APPROVAL LETTER

ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

As members of the examining Board of the Final M.Sc. Open Defense, we certify that we have read and evaluated the thesis prepared by Fikadu Kifle entitled““ studies on the management of *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) on Common beans (*Phaseolus vulgaris.L*) using Resistant Varieties and Botanicals““ and recommend that it is accepted as fulfilling the thesis requirement for the Degree of Master of Science in Zoological Sciences.

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Advisor	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the Council of Graduate Studies through the Departmental Graduate Committee of the candidate's major Department.

I hereby certify that I have read this thesis prepared under my direction and recommend that it is accepted as fulfilling the thesis requirement.

Emana Getu (Ph.D) (Prof.)	____________________	_____________
Name of Thesis Advisor	Signature	Date

LIST OF ABBREVIATIONS

CPC	Crop protection Compendium
CRD	Completely Randomized Design
CSA	Central Stastical of Authority
EU	European Union
MOA	Ministry of Agriculture
SAS	Stastical Analysis System
SNNPR	South Nation and Nationalities People of Republic

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Abstract

Protections of stored products from insect pests using resistant varieties and botanicals have been utilized for centuries. Studies were conducted to evaluate different botanicals and local common bean varieties against Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) at Melkassa Agriculture Research Center in 2016/2017. Twenty local common bean varieties were evaluated twice against *Z. subfasciatus* in completely randomized design in three replications. The result indicated that, RAZ White and Round yellow were resistant in the first and second round experiments. The botanical experiment consists of Jatroph seed powders, Parthenium seed powders and Neem leaves powders at the rate of 0.2g/kg of seed for each experiment were evaluated against *Z. subfasciatus.* Malathion 0.2g/kg of seed was used as a standard check. Jatroph seed powder was the most effective against Z. *subfasciatus.* Parthenium seed powders and neem leaves powders also showed promising result. Germination test was conducted on bean seed treated with different botanicals. Common bean treated with botanicals significantly ($p<0.05$) had higher germination percentage than that of the untreated seed. In general, the results obtained indicated that using bean Varieties (RAZ white and Round yellow) resistant varieties and botanicals (Jatropha) seed powder gave the best control of Z. *subfasciatus.*

KEYWORDS: *Botanical, Bruchids, Malathion, Resistant varieties and Z. subfasciatus*

1. Introduction

1.1. Background and Justification

Common bean (*Phaseolus vulgaris* L.) is a legume crop, which is originated in South America and Central America (Purseglove, 1988 cited in Batureine, 2009). It belongs to the class Magnoliopsida in the Leguminoceae family. The crop grows in many environments ranging from tropical and subtropical to the temperate regions of the world (Batureine, 2009).

Common bean is the most important legume crop and a vital source of nutrition worldwide for direct human consumption (Sileshi Fistum *et al*., 2014). It is used for food predominantly as form of mature beans (dry), shell beans, and green pods. Beans constitute an important source of dietary protein which accounts for 22% of seed weight that match carbohydrate rich crop such as rice, maize, and cassava. It is a rich source of Macro and micronutrients (Dawit Kidane, 2005)

Common bean is grown and consumed in developing countries like Latin America, Africa, and Asia (Sileshi Fistum *et al*., 2014). Half of the world"s common bean production occurs in countries which have low income and food deficit, whereas, the other half is produced in countries where common bean is an important economic crop, like in U.S.A. Brazil, Mexico and China are the largest producers of dry beans. Annual production of green beans is estimated to 4.5 million tonnes, with the largest production around the Mediterranean and in the USA (Mwanauta *et al*., 2015).

Common bean was introduced into East Africa by the Portuguese in the sixteenth century and spread into many parts of Africa (Gepts *et al*., 2008). According to CSA (2014/15), in Ethiopia, Common bean national annual yield ranged from 1.58 to 1.6 tonnes/ha. Low productivity of common bean in Ethiopia is due to abiotic and biotic factors. Bean bruchids is a major production constraint in bean growing regions of Ethiopia (Sileshi Fistum *et al.,* 2014).

The dominant species in the major bean growing parts of Ethiopia are the Mexican bean weevil (*Zabrotes subfasciatus*) (Boheman) and *Acanthoscelides obtectus* (Say) (Ferede Negasi and Tsedeke Abbate, 1995). Ferede Negasi and Tsedeke Abbate (1995) reported that these two species are the principal insect pests of stored beans in Ethiopia. Stored bean is used as food and planting material the next season. However, farmers are unable to store common bean seed for long period due to bruchids which damage and increase losses of quantity and quality.

Control of bruchids infestation were done by treating stored common bean with Malathion, methyl bromide, carbon disulfide, and several other chemicals. These are considered environmentally undesirable and are too expensive for subsistence farmers. To increase the resistance of cultivated varieties, plant breeders are interested in understanding resistance mechanisms that operate in local variety. Therefore, these studies aimed to determine level of resistance of local varieties and see the effect of botanicals on *Z. subfasciatus.*

1.2. Statement of the Problem

Bean storage over long periods at small-scale subsistence farming levels in Ethiopia is limited due to two common bean bruchid species *(Z. subfasciatus* and *A. obtectus)* infestation that result in heavy losses in terms of quality, weight and nutritional value. To reduce such excessive losses, most farmers are forced to sell off bulk of excess common bean immediately after they harvest when the prices in the local market is very low. This ultimately leads to food in security, hunger and low farm income. This situation reduces farmer‟s motivation to increase production and productivity of bean.

Thus, looking for environmentally friendly and economically feasible control option is mandatory that is the aim of the current experiment.

1.3. Objective

1.3.1. General Objective

- To screen the resistant varieties and botanicals against Z. subfasciatus on Common bean

1.3.2. Specific Objectives

- ✓ To screen local common bean varieties against *Z. subfasciatus*
- ✓ To evaluate the efficacy of powders *Jatropha seed, Neem leaf and Parthenium* seed against *Z. subfasciatus*
- ✓ To see the effect of powder treatment of different botanicals on the germination of common bean seeds

2. Literature Review

2.1. Common Bean Production in Ethiopia

Phaseolus vulgaris is highly cultivated for its delicious seeds that add flavour and protein to the diets of millions of people throughout the world. This ancient crop, like many other legumes, has an ability to fix nitrogen from the air through a symbiotic relationship with bacteria housed in the root nodules (Ferris and Kaganzi, 2008). Protein is high in common bean and considered the „meat of the poor‟. The impressive diversity of colours, textures and tastes of the common bean make it a popular choice for people (Gepts, 2008). Common bean originated in Central and South America where small seeded wild types can still be found growing today (Batureine, 2009).

Common bean is an ancient crop and archaeological evidence indicates that it was being cultivated as early as 6,000 BC. Domestication occurred in Central America (Mexico and Guatemala) and in South America (Peru) independently leading to two distinct gene pools (Purseglove, 1988 cited in Batureine, 2009). Today, common bean is a globally important crop, especially in North and South America, Europe and Africa. The crop was brought to East Africa in the 16th century by Portuguese traders and carried to high altitude regions by slave trading caravans and merchants (Firdissa Iticha *et al.*, 2000).

It is a highly nutritious pulse cultivated throughout the world and is placed third in the importance list of the food legumes and it contains 38% to 59% carbohydrates and 25.3% to 28.9% proteins (Shukla *et al*., 2007). The crop is grown worldwide for its edible bean, popular as dry, fresh and green beans. Production is expanding slowly based on population growth with the highest usage in poor developing countries. Rift valley area, Oromia and Southern Nations, Nationalities and People‟s Region (SNNPR) are the major bean producing regions in Ethiopia.

They produce 70 and 60 thousand tones, respectively. These two regions make up about 85% of common bean production in the country. The addition of the Afar region brings the total production to 97% (Nedumaran *et al,* 2015).

According to CSA (2014/15), in Ethiopia, Common bean national annual yield is ranging from1.58 to 1.60 tonnes/ha).The level of production and sales of beans are highly dependent upon rainfall and drought. Common Bean production during drought season is approximately 200 to 300 kgs. This level increases to 600 to 800 kgs/timad in the long rain season, Levels of sales also increase from 55% to 80% from the low to high rainy season.

2.1. 1. Importance of Common Bean

Common bean is high in starch, protein and dietary fiber and an outstanding source of minerals and vitamins including Iron, Potassium, Selenium, Molybdenum, Thiamine, vitamin B6, and Folic acid. It can be eaten as mashed or made into soup (Ferris and Asganzi, 2008). The young leaves of the plant are eaten as a salad. Common bean are canned in many parts of the world, either alone or in tomato sauce. It plays an important role in the preparation of traditional medicine and used to relieve a variety of ailments from acne, diarrhea, eczema, chronic rheumatism, kidney and bladder problems, uric acid accumulations and hiccups.Bean pods are effective in lowing blood sugar levels and can be used for mild cases of diabetes (Nedumaran *e tal,* 2015).

It is important in fixation of Nitrogen in soil which is vital to the environment. The leave of common bean is used to trap bedbugs in houses. Adaptable for intercropping that is cultural control methods. It is an important crop in the provision of food security and commercial product in Ethiopia.

In addition to the domestic markets, Ethiopia is supplying white beans into the export canning industry in European Union (EU) and other Eastern European markets. Ethiopia has also been a major supplier of red beans into northern Kenya. White beans are almost solely grown to supply a longstanding export market from Ethiopia. This market is a valued source of foreign exchange with an annual value range 25 to 30 million USA dollar (Ferris and Kaganzi, 2008).

2.2. Storage insect pest of common Bean

2.2.1. Bruchids (Coleoptera: Bruchidae)

Bruchids are the major problem to beans both in the field and storage. Bruchids are the most economically important insect pest of beans specifically in store. The major pests of stored beans are Mexican bean weevil Zabrotes *subfasciatus* and *Acanthoscelides obtectus.* According to Ferede Negasi and Tsedeke Abbate (1995), these two species are the major pests of stored legumes in Ethiopia.

These insect pests damage stored legumes throughout the world, particularly in the tropical area (Abate and Ampofo, 1996; Schmale *et al*., 2002; Nchimbi-Msolla and Misangu, 2002). Mexican bean weevil is recent introduction to Ethiopia (Emana Getu *et al*., 2003). Bruchids of economic importance in Ethiopia include the bean bruchids, the Mexican bean weevil or spotted bean weevil and the cowpea bruchids (Emana Getu *et al*., 2003).

2.2.2. Descriptions and Identification of Bruchids

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 originated 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; Rodriguez *et al*., 2002). The adult *Z. subfasciatus* is about 1.8 to 3.5 mm in body length, which makes it the smallest of the bruchids commonly infesting stored legume seeds (Dendy and Credland, 1991).

The adults have 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 and Graham, 2006). On the other hand, the more closely related bruchid *A. obtectus* possess no teeth on the hind femur. Moreover, it has long, filiform antennae that are black with basal segments reddish yellow (CPC, 2004).

2.2.3. Biology and Behavior

The most important host for Bruchids, bean beetle is common bean and the biology of *Z. subfasciatus is* similar to other Bruchids. Adults are small about 3.5 mm. long, brownish in colour (Awasthi, 2007). They are robust beetles that appear square at the posterior and narrow at the front and elytra do not cover the tip of the abdomen (Robinson, 2005). The shape of the antennae in males, the fourth through apical segments are pectinate to strongly pectinate whereas in females these segments are serrate. The other common character to distinguishing *is* the end femur hence, in *C. chinensis* it is ventrally bicarinate with a denticle situated on each carina near the apex. The outer tooth is blunt and the inner tooth is long and straight, and rounded at the tip (Talekar, 1988). Anton (2000) further identified it has the median lobe and lateral lobes usually strongly elongate, median lobe with ventral valve more or less spear-shaped, internal sac with a single pair of denticulate plates usually sub-basal.

The females lay their eggs in field or storage on the seed pods of legumes and the first-stage larvae bore the seed with holes then the adults often emerge after harvest in the storage where subsequent generations may develop. Adults mate within an hour after coming out from seed and mating lasts 5 to 8 minutes. Although the insects mate several times, to ensure egg laying only one mating is sufficient (Talekar, 1988).

At the time of egg laying, the female deposit a chemical „oviposition marker‟ on the seed surface which has an ovicidal and arrestant action (Oshima *et al*., 1973; Yamamoto and Honda, 1977). This chemical consists a mixture of fatty acids, triglycerides and hydrocarbons. It‟s serve to prevent the hatching of more than one or two eggs per seed and assist to regulate the pest population and maximize use of the food. Yamamoto (1976) suggested that this chemical can be used as a possible oviposition inhibitor to control the bruchids.

The eggs are elongate, oval in shape and translucent white in color, flattened on the side of attachment to the seed. Oviposition period ranges between 3 to 6 days and ovipost an average of 78 eggs and the duration for the 1st to 4th instar larva and pupal stage is 7-12,12-16, 16-19, 19-22 and 22-27 day of oviposition, respectively (Ahmed Ibrahim *et al*., 2003). The optimal condition for development is temperature of 30 ^{0c} and relative humidity (RH) of 70% (Raina, 1970). Talekar (1988) state that there is no difference in developmental time and life span between male and female under similar environmental conditions and the sex ratio is 6 to5 males to females.

2.3. Damage symptom and levels of loss

Losses of common bean is proportion with length of storage, the longer the beans are stored, the greater the loss. There are two ways of losses: quantitative or the number of seeds or parts of seeds eaten by insects; and qualitative, or the grains contaminated by excrement or insect bodies. These losses may be increased by subsequent attacks from fungi or bacteria because larval stage completion elevates temperature and relative humidity, inviting secondary rotting by micro-organism attack. Damage shows in the circular holes or "windows" left in the bean when bruchids emerge. Unclean storage conditions are the main cause of bruchid infestation. Storing beans with other grains, or newly harvested beans with the infested residue from other harvests, encourages bruchids to flourish (Van Schoonhoven and Cardona, 1986). In Ethiopia, *Z. subfasciatus* and *A. obtectus* are the major insect pests of stored beans causing average grain losses of 60% within 3 to 6 months of storage period (Emana Getu *et al*., 2003).

The word loss is often expressed in different ways and it refers to the amount of damage or total amount of crop lost. In the same way, 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 (Salunkhe *et al*., 1985). All grain produced in the tropical countries is stored at farm level has been estimated to 60% to 80 % (Boxall, 1998).

Crop products must be stored in the way that the quality does not depreciate 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. It was frequently, reported that worldwide a minimum of 10 % of cereals and legumes are lost after harvest (Boxall *et al*., 2002).

Likewise, in Ethiopia loss of stored produce reached up to 20-30 % (Abraham Tadesse, 1996, Emana Getu, 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) indicated that 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 ranged from 5.5% to 8.5% (Espinal, 1993). FAO (2014) estimated losses on pulses in the range of 25 to 50 % due to infestations by 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 up to reach 3.2 %. *Z. subfasciatus* and *A. obtectus* are very closely related to bean bruchids. 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 Wwubet and Dawit kidane, 2005).

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. Weight loss of pulses ranges from 4 to 29 % in Eritrea (Adugna Haile, 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 Andargie, 2004; Emana Getu, 2003). In Ethiopia, the structural condition of the traditional granaries used for storage of beans varies from one farm to another. It includes “Koffo”, “Debignet”, and “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 which are not repaired permit easy access to rodents, insects and flooding (Teshale Asefa, 2010).

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 example, in Rift valley zones of bean producing areas percent weight loss of seeds varied with the type of seed storage structures. (Williamson, 2003)

According to Mebeasilassie Andargie (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, (1985) 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). 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) demonstrated that a reduction in 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, up to 25% of the dry matter may be crude protein (FAO, 2014).

Further, contamination from uric acid by bruchids is correlated with negative changes in nutritive composition of pulses causing an increased fatty acid 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.

2.4. Management Options of Bruchids

To reduce the impact of insect pest, there should be an integrated pest management approach: including appropriate cultural practice, host plant resistance, Botanical control, and biological and use chemicals with appropriate rate and frequency applications. Integrating of different management options helps in reducing the risk of pesticide resistance development, reduce the impact of the insecticide to the environment, non target organism, beneficial insects such as natural enemies and human hazards (Abiy Tilahun, 2012).

2.4.1. Host plant resistance (Varietal resistance)

Plant resistance is a principal method for insect pest control method which is effective, practical, and low cost to farmers. Cultivars with genetic resistance to the Mexican bean weevil have been identified. High levels of resistance to these weevils have recently become available in commercial bean types. Resistance is simply inherited dominant gene that can be rapidly backcrossed into local varieties (Tigist Shiferw, 2004)

Wortmann and Allen, (1994); and Fivawo and Msolla, (2011) stated that to have the highest level of variation in seed characteristics. The common bean variety is classified based on the size, shape and color. About 40,000 varieties of common bean available and all give identical total calories per gram. Plant resistance to insects consists of inherited genetic trait that result in plants being less damaged than another (susceptible one) that is subjected to the same conditions. The production of plant resistant to particular insect pests is accomplished by selective breeding for resistance traits (Kiruba *et al*., 2006).

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. 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 germplasm 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. 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 anti metabolic to larvae of *A. obtectus* and *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 indigenous 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,24"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. 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* (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).

Plant Resistant varieties can be used as the primary method of insect control, or as a component of an integrated pest management program. Common beans varieties resistant to pre and postharvest damage by beetles and varieties showing multiple resistances to insect attack are being selected. In general, host-plant resistance is a method of combating pest depredation in storage.

2.4.2. Cultural control methods

Cultural methods are the most important control method that serves in the prevention, inhibition, or eradication of insect pests in stored bean. The principle involved in the cultural control of insect pests are 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 (Belmain and Stevenson, 2001)

The most susceptible stage or stages of the insect pests 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 most important 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 Getu *et al*. (2003) also recommended 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 without cleaning. Sanitation practices can bring satisfactory output if it is coupled with appropriate and adequate drying technologies (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 to control insect pest infestation for many decades. 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 seed, 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: no 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, 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 Getu and Assefa Gebre Amlak (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 bruchids 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. subfasciatus*, 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 (Songa and Rono, 1998; Kiruba *et al*., 2006) 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).

2.4.3. Botanical Control

At present, to control insect pest infestation in common bean 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 (Srinivasan, 2008).

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 (Rajapakse and 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).

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 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 *e tal*., 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, Emana Getu *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.

In Ethiopia, Mekuria Tadesse (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. Moreover, Mebeasilassie Andargie (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 kidane (2005) also demonstrated that orange peel oil applied at the rate of 0.3% w/w caused 100 % mortality of *Z. subfasciatus*. Emana Getu (1999) and Asmare Dejene (2002) evaluated the efficacy of local plant materials including datura, chenopodium, endod, neem, croton, and castoragainst maize weevil.

They reported that the treatments resulted in high percentage of adult mortality, reduced progeny emergence and low percent grain damage. 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. 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 F1 and F2 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 Ibrahim, 2002 and Shaheen (2006)).

2.4.4. Chemicals control Methods

Even though 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 bruchids infestation in stored grain legumes include organophosphates, carbamates and synthetic pyrethroid.

Bruchids Control infestation is done by treating stored seeds with methyl bromide, carbon disulfide, Malathion and several other chemicals. Malathion kills 85 percentages to 99 percentages of *Z. subfasciatus* adults in the 24 hours after applications. These groups of insecticides have been used for over six 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 all over 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 (Salunkhe *et al.*, 1985, Gwinner *et al*., 1990).

For instance Emana Getu *et al*. (2003) reviewed that pirimiphos-methyl at the rate of 4-5 ppm provided effective control of bruchids in Ethiopia and are widely used at present. 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 all over the world in the control of bruchids, 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 (Beebe *et al*., 2013)

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 (Faruki *et al*., 2004). El-Nahal *et al*. (1984) cited by Mebeasilassie Andargie (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 (Mwanauta *et al.*, 2015).

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).

3. MATERIALS AND METHODS

3.1. Description of the experimental site

The experiments were conducted at Melkassa agricultural research center (MARC), located 128km west of Addis Ababa, between 90‟02 N latitude h 38‟34‟E longitude. The altitudinal range is 2060 to 3380 m above sea level. Average annual temperature is $22c^0$ and annual rainfall is 900-1100mm. The experiments were conducted in 2016/2017.

3.2. Description of the botanicals plant used in the Experiments

Parthenium hysterophorus (L.) Commonly known as Congress weeds, Faramisesa in Amharic and Carrot weed. Parthenium is an herbaceous annual or short-lived member of the Asteraceae. It has a length of 2 m above ground at favorable condition and flowering within 4 to 6 weeks of germination. The plant produces allelopathic chemicals that suppress crop and pasture plants, and allergens that affect humans and livestock. It also frequently causes pollen allergies. 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 (Gebeyehu Gebre Amlak and Adane Kassa, (2008). Although, *Parthenium* is considered as toxic plant, its 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) studied the repellent and toxicant property of *P. hysterophorus* extracts against *C. chinensis.*

Jatropha curcas (L.) commonly called as physic nut and "Ayderke" in Amharic The physic nut is a drought. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. It reaches a height of up to 8m and is mainly cultivated for the production of seeds with oil content of 55% to 60 % seed yields (Adebowale, and Adedire, 2006).The seed 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. 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 Dejene, 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).

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). *A. indica* is a multi-purpose tree whose products have been used traditionally for centuries for insecticidal, antiseptic, contraceptive, antipyretic and antiparasitic purposes (Martinez and Emden, 1999).

In addition, it is used for reforestation and as a source of wood and provider of shade. The fruit produces oil which is used in soaps and detergents while other by-products are used for fertiliser and soil amendments. HabteTekie (1999) state 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.

Table 1. Dosages of botanicals used in the experiment

No	Treatment	Local name	Common Name	Name part used	Dosage used in g/kg
1	*Jatropha seed powder*	Ayderke	Physic	seed powder	0.20
2	*Parthenium* Seed powders	Faramisesa	Congress weed	Seed powder	0.20
3	*Azadirachta indica*	Kinini	Neem	leave powder	0.20
4	Malathion 5 % dust	-	-	-	0.20

3.3. Rearing of Bruchids, *Zabrotes subfasciatus* for the experiment

The adult bruchids, *Zabrotes subfasciatus* were brought from Melkassa Agricultural Research Center where bean seeds were stored. Rearing took place in the Entomology laboratory of Melkassa Agricultural Research Center. The *Z. subfasciatus* were reared on different common bean varieties, which are commonly grown by farmers in major bean producing areas of Ethiopia.

Before rearing, the seeds were disinfested by keeping them in refrigerator at $0c^o$ for seven days. Rearing was conducted under ambient laboratory condition where the average temperature between $27c^o$ and $30c^o$and the relative humidity between 35% and 60%. Seeds moisture content was determined. Moisture content was determined using moisture meter and ranged between 9.25% and 11.24%.

Three kilograms of the disinfested seeds were placed in bag as a container. The bag was agitated every 2 to 3 days to facilitate aeration and prevent the development of pathogenic organisms. Then, in none choice methods 100 pairs of *Z. subfasciatus* were infested the local common bean and allowed to oviposite under normal laboratory condition. After seven day, the eggs were laid, adult *Z. subfasciatus* were removed and discarded from the bag. Adult emergence was monitored daily and those emerged on the same day were transferred to fresh seed in a plastic jar containers and kept until a sufficient number of bean bruchids were obtained for the experiment. The culture medium was sieved to remove the beetles in order to obtain adults of the same age, and the insects that had emerged the following day were collected for experiments. Newly emerged adults of 1 day old were used for each experiment.

3.4. Host plant resistance experiment

The experiments were conducted twice with a total of twenty varieties (Table 1), that is, 17 varieties of common bean were collected from Rift valley of Adama Town local market and three varieties were from Melkassa Agriculture Research Center for comparison. Seeds name were derived from local market. The seeds had been cleaned and disinfested before the experiment. Hundred seeds of common bean from each variety were placed in 60 plastic jars. The experiments were replicated three times. Seeds were artificially infested in non choice methods with ten pairs of unsexed adult of *Z. subfasciatus* from the laboratory.

After ten days, the eggs laid on the seed were counted and kept up to the adult emergence. This continues for two months. Then, data number of eggs laid, number of adult emerged and percent adult emergence and damaged seeds were collected. In the second experiment, retesting of the same varieties (a total of 20 varieties) was done in order to confirm the result. Data on development period from egg laying to adult emergence was additionally recorded in the second round experiment.

The experimental procedures employed were similar with the first round experiment. Periodic monitoring of the seed was done for Z. *subfasciatus*. When the exit holes began to be externally visible on each variety, the adults that emerged were counted and removed at every checking time. Removal of newly emerged adults continued for about two months. Percentage of hatched eggs, mean developmental period days of Z. *subfasciatus* from oviposition to adult emergence, and percent damaged seed were recorded.

Table 2. List of common bean varieties tested in the experiments

No.	Name of local common bean varieties	Commercial name Common Bean	Seed colour
1	Aregonde	KAT-B1	Green yellow
2	Small white	Mexican142	White
3	Pinto	Pinto	Pinto
4	Large yellow	-	Yellow
5	Yellow round	-	Yellow
6	Large red	DRK	Red
7	Cerem medium	Bolonda	Cerem
8	Cerem	Gofta	Medium cream
9	Walkite medium red	Melka dimaa	Dark red
10	Walkite small red	Nasir	Dark red
11	Red renger	-	Red speckled
12	Black	Black	Black bean kidney
13	Batu	Batu	Large White
14	Gojam red large	Mixed Dinkinesh	Red
15	Gojam red small	Agere	Red
16	White renger	Cranscpoe	Red speckled
17	Society	Society	White speckled
18	RAZ white	RAZ-11	White
19	Gojam red medium	Nasir	Red

20	Awash white	Awash-1	White

3.5. Preparation of botanical powders

Different parts of three species of botanicals were evaluated for their efficacy against *Z. subfasciatus.* The botanical treatments used for this study were neem leaves powder, Jatropha seed powder, and Parthenium seed powder. The botanicals were collected from Melkasa Agricultural Research Center and air-dried for about 21 to 35 days under shade. Then, they have been crushed using pestle and mortar to get fine powder. Malathion 5% dust at the rate of 0.2g/ kg of seeds was used.

Susceptible common bean varieties were selected from first and second experiment of this studies were used. The susceptible seeds were treated with botanical powders in the jars. The procedure used for local Common bean disinfestations, equilibrating and insect rearing method were similar with the method used above. After disinfestations, hundred seed of the common bean varieties were placed in plastic jars and covered. Perforations were made on the jar cover for ventilation and prevent pathogenic organism.

The botanical powders plant parts were introduced to each plastics jar. Malathion 5% dust was applied as standard check. The doses were selected based on reports in literature (Teshome Lemma, 1991). The doses of each product were mixed and shaken for five minutes, so that the entire surface of each seed could get uniform coating of the protectants. The treatments were replicated three times in completely randomized design (CRD). About one days old ten unsexed adults of Z. *subfasciatus* adults were introduced in each plastic jar.

After one week, seeds of infested common beans varieties were sieved in order to remove dead and alive adult *Z. subfasciatus.* Then, counted and later discarded and seeds were kept for further assessment. After ten days, total numbers of eggs laid on the seeds of each treatment were counted. The seeds were kept for F1 progenies emergence. Daily monitoring of the seed were done. When the exit holes began to be externally visible on each seed, the adults that emerged were counted and removed at every checking time. This continued for two months. Total eggs and mean developmental period of Z. *subfasciatus* were counted calculated on each variety. Damaged and undamaged seed were counted and separately weighed and the result data was used to calculate the estimated percent weight loss. Percent weight loss was calculated following the equation of Adams and Schlten (1978) as follows:

$$\% \text{ weight loss} = \frac{U*ND - D*NU \times 100}{U\,(ND+NU)}$$

Where, U= Weight of undamaged seed

NU= Number of undamaged seed

D= Weight of damaged seed, and

ND= Number of damaged seed.

3.6. Germination Test

Germination test were carried out at the end of the experimental period when collection data of other was completed. Ten seeds were taken randomly from each replication of all the treatments and malathion 5% dust treated seeds. The sampled seeds were placed on moist filter paper in petridishes in a CRD in three replications. Healthy untreated seeds were used as a control. The number of emergence of seedlings from each Petri dish were counted and recorded after seven days. Percent germinations were computed according to Ogendo *et al*. (2004) as follows:

$$\text{Germination (\%)} = (NG*100)/TG$$

Where NG = number of seeds germinated

TG = total number of seeds tested in each Petri dish.

3.7. Data Analysis

Data entry and analysis were done using Microsoft Excel and JMP 10 SAS. Data were transformed using square root transformation when necessary. To observe the effects of the treatment on number of egg laid, % number adult emergence, % weight loss and Percentage of germination ANOVA was run. In cases where the significant result were obtained, means separation were conducted using Tukeys studentzed (HSD) test at 5% level of significance.

4. RESULTS

4.1. Host plant resistance

Mean (± SE) number of eggs, % adult emergence and damaged seed of bean varieties by *Z.subfasciatus* of the first experiment are given in Table 3.

All varieties did not show significant variations (P>0.05) with regard to mean number of adult Z. *subfasciatus* eggs laid. However, no adult emergence and damaged seeds were recorded on RAZ white and yellow round varieties. Likewise, the highest adult emergence and damaged seeds were emerged from Black bean and Batu varieties (Table 3 and 4).

From all varieties, RAZ white and yellow round (Plate 1a) are gave major resistance performance against *Z. subfasciatus* while Batu and Black bean (Plate 2) were the most susceptible against *Z. subfasciatus.*

Table 3. Mean number of eggs, % adult emergence and damaged seeds due to Z. *subfasciatus* on bean varieties

No	Local variety name	Mean Number Of egg laid	Mean Number of Adult	% Mean adult emerged	% Mean Damaged seed
1	Arengode	175.3± 27a	87± 5.8a	53.5±13.1ab	55 ± 5.96ab
2	Small white	110±00a	93±3.5ab	84.55± 3.19ab	34.7±1.38ab
3	Pinto	152.7±39.8 a	23.33±1.67a	17.25± 4.333cd	34.7± 1.3ab
4	Large yellow	127 ±00a	40.7±28.2abc	32±22.2ab	45.3±10.9ab
5	Yellow round	148± 9.7a	00±00d	00±0.00d	0±0.00c
6	Large red	156±48.5a	60±00a	50.4±19.8ab	60± 16ab
7	Cerem medium	103± 65.5a	25±7.5bc	59.3± 27.4ab	86.67±16.ab
8	Cerem	128±8.29a	34±20.5bc	27.3± 16.4bc	56±19.7ab
9	Walkite medium red	197± 42.4a	69±43.6ab	38±24.9ab	78. ±10.5ab
10	Walkite small red	152±39.4a	12.67±4.91b	11.78±6.7ab	42.7±16.2ab
11	Red renger	150± 53.5a	45.3±35.8c	34.25± 5.96abc	36±11.5ab
12	Black	203.3±34.3a	196± 35.8a	95.85± 1.38a	93.6±0.06ab
13	Batu	189.3± 25.3a	182± 26.5a	95.88± 1.38a	94.3±1.15ab
14	Gojam red large	103±10.0a	81±4.58ac	79.23±1.05ab	46.7± 15.ab
15	Gojam red small	152.3± 67.3a	24± 2.ab	34.9± 5.96abc	22±4.6bc
16	White renger	159.3±50.0a	62±6.24dc	47.9±14.6abc	38± 7.02ab
17	Society	166±45.6a	20±12.5dc	10.32± 4.7ab	40±15.5ab
18	RAZ white	88±34.0a	0±0d	0±.00	00±c
19	Gojam red medium	116.7± 31.8a	75±5.6a	72.9±14.1ab	82.6±5.29ab
20	Awash -1	130.33±7.3a	104±5.6ab	79.5±8.8ab	76.67±4.81ab

Means followed by the same letters (s) with in column are not significantly different at 5%, HSD

M ± SE of eggs, % adult emergence, developmental period of days of adult emergence and damaged seed of bean varieties by *Z.subfasciatus* of the second round experiment are given in Table 4.

Local common beans were resistant for all the parameters measured in the second round experiment as well (Table 4).

Developmental period of days *Z. subfasciatus* (from eggs-adults) was significant ($p<0.05$) longer in resistant varieties and shorter in susceptible bean varieties.

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Table 4. Mean (± SE) number of eggs, % adult emerged, damaged seed, & *Z. subfasciatus* development period days in *Z. subfasciatus* infested common bean varieties.

No	Local variety name	Number of egg laid	% adult emerged	% damaged seed	Development period/days
1	Gojam red medium	168	88.7±9.4a	88.7±9.4a	43.2±1.0 cb
2	Gojam red large	175	55.4±7.3abc	56.7±7.5ab	41.2±1.0b
3	Gojam red small	203	13.7±3.6bcde	22.0±4.6ab	34.2±0.4b
4	Small white	110	88.4±9.4a	52.0±7.1ab	41.2±1.0 ed
5	Aregonde	189	53.5±7.2a	71.3±8.4ab	44.2±0.7 cb
6	Awash-1	178	76.7±8.6ab	76.0±8.7ab	44.2±0.7 cb
7	Black	175	94.5±9.7a	90.0±9.5ab	35±0.6b
8	Cream	123	28.9±4.9abcde	76.7±8.7ab	44.2±0.7 cb
9	Cream medium	213	26.5±5abcde	83.3±9.1ab	45.5±0.9 b
10	Batu	263	40.2±6.2abcd	90.7±9.5a	33±0.6b
11	Large red	182	33.3±5.8abcde	60.0±7.6ab	40.2±0.6 e
12	Large yellow	127	38.0±5.3abcde	45.3±6.6ab	39.2±0.7 cd
13	Pinto	240	9.7±3.1cde	34.7±5.6ab	38.2±0.7 cd
14	RAZ-white	207	0.80±.5e	0.0±0.0ab	48.7±0.9 a
15	Red renger	207	21.3±4.6abcde	36.0±5.8ab	35.2±0.7 b
16	Society	206	2.8±1.6de	35.3±5.3ab	35±0.6b
17	Walkite medium red	185	26.6±4.4abcd	58.0±7.3ab	40.2±0.4 e
18	Walkite small red	234	6.8±2.6abcde	72.0±8.5ab	44.5±0.9 b
19	white renger	194	38.1±6.1abcd	38.0±6.1ab	34.5±1.5b
20	Yellow round	171	0.7±.5e	0.0±0.0ab	47.5±0.3 a

Means followed by the same letters (s) within column are not significantly different at 5%, HSD

4.2. Botanical control of *Z. subfasciatus*

4.2.1. Effect of Botanicals on Egg Laying* and *Adults emergence: Table 5 summarizes the mean number of eggs laid by female *Z. subfasciatus* after being exposed to the different botanicals. Females of *Z. subfasciatus* laid significantly (P < 0.05) low number of eggs on seeds treated with malathion, Jatropha seed, Parthenium seed powder and neem leaf powder as compared to untreated check.

The lowest numbers of eggs were laid on common bean treated with malathion and Jatropha seed powder.

Malathion, Jatropha and Parthenium seed powder were significantly (P < 0.05) yielded low adult emergence as compared to the other treatments (Table 5). Jatropha seed powder was as effective as malathion in terms of adult emergence.

Significantly (P < 0.05) the highest percentage of adult *Z. subfasciatus* was emerged from the untreated check (88% and 89 %) as compared to the lowest percentage of adult *Z. subfasciatus* emergence from Jatropha seed powder (3.49% and 9.35 %) and Parthenium seed powder (12.1% and 19.17%) on Black and Batu variety respectively, (Table 5).

Table 5. Effect of botanicals on Mean number of eggs and % adult emergence of *Z. subfasciatus* on variety (Batu and Black)

Variety	Treatment	No of egg	% mean adult emerge
Batu	Untreated	221 ± 16.1a	89.42±2.7 a
	Neem leaf	113± 10.4 ab	24.35±6.3b
	Jatropha seed	44± 20.5ab	9.35±4.45bcd
	Parthenium seed	64± 9.7ab	19.17±2.02bc
	Malathion 5%dust	14±5.2cd	0.00±00d
Black	Untreated	219±26.4a	88.53± 2.57a
	Neem leaf	133±8.41a	24.62± 6.17b
	Jatropha seed	100± 22.5ab	3.49± 1.11cd
	Parthenium seed	204±41.2a	12.10± 3.32bcd
	Malathion 5 %dust	4± 1.15d	0.00± 00d

Means followed by the same letters (s) within column are not different significantly at 5%, HSD,

***4.2.2. Effect of botanicals on seed damage and weight loss*:** Effect of botanicals on Mean (± SE) % damaged seed and weight loss by *Z. subfasciatus* is presented in Table 6.

Mean percentage seed damage of common beans (Black and Batu) in the untreated check ranged between 80% and 82%. Malathion and Jatropha treated common beans (Black and Batu) significantly had the lowest percent seed damage .Mean percentage weight loss of common bean (Black and Batu)on the untreated check ranged between 29% and 19.9%.No losses were recorded on seeds treated with malathion. Jatropha seed treated common bean ss significantly ($p<0.05$) showed the lowest percent weight loss.

Table 6. Effect of botanicals on Mean (± SE) % damaged seed and weight loss by *Zabrotes subfasciatus*.

Variety	Treatment	% mean seed damage	% mean weight loss
Batu	Control	82±1.15a	29.1±3.09a
	Neem leave	18±5.36bcd	8±0.53b
	Jatropha seed	3±1.2de	1.1± 0.6b
	Parthenium	14±3.61cde	1.9± 0.32b
	Malathion	0± 00e	0±00b
Black	Control	80±1.15a	19.9± 3.44 a
	Neem leave	21± 7.36bc	1.16± 0.62b
	Jatropha seed	1.6± .33de	0.6± 0.32b
	Parthenium	32±5.04b	6.1±0.53b
	Malathion	0.00±00e	0±00b

Means followed by the same letters (s) with in a column are not significantly different at 5%, HSD

4.3. Germination Test

4.3.1. *Effect of Botanicals on the germination of bean Seeds*:

Mean percent seed germination of bean seeds treated with different botanical is shown in Table 7.

Percent germination of local common bean varieties treated with different type of powder within two susceptible varieties presented in Tables 7. Significantly ($P < 0.05$) difference on germination capacity of Batu and Black bean varieties treated with different powders and untreated control (Table 7) were observed. However, No significant ($P > 0.05$) difference within bean varieties.

Table 7. Mean percent common bean germination treated with different botanicals Powders

NO.	Varieties	Botanicals	% Mean of G
1	Batu	Control	70±5.7bc
2		Neem leave	86.6±3.3ab
3		Jatropha seed	90±5.7ab
4		Parthenium	86.6±2.5ab
5		Malathion	90±0ab
6	Black bean	Control	60 ±5.7c
7		Neem leave	90±3.3ab
8		Jatropha seed	90±5.7ab
9		Parthenium	90±3.33ab
10		Malathion	93±0a

Means followed by the same letters (s) with in a column are not significantly different at 5%, HSD

5. Discussion

Common bean varieties should remarkable variability towards reaction to *Z. subfasciatus.* Varieties like RAZ white and yellow round found to be highly resistant to the *Z. subfasciatus*, while black bean and Batu bean varieties found to be very susceptible. The existence of resistant gene(s) opens up opportunities for the breeders to transfer this gene to varieties with good agronomic performance and/or Entomologists or stakeholders can use the resistant varieties directly for the protection of *Z. Subfasciatus*.

The mechanisms of resistance to storage insect pests like Z. *Subfasciatus* could be categorized into antibiosis and anxizenosis. Lara (1997), Goossens *et al*. (2000),Mazzoneto and Vendramim (2002) reported that resistance to *Z. Subfasciatus* is related to the concentration of a protein known as arcelin such that the higher the protein the more antibiosis effect on the pests. This implies that resistant varieties possess high concentration of arcelin, unlike the susceptible varieties which has low concentration of arcelin.

Goossens *et al.* (2000) demonstrated that in addition to arcelin concentration morphological characters such as seed size, seed coat, texture and color contributes to resistance of bean varieties to *Z. Subfasciatus.*

Abate and Ampofo (1996), Shaaya and Kostyukovysky (2006), Schoonhoven *et al*. (1983) and Abiy Tilahun (2012) demonstrated that resistant bean varieties lead to reduced oviposition, prolonged larval development and low number of adult emergence which is in line with the current findings.

Application of malathion 5% dust were effective to control Z. *subfasciatus*. However, the use of malathion is costy, environmentally unfriendly and toxic to the applicator and the consumers in addition to other side effects such as resistance development.

A number of scientists recommend the use of botanicals to avoid or minimize the crisis due to chemicals (Abiy Tilahun, 2012) Hall, 1990, Ivbijaro, 1990, Ofuya, 1990., Araya G.Selassie and Emana Getu, 2009).

In the current experiment botanicals such as Jatropha seed powder, Prathneium seed power and neem leaf powder gave comparable results with malathion 5% dust which gave 100% control of Z. Subfasciatus. This result is in agreement with Williamson (2003), Tigist (2004) and Mulungu *et al*. (2007).

The botanicals also have no negative effect on seed germination to the common bean which is agreement with Wortmann and Allen (1994) and Singh, 2006 and Jackai (1985).

6. Conclusion

- Remarkable variabilities were recorded among bean varieties in terms of *Z. subfasciatus* reaction.
- RAZ white and yellow round bean varieties were found to be resistant to Z. *Subfasciatus.*
- Jatropha seed powder, Prathneium seed powder and neem leaf powder gave comparable control of the *Z. subfasciatus* with malathion 5% dust.
- Effective botanicals (Jatropha seed, Parthenium seed, and Neem leaf) against the Z. *subfasciatus* had no negative effect on bean seed germination.

Recommendation

- Breeders should be communicated to explore the resistant gene (s) found in bean varieties against *Z. subfasciatus*
- Entomologists should make use of the resistant varieties for the control of the *Z. subfasciatus.*
- Resistance mechanisms of the resistant varieties need to be studied.
- Jatropha seed powder, Prathneium seed powder and neem leaf should be used by bean farmers for the control of the *Z. subfasciatus.* may be together with resistant varieties.
- Chemicals responsible for the control of the *Z. subfasciatus* in the botanicals should be identified

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plate 3. Local common bean variety used in the experiment

Table 8 . summary Results of data analysis for table 3, 5,6 and 7

Descriptive Statistics: No. of egg l, No.Adult Eme, %emergence o, % damage

Variable	Treat.	Mean	SE Mean
No. of egg laid	1	175.3	27.0
	2	110.00	0.000000000
	3	152.7	39.8
	4	127.00	0.000000000
	5	148.67	9.70
	6	156.0	48.5
	7	103.0	65.5
	8	128.67	8.29
	9	197.0	42.4
	10	152.3	39.4
	11	150.0	53.5
	12	203.3	34.9
	13	189.3	25.3
	14	103.0	10.0
	15	152.3	67.3
	16	159.3	50.0
	17	166.0	45.6
	18	88.3	34.0
	19	116.7	31.8
	20	130.33	7.33
No.Adult Emergen	1	87.00	5.86
	2	93.00	3.51
	3	23.33	1.67
	4	40.7	28.2
	5	0.000000000	0.000000000
	6	60.000	0.000000000
	7	25.00	7.64
	8	34.0	20.5
	9	69.0	43.6
	10	12.67	4.91
	11	45.3	10.2
	12	196.0	35.8
	13	182.0	26.5
	14	81.00	4.58
	15	24.00	2.00
	16	62.00	6.24
	17	20.3	12.5
	18	1.33	1.33
	19	75.000	0.000000000
	20	104.0	10.0
%emergence of ad	1	53.5	13.1
	2	84.55	3.19
	3	17.25	4.33
	4	32.0	22.2
	5	0.000000000	0.000000000
	6	50.4	19.8
	7	59.2	27.4
	8	27.3	16.9
	9	38.0	24.9
	10	11.78	6.71
	11	34.25	5.96
	12	95.93	1.38
	13	95.85	1.05
	14	79.23	3.17
	15	34.9	23.8
	16	47.9	14.6
	17	10.32	4.59
	18	3.92	3.92
	19	72.9	15.9
	20	79.50	3.71

% damage	1	55.3	14.7
	2	34.7	13.1
	3	34.7	16.8
	4	45.3	10.9
	5	0.000000000	0.000000000
	6	60.0	16.2
	7	86.67	1.76
	8	56.0	19.7
	9	78.7	10.5
	10	42.7	16.2
	11	36.0	11.5
	12	93.333	0.667
	13	94.00	1.15
	14	46.7	15.6
	15	22.00	4.62
	16	38.00	7.02
	17	40.0	15.5
	18	1.33	1.33
	19	82.00	5.29
	20	76.67	4.81

Results for: Worksheet 2

Descriptive Statistics: No.of egg, % adult emergence, %wl, % damage

Variable	Botanicals	Mean	SE Mean
No.of egg	Ctrl	219.3	26.4
	Jatro	100.0	22.5
	Malathion	4.00	1.15
	Neem	133.33	8.41
	Part	204.3	41.2
% adult emergenc	Ctrl	88.53	2.57
	Jatro	3.49	1.11
	Malathion	0.000000000	0.000000000
	Neem	24.62	6.17
	Part	12.10	3.32
%wl	Ctrl	19.94	3.44
	Jatro	0.605	0.326
	Malathion	0.000000000	0.000000000
	Neem	1.562	0.621
	Part	6.062	0.531
% damage	Ctrl	80.00	1.15
	Jatro	1.667	0.333
	Malathion	0.000000000	0.000000000
	Neem	21.67	7.36
	Part	32.67	5.04

Descriptive Statistics: No.of egg, Adult emerge, % adult emergence, % damage for batu

Variable	Botanicals	Mean	SE Mean
No.of egg	Ctrl	221.0	16.1
	Jatro	44.0	20.5
	Malathion	14.33	5.21
	Neem	113.3	10.4
	Part	64.33	9.70

Adult emerge	Ctrl	198.3	19.2
	Jatro	3.33	1.20
	Malathion	0.000000000	0.000000000
	Neem	26.33	4.48
	Part	12.00	1.15
% adult emergenc	Ctrl	89.42	2.70
	Jatro	9.35	4.45
	Malathion	0.000000000	0.000000000
	Neem	24.35	6.32
	Part	19.17	2.02
% damage	Ctrl	82.00	1.15
	Jatro	3.33	1.20
	Malathion	0.000000000	0.000000000
	Neem	18.67	5.36
	Part	14.00	3.61

Descriptive Statistics: %G

Variable	Botanicals	N	N*	Mean	SE Mean	Minimum	Maximum
%G	Ctrl	6	0	65.00	4.28	50.00	80.00
	Jatro	6	0	90.00	3.65	80.00	100.00
	Malathion	6	0	91.67	1.67	90.00	100.00
	Neem	6	0	88.33	1.67	80.00	90.00
	Part	6	0	86.67	2.11	80.00	90.00

Descriptive Statistics: %G

Variable	Botanicals	N	N*	Mean	SE Mean	Minimum	Maximum
%G	Ctrl	3	0	70.00	5.77	60.00	80.00
	Jatro	3	0	90.00	5.77	80.00	100.00
	Malathion	3	0	90.000	0.000000000	90.000	90.000
	Neem	4	0	87.50	2.50	80.00	90.00
	Part	3	0	86.67	3.33	80.00	90.00

Results for: Worksheet 2

Descriptive Statistics: %G

Variable	Botanicals	N	N*	Mean	SE Mean	Minimum	Maximum
%G	Ctrl	3	0	60.00	5.77	50.00	70.00
	Jatro	3	0	90.00	5.77	80.00	100.00
	Malathion	3	0	93.33	3.33	90.00	100.00
	Neem	3	0	90.000	0.000000000	90.000	90.000
	Part	3	0	86.67	3.33	80.00	90.00