z. subfasciatus.* But it was not toxic to *Sitophilus spp*., showing Mexican bean weevil as the more susceptible to extracts of the two plant species.

All the plant materials admixed with a haricot bean caused more significant adult mortality of *Z. subfasciatus*. Four days after introduction, adult Mexican bean weevil mortality was 83 (acetone extract), 67% (orange peel powder), 66 (orange peel powder) and 64% (ethanol extracts and garlic powder) at maximum dose of each treatment. Pirimiphos-methyl caused 100% mortality with in 24 hour. Among the treatments the toxicity of garlic powder and acetone extracts was not significantly observed with in 24 hours.

The major active constituent in garlic responsible for its toxicity could be diallyl trisulphide (Williamson, 2003). Huang *et al.* (2000) evaluated the toxicity of diallyl trisulphide from garlic (*A. sativum*) and observed LD_{50} values of 1.02 and 5.54µg/mg toward red flour beetle, *Tribolium castaneum* Hebst and maize weevil, *Sitophilus zeamis*. The toxicity of *C. sinesis* peel oil may be attributed to d-limonene (Sharaby, 1988b). Tripathi *et al.,* (2003) reported the contact toxicity of d-limonene with LD_{50} 74.73, 85.37 and 79.78 for *R. dominica, S. oryzae* and *T. castaneum*.

An over-all test of efficacy between the treatments has shown that mortality was directly related to the dosage and time. This indicated higher dosage is more efficient in management of pests. In case of the sun dried powder of garlic and orange peel, the orange peel powder seems

very promising though significant result is gained at higher dosage than the standard rates 5% suggested for most botanicals for storage pest management. Belmain and Stevenson (2001) also reported effective use of *C. sinesis* powder against legume pests. The effectiveness of the orange peel powder is probably due to silica or silica like component, which are abrasive and the ability of the particles to adhere to the grain.

All the treatments caused significant reduction of F1 adult emergence compared to control. The extent to which the botanicals affected the survival of the subsequent progeny has found to vary among them. In progeny count, only live newly emerged adults were observed. This indicated that the active ingredients of botanicals which are responsible for the toxicity of the plant kill the insects gradually. *Citrus sinesis* peel oil was superior to untreated and other extracts causing 100, 99 and 99% reduction in adult emergence at 0.75, 0.15 and 0.03g/250g of haricot bean. The study showed that orange peel oil is even better than pirimiphos-methyl causing 100% reduction of F1 emergence at lower dose. The current findings is in agreement with Tripathi *et al.* (2003) who also reported oviposition reduction effect of orange peel oil against *T. castaneum* by 94.5%. Sharaby (1988b) also reported reduced oviposition and egg hatching of potato tuber moth, *Phthorimaea operculella* exposed to 220µl of the oil. Levinson *et al*. (2003) also reported orange peel oil at 1ml suppressed oviposition of Mediterianin fruit fly, *Ceratitis capitata*. The present study also shows that orange peel oil has strong oviposition deterrent effect against *Z. subfasciatus*. Powdered sun dried orange powder were also effective in reducing F1 adult emergence though not effective as orange peel oil and pirimiphos-methyl.

Both acetone and ethanol extracts of *A. sativum* also gave significant reduction of F1 progeny. Acetone and ethanol extract at a dose (30g/100ml) applied at 15ml/250g of grain reduced emergence of *Z. subfasciatus* by 98 and 96% respectively. Powdered sun dried garlic was only better than untreated check. The study made by Mekuria (1995) also suggested garlic powder was less toxic to *Sitophilus spp*. The possible explanation for failure of the garlic in the powder forms was that when the seeds are shacked to admix the botanical they settle down.

Significant reduction in feeding damage which indicate the higher protectant potential of these materials against insect damage were observed. *Citrus sinesis* was highly effective than other treatments which in turn significantly reduced damage to haricot bean when compared to

control. It appears therefore that orange peel oil has insecticidal properties which accounts for much higher levels of effectiveness. Furthermore, orange peel powder at 15g/250g of haricot bean seed also protected the grain from damage by hundred percent, strongly suggesting the presence of physical interfering agents in orange peel powder. The garlic powder and solvent extracts reduced damage of *Z. subfasciatus* to haricot bean in this study. Though the mean percent weight loss in garlic powder treated seed were not sufficient compared to extracts of garlic suggesting its low effectiveness of absorptive and abrasive properties.

Orange peel oil showed highest fumigant toxicity causing 100 and 61% mortality toward *Z. subfasciatus* and *Sitophilus spp.* at 0.3g/100g of haricot bean applied at 3ml adjusted to 24h exposure. The fumigant toxicity decreased with decreasing concentration. The orange peel oil has been reported to have fumigant toxicity 13 times more than that of methyl bromide (Tripathi *et al.*, 2003). The present study also showed that orange peel has strong fumigant toxicity effect against the *Z. subfasciatus.* Keita *et al.* (2001) reported the mode of the action of fumigant toxicity of essential oil against insects might be the inhibition of acetylcholinesterase.

Using essential oils as a fumigant for stored grain and legumes could be particularly relevant as methyl-bromide is removed from use. However, there is only very limited evidence demonstrating their ability to penetrate through grain bulks which must occur if plant extracts are to emulate fumigant gases. In addition, the understanding of sorption and residue on target grain are important issue to use essential oils as a potential fumigant (Lee *et al.*, 2003).

From the germination test, it was concluded that the plant materials have no significant effect on seed germination except ethanol extracts of *A. sativum*. During the germination test, some of the treatments were affected by fungus resulting in reduced germination percentage. However, all the botanical treatments including pirimiphos-methyl show percentage germination ranged between 91-96% which is not significant from the control indicating most of the treatments do not interfere with germination of the seed and can be applied for grains stored for food and seed purpose.

The use of plants as repellents is very old, but has not received the necessary attention for proper development. The results of the present experiment indicates that all the treatments repelled the two insect species in a dose-dependent manner. The strong repellent effect of fresh

chopped garlic and ethanol extracts of garlic in this study was observed against *Sitophilus spp.* and *Z. subfasciatus,* respectively. Further more, the result of the study showed different insects differ in their sensitivity to different treatments which may on species susceptibility (Peterson and Coats, 2001).

An insect repellent has been defined as a chemical substance that caused the insect to make oriented movements away from the source (Roomi and Antiquiddin, 1977). More repellency effect of the botanicals was observed with choice repellency test rather than with out choice test indicating the seeds have an attraction power to the insects. However, some of the treatments which showed significant toxicity were found to be pure in repellency. *Citrus sinesis* peel oil had the highest fumigant toxicity on both insects, but its repellency effect was significantly lower than garlic extracts. This suggests that the active compounds which acted as repellent and fumigant might be chemically different.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study made the two botanicals posses toxicity, feeding and ovipositional deterrent effect on the *Z. subfasciatus* and *Sitophilus spp*. Overall, *Z. subfasciatus* was the most susceptible than *Sitophilus spp*. The susceptibility of each species to the toxic and repellent effects of plant materials was different and this may reflect the complexity of the chemical composition of the materials. For example, ethanol and acetone extracts of *A. sativum* was not repellent to *Sitophilus spp.,* but had significant repellent effect on *Z. subfasciatus.*

The protection of the haricot beans against *Z. subfasciatus* damage provided by garlic powder was less effective compared to solvent extracts of garlic. This is may be due to high content of the active ingredients in solvent extracts rather than in the powder.

The *C. sinesis* peel oil and its sun dried powder significantly reduced the F1 progeny and weight loss of *Z. subfasciatus*. Further more, the study showed the peel oil posses contact and fumigant toxicity on the *Z. subfasciatus* and *Sitophilus spp*. The overall activity of the orange peel should be of major interest because all the result of this study indicated that the orange peel oil, particularly, may be useful as a potential stored grain protectant.

6.2 Recommendations

- The trials on garlic and orange peel are laboratory-based and of short duration and they do not necessarily reflect responses which would be under real farm condition. So this is an area of research which must be considered as priority.

- For the majority of farmers in developing countries like Ethiopia, commercial insecticides are often too costly or unavailable. Similarly, many uneducated farmers use synthetic pesticides inappropriately, leading to environmental and human safety hazards as well as promoting insecticide resistance. There fore research on post-harvest protection using botanicals should be encouraged.

- For small-scale farmers orange peel powder would be very effective. Therefore more research on its use is recommended.

- Extensive survey should be made to identify these botanical tested with standardized assessment of residual effectiveness on all stages of the insect life cycle and the stored commodities.

- Based on the high efficacy of these botanicals tested in controlling *Z. subfasiatus* and *Sitophilus spp.* in the present study and because they are cheap to prepare and environmentally safe and friendly, their wide use is recommended. It is also recommended to extract botanicals rather than to use powder without extraction.

- Generally, stored product pest management in subsistence agriculture like Ethiopia should highly focus on utilizing plant materials which have insecticidal effect. There are with out doubt, very many plants used as grain protectants by rural communities, which have yet to be identified or developed. With the continual problems of using synthetic insecticides the potential of using botanical insecticides are for the future is huge.

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APPENDICES

Annex 1: Summary table of analysis of variance (ANOVA) for mean %mortality of *Zabrotes subfaciatus* and *Sitophilus spp.* by different plant extracts at different time of interval.

Annex 1.1: Toxicity of Petrolumether extract of *A. sativum.* at the rate of 30g/100ml and applied at the dose of 1, 2 and 3ml after different time of exposure.

Source of error		Sum of Squares	df	Mean Square	F	P value
Mortality after 24hrs	Between Groups	339.574	3	113.191	4.005	0.052
	Within Groups	226.075	8	28.259		
	Total	565.649	11			
Mortality after 48hrs	Between Groups	732.887	3	244.296	2.213	0.164
	Within Groups	883.078	8	110.385		
	Total	1615.966	11			
Mortality after 72hrs	Between Groups	909.991	3	303.330	2.748	0.112
	Within Groups	883.078	8	110.385		
	Total	1793.069	11			
Mortality after 96hrs	Between Groups	1175.502	3	391.834	3.339	0.077
	Within Groups	938.808	8	117.351		
	Total	2114.310	11			

* = Significant at P > 0.05.

Annex 1.2: Summary table for analysis of variance (ANOVA) for mean % mortality of *Z. subfasciatus* due to essential oil of *C. sinesis* at 1, 2 and 3ml concentration respectively.

Source of error		Sum of Squares	df	Mean Square	F	P value
Mortality after 24hrs	Between Groups	1661.024	3	553.675	14.789	0.001*
	Within Groups	299.513	8	37.439		
	Total	1960.536	11			
Mortality after 48hrs	Between Groups	2968.147	3	989.382	17.259	0.001*
	Within Groups	458.602	8	57.325		
	Total	3426.749	11			
Mortality after 72hrs	Between Groups	6693.430	3	2231.143	30.899	0.000*
	Within Groups	577.667	8	72.208		
	Total	7271.097	11			
Mortality after 96hrs	Between Groups	6693.430	3	2231.143	30.899	0.000*
	Within Groups	577.667	8	72.208		
	Total	7271.097	11			

* = Significant at $P < 0.05$.

Annex 1.3: Summary table for analysis of variance (ANOVA) for mean % mortality of *Sitophilus spp*. due to essential oil of *C. sinesis* at 1, 2 and 3ml concentration respectively.

Source of error		Sum of Squares	df	Mean Square	F	P value
Mortality after 24hrs	Between Groups	348.865	3	116.288	0.873	0.494
	Within Groups	1065.197	8	133.150		
	Total	1414.061	11			
Mortality after 48hrs	Between Groups	459.643	3	153.214	0.982	0.448
	Within Groups	1248.315	8	156.039		
	Total	1707.958	11			
Mortality after 72hrs	Between Groups	1219.412	3	406.471	2.800	0.109
	Within Groups	1161.537	8	145.192		
	Total	2380.949	11			
Mortality after 96hrs	Between Groups	1907.133	3	635.711	4.153	0.048*
	Within Groups	1224.600	8	153.075		
	Total	3131.732	11			

* = Significant at $P < 0.05$.

Annex 2: Summary table for analysis of variance (ANOVA) for mean percent mortality of *Z. subfasciatus* due to different extracts of *A. stavium* over different period of time on treated haricot bean seeds.

Source of error		Sum of Squares	df	Mean Square	F	P value
Mortality after24hrs	Between Groups	134883.491	36	3746.764	20.802	0.000*
	Within Groups	13328.267	74	180.112		
	Total	148211.758	110			
Mortality after 48hrs	Between Groups	116482.985	36	3235.638	21.922	0.000*
	Within Groups	10922.108	74	147.596		
	Total	127405.093	110			
Mortality after 72hrs	Between Groups	105668.264	36	2935.230	29.700	0.000*
	Within Groups	7313.328	74	98.829		
	Total	112981.593	110			
Mortality after 96hrs	Between Groups	96978.777	36	2693.855	30.043	0.000*
	Within Groups	6635.332	74	89.667		
	Total	103614.109	110			

* = Significant at $P < 0.05$.

Annex 3: Summary table for analysis of variance (ANOVA) for mean percent mortality of *Z. subfasciatus* due to *A. sativum* powder, *C. sinesis* powder and essential oil over different period of time on treated haricot bean seeds.

Source of error		Sum of Squares	df	Mean Square	F	P value.
Mortality after 24hrs	Between Groups	26366.900	10	2636.690	252.309	0.000*
	Within Groups	229.906	22	10.450		
	Total	26596.806	32			
Mortality after 48hrs	Between Groups	19576.461	10	1957.646	82.303	0.000*
	Within Groups	523.287	22	23.786		
	Total	20099.748	32			
Mortality after 72hrs	Between Groups	18013.181	10	1801.318	51.994	0.000*
	Within Groups	762.189	22	34.645		
	Total	18775.369	32			
Mortality after 96hrs	Between Groups	16905.083	10	1690.508	137.203	0.000*
	Within Groups	271.068	22	12.321		
	Total	17176.150	32			

* = Significant at $P < 0.05$.

Annex 4: Summary table for analysis of variance (ANOVA) for mean F_1 progeny emergence of *Z.subfaciatus* on seeds treated with different extracts of *A. sativum.*

Source of error	Sum of Squares	df	Mean Square	F	P-value
Between Groups	67255.212	10	6725.521	112.205	0.000*
Within Groups	1318.667	22	59.939		
Total	68573.879	32			

* = Significant at P < 0.05.

Annex 5: Summary table for analysis of variance (ANOVA) for mean F_1 progeny emergence of *Z.subfaciatus* on seeds treated with *A. sativum* powder and *C. sinesis* powder and essential oil.

Source of variation	Sum of Squares	df	Mean Square	F	P value
Between Groups	67255.212	10	6725.521	112.205	0.000*
Within Groups	1318.667	22	59.939		
Total	68573.879	32			

* = Significant at P < 0.05.

Annex 6: Summary table for analysis of variance (ANOVA) for a weight loss produced by *Z. subfascatus* on seeds treated by different plant materials.

A) Ethanol at a rate of 10g/ 100ml

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	34.543	3	11.514	150.094	0.000*
Within Groups	4.296	56	7.671E-02		
Total	38.839	59			

* = Significant at P < 0.05.

B) Ethanol at a rate of 20g/ 100ml

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	41.587	3	13.862	182.815	0.000*
Within Groups	4.246	56	7.583E-02		
Total	45.833	59			

* = Significant at P < 0.05.

C) Ethanol at a rate of 310g/ 100ml

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	57.482	3	19.161	359.544	0.000*
Within Groups	2.984	56	5.329E-02		
Total	60.466	59			

* = Significant at P < 0.05.

D) Acetone at a rate of 10g/ 100ml

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	49.850	4	12.462	78.897	0.000*
Within Groups	11.057	70	.158		
Total	60.907	74			

* = Significant at P < 0.05.

E) Acetone at a rate of 20g/ 100ml

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	60.204	4	15.051	123.624	0.000*
Within Groups	8.522	70	.122		
Total	68.726	74			

* = Significant at P < 0.05.

F) Acetone at a rate of 30g/ 100ml

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	75.813	4	18.953	225.351	0.000*
Within Groups	5.887	70	8.411E-02		
Total	81.700	74			

* = Significant at P < 0.05.

G) Orange powder

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	48.697	3	16.232	178.319	0.000*
Within Groups	5.098	56	9.103E-02		
Total	53.795	59			

* = Significant at P < 0.05.

H) Garlic powder

Source of error	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.317	2	3.159	10.667	0.000*
Within Groups	12.437	42	.296		
Total	18.754	44			

* = Significant at P < 0.05.

Annex 7: Summary table for analysis of variance (ANOVA) for percent mortality of *Z. subfasciatus* due to fumigant toxicity of *C. sinesis* essential oil at 1, 2 and 3ml level of application after 24 hrs.

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	49792.714	6	8298.786	1974.782	0.000
Within Groups	88.250	21	4.202		
Total	49880.964	27			

* = Significant at P < 0.05.

Annex 8: Summary table for analysis of variance (ANOVA) for percent mortality of *Sitophilus spp*. due to fumigant toxicity of *C. sinesis* essential oil at 1, 2 and 3ml level of application after 24 hrs.

Source of error	Sum of Squares	df	Mean Square	F	P value
Between Groups	10092.188	3	3364.063	239.222	0.000
Within Groups	168.750	12	14.063		
Total	10260.938	15			

* = Significant at P < 0.05.

Annex 9: Summary table for analysis of variance (ANOVA) for mean germination test of haricot bean treated by different materials of *C. sinesis* and *A. sativum*.

Source of error	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2863.733	6	715.933	55.642	0.000
Within Groups	128.667	10	12.867		
Total	2992.400	14			

* = Significant at P < 0.05.

Annex 10: Summary table for analysis of variance (ANOVA) for mean percentage repellency of powders and chopped plant materials to S*itophilus spp* and *Z. subfasciatus.*

Source of error		Sum of Squares	df	Mean Square	F	P value
No choice *Z. subfasciatus*	Between Groups	12118.223	12	1009.852	80.026	0.000*
	Within Groups	479.524	38	12.619		
	Total	12597.747	50			
With choice *Z. subfasciatus*	Between Groups	13491.729	12	1124.311	99.044	0.000*
	Within Groups	431.360	38	11.352		
	Total	13923.088	50			
No choice *Sitophilus spp.*	Between Groups	14698.125	12	1224.844	82.025	0.000*
	Within Groups	567.437	38	14.933		
	Total	15265.562	50			
With choice *Sitophilus spp.*	Between Groups	25684.494	12	2140.374	183.325	0.000*
	Within Groups	443.663	38	11.675		
	Total	26128.156	50			

* = Significant at $P < 0.05$.

Annex 11: Summary table for analysis of variance (ANOVA) for mean percentage repellency of solvent extracts *A. sativum* and essential of *C. sinesis* oil of plant materials to S*itophilus spp* and *Z. subfasciatus.*

Source of error		Sum of Squares	df	Mean Square	F	P value
No choice *Z. subfasciatus*	Between Groups	37708.177	15	2513.878	91.554	0.000*
	Within Groups	1317.982	48	27.458		
	Total	39026.159	63			
With choice *Z. subfasciatus*	Between Groups	45142.409	15	3009.494	182.285	0.000*
	Within Groups	792.470	48	16.510		
	Total	45934.879	63			
No choice *Sitophilus spp.*	Between Groups	6278.550	15	418.570	165.880	0.000*
	Within Groups	121.120	48	2.523		
	Total	6399.670	63			
With choice *Sitophilus spp*.	Between Groups	2115.801	15	141.053	61.646	0.000*
	Within Groups	109.830	48	2.288		
	Total	2225.631	63			