Status, Species Composition and Management of Stored Maize Grain Insect Pests in Hadiya and Silte zones of Southern Ethiopia

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Abstract

Status, Species Composition and Management of Stored Maize Grain Insect Pests in Southern Ethiopia

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Addis Ababa University, 2018

Maize (*Zea mays* L.) is a major staple food and feed sources for millions of people in Ethiopia in general, and Shashogo and Sankura districts of Hadiya and Silte zones, in particular. However, insect pests, both in the field and in the storage seriously threatened its production and consequently, affect food security of the poor farmers. The most important of these have been proven to be Lepidopteran stalk borer and Coleopteran maize weevil. However, no or few studies were conducted on indigenous methods and practices used by farmers in storing and protecting maize grains, as well as the species composition and the status of insect pests of stored maize and the associated losses in the study area. Thus, the survey using face to face semi-structured interviews and farmer's participatory discussions were conducted in six major maize growing localities of Hadiya and Silte zones of Southern Ethiopia for documenting various indigenous practices and methods used by farmers in protection of their grains from insect pests at different storage periods (3, 6 and 9 months) in 2013/2014 and 2015/2016, using a nested design. Moreover, level of infestation, distribution and the status of insect pests of stored maize, and the associated damage and loss in four farmer's traditional storage methods were conducted in six localities or peasant-associations for three storage periods (3, 6 and 9 months) in the same year. Besides, laboratory studies were conducted on botanicals, inert dusts, varietal resistance and their integration in the management of maize weevil during the period/ year of 2016/2017. The survey result demonstrated that farmers harvested maize in between November and January. Hand picking was the most commonly used harvesting method. Maize harvesting was commonly done in December. Farmers used different traditional storage methods (facilities) and store their maize either shelled or unshelled. However, most farmers ($\geq$ 82%) store their maize grain unshelled for 7-9 months. Most farmers (52.7%) used outdoor Gotera for storing their maize grain. Through the survey, over thirty arthropods were recorded in stored maize grains from the study area. Of these, nineteen were primary and secondary pests, seven were mold feeders and five were natural enemies. The 19 primary and secondary pests include *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Sitophilus oryzae* (Linnaeus), *Tribolium castaneum* (Herbst), *Tribolium confusum* (Jacquelin duVal), *Cryptolestes ferrugineus* (Stephens), *Cryptolestes pusillus* (Schonherr), *Rhyzopertha dominica* (Fabricius) *Oryzaephilus surinamensis* (Linnaeus), *Tribolium destructor* (Uyttenb.), *Palorus subdepressus* (Wollaston), *P. ratzeburgii* (Wissmann), *Ephestia cautella* (Walker), *Acarus siro* (Linnaeus), *Plodia interpunctella* (Hubner), *Gnatocerus cornutus* (Fabricius), *Tribolium audax* (Halstead), *C.*

pusilloides (Steel and Howe) and *Prostephanus truncatus* (Horn). Of these primary and secondary pests, *S. zeamais*, *S. cerealella*, *S. oryzae*, *T. castaneum*, *T. confusum*, *C. ferrugineus*, *C. pusillus* and *R. dominica* were found to be the most abundant, and frequently occurring and were found to be the major pests. Insects such as *O. surinamensis*, *T. destructor, P. subdepressus*, *P. ratzeburgii*, *E. cautella*, *Acarus siro*, *P. interpunctella* and *G. cornutus* were found to be the next abundant, and frequently appearing and were found to be intermediate pests. However, *T. audax*, *C. pusilloides* and *P. truncates* were found to be the least abundant, least frequently occurring and were minor pests. Besides, from seven species of mold feeders recorded, *Carpophilus dimidiatus* was the most commonly occurring and the most important, followed by *Liposcelis* sp. and *L. entomophila*. But, *Typhaea stercorea*, *C. freeman*, *C. hemipterus* and *Brachypeplus* sp. were found to be the least commonly appearing and the least important. Among the five species of natural enemies recorded, one species of predatory mite, *Cheyletus* sp., two species of hymenopteran parasitic wasps; *Anisopteromalus calandrae* and *Cephalonomia tarsalis* and coleopteran Staphylinidea were the most abundant and frequently occurring. However, *Dactylosternum abdominale* recorded rarely. Significantly ($P < 0.05$) higher level of insect pest infestation, grain damage and losses were recorded in all tested traditional storage methods, the highest being in polyethylene sac, followed by storage in the living house and store room, while the minimum was from in the Gotera out door. Hence, various traditional methods and practices used by farmers were found to be inefficient to provide sufficient protection of grain against insect pests. Thus, there is a need to look for alternative control methods such as varietal screening, botanical control and the integration of compatible control options are paramount importance which are the main focal areas of this PhD thesis. Accordingly, from varietal screening study involving twenty one maize grain varieties, 6 varieties such as Melkasa-6Q, MH-138Q, SPRH, BHQPY-545, AMH-760Q and Gibat (AMH-851) were found to be resistant. Hence, these resistance varieties could be used and adopted as ecologically sound and effective management method to reduce loss by *S. zeamais* under subsistence farmer's storages. However, their mechanisms of resistance need further study. Besides, the laboratory studies also confirmed that all of the different traditional or bio-rational methods tested were significantly ($P < 0.05$) effective in protection stored maize grain against maize weevil. Of which, integration two botanicals (*C. aurea* and *M. ferruginea*) and two colored inert dusts (red and white) with the top three resistant varieties; Melkasa-6Q, MH-138Q and SPRH were the most effective, followed by botanicals and inert dusts, respectively at the rates of 5 and 10%. However, their evaluation regarding their effect on human being, natural enemies and cost effectiveness under subsistence farmer's storage conditions is necessary before wide implementation of the outcomes these studies.

Keywords: Botanicals, Inert dusts, Integrated pest management, Insect pests status, Maize varietal resistance, Stored maize grains, *S. zeamais.*

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Dedication

To my wife Embet Enigida

To my daughter Betelihem Berhanu and

To my mother Gebayanesh Tesfaye

To late my beloved father, Hiruy Yeshitilla

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Chapter 1. General Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops in the world both as food for human being and feed for animals. It is also known as queen of cereals because of its high yielding potential (Nand, 2015). Besides, it is the third after wheat and rice in area coverage and total production in the world (OECD and FAO, 2010; Lale *et al*., 2013). It is also the major staple food in Africa, contributing significantly to the agricultural sector (Tefera *et al*, 2011) and it is the staple crop, accounting for an average of 32% of consumed calories in Eastern and Southern Africa countries, and rising to 51% in some developing countries like Ethiopia (FAOSTAT, 2010).

In sub-Saharan Africa (SSA), maize is one of the most important grain staples for agricultural income and caloric intake accounting for nearly 20% of the plant-based food supply (Jones *et al*., 2011). In Ethiopia, maize is one of the major cereal crop grown for its food, feed, firewood and construction purposes (Sori, 2014; Abate *et al*., 2015). However, maize production and yield in Ethiopia, in particular, and Africa, in general have been highly affected by an array of biotic and abiotic stresses (Tefera *et al*., 2010; 2011). Among biotic constraints, insect pests are the most important one (Adams and Schulter, 1978; Tadesse, 1997; Ali *et al*., 2007). The most important of these insect pests in the field and storage are lepidopterous stem borers and Coleopterous weevils, respectively (Emana and Tesdeke, 1999; Demissie *et al*., 2008; Hiruy and Emana, 2018). Besides, the storage insect pests are the most detrimental, as the loss is none compensated (Chomchalow, 2003).

In Ethiopia, food security is more threatened by excessive post-harvest losses of grains caused by stored grain insect pests, at household and at a nation level (Worku *et al*., 2012).

The primary causes of losses of stored maize grain are maize weevil and Angoumois grain moth (Worku *et al*., 2012). The lack of suitable grain storage structures and absence of storage management technologies in the tropics forced growers to sell their produce immediately after harvest (Emana, 1995). Consequently, farmers receive low market prices for any surplus grain they may produce (Tefera *et al*., 2011).

Hence, farmers are not the beneficiaries of surplus production of crops like maize (Tadesse, 1995; Demissie *et al*., 2008). As a result, there is an urgent need to maintain the quality of stored grains such as maize through proper management of stored grain insect pests (Upadhyay and Ahmad, 2011). However, traditional storage methods and practices that farmers use to protect stored maize grains against pests, species composition, status of insect pests, and the associated damage and losses have never been studied before in the study area. Besides, due to environmental, health and other concerns that have been associated with repeated use of synthetic pesticides over the past years, there is an urgent need for searching for cheap, safe and effective management options.

1.1 General Objective

To know the status and species composition of stored maize grains insect pests in Hadyia and Silte zones and develop some control options.

Chapter 2. Literature Review

2.1 Origin and Importance of Maize

About 700 years ago, maize (*Zea mays* L.) is believed to be originated in central Mexico from a wild grass and was transformed into a better source of food by Native Americans (Ranum *et al*., 2014). Nutritionally, it contains approximately 72% starch, 10% protein and 4% fat, supplying an energy density of 365 Kcal/100 g (Ranum *et al*., 2014). In addition, it has higher content of protein and fat as compared to other cereals. It also contains vitamin B-complex such as B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) and B6 that makes it commendable for hair, digestion, skin, heart and brain. Furthermore, it contains vitamin A, C and K together with large amount of beta-carotene and fair amount of selenium that helps to improve thyroid gland and play important role in proper functioning of immune system (Kumar and Jhariya, 2013).

Accordingly, maize (*Zea mays* L.) is one of the most important cereal crop in the world both as food for man and feed for animals (Nand, 2015). United States, China, and Brazil are the top three maize-producing countries in the world (Ranum *et al*., 2014). It is also known as queen of cereals because of a very high yielding potential (Nand, 2015). Besides, it is the third after wheat and rice in area coverage and total production in the world (OECD and FAO, 2010; Lale *et al*., 2013). It is produced on nearly 100 million hectares in developing countries, with almost 70% of its total production coming from low and middle-income countries (FAOSTAT, 2010).

Along with the aforementioned importance, the current inevitable world population growth and seriousness of food insecurity issue in Africa (Nukenine, 2010), especially in SSA will place an increasing demand on the production and productivity of cereals like maize (FAO, 2009). As a result, increasing and improving maize production and utilization have been suggested as one major strategy for alleviating the danger of hunger and malnutrition. Accordingly, maize has been spread out quickly and changed production systems in Africa as a popular and widely cultivated food crop (McCann, 2005). Now a days, it is a major staple food crop grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socioeconomic backgrounds in SSA (Macauley, 2015). Besides, among cereals, it is the most important food staple, providing food and income to millions of resource poor smallholders in Eastern and Southern Africa (ESA) (Tefera *et al*., 2011). Furthermore, it is a staple food crop, which plays a great role in food security in Ethiopia at the moment (Mitiku *et al*., 2015).

2.2 Maize Storage Conditions, Associated Insect Pests and Their Status

2.2.1 Maize Storage Conditions

Storage is one part of the post-harvest system through which food grains like maize passes on its way from the field to the consumer (http://www.acdivoca.org/Storage Handbook, browsed on 16/4/2016). It is particularly important in agriculture, because agricultural production is seasonal, while the demands for agricultural commodities are more evenly spread throughout the year. Thus, there is a need to meet average demand by storing excess supply during the harvesting season for gradual release to market during off-season periods (Okoruwa *et al*., 2009). As a result, storage helps to smooth out variations in market supply both from one year to the next and from one season to the next by taking produce off the market in surplus seasons and releasing it back onto the market in lean seasons (Proctor, 1994; Komen *et al*., 2006; Kimenju *et al*., 2009). Accordingly, grains are stored by farmers for their consumption and/or for seed purposes, and by traders as well as marketing agencies for financial gain in general (Chakraverty *et al*., 2003).

Grain storage periods across Africa, generally range between three and twelve months and their length depends on the agro-ecological zone, ethnic group, the quantity of commodity stored, the storage condition, the crop variety stored and so on (Nukenine, 2010). A number of methods, practices and facilities were used traditionally by farmers in order to store their grains. For instance, maize could be stored either shelled or unshelled and in the latter case, maize is shelled manually in a staggered manner when required for consumption and/ or for sale. Shelling of maize is also done by using hired machines especially when production is large (Gabriel and Hundie, 2006). In addition, the structures used for grain storage depends on the level of storage such as on-farm, village and city (central storage's). On farm storage involves individuals, while village storage may implicate individuals (family granary) or a group of individuals (community stores). The city and central storage facilities include large warehouses and are usually owned by government agencies or non-governmental organizations (national or international) and are usually built with expertise from the developed world (Nukenine, 2010).

Generally, grains are stored in distinct entities such as silos, warehouses, bags, containers and even in piles on the ground (Jayas, 2012). However, the use of traditional storage structures, as well as storage of cereals like maize in the houses by small scale and subsistence farmers in Africa leads to considerable losses. On the contrary, airtight storage technologies like the metal silos, which are said to have zero storage loss, are expensive for the individual farmer to afford. There are, therefore costs and benefits to storage regardless of the storage structure used (Tabitha *et al*., 2013).

Accordingly, proper storage of grains continues to be a challenge for subsistence farmers in SSA, including Ethiopia (Govender *et al*., 2008). The lack of suitable storage structures for storing grains and absence of storage management technologies often forces the smallholder farmers to sell their produce immediately after harvest. Consequently, farmers receive low market prices for any surplus of grains they may produce (Emana, 1995; Tefera *et al*., 2011). Besides, tropical climatic conditions and poor sanitation of grain storage such as maize (that are highly favorable for insect growth and development) in SSA, also encourage insect pest attack and have been compounding the problem (Talukder, 1995). As a result, farmers are not as such the beneficiaries of this increased production and productivity potential of new varieties of maize (Sori and Ayana, 2012). Hence, safe storage of grains like maize is very crucial, as it has directly impacts on poverty alleviation, food and income security, as well as prosperity for the smallholder farmers (Gitonga *et al*., 2015).

2.2.2 Insect Pests Associated to Stored Maize Grain

Insect pests that affect stored grains like maize are referred to as post-harvest insect pests or storage insect pests. They are generally members of two major groups or insect orders such as Coleoptera (beetles) and Lepidoptera (moths) (Emana and Tesdeke, 1999; Upadhyay and Ahmad, 2011). Of these two groups of insect pests, beetles in which both the larva and adults are responsible for damage (loss), are more diversified and highly destructive, in comparison to moths, in which only the caterpillars are harmful life stage that causes damage (Upadhyay and Ahmad, 2011). According to Pedersen and Lee (1996), the species from both orders can complete their life cycles in less than 30 to 35 days and lay many eggs, which results in rapid build-up of populations that consume and contaminate various stored products, and they undergo complete metamorphosis.

Storage insect pests can be broadly grouped in to two groups such as internal feeders (primary pests) and external feeders (secondary pests or bran bugs). That means, insects that attack sound grains such as maize and cause damage to them are called primary pests. In the contrary, those that attack already damaged grains and cause further damage are called secondary pests (Navarajan Paul, 2007; Rees, 2007). However, according to Semple *et al*. (2011), it must be noticed (emphasized) that primary pests does not necessarily refers to the importance of the pests, but simply implies to the dynamic processes involved, whereby secondary pests can cohabitate (follow) primary pests and inflict serious and economic losses, especially under long-term storage. Examples, of primary pests include the maize weevil, rice weevil, lesser grain borer and larvae of the Angoumois grain moth. Examples of secondary pests include Indian meal moth, yellow mealworm, red and confused flour beetles and flat grain beetle (Ebeling, 2002). Besides, there is also a third group of insects that infest stored grains that are known as mold feeders. This group does not directly damage the grain through feeding; instead, they contaminate the grain mass through their presence and their metabolic activity (Mason and McDonough, 2012).

Mold feeders feed on molds (fungi) growing on grain stored at excessive moisture levels (https://www.extension.entm.purdue.edu/publications/E-66.pdf, browsed on 21/5/2016). According to Mason and McDonough (2012), the presence of mold feeders in grain mass usually indicate that the grain is going out of condition and that some mold growth has occurred. According to him, examples of mold feeders include foreign grain beetle, rusty grain beetle, hairy fungus beetle and psocids. Generally, according to Obeng-Ofori, (2011), a myriad of insect pests attack stored grains such as maize in Africa, in general and in SSA including Ethiopia, in particular. Of these insect pests species, Angoumois grain moth (*Sitotroga cerealella* (Olivier)), lesser grain borer (*Rhyzopertha dominica* (Fabricius)), larger grain borer (*Prostephanus truncates* (Horn)), rice weevil (*Sitophilus oryzae* (Linnaeus)) and maize weevil (*Sitophilus zeamais* (Motschulsky)) have been recognized as an increasingly important problem to maize storage in Africa (Jembere, 1995; Abebe *et al*., 2009; Tefera *et al*., 2011).

2.2.3 Status of Insect Pests Associated to Stored Maize Grain

Post-harvest insect pests associated to grains such as maize are the first from the invasive forces to begin the interaction with the grain. Consequently, they are one of the major threats to the grains quality maintenance during storage (Chakraverty *et al*., 2003). Besides, they are the most damaging of all other pests and the most difficult to control due to their small size, feeding behavior and ability to attack grain before harvest (Talukder, 2009). As reported by Chimoya and Abdullahi (2011), the majority of these insect pests are cosmopolitan and polyphagus in their feeding behaviors. The reasons for such widespread occurrence of them according to Pugazhvendan *et al*. (2009) range from evolutionary adaptations to the actions of humans who contribute to transportation of them throughout the world and provide a protected habitat within stored food stuffs. This always compounded the food loss problem, as it tends to raise the incidence as well as seriousness of the attack (Chimoya and Abdullahi, 2011).

Insect pests of stored grains like maize cause their damage (loss) on stored grains mainly by direct feeding in both field and storage. In addition to direct consumption, they also contaminate their feeding media through excretion, molting, their own existence, leaving their dead bodies, body fragments, webbing and an unwanted odor or flavor (Offor *et al*., 2014). According to Weaver and Petrof, (2005) and Tefera *et al*. (2010), in most cases, insects also pre-dispose the stored grains like maize to secondary attack by disease causing pathogens such as fungi. Besides, as reported by Mason and McDonoug (2012), a major concern with the presence of insects in storage's is a potential to vector disease organisms. This is because many of them possess hairs and indentations on their exoskeletons that can serve for mechanical vectoring of pathogens. For instance, maize weevils have been reported to carry or vector several fungi species, including *Aspergillus niger*, *A. glaucus*, *A. candidus*, *Penicillium islandicum*, *P. citrinum*, *Fusarium semitectum* and yeasts (Smalley, 1989 cited in Hagstrum *et al.*, 2012).

Hence, in terms of economic importance in general, storage insect pests mainly and fungi to a lesser degree reduce the quality, as well as value of grain in storage. However, losses due to rodents and birds are typically quite infrequent and minor (Weaver and Petroff, 2005). Consequently, infestation by the insect pests in grain storage such as maize, and damage and loss that result from them poses a major threat in food security not only to farmers in SSA, including Ethiopia, but also globally, especially in resource poor nations (Obeng-Ofori, 2008; Chimoya and Abdullahi, 2011).

2.3 Management Options of Stored Maize Grain Insect Pests

2.3.1 Botanical Control

Botanicals refer to the chemicals that are produced by plants, and repel approaching insects, deter feeding and oviposition on the plant or disrupt the behavior and physiology of insects in various ways (Shankar and Abrol, 2012). These include spices, medicinal, weeds and other plants (Chomchalow, 2003; Said and Pashte, 2015). These pesticidal plants are utilized in two main ways in post-harvest protection; first the use of plant tissue or crude plants products such as aqueous or organic solvent extracts are directly used (Golob *et al.*, 2002; Talukder, 2006). The second approach is the use of active compounds isolated, identified and chemically synthesized from plants. If feasible, these compounds or their active analogues are synthesized and marketed by the chemical industry (Golob *et al.*, 2002; Talukder, 2006). The later method is inappropriate for small-scale subsistence farmers, but they may eventually have commercial applications for large-scale storage's (Golob *et al.*, 2002).

The utilization of botanicals to protect stored commodities against insect pest attack have a very long history (Belmain and Stevenson, 2001; Isman, 2006). The use of such locally available plant materials for stored-product protection is a common practice and has been believed to have more potential in subsistence and traditional farm storage conditions in developing countries like Ethiopia (Obeng-Ofori, 2007). It has been also well argued that botanicals are appropriate technology for resource poor smallholder farmers (Isman, 2008), especially in SSA for their several advantages over synthetic pesticides. These include they are one of the most important locally available and biodegradable methods (Mishra *et al*., 2012). Besides, they are easily produced by farmers, small scale industries and are potentially less expensive (Nikkon *et al*., 2009). Furthermore, unlike synthetic pesticides, they often have several modes of action, and their toxicity against insect pests are expressed by: (1) directly killing particular life stages of the insect, (2) interfering with mating or suppressing reproduction, (3) acting as a repellent or affecting host findings and selection in a way that prevents infestation or (4) reducing or preventing feeding (Golob *et al*., 2002).

Having more than one mode of action by many botanicals contribute to the efficacy of them in reducing insect pests damage of food grains like maize. Another merit could be that the potential for insects to develop resistance to botanical with many modes of action is reduced (Golob *et al*., 2002). Botanicals also pose little threat to the environment &/or to human health than synthetic pesticides (Dubey *et al* 2008). They are also nontoxic to non-target organisms (Rajashekar *et al*., 2012). Consequently, they maintain biological diversity of natural enemies, which make their use a sustainable pest management alternative in agriculture (Sola *et al*., 2014). Resource poor farmers typically also use them as crude extracts which exposes them to lower concentrations of active ingredients compared to synthetic pesticides (Isman, 2008). The successful utilization of them can also help to control many destructive pests and diseases of the world, as well as reduce desertification, deforestation and erosion (Rajashekar *et al*., 2012).

Accordingly, the use of green pesticides, particularly for stored grains such as maize insect pests is being recommended globally (Dubey *et al*., 2008). However, only few botanicals are used on a commercial scale, that means only few plants have led to major commercial pesticidal products similar to those produced by the synthetic pesticides industry (Phillips and Throne, 2010; Sola *et al*., 2014). Neem products from *Azadirachta indica*, Pyrethrum from *Tanacetum cinerariifolium* and rotenone from *Derris* and *Lonchocarpus* sp. are commercial examples of botanical pesticides that have been developed and are being traded globally (Phillips and Throne, 2010; Sola *et al*., 2014). However, subsistence farmers throughout SSA often lack the financial resources to buy good quality commercial insecticides to protect their stored grain and their inappropriate use of conventional pesticides can result in the risks to human and environment. As a result, traditional storage methods using indigenous plant materials that posse's insecticidal properties could, if improved, offer a low-cost, safer and more dependable method of storage protection, while reducing the increasing reliance upon conventional pesticides. Thus, botanicals could offer a solution for the problems of availability, health risks, costs and resistance in the case of synthetic pesticides, and for the lack of equipment for hermetic storage, gamma irradiation and controlled atmospheres (Shankar and Abrol, 2012).

However, problems with botanical insecticides are lack of consistency (maintaining a particular standard), safety concerns and sometimes odor (Phillips and Throne, 2010). Traditional methods of their preparation are often variable and lead to inconsistent efficacy. This problem is compounded by inherent differences in plant chemistries that may be genotypic or spatio-temporal variations caused by abiotic factors such as altitude, rainfall and soil type or different chemistries expressed in different plant parts (Sarasan *et al*., 2011; Belmain *et al*., 2012). This inconsistency in efficacy remains one of the major difficulties that stand in the way of exploitation of pesticidal plants (Sola *et al*., 2014). Besides, other obstacles of them include doubts to their efficacy due to their slow action and lack of rapid knockdown effect, instability of the active ingredients when exposed to direct sunlight and high cost of commercially formulated botanical pesticides (Obeng-Ofori, 2007). Thus, the promotion of these botanicals, particularly with optimized applications based on knowledge of the active plant chemicals would greatly benefit resource poor farmers in SSA (Dubey, 2011).

2.3.2 Management Using Resistance Variety

Resistance of plant or plant materials (Host plant resistance (HPR)) to insects are defined as the relative amount of heritable qualities possessed by a plant or its materials (like for instance, its seeds), which influence the ultimate degree of damage done by the insects. However, in the case of stored grains such as maize, resistance refers to the ability of a certain crop variety to produce grains that maintain better quality than commonly cultivated varieties following long storage under similar insect populations (Mbata, 1987). Two mechanisms of resistance have been known in stored grain. These are antibiosis and antixenosis (Kumar *et al*., 2006; Tefera *et al*., 2011). Antibiosis refers to condition where the biology of the pest is adversely affected after feeding on the plant (the seed). However, antixenosis (non-preference) represents a condition where the plant and the seed are not desirable as a host and post-harvest pests seek alternative host (Tefera *et al*., 2011).

The types of resistance factors have been reported as they include both morphological, and biochemical traits and work individually or collectively (Ahmed and Yusuf, 2007; Wanja *et al*., 2015). Nevertheless, resistance is rarely totally dependent on a single mechanism; there are often overlaps between the morphological and biochemical bases of resistance (Dent, 2000; Ahmed *et al*., 2002). For instance, kernel resistance is due to physical barrier through mechanical fortification of the pericarp cell wall and antibiosis through the toxic effects of phenolic acid amides and peroxidase activity localized in the aleurone layer (García-Lara *et al*., 2007). In addition, synergistic act the aforementioned both factors have been reported in several wheat, rice and maize varieties to protect them against *Tribolium castaneum*, *Rhizopertha dominica*, *Trogoderma granarium*, *Sitophilus* spp., *Plodia interpunctella* and *Sitotroga cerealella* (Ahmed *et al*., 2002; Shafique and Chaudry, 2007).

But, breeding for the resistance to stored grain insect pests was ignored initially (in the past), probably due to the long duration from crop establishment to post-harvest screening for resistance and the high cost involved (Derera *et al*., 2001; Mwololo *et* *al*., 2010). However, because of the widespread use of insecticide and its associated risks, it has been recommended recently as alternative methods of control. This is because it is selective (Kanyamasoro *et al*., 2012), the cheapest, effective and ecologically safe method of protecting grains such as maize against insect pests in Africa (Tefera *et al*., 2011). It also helps to avoid health risks and requires little or no scientific knowledge by the farmers (Ahmed and Yusuf, 2007). Moreover, it is also maintain high levels of resistance for a long time despite upsurge of biotypes (Mwololo *et al*., 2012). It is therefore, evident that breeding for resistance to post-harvest insect pests is important for small and large-scale farmers alike (Ahmed and Yusuf, 2007).

2.3.3 Inert Dusts

Inert dusts are dusts that are chemically unreactive and thus, used for managing insects of stored grains such as maize by physical rather than chemical means (Abd EL-Aziz, 2011). Inert dusts act as a desiccant, absorbing water from the insect body and may have an abrasive action. Water is lost because the dusts remove the waxy layer of the cuticle of the exoskeleton by adsorption. Accordingly, insects pests coated with inert dusts show massive dehydration and die very soon (Upadhyay and Ahmad, 2011). The use of chemically inert materials such as sand, wood ashes or minerals in large amounts fill up the interstitial space in grain bulks and offer an obstacle to insect movement (FAO, 1999).

According to Golob (1997), there are five types of inert dusts: (a) non silica dusts (e.g., limestone, lime, katelsons); (b) ash, clays, sand; (c) diatomaceous earths, which are the fossilized diatoms of marine or freshwater origin and are composed mainly of amorphous hydrated silica; (d) synthetic silicates and precipitated silicas; and (e) silica aerogels obtained by drying aqueous solutions of sodium silicate. Because the effect of inert dusts is through desiccation, their effectiveness decreases as relative humidity increases (Abd EL-Aziz, 2011). They also act slowly and take 20 or more days to cause insect mortality. Besides, they affect grain bulk density, flow ability and grain handling properties, and the dusts containing crystalline silica may cause silicosis and other respiratory diseases (Chakraverty *et al*., 2003). However, the main advantage of using them is that they are non-toxic to humans and animals. For instance, diatomaceous earths are registered as a food additive in the USA (Abd EL-Aziz, 2011).

There has been a considerable amount of historical data concerning desiccant dusts and their insecticidal effects on stored-product insects (Subramanyam *et al*., 1994). Most of the early formulations, however, were not widely accepted by the grain industries in developed countries for a variety of reasons, including the high rates required for mortality, variation in toxicity among target species, damage to grain handling equipment and health problems with worker exposure to dusts. However, with the current concerns of protectant resistance and the desire of consumers for residue free grain, these dusts have been receiving increased attention (Arthur, 1997). Thus, detecting and isolating valuable locally existing inert materials against post-harvest pests of maize could provide significant input in management of storage pests in Ethiopia in particular and Africa in general.

2.3.4 Chemical Control

Two major chemical methods used for managing insect pests of stored grains such as maize recently are fumigation and grain protection by contact insecticides according to Kostyukovsky *et al*. (2016).

Fumigation: It is one of the most effective management method, in which insect pests are exposed to a poisonous gaseous environment, by applying a grain fumigant. Fumigants are chemicals available as gases, liquids and in solid formulations, but act on the insect pests of stored grains such as maize in gaseous state (Chakraverty *et al*., 2003). Fumigation is applied in buildings, warehouses; small bags, soil, seed and stored products, and fumes generated by fumigants enter the body of insect through the spiracles and spread to trachea and tracheoles and bind to the hemolymph components (Upadhyay and Ahmad, 2011). Accordingly, fumigation plays a key role in grain preservation as it controls insects developing inside and outside the grain, and crawling and hidden pests (Chakraverty *et al*., 2003).

Currently, phosphine and methyl bromide are the two common fumigants used for stored-product protection over the world (Boyer *et al*., 2012). Among these fumigants, the usage of methyl bromide was phased out in developed countries due to its ozone depletion effects and instead, phosphine is widely used now a day (Daglish *et al*., 2014). Thus, phosphine is the most widely used fumigant not only in Africa, but also in the world due to its low cost and ease of application. It is also the preferred chemical for routine grain disinfestations in the developing countries such as SSA, including Ethiopia where other alternative techniques such as controlled atmosphere storages, are expensive or cannot be readily adopted (Chakraverty *et al*., 2003).

Use of Contact Insecticides: Contact insecticides are solid or liquid formulations of manmade insecticides, which are toxic to insects and exert their effect when insect pests of stored grain such as maize come into direct contact with them (Golob *et al*., 2002). Solid formulations include dust, granules, baits, wettable powders and soluble powders, whereas liquid formulations include emulsifiable concentrate, oils, solutions, fumigants, aerosols and ultra-low volume (Semple *et al*., 1992). Most of these available for post-harvest use were originally developed to protect field crops and then found to be useful for stored products (Golob *et al*., 2002). They play a significant role in grains preservation along with other control measures, regular hygiene and sanitation measures. Insecticides kill insects previously exist, as well as hinder cross-infestation and re-infestation of non-infested grains (Chakraverty *et al*., 2003).

Synthetic organic chemicals that are currently approved for use in stored gain insect pest's management fall into one of three groups such as organophosphates (OPs), carbamates (Cs) and synthetic pyrethroids (SPs) (Golob *et al*., 2002). Example of these chemicals include malathion, dichlorvos, fenitrothion, pirimiphos methyl and chlorpyrifos methyl from Ops and biores-methrin, deltamethrin, permethrin, fenvalerate and phenothrin from SPs and carbaryl, primicarb, indoxacarb, alanycarb and furathiocarb among Cs (Semple *et al*., 1992). These insecticides have been used in management of stored grains as grain admixture treatments, residual surface and space treatments (Proctor, 1994; Golob *et al*., 2002). But, use of contact insecticides as protectants by direct admixture with grain has been decreasing in recent years and instead use of them as with residual sprays, fogging and aerosol applications is likely to continue in grain storage (Chakraverty *et al*., 2003). Nevertheless, synthetic pesticides have been used by the majority of smallholder farmers in many parts of Africa for protection grains such as maize against storage insect pests (Mvumi *et al*., 1995; Mvumi and Stathers, 2003). Besides, it was also reported that as they have been the most efficient and effective methods for protection of stored products in Ethiopia and elsewhere (Emana, 1999).

However, over the years, negative attributes have been associated with synthetic pesticide use. These includes the presence of toxic residue in food products, toxicity against non-target species, development of resistance by targeted species (Guedes *et al*., 1996; Subramanyam and Hagstrum, 1996; Harish *et al*., 2013), high persistence and its associated environmental pollution, direct toxicity to users and increased risk to workers safety (Ofuya and Longe, 2009). Their indiscriminate use also leads to give raise of secondary insect species due to the destruction of their natural enemies in storage ecosystem according to Gu *et al*. (2008). These problematic situations of insecticides, along with possibility of misuses of pesticides have been making them less attractive and demand a vigorous search for alternative insect pest control practices (Owusu *et al*., 2007). These situations hence inspired the search for safe, effective, pest specific and economical alternative methods including inert dusts, botanicals, varietal resistance, biological control and others (Smith *et al*., 2006).

Accordingly, in recent years, the aforementioned bio-rational options have been suggested as alternative to chemical pesticides for protection of grains against insect pests (Kostyukovsky *et al.,* 2016). In addition to aforementioned problematic situation, none of the existing products will entirely fulfill all of the criteria for use in storage ecosystems (Obeng-Ofori, 2011). Hence, these chemicals do not have very promising future for food grains such as maize and they may be used with hazard restrictions (Mohapatra *et al.*, 2015).

2.3.5 Integrated Pest Management (IPM)

Integrated pest management (IPM) has been defined as a pest management system which takes in to account the environment, the population dynamics of the pest, and uses all suitable techniques and methods in the most compatible manner as possible to maintain the pest population below levels that would cause economic injury (Golob *et al*., 2002; Capinera, 2008). IPM gives priority to non-chemical control measures and only defaults to the use of chemical controls is when other options are unlikely to affords sufficient protection of stored grains like maize from insect pests (in IPM strategies, chemical control is used as a last resort to control insect pests) (Stejskal, 2003; Savoldelli and Trematerr, 2011).

In grain storage, good commodity management (CM) and good store management (SM) are the major prerequisites of IPM. SM involves the controls on in store climate and infestation pressure, which can be achieved by technically sound store design and construction. It also involve the climatic control that could be attainable by scientific management of the commodity to ensure that the stored grain itself is both dry and cool when loaded or in ventilated stores and bins with aeration equipment (Semple *et al*., 1992).

CM can also control the initial insect pest's infestation level in the stored grain to a considerable extent. However, in tropical countries where pre-harvest infestation by storage insect pests is hardly ever completely preventable, the ideal of loading insect free grain into the store is often unattainable. Thus, the common alternatives are to treat the grain at intake with a suitable admixed insecticide or to disinfest the loaded grain by in store fumigation (Semple *et al*., 1992).

Thus, judicious use of chemical insecticides following knowledge-based decision-making is strongly advocated in IPM of grain storages (Phillips and Throne, 2010). As a result, IPM leads to a reduction in insecticide usage and consequently, limits the opportunity for the development of resistance by insect pests and the dangers that acquired to consumers, pest control staff and the environment (Golob *et al*., 2002). Accordingly, in tropical agricultural systems, including Ethiopia where pesticides are increasingly expensive and pose risks to farmers, consumers and their environment, reduction of pesticide use through IPM has many economic, social and environmental advantages (http://www.carana.com/about/storage-handbook, browsed on 17/8/2016). Consequently, there has been growing acceptance of IPM and it has been shown as an increasingly important approach (Capinera, 2008). Besides, it has been reported as the best option for the future, as it guarantees yields, reduces costs, is environmentally friendly and contributes to the sustainability of agriculture (Dent, 2000).

No report has been available so far on integrated management of post-harvest insect pests under storage condition in Ethiopia (Tadesse, 2008). However, the followings are some effective and reported cases regarding grain storage IPM in Ethiopia and oust side under laboratory condition. In his preliminary study, Demissie (2006) reported that the combined use of minimum rates of *Chenopodium* plant powder, botanical triplex, silicosec, and filter cakes with weevil tolerant varieties has reduced grain damage at Bako of Ethiopia. Ibrahim (2017) also indicated that integrating neem seed and Mexican tea powder provided significant protection to maize from the maize weevil. Bayih (2014) also reported that the unitary and binary formulations of botanical at lower and higher rates were effective against *Z. subfasciatus*. Similarly, it was also reported that for the reason of their synergetic effects, integration of one management method with other sustainable method could provide long lasting solution to losses in storage (Dobie, 1977). Besides, it was also reported that combining (integrating) of different botanicals has enhanced their potency in controlling stored grain insect pests than those applied unitarily (Agona and Muyinza, 2003; Bayih, 2014).

Accordingly, IPM has been much less well developed for the protection of stored grain, though it has been well-developed approach for the protection of crops before harvest (Golob *et al*., 2002). Thus, the development and implementation of IPM strategies for insect pests of maize in traditional small-farmers storage in Africa, including Ethiopia will undoubtedly require a major research and extension effort by national and international agencies as well as researchers (Dick, 1988).

Chapter 3. Traditional Methods and Practices used by Farmers for Protecting Stored Maize Grains from Insect Pests

3.1 Introduction

Storage is one part of post-harvest system through which food grains like maize passes on its way from the field to the consumer (http://www.acdivoca.org/StorageHandbook, browsed on 16/4/2016). About 70 to 90% of the grains harvested by resource-poor farmers in sub-Saharan Africa (SSA), including Ethiopia store their maize grains for home consumption and/or for marketing purposes (Golob *et al*., 1999). Grain storage periods across Africa, generally range between 3 and 12 months and their length depend on tradition, agro-ecology, crop variety, quantity of commodity to be stored and the storage conditions (Nukenine, 2010). Farmers also used various methods and facilities to store their crops (Gabriel and Hundie, 2006). Smallholder farmers in Africa also have different local knowledge to tackle pest problems in traditional storage systems, which are generally adapted to the existing social, economic and climatic conditions as shown by Obeng-Ofori (2011).

Farmer's perceptions of pests and their control may provide essential data for designing pest management strategy (Obeng-Ofori, 2011). An understanding of farmer's knowledge systems has been recognized as a basis for development of pest management technologies that could be adapted to local farmers' situations (Van Huis and Meerman, 1997; Norton *et al*., 1999 cited in Majeed *et al.,* 2016). Accordingly, the knweldge of current status of the different strategies and practices used by farmers in protection of their stored maize grains from insect pests is very imperative for obtaining baseline information that aid in designing and implementing any management strategies that could be adoptable by farmers under their storage environment. Furthermore, prior to this study no one had attempted to investigate indigenous methods and practices used by farmers for protecting their stored maize grains from insect pests in the study area. Therefore, this study was initiated with the following objectives:

1) To know the tradational methods and practices used by farmers for protecting their stored maize grains from insect pests in the study area

2) To study the effect of indigenous methods and practices on the management of stored maize grain insect pests

3.2 Materials and Methods

Description of the Study Areas

Survey was conducted in major maize growing areas of six localities or kebeles or peasant associations of Shashogo and Sankura districts of Hadiya and Silte zone, respectively (Figure 3.1). The localities were Bonosha, Shayamba and Hulla Gutancho from shashogo district of Hadiya zone, and Kora, Gutancho and Bonosha from Sankura district of Silte zone (Emana, 1993; Tadesse, 1995). Survey was conducted between December 2013 and November 2014 with the interval of three months, which was also repeated between December 2014 and November 2015 with the same interval. The study was done using semi-structured questionnaires as well as farmers' participatory discussion.

The Study Design and Sampling Protocols

From each locality or kebele, about three sub-localities were randomly selected and from each sub-locality, four villages were selected at random with the assistance of the Ministry of Agriculture (MOA) sub-kebele staff using a nested design (Emana, 1993). Kebeles were selected purposefully based on abundance of maize production, such that all kebeles growing maize were selected for the survey. From each village, five households were selected randomly and presented with semi-structured questionnaires. At each village, seven farmers of different age groups, wealth and gender were invited for the study of the participatory discussion and open-ended topics were raised to generate free flow of ideas. Selection of the survey sites and storage methods were made in such a way that they were the representative of each kebeles of the two woredas or districts at random. Prior to data collection or questionnaire filling, enumerators were employed and hands-on training was given to both farmers/households and the extension workers on the objective of the study, proper filling of the questionnaires and data collections (Emana, 1993; Tadesse, 1995).

Data were collected on storage methods, form of maize stored, the period and purposes of storage, insect pest problems in storage and management practices used by farmers. Besides, data were collected on the effect of storage methods, form of maize stored, periods of storage and management practices on the level of insect pest's infestation.

Data Analysis

Statistical analysis was performed using Microsoft Excel version 2013 and Statistical Program for Social Sciences (SPSS) version 16. Descriptive statistics were used for compiling and computing data on storage methods used, form of maize stored, periods and purposes of storage of maize grain, insect pest problems in storage and management practices used by farmers, as well as the effect of storage methods, form of maize stored, periods of storage and management practices on the level of insect pest's infestation.

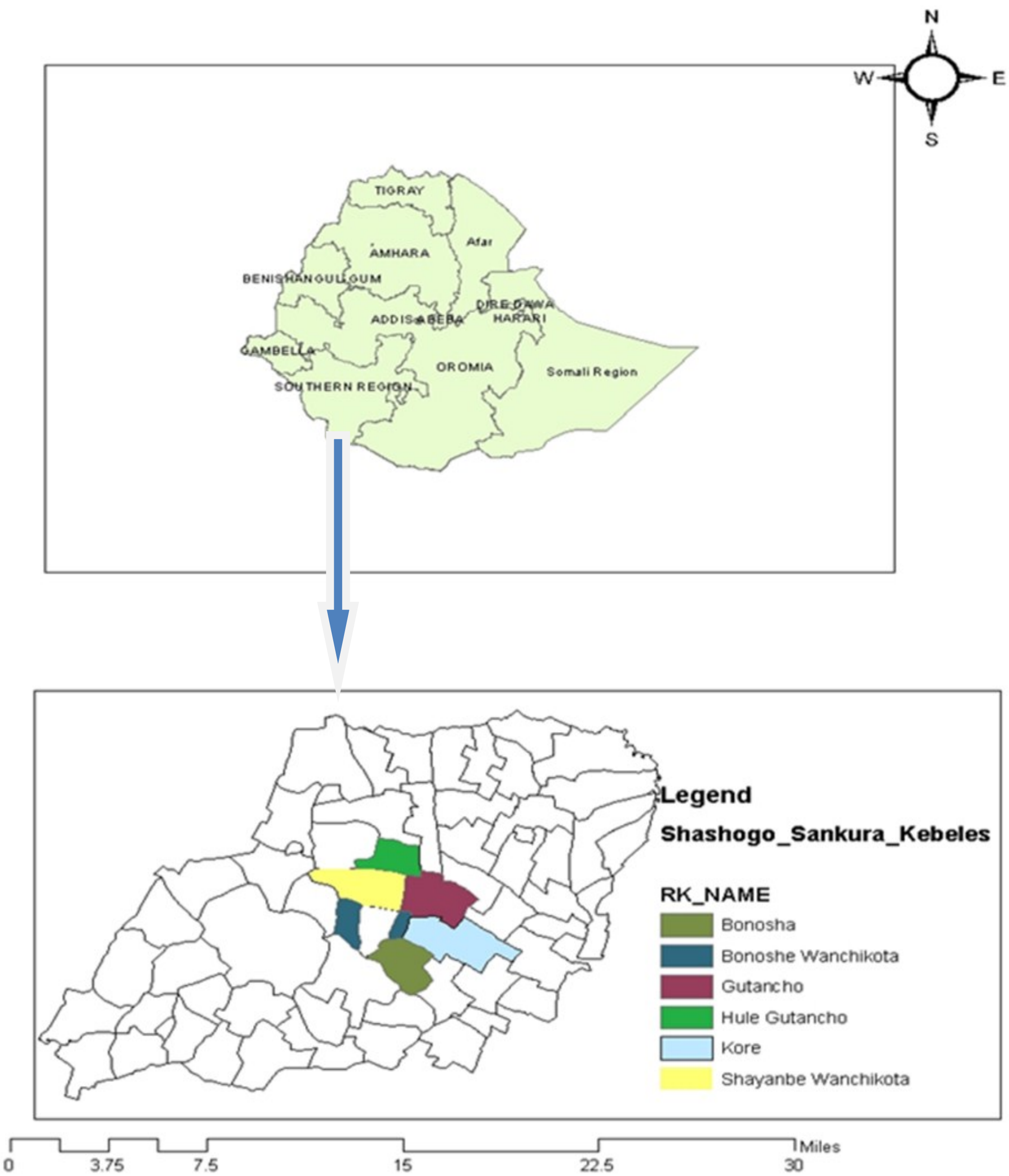


Figure 3. 1 Maps of the survey sites in Hadiya and Silte zones in southern Ethiopia

3.3 Results

Maize Harvesting Time and Methods

Farmers in Shashogo and Sankura woredas harvested maize between November and January for different reasons like fearing loss of grain by animals, pest's, unexpected rain and theft as shown in the Figure 3.2. Among these three months, many farmers (61.39%) harvested their maize in December, followed by some farmers (27%) harvested their maize in November in the survey sites. However, only few farmers (6.67%) harvested their maize in January as compared to those harvested in aforementioned months according to the respondent farmers (Figure 3.2). In other words, the majority of farmers harvested their grain in usual or optimum time (in December), fearing of insect infestation in the field and subsequent loss in storage that they experienced in the past. However, some farmers harvested their grain earlier, in November because of fearing of losses due to animal, theft and untimely rain. Besides, only few farmers harvested their maize later because of lack of money and labor to handle harvesting of their grain at optimum time.

Harvesting of maize was done more commonly by picking the cobs by hand (95%), and rarely by cutting the whole plant part (4.59%) and collected in the field to dry for some days or weeks in the survey site (Figure 3.2). The reasons mentioned for harvesting by cutting the whole plant part by farmers were for saving the stalk of maize for construction of Gotera, for animal feed in dry season and for firewood. Harvesting by cutting the whole plant also is not labor intensive. No reason was mentioned regarding harvesting of maize grains by picking the cobs by hand.

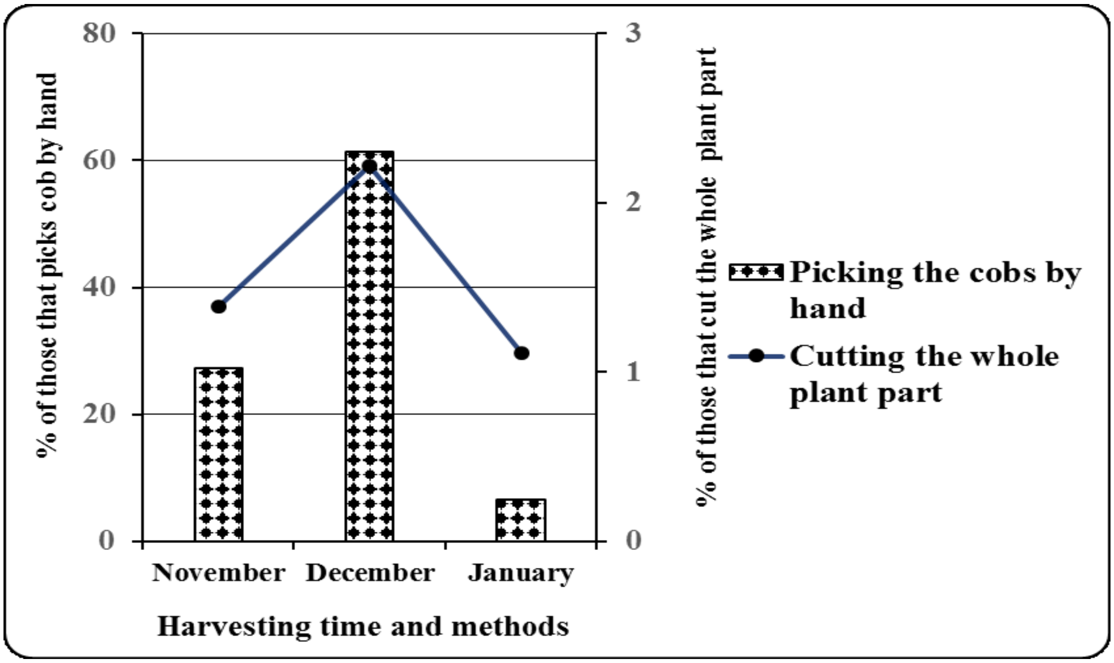


Figure 3. 2 Maize harvesting time and methods in the study area

Pest problem under farmer's traditional storage conditions

Regarding pest problem, about 60% of the farmers mentioned that insect pests were major problem of stored maize grain under traditional storage condition. However, 20% of the farmers put rodent as the major pest of stored maize grain. Less than 13% of the farmers indicated that both insects and rodents are the most important pests of stored maize grain (Figure 3.3). Among insect pests, weevils and moths were the most frequently occurring insect pests on stored maize grain in their localities.

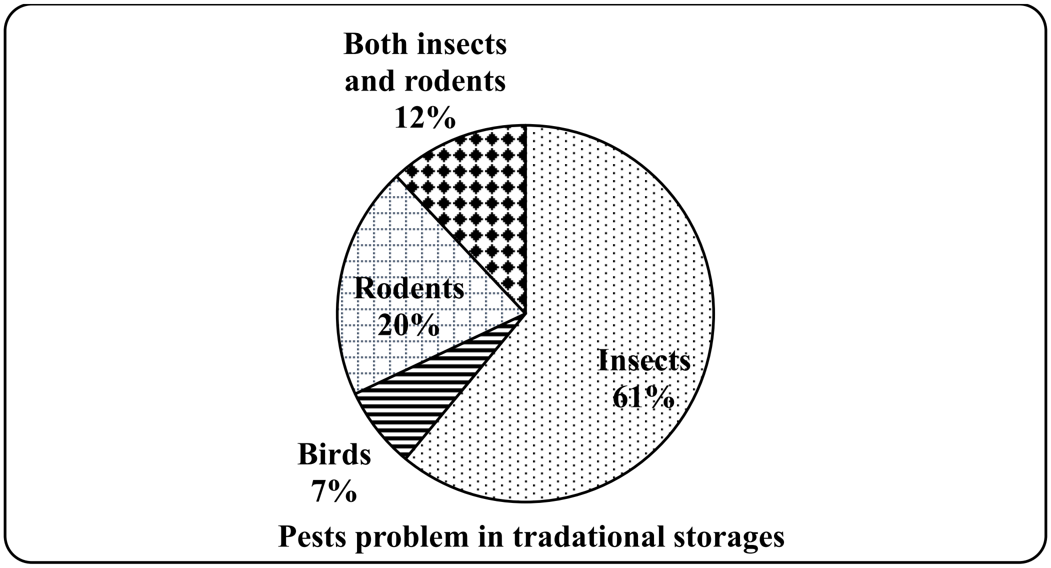


Figure 3. 3 Pests problem in the survey site

Storage Methods used by Farmers and Forms of maize Stored

The maize storage methods identified in the study areas include Gotera outdoor (grain pits), polyethylene bags (sacs) exclusively or in combination with Gotera, Gotera in living house, Kot, Elpingn (storeroom) and inside the living house on the floor (Figure 3.4). Gotera is cylindrical in shape and the wall is made up of dried maize plant stem and woven sticks, and its roof is conical shaded with grass and sometimes with straw. However, Elpingn is a kind of small cottage (small house) behind the main house. Its wall is made of wood, and mud and its conical roof is covered usually by grass or aluminum sheet.

About 52.7% of the farmers stored their maize grains in Gotera outdoor, followed by in living house including indoor Gotera and kot, which were about 24% together. Polyethylene sacs exclusively and in combinations with Gotera indoor comprised of 17%. The other storage methods were rarely used by farmers in the study area (Figure 3.4). Farmers in the survey sites stored their maize grains in various forms such as unshelled cobs with husk intact and shelled maize. In some places, farmers shell their grain only when they want to store the grain in Sacs either for short or long term storage. Most farmers prefer unshelled maize as it is conducive for storage methods such as Gotera outdoor, Gotera indoor, Elipign and inside the living house. Shelling was done most commonly by hand (45%), followed by beating with sticks (30%) and combination of both (25%).

Accordingly, most farmers (≥ 82%) that used storage methods such as outdoor Gotera, indoor Gotera, Elipign and inside the living house exclusively stored their maize grains in the form of unshelled cobs with husks intact for 7-9 months. Farmers who used sacs for storing their maize grain normally stored shelled grain (Figure 3.4).

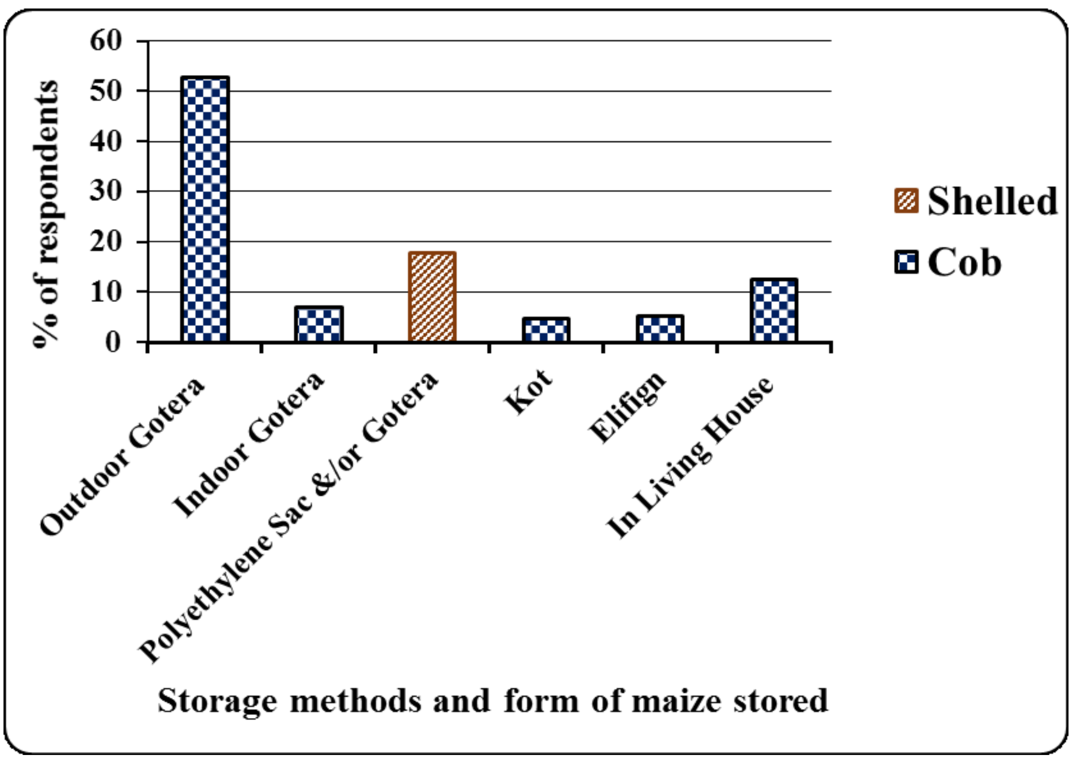


Figure 3. 4 Storage methods used by farmers and form of maize grain stored in the survey site

Periods and Purpose of Storage of Maize in the study area

Of the surveyed farmers or households, the majority of them (50.82%) begunn to store their maize grains in their different storage methods or facilities, starting from either December or November or January to seven to nine months depending on their different conditions in the study area. However, relatively low proportion of farmers (29.33%) stored their maize grain up to 3-6 months long, while relatively few farmers (20.00%) stored it up to 10 months to one year long in the survey sites (Figure 3.5.)

Among all surveyed farmers, high proportion of them (61.59%) gave home consumption as the main purposes for storing their maize grain in 3-12 months storage periods, while relatively low proportion (26.5%) of them indicated a combination of home consumption and expecting better price as the main purposes for storing their maize grain in the survey site. However, only few (12.06%) of them said expecting better price as the main purposes for storing their maize grain in different storage structures in the survey site (Figure 3.5).

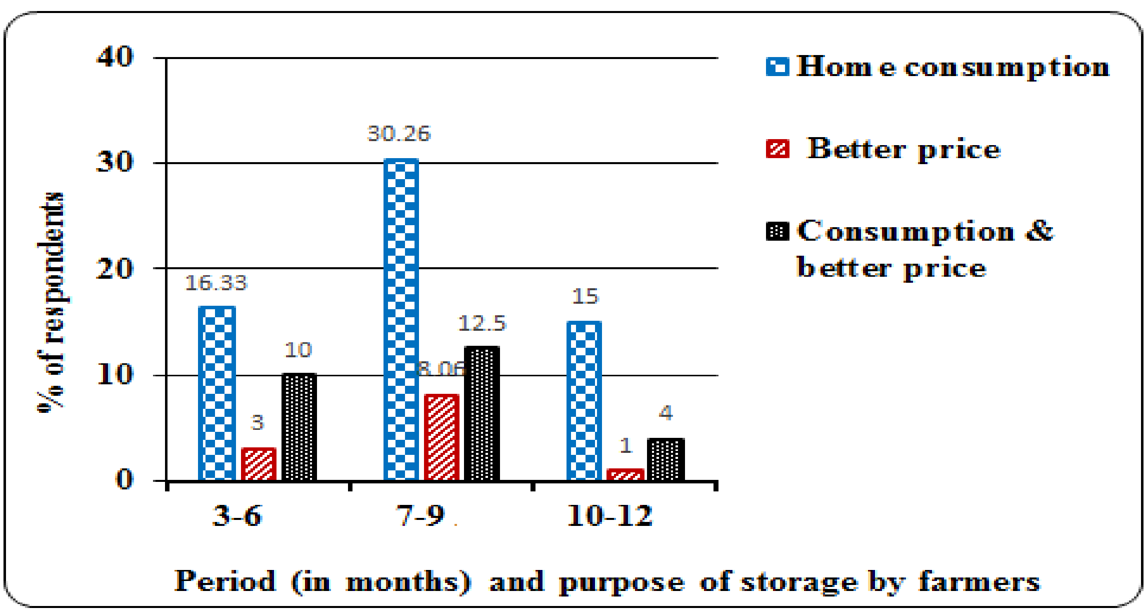


Figure 3. 5 Period and purpose of storage of maize grain in the survey site

Storage period, methods and Form of Maize Stored in the Level of Insect Pest's Infestation

The level of insect infestation was varied both with periods of storage and form of maize grain stored by farmers. According to most of the surveyed farmers (90.9% and 86%), high level of infestation happened when maize grain in shelled form was stored for both 7-9 and 10-12 months periods than in an unshelled form in which high level of infestation occurred only when it was stored for 10-12 months period (86.7%). Besides, most farmers also reported (82%) that low-level infestation occurred when unshelled form maize was stored for 3-6 months periods than shelled forms in which medium level infestation was happened for the same period (81.8%) (Figure 3.6).

Furthermore, most of the surveyed farmers (76%) also said that no level of infestation happened when maize grain in an unshelled form was stored for 1-2 months period than in shelled form in which it was low (83%). Moreover, higher proportion (86%) of the farmers also indicated that unshelled form of maize with the husk intact stored for about 7-9 months had only medium levels of infestation than shelled form in which it was high (Figure 3.6). As mentioned before, the form of maize is somewhat associated with the storage methods used and thus, storage methods such as Gotera outdoor in which maize was stored with husk intact were relatively better for keeping maize with medium level infestation for about 7-9 months period than sac storages according to respondent farmers.

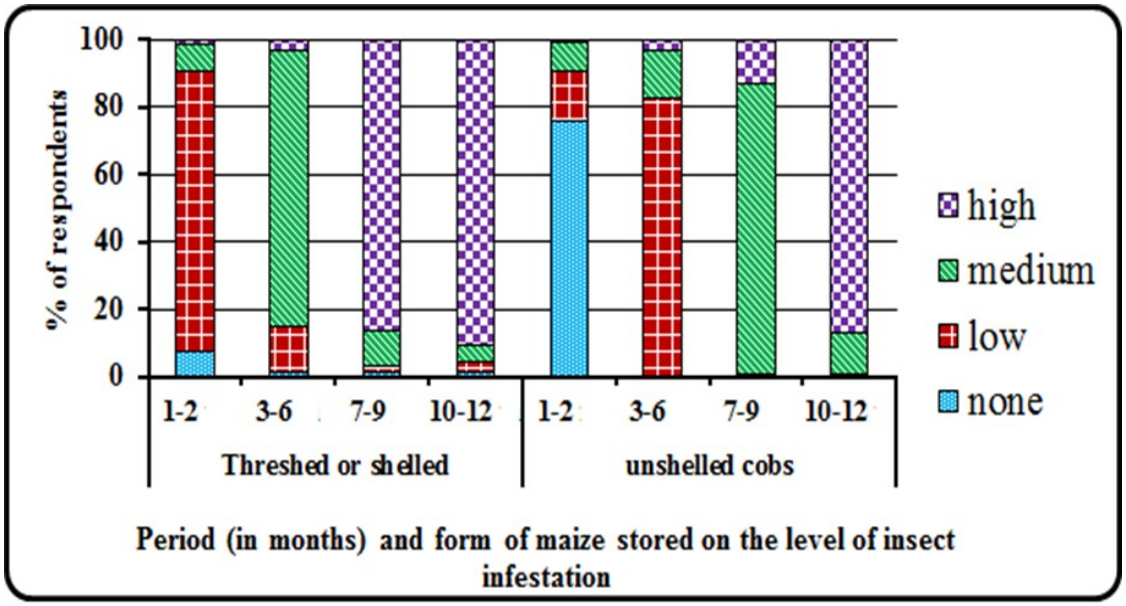


Figure 3. 6 Storage period and form of maize stored in the level of insect infestation in the survey site

Management Practices used against Insect Pests of Stored Maize

A variety of insect pest management methods were used in the survey site so as to protect and preserve their grain against insect pests. These include synthetic chemical pesticides (three fumigant insecticides, namely Quicphos, Alphos and Celphos tablets, and Malathion 5% dust and Malathion 50% (EC), botanicals (*Eucalyptus globlus* and *Croton macrostachyus*) and cultural methods (Putting maize in the sun as soon as the infestation begins, using wood ash, using dang in storage floor, isolating the damaged or infested cobs), while others used no insect pest management measures at all. According to the surveyed farmers, one tablet of the aforementioned fumigant insecticides was used for 100 kg of maize grains. But, 100 g of Malathion 5% dust for 3 quintals (300 kg) and 1 liter of Malathion 50% EC for 140 quintals (14000 kg) of maize grains were used (Figure 3.7).

The majority of farmers (50.56%) that participated in this study reported that they didn't use any of the management methods (for reasons like cost and lack of access), followed by some percentage (35.56%) of them that used synthetic chemicals or pesticides to manage insect pests of stored maize grain in the survey site. Besides, relatively lower percentage of the farmers (13.88%) used botanicals (7.22%) and cultural methods (6.66 %) to manage insect pests of stored maize grain. However, only few farmers (0.83%) used combination of management tactics (Figure 3.7).

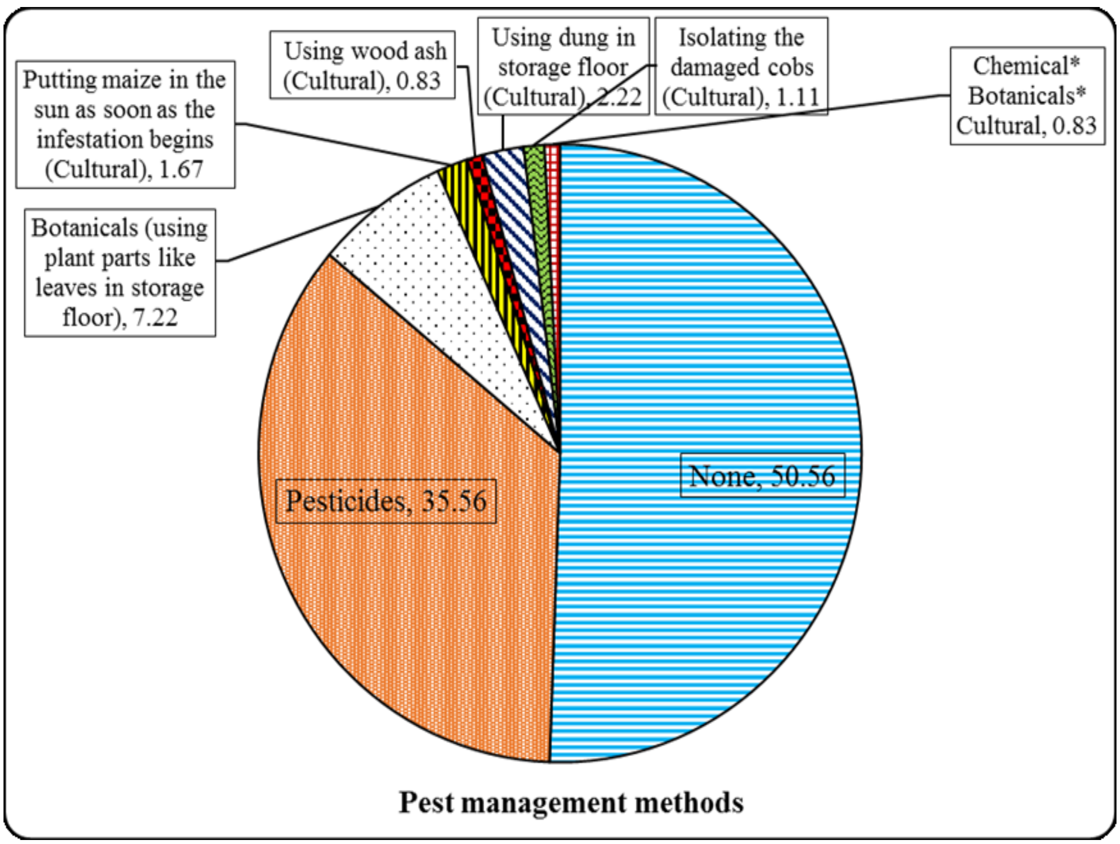


Figure 3. 7 Management practices used against insect pests of stored maize grain in the study areas

Effect of Farmers Management Practices on the Level of Infestation of Insect Pests across the storage periods of 3-9 months

According to the surveyed farmer's, the level of insect pests infestation varied with the different management methods used by farmers. Most of the farmers (84.72%) reported that their grain had low levels of infestation when they used a chemical control method in 3-6 months storage period. Most farmers also indicated that their grain had (≥ 80.33%) high level of insect infestation (≥ 80.33%) when no management measures are taken in both 3-6 and 7-9 months storage period. Besides, most farmers (> 78%) also reported that their grain had medium level of infestation using botanical and cultural methods in 3-6 months storage periods of maize. Furthermore, most farmers (> 77%) also reported that high level insect infestation was occurred when botanical and cultural methods were used in 7-9 months storage period of maize than chemicals in which the level of infestation was medium (82.61%) for the same period (Figure 3.8).

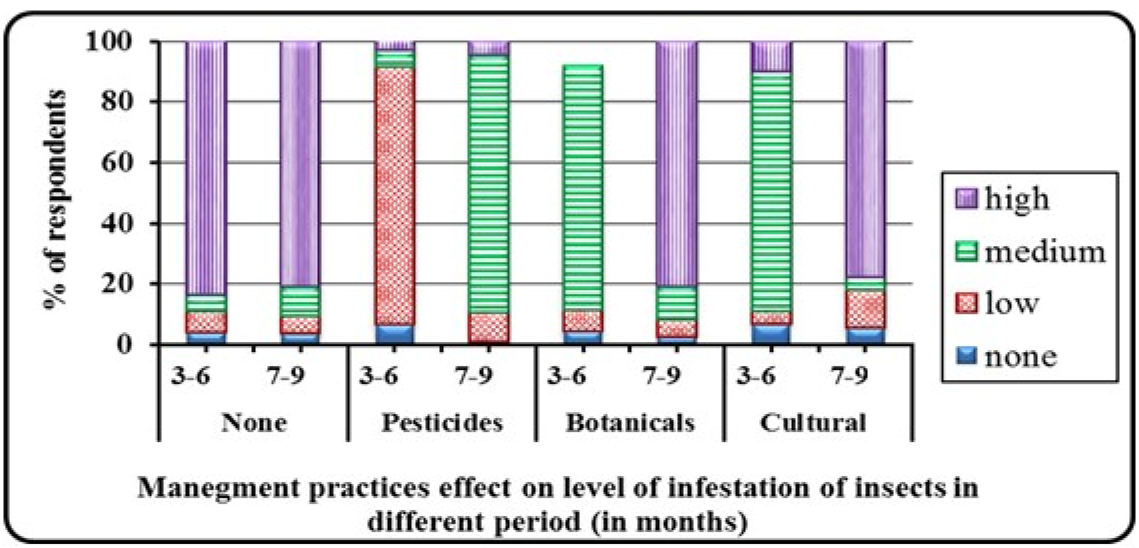


Figure 3. 8 Effect of farmer's management practices on the level of insect pest infestation of stored maize grain stored for different storage periods in the study area

3.4 Discussion

In the present study, farmers harvested maize in between November, and January and hand picking was the most commonly used harvesting method. Similarly, Gabriel and Hundie (2006) indicated that farmers did a dominant harvest of maize grain around November and December by removing the ears by hand at Hetosa, Ada and Bako of Oromiya Regional State.

The reason behind harvesting of maize by majority of farmers in usual or optimum time, which was in December, was fearing of insect infestation in the field and subsequent loss in storage that they experienced in past years. Tadesse (2003) recommended prompt harvesting as prevention method of stored maize grain insect pests. Some farmers harvested their grain earlier, in November at physiological maturity of the crop before the grain moisture content is reduced to the recommended moisture content, which is 12-13%. If such grains are taken to store before they dry to the required moisture content of 12-13%, they can be easily infected by fungi, the condition which also favor insect pests infestation, which is in agreement with Dick (1988), Golob *et al*. (2002), and Kumar and Kalita (2017). Few farmers harvested their grain later than optimum time, which was in January that could favor extended exposure of the grain to pest's infestation. Similarly, Sauer (1978) and Kaaya *et al*. (2005) indicated that delayed harvesting is not recommended, since it leads to long-term exposure of the crop to infestation and damage by insect pests, among others.

About 60% of the farmers mentioned that insect pests were major problem of stored maize grain under traditional storage condition in the present study, which is in accordance with Tadesse (1997 & 2003). Besides, the frequent occurrence of weevils and moths in storages of farmers among insect pests in current study is in line with finding of Emana (1993).

Farmers used different storage methods (facilities) and stored their maize either shelled or unshelled in the present study. This findings are in agreement with finding of earlier researchers (Emana, 1993; Tadesse, 2003; Gabriel and Hundie, 2006; Utono *et al*., 2013; Niamketchi *et al*., 2015). Being the most commonly used storage method of outdoor Gotera by farmers for storing and protecting their grains against insect pests, since it was ventilated outdoor than the rest of storages in the current study, is also in line with findings of previous researchers (Tadesse and Basedow 2005; Tadesse, 2003; Gabriel and Hundie 2006) in which Gotera was shown to be used by the large majority of farmers, among others.

About 82% of farmers stored their maize grains in the form of unshelled cobs with husks intact for protection of their grain against insect pests for 7-9 months as the husks and cobs provide better protection in the current study. This finding agrees with finding of Utono *et al*. (2013) in which a large proportion of respondents stored their grain un threshed than the threshed in Kebbi state of Nigeria. Niamketchi *et al*. (2015) also revealed that conservation in cobs is the most used form for the fact that cobs allow better conservation, of various forms of maize stored by farmers. However, this finding disagrees with result of Tadesse (2003), in which large proportion of respondents stored their grain shelled.

The majority of farmers stored their maize grain for about 7-9 months starting from December to January in the present study, which is in accordance with finding of previous scientists (Utono *et al*., 2013; Nukenine, 2010). Home consumption mentioned as the main purpose for storing their maize by most farmers, followed by a combination of home consumption and expecting better price in the present study, is in agreement with earlier scientists (Golob *et al*., 1999; Chakraverty *et al*., 2003; Utono *et al.*, 2013). Earlier researchers (Nukenine, 2010; Kasozi, 2013; Niamketchi *et al*., 2015) have also shown the variation in the level of insect pest's infestations both with periods of storage and forms of maize stored in the present study.

The present results have showed that unshelled form of maize with the husk intact stored for 7-9 months period had only medium levels of insect pest's infestation as the cobs covered with husks provide better protection than de husked & shelled form. This finding suggest that if maize is stored well in unshelled forms with the husk intact, it can be kept for 7-9 months with only medium levels of insect pests infestation developing. It also suggests that storage methods like Gotera outdoor in which maize was stored in unshelled form with husk intact were relatively better storage methods than sac storage methods to protect grains from insect pest's attack. It also suggests that maize stored in unshelled forms with husk intact in storage methods such as Gotera outdoor can be kept with medium level of insect pest's infestation for longer periods than 7-9 months, if some modification on outdoor Gotera will be applied by professionals to make it ventilated well. These findings are in accordance with the findings of Niamketchi *et al*. (2015) and Utono *et al*. (2013) in which conservation in cobs was indicated to be the most commonly stored form of maize for the fact that cobs allow better conservation against insect pests, of various forms of maize stored by farmers. Nukenine (2010) also reported that different factors such as the type of storage structures used, the duration of storage and the storage management implemented prior to, and during storage could influence storage losses. Tadesse (2003) also suggested that a little improvement in storage structures coupled with sound hygienic measures and other cultural practices can lead to significant reduction in storage loss.

Different pervious researchers (Emana, 1993; Tadesse and Basedow, 2005; Tadesse, 2003; Gabriel and Hundie, 2006; Mboya, 2011) have also indicted a variety of insect pest management methods used by farmers to protect and preserve their grain against insect pests in the present study. Being the most commonly used methods of no management measures, followed by application of pesticides in stored maize for protection of it against insect pests in the current study, is in agreement with the finding of Utono *et al*. (2013) in which the most frequently used methods of insect control were either nothing or the application of chemical pesticides. The variation of level of insect infestation with the different management methods used in the present study, is also agrees with finding of Utono *et al*. (2013).

The current result has also shown that high level of insect pest's infestations was faced by the majority of the farmers using all control tactics, except chemicals in 7-9 storage periods and when no insect control measures were used in both 3-6 and 7-9 months storage periods. This suggests the possibility of reduction of the grains that farmers can have (access) and hence, their food security. In accordance with this finding, Mboya (2011) shown that traditional storage methods reduced stability, promoted vulnerability and increased food insecurity of the farm households.

The fact that farmers encountered only medium level of insect pests infestation in maize stored for about 3-6 months periods, using botanicals and cultural control measures, which are safe, affordable and eco-friendly in the current study, implies these indigenous management methods had important contribution in protection of maize grains against insect pests in farmer's traditional storage's. It also implies as they could be possibly used as vital component of integrated pest management of insect pests of stored grains. Similarly, Utono *et al*. (2013) indicated that improvements to the main storage structure used by farmers and using locally available plant materials and cultural methods instead of chemicals could help to improve farmer's food security.

3.5 Conclusion

From the current study, it is possible to conclude that unshelled form of maize stored and outdoor Gotera storage method were relatively better for keeping maize with medium level of insect pests infestation for 7-9 months. Thus, it is better to use such storage form and method than others to reduce loss of maize grains by insect pests in the storage. This result also calls for the need for improving storage methods like outdoor Gotera to get better protection of stored maize grain against insect pests for longer period. Besides, indigenous management methods such as botanicals and cultural methods were also promising in the protection of stored maize grains against insect pests for about 3-6 months. Therefore, it is important to use these methods as benign component of IPM by determining standard rates to reduce a sole relines on synthetic pesticides.

In the present study, the level of insect pest's infestations were found to be high, despite the different traditional methods and practices in use by farmers so as to protect their stored maize grains from insect pests in about 7-9 months. Accordingly, the traditional methods and practices used by farmers were inefficient for providing adequate protection of their stored maize grains against insect pests. Therefore, it is important to design, and implement safe and effective management strategies to reduce loss of stored maize grains in the study areas. Besides, provision of training to farmers and extension workers on safe handling of grains and management of insect pests of stored maize grains under traditional storage conditions are urgently needed by any concerned bodies.

Chapter 4. Status and Species Composition of Stored Maize Grains Insect Pests in Sankura and Shashogo districts

4.1 Introduction

Maize is a major staple food and feed source for millions of people of Ethiopia (Sori and Ayana, 2012). In southern Ethiopia, in general and in Shashogo and Sankura districts of Hadiya and Silte zones, in particular, it is one of the major cereal crop grown for food, feed, firewood and construction (Gemu *et al*., 2013). However, insect pests are among the most economically important biotic constraints in its production and storage (Tefera *et al*., 2010 & 2011). The most important of these insect pests in the field and storage are lepidopterous stalk borers and Coleopterous weevils, respectively (Emana and Tesdeke, 1999; Demissie *et al*., 2008). Besides, the level of infestation and amount of damage caused by insect pests was found to be influenced by many factors, including storage system, the grain type, length of time stored and pest management practices (Utono *et al*., 2013).

Farmer's perceptions of pests and their control may provide essential data for designing pest management strategy (Obeng-Ofori, 2011). It is therefore, these areas stored maize very crucial to design any management strategies to minimize loss stored maize grain and require great attention by researchers and any concerned bodies. Besides, prior to this study no one had attempted to investigate species composition, relative abundance, level of infestation insect pests, as well as associated grain damage and losses of stored maize in study area. Thus, the current study was initiated with the following objectives:

1) To determine the species composition, abundance, distribution and status of insect pests of stored maize grain under farmer's traditional storage conditions

2) To determine the level of infestation by major insect pests, their associated damage and losses of stored maize in four different traditional storage methods over 3-9 months storage period

4.2 Materials and Methods

Description of the study area: the same study site described in chapter 3 was also used.

Study Design and Sampling Protocol: One kilogram of maize grain was sampled from each stores (a total of 288 stores were considered) using a nested design, such that Districts were nested under Zones and Kebeles were nested under Districts, and sampling stores were nested under Kebeles. Zones and Districts were purposively selected, while Kebeles and sampling stores were selected randomly (Emana, 1993). The samples were taken from top, sides, center and bottom of the storage structures using different sampling tools such as sampling spear, sampling scoop and human hands among others. Samples taken from different positions of the stores were thoroughly mixed and 1 kg was taken as a final working sample. Semi structured questionnaire was issued to each store owner during sampling. Surveys were conducted from December 2013 to August 2015 starting from three months after storage up to nine months at three months interval.

Collected samples were both in the form of cobs and shelled grains from four different farmers traditional storages such as Gotera outdoor, Polyethylene sac, Store room (Elifign) and Living house including Gotera indoor and Kot. The later was from sac storage, whereas the former was from other storage methods. In the case of grain stored on cobs 3-5 cobs from each sections of the store were taken as sample and manually shelled. After shelling they were thoroughly mixed and 1 kg final sample were obtained. In cases where storage methods had shelled forms, grain samples were taken by mixing thoroughly those that taken from different parts of the Sac storages using grain sampling tools. Besides, the samples were collected two times in the middle and end of each storage seasons or periods, namely 3, 6 and 9 months from the aforementioned farmer's traditional methods (facilities).

Each sample at each sampling date and months from each of four storage methods at each villages of the sampling site was collected in sampling bag, labeled with necessary information and kept for further identification of insect pests, and to estimate grain damage and weight loss using count and weight method. Selection of the sampling site and storage methods were made in such a way that they are the representative of each kebeles of the two districts at random. The samples at each sampling date and month from each of four different farmers' traditional storage structures were sub sampled further after thoroughly mixing them to come up with a standard of 100 g sample.

Species compositions, abundance and status of insect pests: Samples collected from four different representative farmers' traditional storage of the study areas at each sampling day and month were taken to Insect Science Laboratory of Addis Ababa University and sieved to collect the insects present. Sieves of different size (mm) were used for separating the adult insects from the sample grains. The average of live and dead insects from samples in each storage periods (3, 6 and 9 months) from each of the four farmers traditional methods were collected and immediately preserved in 100 ml capacity bottles and kept for further identification. The subsampled grains were also putted in 1 L glass jars and kept under laboratory conditions (27 ± 3 °C and 55 - 70% RH) to determine species from internal infestation. The procedures and keys of the books related with stored product insect pests and other arthropods by different authors (Hagstrum and Subramanyam, 2006; 2009; Rees, 2004; 2007; FGIS, 2015) were used for identification purpose. Besides, keys and pictures from on line available literatures were also used for identification. Then after, insects were sorted according to their orders, families and species, and counted for each subsample grains from each of four different farmer's traditional storage methods over 3-9 months periods, in each case noting the number. The average of the sum total of species of arthropods (insect pests) collected from sub-sampled grains from all of the four farmer's traditional storage methods over 3-9 months periods were used to determine the species composition and the importance (status) of insect pests.

For assessing insect pest's infestations, the main variables have been included abundance, relative abundance and Constance (frequency) of species found in samples as suggested by Bueno (1991). Abundance refers to the total number individuals of a species divided by the total number of samples (in this case the total number of kilograms) and it is expressed by the following formula:

$$\text{Abundance of species} = \frac{\text{Total number of individuals of species}}{\text{Total number of samples}}$$

The relative abundance of species is expressed by the percentage of individuals of the species in a total number of observed individuals as shown in the following formula:

$$\text{Relative Abundance of species} = \frac{\text{Number of individuals of a species}}{\text{Total number of observed individuals}} \times 100$$

Constance (frequency) expresses the percentage of species occurrence. It is obtained by the relationship between the number of samples containing the species and the total number of samples. The following formula is expressing this relationship.

$$\text{Constance of species} = \frac{\text{Number of samples in which the species occurred}}{\text{Total number of samples}} \times 100$$

Assessment of Level of Infestation, Damage and Losses

Assessment of Level of Infestation of Major Insect Pests: This was done as indicated before in assessment of species compositions, abundance, distribution and status part. However, the average of the sum total of species of insect pests in each of four storage methods over three to nine storage periods were considered in this case.

Percent Grain Damage and Weight Loss Assessment: One hundred seeds were randomly taken from each maize grains sub-samples and counted as damaged and undamaged and their weight was taken. The percentages of insect damaged grains were then calculated following similar methods by Sori and Ayana (2012) as follows:

$$\text{Percent of insect damaged grains (\%)} = \frac{\text{Number of insect damaged grains}}{\text{Total number of grains}} \times 100$$

Percent weight loss was also determined using a gravimetric method or count and weight method (FAO, 1985; Boxall, 1986; Adams and Schulter, 1978). This method was chosen because, losses were determined or estimated in a rural area of the study site where different varieties of grain were grown under different conditions, such as with or without fertilizer, or on poor or good soils, and that may affect the size of grains and, consequently, the volume/weight ratio. Application of insecticide dusts may also affect the settling of the grains in the standard volume and increase the volume occupied by the grain. Because of these various conditions, a distinct baseline may have to be determined for each individual storage situations. This is often difficult to achieve, and consequently, volumetric method is not recommended to estimate damage and loss in the such different cases (back grounds) in which grains are grown by farmers (Harris and Lindblad, 1978).

According to Boxall (1986) again, not to receive false results, damaged maize kernels were grouped according to size before weighing in comparison to undamaged kernels and relatively large sized grains were taken. A triple beam balance with precision of 5 grams was used in weighting the maize grain. Besides, to overcome the problem caused by hidden infestation, undamaged grains that were separated from severely attacked grains superficially through naked eye and hand lens were also kept for 3-7 days before a second assessment, for the emergence of hidden infestation as indicted by Ratnadass and Fleurat-Lessard (1991). Then, the percentage of weight loss of maize grains due to insect pests was calculated using a gravimetric or count and weight method (FAO, 1985; Boxall, 1986, Emana, 1993; Gebre-Selase and Emana, 2009; Zewde and Jembere, 2010) as follow:

$$\% \text{ Loss in weight} = \frac{UNd - DNu}{U(Nd + Nu)} \times 100$$

Where U = weight of undamaged grain, D = weight of damaged grain, Nd = number of damaged grain and Nu = number of undamaged grain.

Assessment of Germination Loss: Maize grain germination test was conducted using standard procedures (ISTA 1996). From each damaged and undamaged grain of 100 g subsamples, 20 grains were taken randomly and placed in petri dishes lined with filter paper, moistened with distilled water of about 4 ml and then putted in a germination cabinet in four replications in a completely randomized design for about 5 to 7 days to determine the germination percent at room temperature. Percent germination was calculated following Dubale *et al*. (2012) method as follow:

$$\text{Germination percent (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Data Analysis: The data collected for this study was complemented by the Microsoft Excel package 2013 and analyzed using the Statistical Program for Social Sciences (SPSS) version 16. Descriptive statistics (means and percentage) were used to investigate species compositions, abundance, status, their relative abundance and distribution (frequency of occurrence) of insect pests of stored maize. Besides, data on level of infestation of major insect pests, the associated damage and losses per 100 g of sub-sampled maize grains over three storage periods (3, 6 and 9 months) were performed by Univariate analysis or two-way analysis of variances (ANOVA). Significant differences between the means were separated using Tukey's honestly significant difference (THSD) test. Difference among means were stated significant when $p < 0.05$ and highly significant when $p < 0.01$. Standard errors (±se) are given following means in tables.

4.3 Results

Species compositions, abundance and status of arthropod pests associated to stored maize grains

Tables 4.1, 4.2, 4.3 and 4.4 demonstrated different categories of insects and mites recorded on maize grain stored for different storage periods in different storage structures. Accordingly, thirty arthropod species consisting of nineteen primary and secondary pests, seven mold feeders and five natural enemies (predators and parasitoids) were recorded. Of the primary and secondary pests, 84.21% were from the order Coleopteran in five families. These include *S. zeamais* (Motschulsky) and *S. oryzae* (Linnaeus) from the family of Curculionidae; *T. castaneum* (Herbst), *T. confusum* (Jacquelin duVal), *T. destructor* (Uyttenb.), *P. subdepressus* (Wollaston), *P. ratzeburgi* (Wissmann) and *G. cornutus* (Fabricius) from Tenebrionidae; *C. ferrugineus* (Stephens) and *C. pusillus* (Schonherr) from Cucujidae; *R. dominica* (Fabricius) from Bostrichidae and *Oryzaephilus surinamensis* (Linnaeus) from Silvanidae. However, only three species (15.79%) were from order Lepidopteran (moths) in two families such as *Sitotroga cerealella* (Olivier) from Gelechiidiae and *Ephestia cautella* (Walker) and *Plodia interpunctella* (Hubner) from Pyralidae, while only one species, *Acarus siro* (Linnaeus) (5.26%) was from the family of Acaridae of order Acarina (Table 4.2).

Besides, among primary and secondary pests identified from farmers traditional storage methods, eight species such as *S. zeamais*, *S. cerealella*, *S. oryzae*, *T. castaneum*, *T. confusum*, *C. ferrugineus, C. pusillus* and *R. dominica*, respectively were found to be the most abundant as they appeared between 15.58 and 60 individuals per 100 g of sampled grains. They were also found to be the most frequently occurring as they occurred in the range between 96.95 and 55.40% per 100 g of the sampled grains and were major pests. Following these species, eight species such as *O. surinamensis*, *T. destructor*, *P.* subdepressus*, P. ratzeburgii*, *E. cautella*, *Acarus siro*, *P. interpunctella* and *G. cornutus*, respectively were found to be the next abundant as they appeared in between 7.09 and 4.01 individuals per 100 g of sampled grains. They were also found to be next frequently occurring as they occurred in between 38.09% & 17.01% per 100 g of the sampled grains and were intermediate insect pests. However, *T. audax, C. pusilloides* and *P. truncates* were found to be least abundant as they occurred in < 1 individuals per 100 g of the sampled grains (in average), least frequently appearing as they appeared in < 11% per 100 g of sampled grain and were minor pests (Tables 4.1).

Generally, in terms of abundance, relative abundance, frequency of occurrence and status (economic importance), the 19 primary and secondary pests were found to be in the following orders, i.e., *S. zeamais* > *S. cerealella* > *S. oryzae* > *T. castaneum* > *T. confusum* > *C. ferrugineus* > *C. pusillus* > *R. dominica* > *O. surinamensis* > *T. destructor* > *P. subdepressus* > *P. ratzeburgii* > *E. cautella* > *Acarus siro* > *P. interpunctella* > *G. cornutus* > *T. audax* > *C. pusilloides* > *P. truncates* (Tables 4.1).

From seven species of mold feeders identified and recorded, *Carpophilus dimidiatus* was the most commonly occurring, as they appeared 58.86% per 100 g of the sampled grains well as the most important as they appeared in 38.46% of all mold feeders recorded, followed by *Liposcelis* species and *L. entomophila* which were occurred in 45.01% and 31.16% per 100 g of the sampled grains as well as in 31.83% and 16.97% among all mold feeders recorded, respectively. However, *Typhaea stercorea*, *C. freeman*, *C. hemipterus* and *Brachypeplus* sp. were found to be the least commonly occurring as they appeared in between 6.93% & 13.85% per 100 g sampled grains and the least important as they occurred in < 5% of all mold feeders recorded (Table 4.3). Of five species of natural enemies recorded, predatory mite; *Cheyletus* sp., two species of hymenopteran parasitic wasps; *Anisopteromalus calandrae* and *Cephalonomia tarsalis* and coleopteran Staphylinidea were the most commonly occurring as they appeared in between 19.60% & 30.15% per 100 g of sampled grains. However, *Dactylosternum abdominale* was rarely appeared in terms of frequency occurrence as they occurred < 3 percentage per 100 g of sampled grains (Table 4.4).

Table 4.1 Species compositions, abundance and status of primary and secondary pests associated to Stored Maize grains over 3-9 Months Period in the Study Areas

Species of pests	Total number of adult insects	Abundance (average no. insects/ 100 g of grain sample)	Relative abundance (%)	Frequency (% of samples containing each species)	status
Sitophilus zeamais	1733	60.00	20.48	96.95	major
Sitotroga cerealella	1188	41.13	14.04	93.49	major
Sitophilus oryzae	1013	35.08	11.97	90.03	major
Tribolium castaneum	850	29.43	10.05	86.57	major
Tribolium confusum	760	26.32	8.98	83.10	major
Cryptolestes ferrugineus	690	23.89	8.16	65.79	major
Cryptolestes pusillus	536	18.56	6.34	58.86	major
Rhyzopertha dominica	450	15.58	5.32	55.40	major
Oryzaephilus surinamensis	205	7.10	2.42	38.09	inter
Tribolium destructor	188	6.51	2.22	34.63	inter
P. subdepressus	174	6.02	2.06	31.16	inter
P. ratzeburgii	162	5.60	1.91	27.70	inter
Ephestia cautella	123	4.26	1.45	24.24	inter
Plodia interpunctella	119	4.12	1.41	20.78	inter
Gnatocerus cornutus	116	4.02	1.38	17.31	inter
Tribolium audax	13	0.45	0.15	10.39	minor
Cryptolestes pusilloides	10	0.35	0.12	10.39	minor
Prostephanus truncatus	8	0.28	0.09	6.92	minor
Acarus siro	122	4.22	1.44	24.24	inter
Total	8460				

Inter= intermediate

Table 4. 2 Taxonomic position and common name of primary and secondary pests identified in stored maize grains in 3-9 months storage period in the study areas

Scientific name of pest species	**Order**	**Families**	**Common name**	**Pest type**
Sitophilus zeamais	Coleoptera	Curculionidae	Maize weevil	Primary
Sitotroga cerealella	Lepidoptera	Gelechiidiae	Angoumois grain moth	Primary
Sitophilus oryzae	Coleoptera	Curculionidae	Rice weevil	Primary
Tribolium castaneum	Coleoptera	Tenebrionidae	Rust red flour beetle	Secondary
Tribolium confusum	Coleoptera	Tenebrionidae	Confused flour beetle	Secondary
Cryptolestes ferrugineus	Coleoptera	Cucujidae	Flat grain beetles	Secondary
Cryptolestes pusillus	Coleoptera	Cucujidae	Merchant grain beetles	Secondary
Rhyzopertha dominica	Coleoptera	Bostrichidae	Lesser grain borer	Primary
Oryzaephilus surinamensis	Coleoptera	Silvanidae	Saw toothed grain beetle	Secondary
Tribolium destructor	Coleoptera	Tenebrionidae	False black flour beetle	Secondary
P. subdepressus	Coleoptera	Tenebrionidae	Depressed flour beetle	Secondary
P. ratzeburgii	Coleoptera	Tenebrionidae	Small eyed flour beetle	Secondary
Ephestia cautella	Lepidoptera	Pyralidae	Tropical warehouse moth	Secondary
Plodia interpunctella	Lepidoptera	Pyralidae	Indian meal moth	Secondary
Gnatocerus cornutus	Coleoptera	Tenebrionidae	Broad horned flour beetle	Secondary
Tribolium audax	Coleoptera	Tenebrionidae	American black flour beetle	Secondary
Cryptolestes pusilloides	Coleoptera	Cucujidae	Lined flat bark beetle	Secondary
Prostephanus truncatus	Coleoptera	Bostrichidae	Larger grain borer	Primary
Acarus siro	Acarina	Acaridae	Grain or flour mite	Secondary

Inter= intermediate

Table 4. 3 Species composition and status of mold feeder insect pests recorded in stored maize grains in 3-9 months storage period in the study areas

Species of mold feeder insect pests	Total number of adult insects	Abundance (average no. insects/ 100 g of grain sample)	Relative abundance (%)	Frequency (% of samples containing each species)	status
Typhaea stercorea	5	0.17	1.33	6.93	minor
Carpophilus freemani	16	0.55	4.24	13.85	minor
C. dimidiatus	145	5.02	38.46	58.86	major
C. hemipterus	13	0.45	3.45	10.38	minor
Liposcelis sp.	120	4.15	31.83	45.01	inter
Liposcelis entomophila	64	2.22	16.97	31.16	inter
Brachypeplus sp.	14	0.48	3.71	13.85	minor
Total	377				

Inter= intermediate

Table 4. 4 Species composition and status of natural enemies (predators and parasitoids) recorded in stored maize grains in 3-9 months storage period in the study areas

Species of natural enemies	Total number of adult insects	Abundance (average no. insects/ 100 g of sample)	Relative abundance (%)	Frequency (% of samples containing each species)	status
Cheyletus sp.	39	1.35	19.60	20.78	inter
Anisopteromalus calandrae	60	2.08	30.15	31.16	inter
Cephalonomia tarsalis	51	1.77	25.63	24.24	inter
Staphylinidae	45	1.56	22.61	27.70	inter
Dactylosternum abdominale	4	0.14	2.01	6.93	Un common
Total	199				

Inter= intermediate

Mean Level of Insect Pests Infestation, and the associated percent Grain Damage, Weight Loss and Germination Loss of Stored Maize in Four different traditional Storage Methods in the Study Area

Significantly ($p < 0.05$) higher mean level of insect pests infestations (48-66 and 84-134), and the associated higher percentage grain damages (58.75 and 72.25%), weight losses (42.65 and 58.04) and germination losses (60 and 84%) of stored maize grain were recorded in all tested traditional storage methods tested in this study in 6 and 9 months period, respectively than in 3 months period in general. Among these higher mean level of infestations, and the associated percentage grain damages and losses, relatively the highest were recorded from Polyethylene sac, followed by storages in the living house and Store room/Ellipign, while relatively the minimums were from the Gotera outdoor, when each of the four storage structures were compared (Figure 4.1 and Table 4.5).

However, significantly ($p < 0.05$) lower mean level of insect pests infestations, and the associated percentage grain damages, weight losses and germination losses were recorded in 3 months period in all of the tested traditional storage methods in general. Mean level of insect pest's infestations, and the associated percentage grain damages, weight losses and germination losses were also found to be increased ($p < 0.05$) significantly as periods of the storage increased from 3 to 6 and 9 months period in all of the tested traditional storage methods in this study. The highest of these were recorded from 9 months period, followed by that of 6 months period in all of the tested traditional storage methods (Figure 4.1 and Table 4.5).

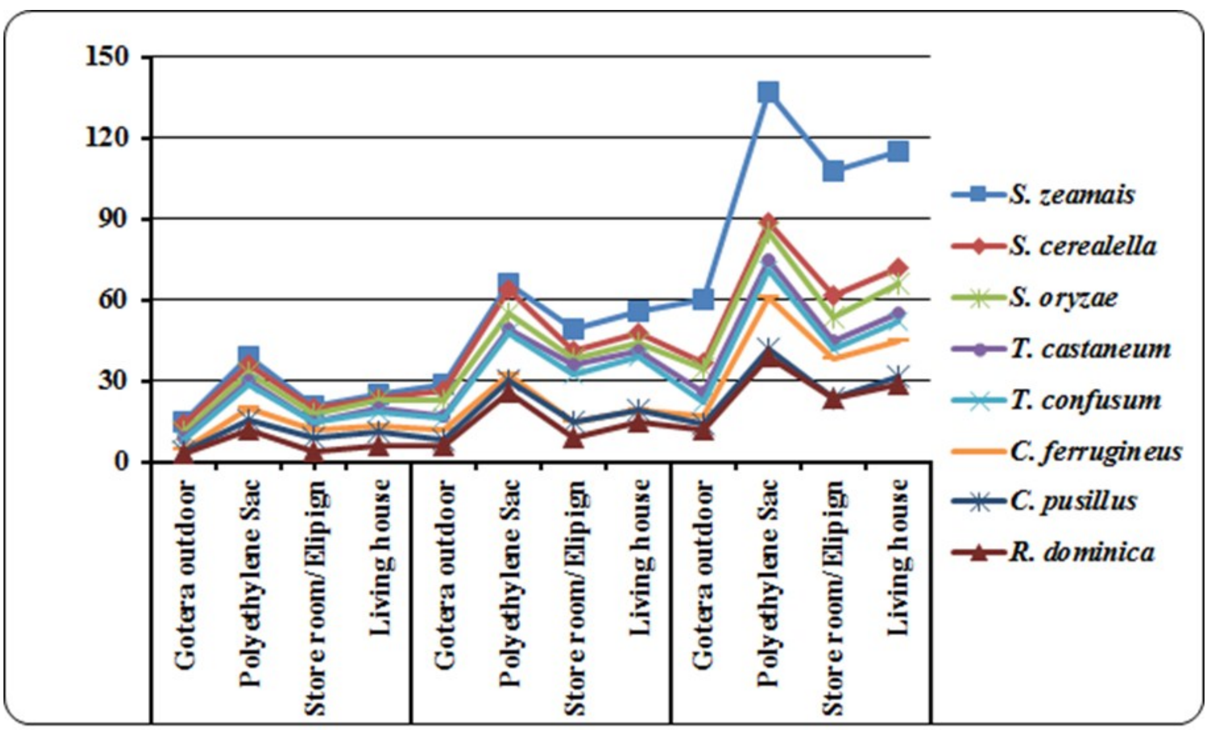


Figure 4. 1 Mean of level of major insect pest's infestation of stored maize in four different farmer's traditional storage methods over 3-9 months storage period

Table 4. 5 Mean percent grain damage, weight and germination loss in four different farmer's traditional storage methods over three storage periods in the study area

Parameters tested	Periods of storage in months	Storage methods			
		Gotera outdoor	Polyethylene Sac	Store room/Elipign	Living house (including Gotera indoor and Kot)
% grain damage	3	3.50±0.29Dc	11.75±0.25Ac	6.50±0.29Cc	10.75±0.25Bc
	6	36.50±0.29Db	58.75±0.25Ab	49.00±0.91Cb	52.75±0.25Bb
	9	45.75±0.48Da	72.25±0.25Aa	58.50±0.29Ca	63.75±0.25Ba
% weight loss	3	1.75±0.41Dc	5.86±0.58Ac	3.25±1.15Cc	5.38±0. 91Bc
	6	26.50±0.41Db	42.65±0.91Ab	35.58±0.58Cb	38.30±0.58Bb
	9	36.75±0.41Da	58.04±0.58Aa	46.35±0.91Ca	50.46±0.63Ba
% germination	3	95.13±0.55Aa	85.00±0.20Da	92.00±0.46Ba	90.00±0.91Ca
	6	55.00±0.20Ab	40.00±0.65Db	50.00±0.41Bb	47.00±0.46Cb
	9	30.00±0.20Ac	16.00±0.58Dc	25.00±0.74Bc	22.00±0.41Cc

Means followed by the different letters within row (upper case letters) and within columns (lower case letters) are significantly different, $p < 0.05\%$ using Turkey's studentized range test (HSD).

4.4 Discussion

Most of the species identified and recorded in the present study were from the order of Coleoptera (beetles). This finding suggests that actual great importance of beetles in grain storages than moths and mites. Similarly, Hill and Waller (1990) cited in Chimoya and

Abdullahi (2011) shown that pests that devastate stored cereals are insects of the order Coleopteran. Upadhyay and Ahmad (2011) also indicated that beetles are more diversified and highly destructive than moths, among post-harvest pests.

The fact that over thirty arthropods associated stored maize were identified & recorded from only six kebeles or localities of the study area in the current study suggestes the conduciveness of the hot and humid environment of the study site similarly to other major maize producing areas of this country, which enable them to proliferate well. Similarly, Tadesse (1997) also recorded 37 species of arthropods associated with stored maize grain in western part of Ethiopia. Emana (1993) also recorded nine insect species belonging to four orders and seven families on stored maize grain in Sidama administrative region. Sori and Ayana (2012) also recorded seventeen arthropod species on stored maize grain in Jimma zone.

Sitophilus zeamais, *S. cerealella*, *S. oryzae*, *T. castaneum*, *T. confusum*, *C. ferrugineus*, *C. pusillus* and *R. dominica*, respectively were found to be the most abundant, frequently occurring and major insect pests, followed by *O. surinamensis*, *T. destructor*, *P. subdepressus*, *P. ratzeburgi*, *E. cautella*, *Acarus siro*, *P. interpunctella* and *G. cornutus*, respectively in the present study. This justifies the actual knowledge of ecology and high economic importance of these insect pests, which is in line different previous scientists (Haines, 1991; Rees, 2007; Hagstrum and Subramanyam, 2009) who indicated that as these pests are cosmopolitan pests in the world. Similarly, Tadesse (1995) also recorded 37 species of arthropods associated with stored maize grain in Ethiopia and of them, he indicated that the Coleopteran *Sitophilu*s sp., *Tribolium* sp., *Cryptolestes* sp., and *Carpophilus* sp. and Lepidopteran *Sitotroga cerealella*, *Plodia interpunctella* and *Ephestia* *cautella* were widespread and common. Emana (1993) also shown that maize in storage has been attacked by numerous Coleopterous and Lepidopterous insect pests. However, this finding disagrees with the finding of Sori and Ayana (2012) in which *T. castaneum*, *C. pusillus* and *R. dominica* were indicated to be intermediate pests, while *C. ferrugineus* and *O. surinamensis* were shown as minor pests.

In this study, *T. audax*, *C. pusilloides* and *P. truncates* were the less abundant, less frequently occurring as well as they were minor insect pests in farmer's traditional storage methods of the study areas suggests the less establishment and the less economic importance of these pests in stored maize grain in the study area for the reason that is not known and needs further research. It might probably be associated with their newness to the area, since these insect pests were recorded probably for the first time in south central Ethiopia, in particular and Ethiopia, in general. In addition to these three species, *P. subdepressus* from primary and secondary pests, *L. entomophila* among mold feeders and *Cephalonomia tarsalis* among natural enemies were also found to be probably new records accordingly to the review Tadesse (2008), in which various insect pests identified & recorded by pervious researchers in Ethiopia were listed. Others like *C. pusillus* and *T. destructor* from pests and *Liposcelis* species among mold feeders that were reported to be uncommon by previous researchers in Ethiopia (Tadesse, 2008) were also confirmed to be commonly occurring and important in the present study.

The fact that the majority of species (fourteen) were secondary pests, five were primary pests and seven species were mold feeder in terms of the type of insect pests, out of twenty six species of pests identified confirms the actual knowledge of their difference in feeding behavior. It also suggests that the maize grains probably might be damaged during harvesting and transportation. Besides, it suggests poor handling practices might be used by farmers and/or the grain might probably be infested with primary pests prior to or during storage, so that it enables secondary pests to dominate. In similar manner, Navarajan Paul (2007) and Rees (2007) indicated that the insect pests of stored grains can be grouped as primary and secondary pests, whereby the former ones can attack sound grains and the later ones require the grains to be damaged to pose attack. Mason and McDonough (2012) also revealed that insect pests of stored grains can be categorized as primary pests, secondary pests and mold feeders, where by the former's two attack grain directly through feeding, while the later ones do not affect the grain directly by feeding, instead, contaminate the grain mass through their presence and their metabolic activity.

The aforementioned findings along with the occurrence of four species of secondary feeders beyond *R. dominica* (primary feeder) in the current study also suggested that great economic importance of secondary pests as that of primary pests. Similarly, Semple *et al*. (2011) indicated that primary pests does not necessarily refers to more importance the pests, but simply implies to the dynamic processes involved whereby secondary pests can cohabitate (follow) primary pests and inflict (cause) serious and economic losses, especially under long-term storage.

The categorization of arthropods in to nineteen primary and secondary pests, five natural enemies species and seven mold feeders species in the present study, is in agreement with the works or reports of previous scientists (Hagstrum and Subramanyam, 2009; Rees, 2004; 2007).

In accordance with species categorized under natural enemy in the present study, *Cheyletus* sp. (like *Cheyletus eruditus*) was indicated to be a predator of all stages of mites, as well as eggs and larvae of moths and book lice, while, *Anisopteromalus calandrae* was shown to be larval parasitoid of weevils and beetles (Reichmuth, 2000; Hagstrum and Subramanyam, 2006) and moths: *Sitotroga* as well (Rees, 2004). Besides, *Cephalonomia tarsalis* was indicated to be a common larval parasitoid of the grain beetles (like *Oryzaephilus*, and *Cryptolestes*) and weevils (like *Sitophilus*) (Rees, 2004; Hagstrum and Subramanyam, 2006; FGIS, 2015). With respect to Staphylinidae, there is no clear cut information on their feeding behavior as pests, except that they have been shown to be associated to stored grains and their storage facilities, but certain species of them were clearly indicated to be predatory natural enemies of stored grain insect pests and mites (Hagstrum and Subramanyam, 2009; Kumar, 2017). Moreover, *D. abdominale* was shown to be a predatory natural enemy of stored product insects (Hagstrum and Subramanyam, 2006; 2009).

Five species out of 31 arthropods recorded were natural enemies, and of which four (*Cheyletus* species, *Anisopteromalus calandrae* and *Cephalonomia tarsalis* and coleopteran Staphylinidea) were occurring commonly in the current study, suggests the possibility of using these natural enemies (predators & parasitoids) as safe pest management alternative to synthetic chemicals in storage ecosystem. Similarly, Tadesse (2003) shown that the existence of considerable individuals of parasitoids and predators in stored maize were indications for the possibility of their use in stored grain insect pest management.

The facts that seven species of mold feeders were found in this study also revealed that the grains were heavily infested. This finding is in line with findings of Mason and McDonough (2012) in which the presence of mold feeders in grain mass shown as indication for grains are going out of condition and as some mold growth has occurred on it.

The higher mean level of major insect pests infestations, and the associated percent grain damages, weight losses and germination losses of stored maize recorded from all traditional storage methods tested in the present study in 6 and 9 months period than in 3 months period confirms the inefficacy of traditional storage methods used in the study areas in protection of grains against insect pests after 3 months storage period. It also indicates the conduciveness of climatic condition of the study area like that of other tropical areas. In accordance with this finding, Nukenine (2010) shown that the type of storages, the duration of storage and the storage management implemented prior to, and during storage affect level of insect infestation and the associated losses. Tefera *et al*. (2011) also shown that farmers of Africa in general and Ethiopia in particular use traditional granaries to store their grains, which are not effective against storage pests. Talukder (1995) also indicated that together with the use of inappropriate storage facilities, tropical climatic conditions and poor sanitation of grain storage in SSA also encourage insect pest attack.

Among the higher mean level of major insect's infestations, and the associated percent grain damages, weight losses and germination losses, relatively the maximums were recorded from Polyethylene sac, followed by storages in the living house and Store room/Elipign, while relatively the minimums were from the Gotera outdoor in 6 and 9 months period in the present study. This finding suggests as variation in temperature and relative humidity in different traditional storage methods could influence the level of insect pest's infestation in different storage methods. Similarly, Manandhar (2001) indicated that high humidity coupled with high temperature allow insects and diseases to establish quickly. Emana (1993) also indicated that the lowest number of *S. Cerealella* and percent grain damage were recorded at outdoor Gotera, while farmers who installed storage structures inside the house had heavy infestation.

The higher level of insect infestations, and the associated percentage grain damages, weight losses and germination losses that recorded from all traditional storages methods tested in the present study also confirms that the quantity, quality, resources, labor and food security of poor farmers was affected in the study site. In agreement with this finding, FAO (1994) indicated that losses of food and/or food grains even as low as 5%, should not be ignored, because such physical losses are usually accompanied by qualitative losses, which affect the whole mass of the grain. Mailafiya *et al*. (2014) also revealed that grain losses of even 1% in the various agro-ecological zones can deprive people of constant supply of quality food (adequate nourishment year round), and income generation and their means of livelihood.

4.5 Conclusion

From the present study, over thirty species of arthropods associated to stored maize grain were recorded & of which, nineteen were primary and secondary pests, seven were mold feeders and five were natural enemies. Among the primary and secondary pests, *S. zeamais*, *S.cerealella*, *S. oryzae*, *T.castaneum*, *T. confusum*, *C. ferrugineus*, *C. pusillus* and *R. dominica* were the most abundant, the most frequently occurring and the most important (major) insect pests that affect long term storability of maize grains under traditional farmers storage methods in the study area.

Most of the species identified in the current study were beetles with dominance of secondary pests than primary pests and mold feeders. Thus, beetles were the most damaging ones than moths and mites in the study area. Outdoor Gotera was also found to be relatively good storage method, among the traditional storage methods tested in this study. The higher infestation of aforementioned insect pests had led to considerable grain damage of up to 72.25 and weight loss up to 58.04, as well as loss of quality through contamination in maize grains stored for about 9 months period. Thus, ultimately food security of resource poor farmers was threatened due to insect pests in the study area.

Accordingly, storage insect pests were found to be the most economically important constraints of storing and utilizing maize grains by farmers in the study areas of Hadiya and Silte zones, respectively. Thus, it is important to design and implement safe and effective management strategies to reduce loss of maize grains and hence food insecurity of poor farmers in the study area. Besides, improvement of the existing farmer's storage facilities, as well as provision of training to farmers and extension workers on safe handling of grains as well as management practices of insect pests of stored maize grains is urgently needed by any concerned bodies.

Chapter 5. Studies on the Efficacy of *Calpurnia aurea* (Ait.) Benth and *Millettia ferruginea* (Hochst) Baker leaves in the management of *S. zeamais* Under Laboratory Condition

5.1 Introduction

Maize is the major staple food in Africa contributing significantly to the agricultural sector (Tefera *et al*, 2011). It is one of the most important grain staples for agricultural income and caloric intake in SSA, including Ethiopia (Jones *et al*., 2011). However, during storage, it is heavily attacked by various insect pests, of which the maize weevil, *Sitophilus zeamais* (Motsch.) is the most economically important. Storage insect pests, in general and *S. zeamais*, in particular have been reported to be responsible for loss ranging from 30% to 90 % in Ethiopia (Emana, 1993) and 40%-100% in Malawi (Denning *et al*., 2009). Thus, maize weevil among others has been recognized as an increasingly important problem in Africa (Markham *et al*., 1994). However, management of this insect pest has been heavily relied on the use of synthetic insecticides, which led to resistance development, environmental and health problems (Ofuya and Longe, 2009). As a result, the search for the development of safe, affordable and ecologically sound management alternatives such as botanicals is crucial.

Millettia ferruginea and *Calpurnia aurea* plants may have protective role of stored maize against weevils. The former one is a large shady tree, which grows up to a length of 35 m, and is endemic to Ethiopia and widely grown at the elevation between 1,000 and 2,500 m above sea level (Jembere, 2002; Emana, 2014). It has been commonly used in traditional medicine. The roots and seeds of this plant are also used as insecticides and pesticides in many parts of the world, and rotenone are responsible for their toxicity (MacLachlan, 2001 cited in Ameha, 2004). *Calpurnia aurea* is a small, multi-stemmed tree, 3-4 m tall plant. It is widely distributed in Ethiopia. It is widely grown in high land areas (Birhanu and Asale, 2015) and is easily cultivated (Germishuizen and Meyer, 2003). The plant has been commonly used in traditional medicine (Fisseha *et al*., 2009; Tilahun and Mirutse, 2007 cited in Alemu *et al*., 2013). Its leaves and powdered roots are used to destroy lice, and to relieve itches and contain terpenoids, saponins, tannins, flavonoids, steroids, glycosides as well as alkaloids (Nega *et al*., 2016). Hence, the current study was conducted with the hope that the chemicals present in these two plants can have toxicity effect on *S. zeamais*. The objectives of this study, thus is to evaluate the toxicity of leaf powder and solvent extracts of *C. aurea* and *M. ferruginea* against *S. zeamais* under laboratory condition

5.2 Materials and Methods

The study was conducted between 1 October and 30 June of 2016/2017.

The Test insect's culture: *Sitophilus zeamais* adults were collected from maize stored in various farmers' traditional storage facilities of the study (survey) site (Figure 3.1) and brought to the laboratory of Insect Science Stream of the Zoological Science Department of Addis Ababa University. Then, the test insects were cultured at 27 ± 3 °C and 55 - 70% RH (Jembere *et al*., 1995; Zewde and Jembere, 2010). Shone variety of maize grains were obtained from farmer's stores of the survey site (Figure 3.1). It was the most commonly grown hybrid in the region and considered to be susceptible to insect infestation. The grains were kept at -20 ± 2 °C for 2 weeks to kill any infesting insects. Broken kernels, and debris were removed and the grains were graded manually, where similar sized grains were selected for the experiment (Gemechu *et al*., 2013). Following Zewde and Jembere (2010) procedures, fifteen pairs of unsexed adults of *S. zeamais* were placed in 12, one-liter glass jars containing 250 g seeds. The jars were then covered with nylon mesh and held in a place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. The parents of the test insects were sieved out after an oviposition time of 14 days. Then, the jars were kept under the aforementioned laboratory condition until F1 progeny emergence. The F1 progeny, which emerged after 30 days were sieved out and used for the experiment.

Description of the Test Plants

***Millettia ferruginea* (Hochst) Baker (commonly called Birbira in Amharic)**: This plant is a member of Sub-family Papilionoideae of the family Fabaceae (Leguminosae) and is a useful species of Ethiopia with a great potential for agro-forestry (Negash, 2002). Besides, about 200 species found in tropical and subtropical Africa, Asia and Australia constitutes the genus *Millettia* (Leguminosae, Papilonoideae) (Thulin, 1983).

According to Jembere *et al*. (2005) and Emana (2014), it is a large shady tree, which grows up to a length of 35 m, and is endemic to Ethiopia and widely grown at the elevation between 1000 and 2500 m above sea level. In other words, most parts of Kola and the whole woinadega of Ethiopia are potential areas for *M. ferruginea* growth. The tree is also commonly named as Sotallo, Kotalu, Sari, Yego (in Afan Oromo), Zaghia (in Wolaita), Enghediksho (in Sidama), Dhadhato (in Gedoffa) languages (Getahun, 1976) and Belawheka (in Hadiya) languages. Therefore, the natural habitat of *M. ferruginea* is rather diverse, as it has advanced mechanism of minimizing water loss, which enabled it to utilize water in a very conservative manner as demonstrated by Gindaba *et al.* (2004). As reported previously by researchers such as Jembere *et al*. (2005) and Emana (2014), two sub-species of it are known to occur in Ethiopia, and these are *M. f. ferruginea* which is confined to the northern part of the country and *M. f. darasana* which occurs in southern provinces, particularly Sidamo region. The mixtures of the two species are shown in trees from central to western Ethiopia (Azene *et al*., 1993 cited in Jembere, 2005). It was also reported that in central south Ethiopia, *M. ferruginea* is frequently found in association with some useful annual and perennial crops like maize, sorghum, barley and coffee, which indicates the usefulness of it in the conservation and improvement of soil fertility and in the productivity of traditional farming systems (Negash, 2002).

As demonstrated by Jembere *et al*. (2005), *M. ferruginea* is the tree with a numerous purpose, and used as tool handles, local construction, firewood, household utensils and aids as shade tree in coffee growing areas. Its products are used for fish poisoning, where mature pods and seeds are ground to fine powder and is spread over the water surface (Siegenthaler, 1980). It is also used as decorative plants that planted along roads and in agroforestry. It has a traditional medicinal value in different parts of Ethiopia in different forms to treat some diseases and skin infections (Mesfin *et al*., 2009). The roots and seeds of this plant are used as insecticides and pesticides in many parts of the world, and rotenone is responsible for the toxicity (MacLachlan, 2001 cited in Ameha, 2004). As a result, recently different parts of it have been extracted and the active chemical components have also been screened against different insect pests, including storage insect pests and have also been reported effective in their management (Jembere, 2002, Gebre-Selase, and Emana, 2009; Zewde and Jembere, 2010; Emana, 2014).

a) b)

Figure 5. 1 Photographs showing *M. ferruginia* (Hochst) Baker: a) tree at the age of farm and b) fresh leaves collected from the study area

***Calpurnia aurea* (Ait.) Benth (commonly called Digita in Amharic)**: This plant is belongs to the subfamily Papilionoideae of the family Fabaceae and it is widely distributed in Ethiopia (Amante, 2016). *Calpurnia aurea* is a genus of flowering plants within the family of fabaceae. It is a small, multi-stemmed tree, 3-4 m tall (Zorloni, 2007). It is also widely distributed in Africa from Cape Province to Eritrea. Besides, it is the most widespread in many parts of sub-Saharan Africa. It is also present in South Africa and southern India (Zorloni, 2007). It is one of the smaller genera consists of some 16 - 17 species, eight of which occur in Southern Africa, where they are found in the eastern and north-eastern parts of the country (Ramurafhi, 2011 cited in Getiye, 2014). *Calpurnia aurea* is widely grown in high land areas of Ethiopia (Birhanu and Asale, 2015). It is often found in forest margins, bush lands or grasslands, especially in overgrazed areas (Zorloni, 2007) and is easily cultivated (Germishuizen and Meyer, 2003). It is also known in different several local names in Ethiopia; Chekata by Oromo people, Cheka by the Borana people and Digita by Amhara people (Amuamuta and Na-Bangchang, 2015) and also called Sena by Hadiya people.

The plant, *C. aurea* has been commonly used in traditional medication to treat various medical disorders and parasitic infestation in animals and humans in different parts of Africa. Its arthropodicidal importance has been reported by different researchers as to treat maggot-infested wounds in South Africa and to treat scabies in Ethiopia (Watt and Breyer-Brandwyk, 1962 and Jansen, 1981 cited in Zorloni *et al*., 2010). The extract of its crumpled leaves is also used through the auricular route to treat earache for about 2 days in humans in southwestern Ethiopia. It is also traditionally used to treat rheumatism in the same area (Yineger *et al*., 2008). Extracts of different parts of it were also served for tick management in southwestern and western Ethiopia (Regassa, 2000). The Borana people of northern Kenya and southern Ethiopia soak its leaves in cold water to treat lice in humans and calves (Heine and Brenzinger, 1988). Furthermore, *C. aurea* is also used for treating stomach disorders, amoebic dysentery and eye diseases in Ethiopia (Abate, 1989). Its antibacterial and antioxidant activity have also been reported (Tadeg *et al*., 2005). Moreover, Blum and Bekele (2002) also reported its use as a natural pesticide for grain storage. However, its properties as insecticide and its efficacy in managing insect pests, particularly those of storages are not studied yet.

a) b) c)

Figure 5. 2 Photographs showing *C. aurea* sp. aurea (Ait.) Benth: a) tree at the age of farm, b) fresh leaves collected and c) tree in the maize farm of the study area

Plant Material Collection and Extraction: Plant materials (i.e. leaves) used for the study were collected from natural habitats of the study area (Figure 3.1) and the identities of the plants were confirmed at the national herbarium of Life Science Faculty of Addis Ababa University.

Dried and Ground Materials: Following methods of Gebre-Selase and Emana (2009), the fresh plant materials (leaves) of a known weight were kept in a well-ventilated room under shade for 2 to 3 weeks depending on weather conditions and allowed to dry. Dried materials were ground to fine powder using mortar, and pestles and they were applied at the rates of 5 g (5%), 10 g (10%) and 15 g (15%) per 100 g of grains, following similar procedures by earlier researchers (Tekie, 1999; Jembere, 2002).

Solvent Extracts of Plants Materials: Ground plant material (powder) from the leaves each of the two test plants was soaked in n-hexane (non-polar), chloroform (partial polar), distilled water, methanol, acetone and ethanol (polar) at the rate of 10 g /100 ml, 20 g /100 ml and 30 g /100 ml of each solvent (Jembere, 2002). The solution was allowed to stand for 24 hours for extraction. After 24 hours, the mixtures were filtered with cheesecloth. Then, the filtrates were ready to be used for the different treatments, following similar procedures by Zewde and Jembere (2010).

Filter Paper Bioassay: Following similar procedures by Zewde and Jembere (2010), different levels of each solvent extracts were applied to a filter paper of 9 mm diameter at the rate of 2 ml and 3 ml per filter paper and placed in a petri dish of 10 cm diameter. Variable exposure times were considered, which were based on the nature of the solvent. In case of acetone and methanol, the exposure time was 30 minutes, while it was 60 minutes for ethanol (Jembere, 2002). Then, 1 ml of distilled water was added to the entire surface of each treated filter papers, as a carrier of the extracts. Other filter papers were treated with two levels of the different solvents were served as control. After treatment, five pairs of 3 to 7 days old unsexed adults of the test insects were introduced into treated and control filter papers in the petri dishes. Mortality of the adult insects was counted after 24, 48, 72 and 96 hrs. When no leg or antennal movements were observed, insects were considered as dead as suggested by Gebre-Selase and Emana (2009). All treatments of filter paper were arranged in a Completely Randomized Design (CRD) in three replications.

Admixture Toxicity Assessment Bioassay with Botanicals Powder and Solvent Extracts: Following methods by Gebre-Selase and Emana (2009), 100 g of disinfected maize grains of shone variety (that were disinfested using the same procedure as indicated in insect culture section) were introduced into 1 L glass jars that were treated differently with the powdered peels of the test plants (i.e. 5, 10 and 15 g of the powder) for treatment of powder. Malathion (5%) dust was used as the positive control at a dosage of 0.05 g / 100 g maize grain and untreated grains were served as the negative control. The jar contents were shaken thoroughly for five minutes to ensure uniform distribution of the treatments with grain surface. After treatment, 20, three to seven days old unsexed experimental insects were introduced to the treated and untreated seeds in the glass jars. Then, the jars were covered with nylon mesh and held in place with rubber bands.

For bioassay of solvent extracts on maize grain, 100 g of disinfested shone variety of maize grains were placed in 1 L glass jars and treated with water, acetone and ethanol extracts of the test botanicals of 10 and 15 ml from the two extraction levels of 20 g /100 ml and 30 g /100 ml, following similar procedures by Zewde and Jembere (2010). The jar contents were shaken thoroughly for five minutes to ensure uniform distribution of the solution over grain surface. Then, the treated grains were kept for 24 hours for acetone extracts and 36 hours for ethanol extracts to allow complete evaporation of the solvents before the conduct of the bioassay. Malathion 5% dust at the recommended dose rate of 0.05 g per 100 g of maize grains (standard check) and solvent treated grains (untreated check) were used for comparison. Then after, 20, three to seven days old unsexed experimental insects were introduced to the treated and untreated grains in each of the glass jars. The jars were covered with nylon mesh and held in place with rubber bands.

Then, the jars of both powder and solvent extract treatments and their control were kept under laboratory condition. All powder treatments and solvent extracts were arranged in a Completely Randomized Design (CRD) in three replications. Mortality were recorded at

1, 2, 3, 4, 7 and 14 days after treatment application in botanicals powder treated experimental units, while mortality were recorded at 24, 48, 72 and 96 hours after treatment application in solvent extract treated jars as recommended by Zewde and Jembere (2010). All live and dead insects were also sieved and discarded after 13 days oviposition.

F1 Progeny Assessment: The treated and control grains were also kept until emergence of F1 progeny under the same experimental condition indicated in insect culture section, after mortality observation. Then the numbers of F1 progeny produced by the experimental insects were counted. Counting was stopped after 56 days from the day of introduction to avoid overlapping of generation (Zewde and Jembere, 2010).

Damage and Weight Loss Assessment: Two days after the last F1 count of 56 days, samples of 30 grains were taken randomly from each jar and the number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Grain damages were conveyed as a percentage of the entire number of grains in each replicate. Percent weight losses were calculated by count and weight method as shown in chapter 4.

Percent protection or inhibition in F1 progeny emergence (% IR): This was calculated using the following formula as adopted by pervious researchers (Gebre-Selase and Emana, 2009):

$$\% \text{ IR} = \frac{\text{Cn} - \text{Tn}}{\text{Cn}} \times 100,$$

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

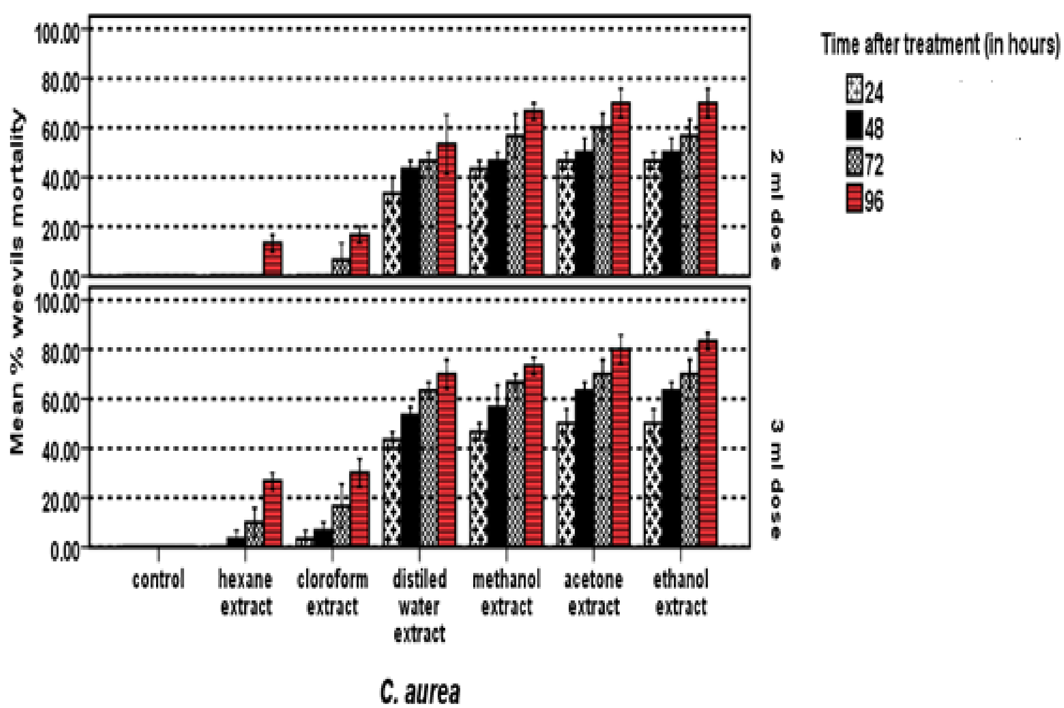
Data Analysis: The data collected for this study were managed by the Microsoft Excel package 2013 and analyzed using the Statistical Program for Social Sciences (SPSS) version 16. Data on adult weevil's mortality were analyzed using Univariate analysis or two way one-way analysis of variance (ANOVA). Besides, data on F1 progeny emergence, percent grain damage and weight loss of maize grains at a particular time were analyzed using one way ANOVA. Significant differences between means were separated using Tukey's studentized (HSD) test at 5% confidence interval. Significant differences among means were stated as indicated in chapter 4. Standard errors (±se) were given following means in tables & in the form of T- shaped error bars in figures. Correlation between the treatments and the efficacy measuring parameters were determined using Pearson's correlation of SPSS program of version 16.

5.3 Results

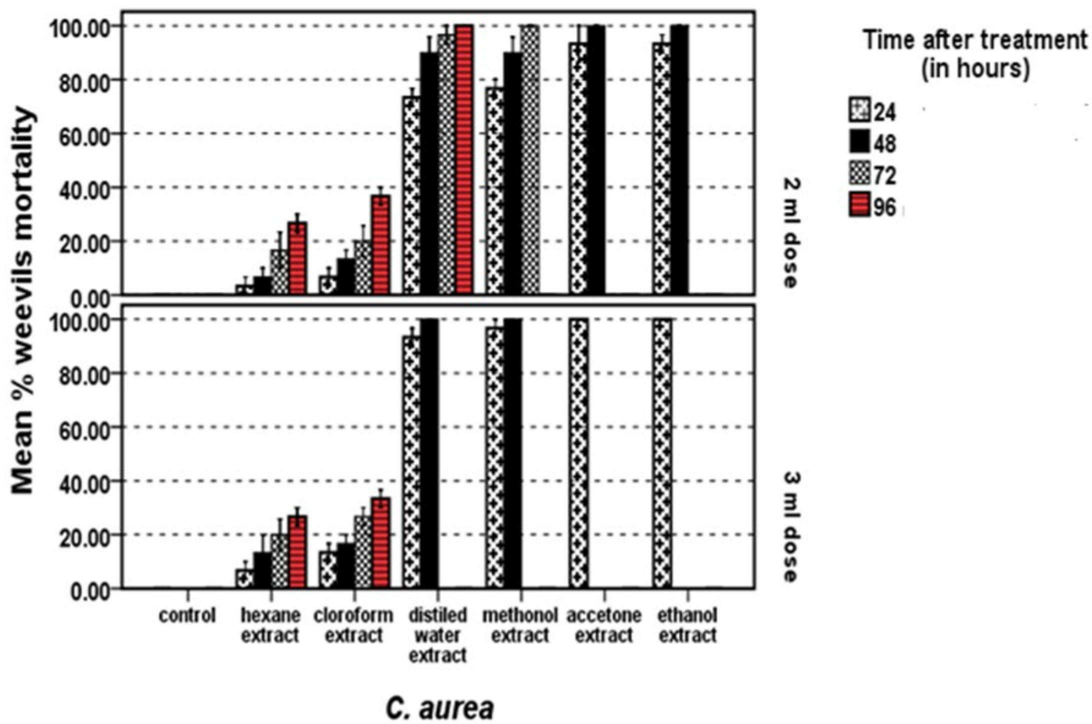
Filter Paper Bioassay

Percentage adult weevil's mortality was increased ($P < 0.05$) significantly with increased dosage, extraction level, polarity and exposure time after treatment for both tested botanicals in general. Polar solvent extracts (distilled water, methanol, ethanol and acetone extracts) of *C. aurea* and *M. ferruginea* applied at all rates (2 and 3 ml) and levels demonstrated significant ($P < 0.05$) toxicity effect compared to non-polar and partial polar solvent extracts, as well as the negative control at all days after treatment application (Figures 5.3 and 5.4).

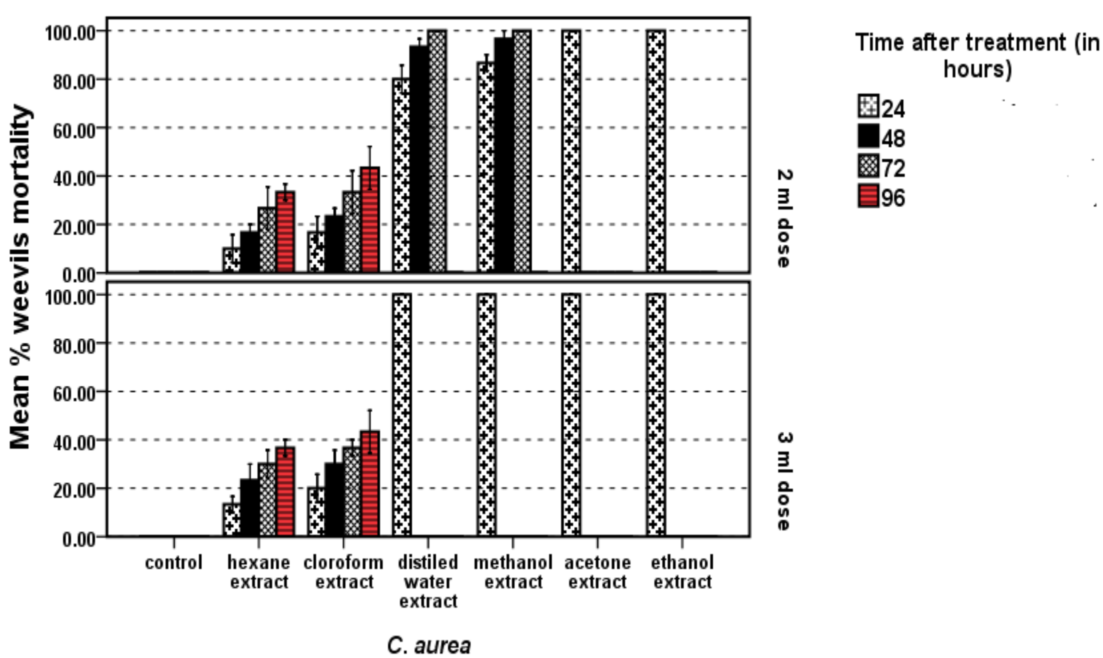
Significantly ($P < 0.05$) higher mean percentage mortality of *S. zeamais* (> 60 and > 80%) were recorded from all polar solvent extracts of the tested plants applied at the rate of 2 and 3 ml per filter paper from the higher level of extraction (20 and 30 g /100 ml), respectively following 24 hours post treatment exposure. Significantly ($P < 0.05$) high mean percentage mortality (> 55%) of *S. zeamais* was also recorded from all polar solvent extracts of the tested plants applied at the rates of 2 and 3 ml per filter paper from 10 g / 100 ml level of extraction, though relatively extended time (96 and 72 hours, respectively) after treatment were required to achieve high percentage mortality than the rest of higher levels of extraction. Furthermore, 100% weevil's mortality was induced by all polar solvent extracts of *C. aurea,* as well as by only ethanol and acetone extracts of *M. ferruginea* applied at the rate of 3 ml per filter paper from 30 g /100 ml level of extraction at 24 hours after treatment application. Hundred percent mortality of weevils was also recorded at 48 hours after treatments application by 2 ml distilled water and methanol extracts of *C. aurea,* and at 96 and 72 hours after treatments application by the same rate (2 ml) of distilled water and methanol extracts, respectively of *M. ferruginea* from 20 g / 100 ml extraction level (Figures 5.3 and 5.4).



a)

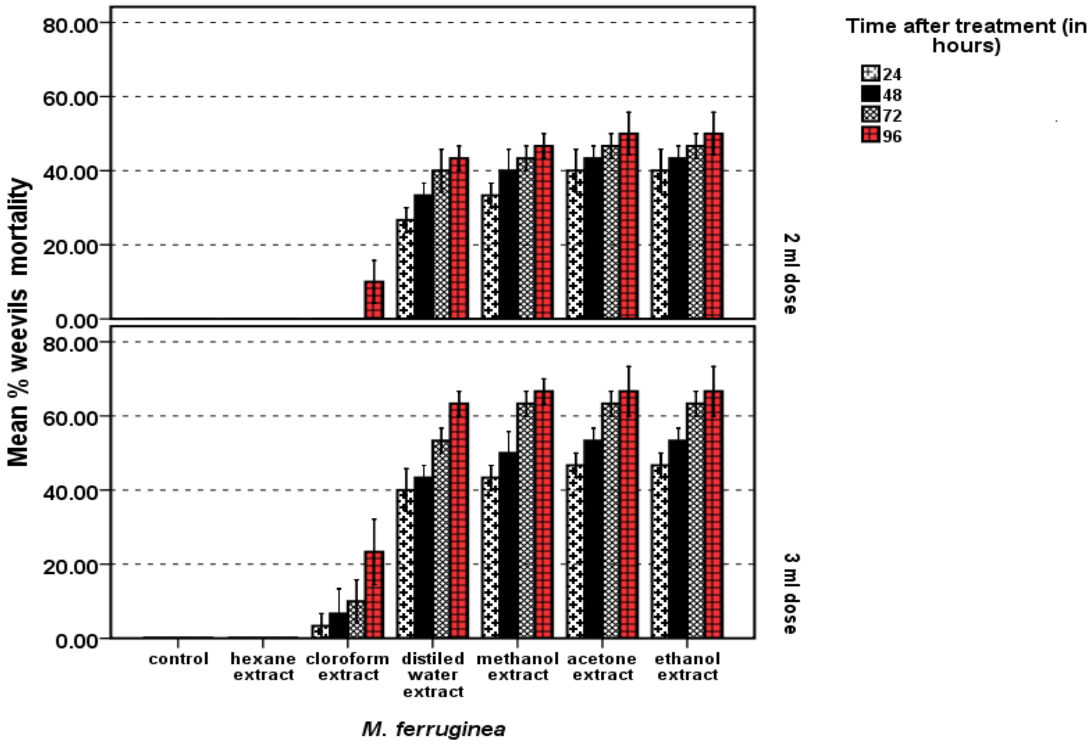


b)

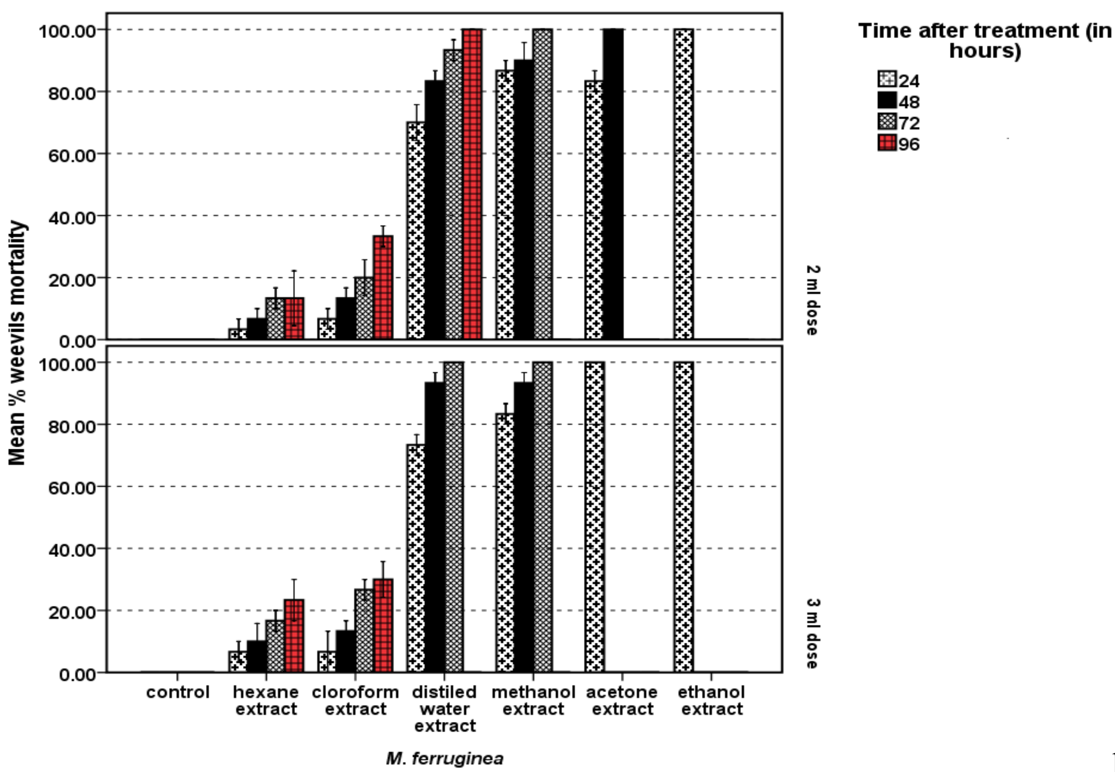


c)

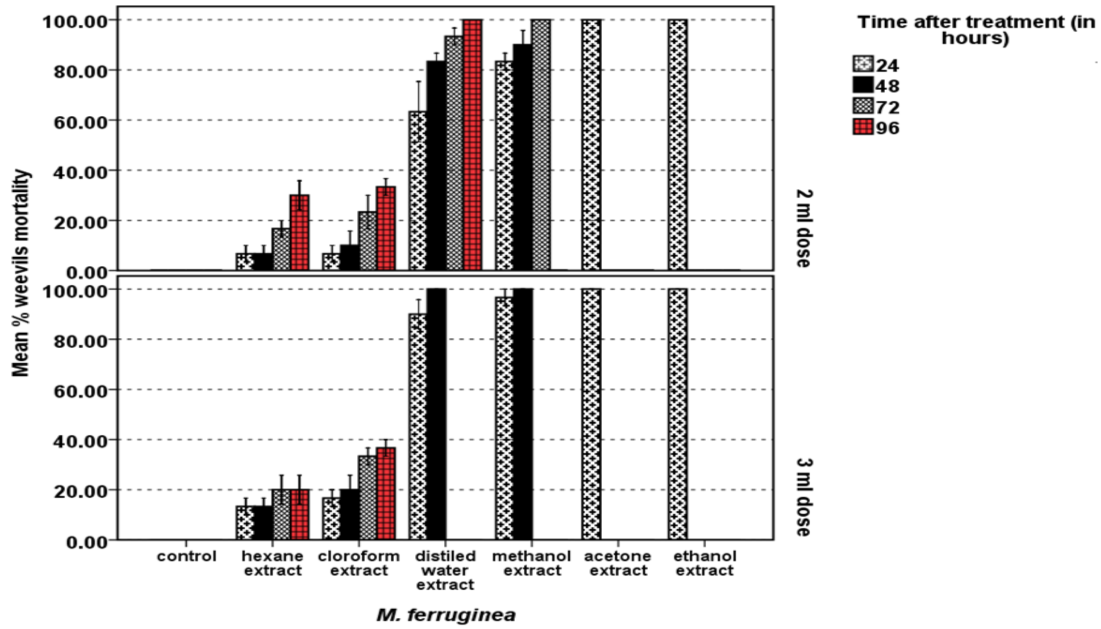
Figure 5. 3 Mean % mortality (mean ± SE) of *S. zeamias* due to solvent extracts of *C. aurea* extracted at the rate of: a) 10 g / 100 ml, b) 20 g / 100 ml and c) 30 g /100 ml and applied at the dose of 2 ml and 3 ml after different post treatment exposure


Time after treatment (in hours)
24
48
72
96
Mean % weevils mortality
80.00
60.00
40.00
20.00
0.00
2 ml dose
3 ml dose
control
hexane extract
cloroform extract
distiled water extract
methanol extract
acetone extract
ethanol extract
M. ferruginea

a)


Time after treatment (in hours)
24
48
72
96
Mean % weevils mortality
100.00
80.00
60.00
40.00
20.00
0.00
2 ml dose
3 ml dose
control
hexane extract
cloroform extract
distiled water extract
methanol extract
acetone extract
ethanol extract
M. ferruginea

b)



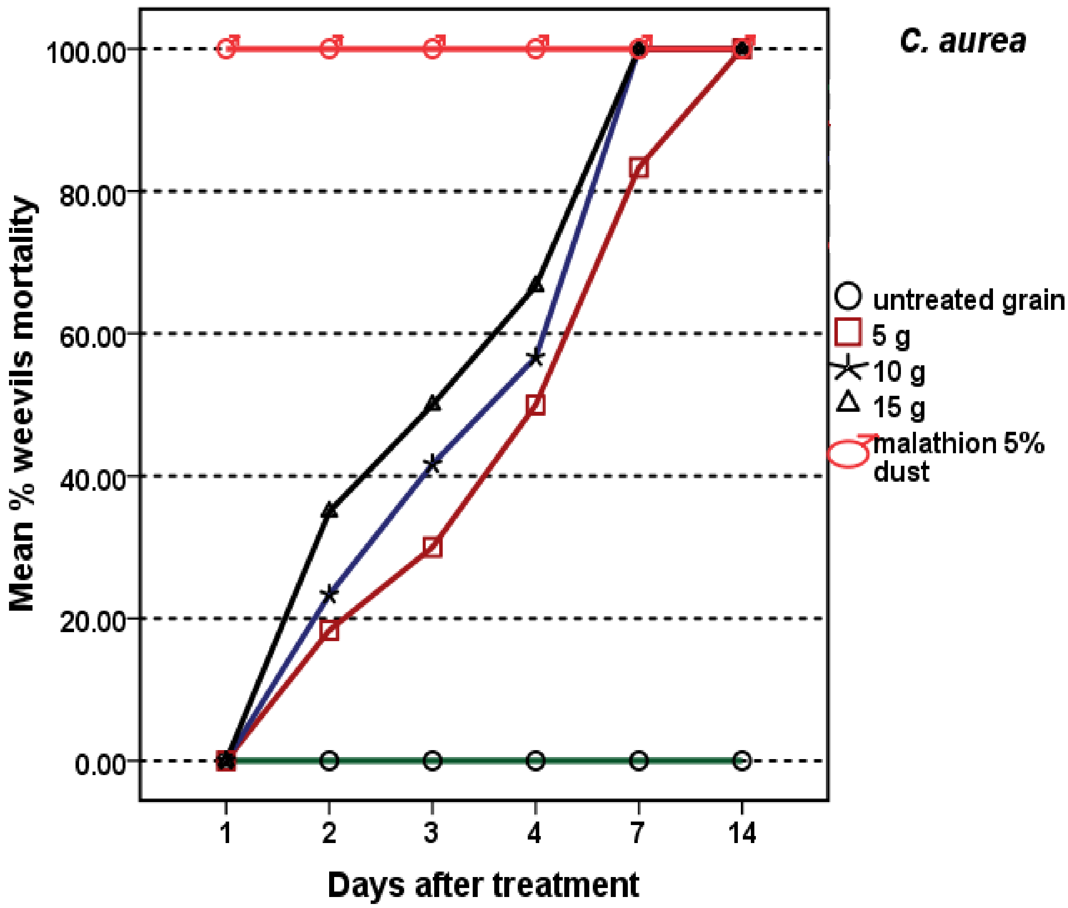
c)

Figure 5. 4 Mean % mortality (mean ± SE) of *S. zeamias* due to solvent extracts of *M. ferruginea* extracted at the rate of: a) 10 g / 100 ml, b) 20 g / 100 ml and c) 30 g / 100 ml and applied at the dose of 2 ml and 3 ml after different post treatment exposure

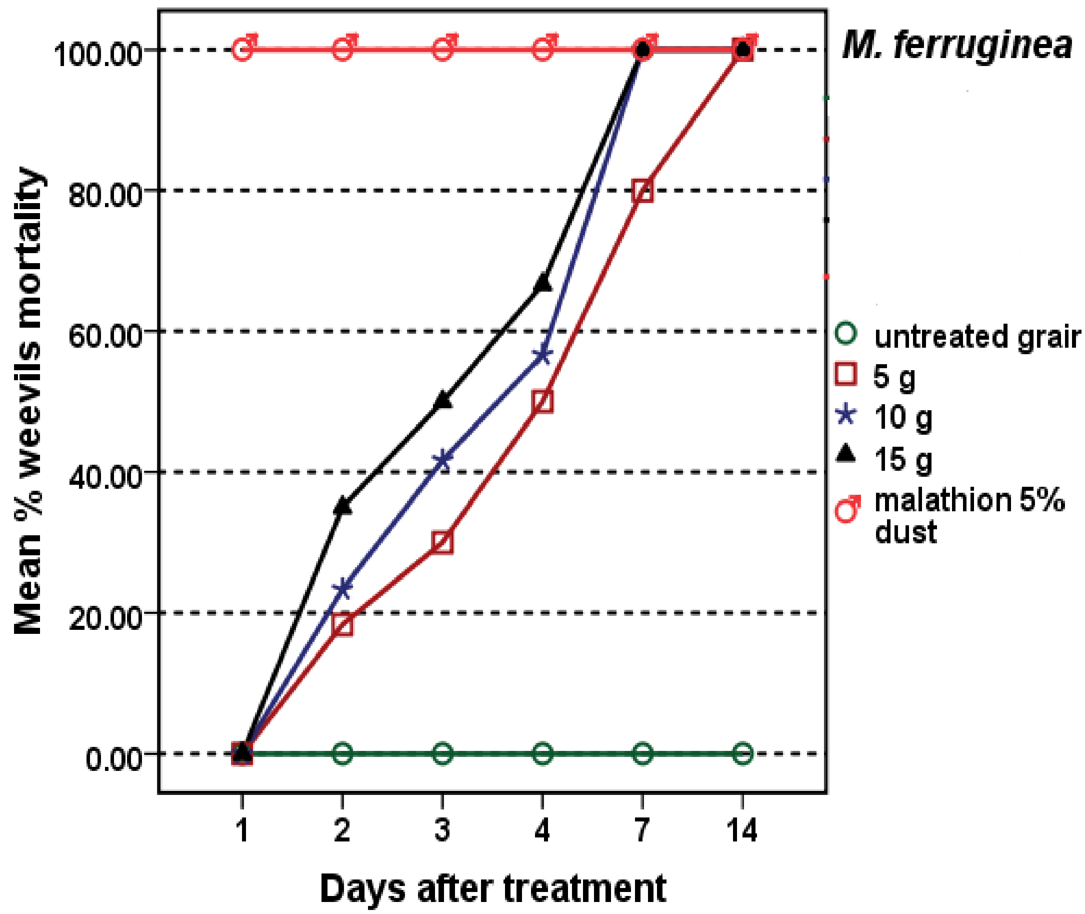
C. aurea **and** ***M. ferruginea*** **leaf powder on the mortality of parental maize weevils**

Percentage adult weevil's mortality was proportionally increased with increase in dosage and time of exposure. Both the tested botanicals had significant ($P < 0.05$) effect on percent mortality of adult weevils in comparison to the untreated control. However, significant percentage weevil's mortality was not happen at 5% and 10% rates of the tested botanicals before four days after treatment application (Figure 5.5).

Significantly ($P < 0.05$) high weevil's mortality (≥ 50%) was recorded from all botanicals leaf powder applied at all dosages at four days after treatment application. Significantly ($p < 0.05$) higher weevil's mortality (≥ 80%) was recorded from all treatments of the tested botanicals leaf powder applied at a dose of 5% at 7 days after treatment application. Besides, 100% weevils morality was recorded from all treatments of the tested plant leaf powder applied at rates of 10 and 15% at 7 days after treatment application. Furthermore, 100% weevils morality was recorded from all treatments of the tested botanicals leaf powder applied at all rates, following 14 days of treatment application likewise that of Malathion 5% dust (Figure 5.5).



a)



b)

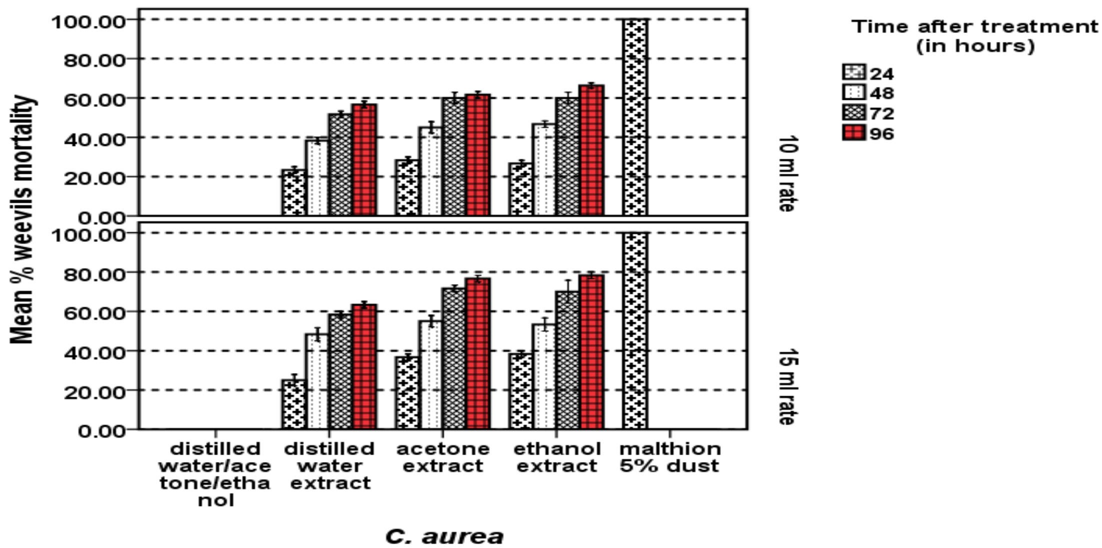
Figure 5. 5 Mean % mortality (mean ± SE) of maize weevil adult exposed in grains treated with: a) *C. aurea* and b) *M. ferruginea* leaf powder

***C. aurea* and *M. ferruginea* Leaf Solvent Extracts on the Mortality of Maize Weevils**

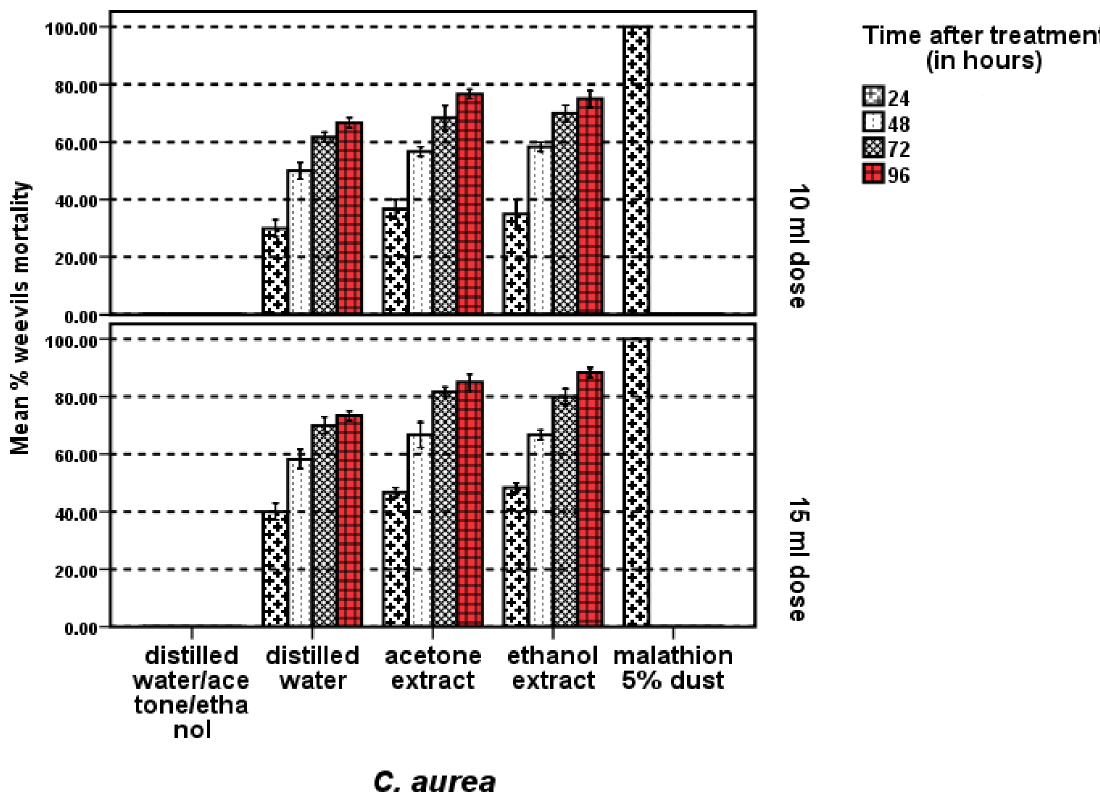
Polar solvent extracts (ethanol, acetone and distilled water extracts) that caused significant ($P < 0.05$) toxicity effect on parental *S. zeamais* adults in filter paper bioassay were screened and applied for this experiment. Significantly ($P < 0.05$) high weevil's mortality was recorded from all botanicals solvent extracts applied at all dosages from the two tested levels of extraction (20 g / 100 ml and 30 g/ 100 ml) at all dates after treatment than the negative control. The efficacy of the solvent extracts of the tested botanicals against weevils was increased with increased dosage, extraction level and exposure time after treatment application. Significantly ($P < 0.05$) higher weevil's mortality (> 50%) was recorded from all botanicals solvent extracts applied at all dosages from the two tested levels of extraction (20 g / 100 ml and 30 g/ 100 ml) at 3 days (72 hours) after treatment application.

The efficacy the tested plants leaves solvent extracts on weevil's morality was also varied ($P < 0.05$) significantly with the type of solvent used for extraction; the highest being from ethanol exacts, followed by extracts in acetone and distilled water in general. Significantly ($P < 0.05$) higher weevils mortality (>75%) was recorded from all polar extracts of the tested botanicals applied at 15 ml dosages from 30 g / 100 ml extraction levels at 96 hours (4 days) after treatment application than at 10 ml rate. Of which, the maximum being from ethanol extract (> 82%), followed by extracts in acetone and distilled water (between 75% & 82%) in general (Figures 5.6 and 5.7).

Significantly ($P < 0.05$) (> 55%) higher mortality of weevils was recorded from all polar solvent extracts of the tested plants applied at a dose 15% from 30 g/ 100 ml level of extraction, following 2 days post treatment exposure than the leaf powder of the tested botanicals, in which significantly higher weevils mortality was recorded, following 4 days of treatment application at the same rate (15%) (Figures 5.5, 5.6 and 5.7).

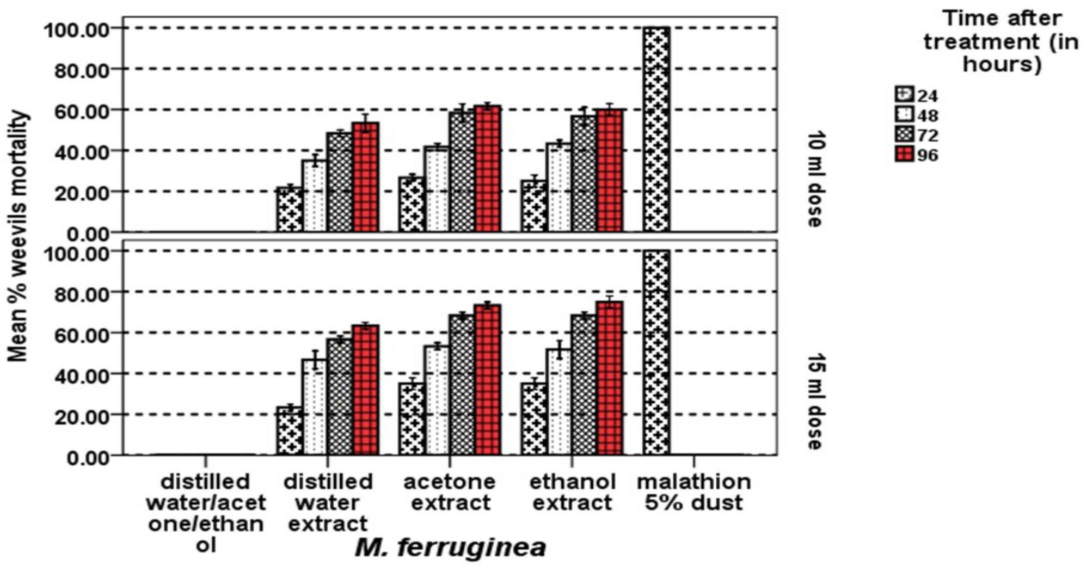


a)

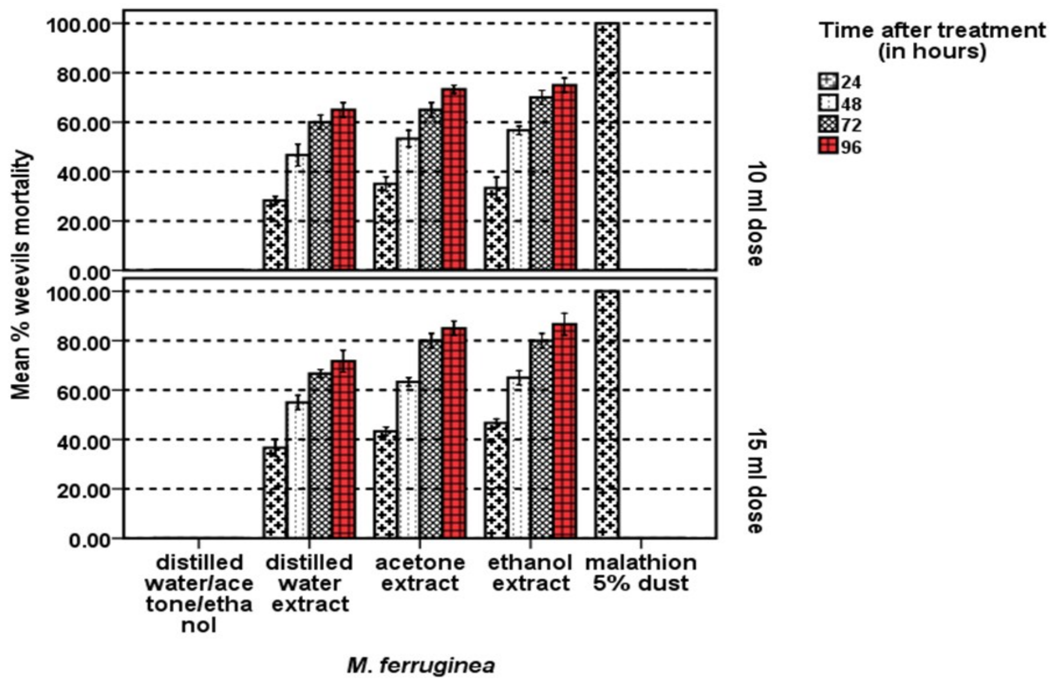


b)

Figure 5. 6 Mean % mortality (mean ± SE) of maize weevil adult exposed in grains treated with *C. aurea* leaf solvent extracts applied at a rate of: (a) 20 g/ 100 ml and (b) 30 g /100 ml



a)



b)

Figure 5. 7 Mean % mortality (mean ± SE) of maize weevil adult exposed in grains treated with *M. ferruginea* leaf solvent extracts applied at a rate of: (a) 20 g / 100 ml and (b) 30 g / 100 ml

C. aurea and _M. ferruginea_ Leaf Powders and Solvent Extracts on F1 Progeny emergence, % Protection, Grain Damage and Weight Loss

Significantly ($P < 0.05$) lower F1 progeny emergence, percentage grain damage and weight losses of weevils were recorded from all treatments of botanicals leaf powder and solvent extracts as compared to negative control.

Significantly ($P < 0.05$) (>73%) higher inhibition of F1 progeny emergence as well as higher reduction of grain damage (≤ 4.33) and weight loss (≤ 0.58) by *S. zeamais* were recorded from all leaf powder and solvent extracts treatments of the two tested botanicals applied at all rates (5, 10 and 15% of powder, and 10 and 15% of solvent extracts) than negative control. Besides, 100% F1 progeny production inhibition, as well as no grain damage and weight loss of maize were observed in all powder and solvent extract treatments of the tested botanicals applied at a rate of 15% likewise that of the positive control (Tables 5.1-5.3).

The correlation among the treatments of leaf powder and solvent extracts of the tested plants applied at different dosage and the efficacy parameters measured (the number of F1 progeny emerged, percentage grain damage and weight loss) was found to be highly significant ($P < 0.01$) and strongly negative. However, the correlation was highly significant and strongly positive between the number of F1 progeny produced, and percent grain damage and weight loss of all treatments of the tested plants powder and solvent extracts (Tables 5.4, 5.5 & 5.6).

Table 5. 1 Mean number of F1 progeny produced, percent protection, grain damage and weight loss caused by *S. zeamais* on maize grains treated with *C. aurea* and *M. ferruginea* leaf powders

Treatments	Dosage (g/100g)	Mean number of F1 progeny	Percentage protection	Mean % grain damage	Mean % weight loss
C. aurea	5	6.67±4.41c	78.93	4.00±0.58b	0.50±0.01b
	10	3.33±1.67b	89.49	1.00±1.00ab	0.22±0.01ab
	15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
M. ferruginea	5	8.33±4.44c	73.70	4.33±0.33b	0.58±0.01b
	10	5.00±2.88b	86.33	1.33±0.88ab	0.25±0.02ab
	15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Control (untreated)	0	31.67±1.67d	0.00	11.67c	5.30±0.17c
Malathion 5%	0.05	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a

Means followed by the same letter in a row are not statistically significantly different at $P < 0.05$ using Tukey's studentized (THSD) test.

Table 5. 2 Mean number of F1 progeny produced, percent protection, grain damage and weight loss caused by *S. zeamais* on maize grains treated with *C. aurea* solvent extracts

Treatments	Level of extraction	Dosage (ml/100 g)	Mean number of F1 progeny	Percent protection	% grain damage	%weight loss
Ethanol	20	10	1.67±1.67ab	94.72	1.00±0.588b	0.13±0.01a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
	30	10	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Acetone	20	10	1.67±1.67ab	94.72	1.00±0.588b	0.13±0.01a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
	30	10	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Distilled water	20	10	3.33±1.67b	89.58	1.33±0.67b	0.28±0.03b
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
	30	10	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Ethanol/acetone/distilled water	0	15	31.67±1.67c	0.00	11.67±0.34c	5.30± 0.14c
Malathion 5% dust	0	0.05	0.00a	100	0.00±0.00a	0.00±0.00a

Means followed by the same letter in a row are not statistically significantly different at $P< 0.05$ using Tukey's studentized (THSD) test.

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Table 5. 3 Mean number of F1 progeny produced, percent protection, grain damage and weight loss caused by *S. zeamais* on maize grains treated with *M. ferruginea* solvent extracts

Treatments	Level of extraction (g/100 ml)	Dosage (ml/100 g)	Mean number of F1 progeny	Percent protection	% grain damage	%weight loss
Ethanol	20	10	3.33±1.67b	89.49	1.67±0.88ab	0.13±0.01a
		15	0.00±0.00	100.00	0.00±0.00a	0.00±0.00a
	30	10	1.67±1.67ab	94.73	0.00±0.00a	0.00±0.00a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Acetone	20	10	3.33±1.67b	89.49	2.33±1.2b	0.23±0.03ab
		15	0.00±0.00a	10.000	0.00±0.00a	0.00±0.00a
	30	10	3.33±1.67b	89.49	0.00±0.00a	0.00±0.00a
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Distilled water	20	10	6.67±1.67d	78.94	2.67±1.33b	0.42±0.01b
		15	3.33±1.67b	89.49	0.00±0.00a	0.00±0.00a
	30	10	5.00±2.89c	84.21	0.00±0.00a	0.32±0.01ab
		15	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Ethanol/acetone/distilled water	0	15	31.67±1.67e	0.00	11.67±0.34c	5.30±0.14c
Malathion 5% dust	0	0.05	0.00a	100	0.00±0.00a	0.00±0.00a

Means followed by the same letter in a row are not statistically significantly different at P< 0.05 using Tukey's studentized (THSD) test.

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Table 5. 4 Correlation among Efficacy determining parameters of CA and MF leaf powder

Efficacy determining parameters	Rate	F1 CA	F1 MF	% GDCA	% GDMF	% WLCA	% WLMF
Rate	1						
F1CA	-.943**	1					
F1MF	-.949**	-	1				
% GD CA	-.920**	.972**	-	1			
% GD MF	-.921**	-	.942**	-	1		
% WL CA	-.866**	.920**	-	.964**	-	1	
% WL MF	-.912**	-	.932**	-	.933**	-	1

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed), with hyphen (-) represent no association and those without asterisk are non-significant

CA = Calpurnia aurea, MF = Millettia ferruginea, GD = grain damage and WL= weight loss

Table 5. 5 Correlation among efficacy determining parameters of CA solvent extracts

Efficacy parameters	CA S. ext dose	F1 ext 1	F1 ext 2	GD ext 1	GD ext 2	WL ext 1	WL ext 2
CA S. ext dose	**1**						
F1 ext 1	-.380*	1					
F1 ext 2	.000	-	1				
GD ext 1	-.073	.652**	-	1			
GD ext 2	.000	-	.966**	-	1		
WL ext 1	-.371*	1.000**	-	.638**	-	1	
WL ext 2	.000	-	.973**	-	.998**	-	1

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed), with hyphen (-) represent no association and those without asterisk are non-significant

CA = Calpurnia aurea, GD = grain damage, WL= weight loss, ext 1= extraction level of 20 g/ 100 ml and ext 2 = extraction level of 30 g/ 100 ml

Table 5. 6 Correlation among efficacy determining parameters of MF solvent extracts

Efficacy parameters	MF S. ext dose	F1 ext 1	F1 ext 2	GDs ext 1	GD ext 2	WL ext 1	WL ext 2
MF S. ext dose	**1**						
F1 ext 1	-.380*	1					
F1 ext 2	.000	-	1				
GD ext 1	-.098	.480**	-	1			
GD ext 2	.000	-	.966**	-	1		
WL ext 1	-.033	.655**	-	.735**	-	1	
WL ext 2	.000	-	.973**	-	.998**	-	1

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed), with hyphen (-) represent no association and those without asterisk are non-significant

MF = Millettia ferruginea, GD = grain damage, WL= weight loss, ext 1= extraction level of 20 g/ 100ml and ext 2 = extraction level of 30 g/ 100ml

5.4 Discussion

Significant (P < 0.05) increment of percentage adult weevil's mortality with increased dosage, extraction level and post treatment exposure time of the tested botanicals leaf crude extracts in all bioassays in the current study, is in agreement with findings of earlier researchers (Gebre-Selase and Emana, 2009; Zewde and Jembere, 2010; Gebre-Egziabiher, 2016).

The present study also revealed that all polar solvent extracts of the tested botanicals at all rates (2 and 3 ml in filter paper, and 10 and 15 ml in admixture bioassay) from all extraction level were induced significant toxic effect against *S. zeamais* than negative control. This suggests the presence of more polar solvent soluble phytochemicals in leaves of *C. aurea* and *M. ferruginea*, which are responsible higher weevil's mortality and as most of them probably might be polar. In agreement with finding, Amante (2016) suggested that the active ingredients in the leaf extract of the plant reside in the polar fractions indicating that the active principles are polar in nature after he studied castor bean plant against ectoparasites of animals. Jembere *et al*. (2005) also indicated that high *Z. subfasciatus* mortality was caused by *M. ferruginea* water extract that probably might be due to the presence high water soluble chemicals in the seeds of it. Emana (2014) also shown that the polar solvent extracts (acetone and water) of *M. ferruginea* seeds caused significantly high toxicity to *Z. subfasciatus*, 48 hours after treatment. Blum and Bekele (2002) also revealed that *C. aurea* has been used as a natural pesticide to improve grain storage. Amante (2016) also indicated that as *C. aurea* possess potent activities (louscidal and acaricidal effects) against ectoparasites of animals.

In the current study, significantly higher mortality of weevils was recorded from all leaf powder and solvent extract treatments of the tested botanicals applied at all rates than negative control, following 4 days after treatment application. Significantly higher inhibition in F1 progeny emergence, as well as significantly higher reduction in grain damage and weight loss were also recorded from all leaf powder and solvent extract treatments of the tested botanicals applied at all rates than negative control in about 2 months storage period. This higher efficacy the crude extracts may be attributed due to either the toxic or repellent effects of phytochemicals in the tested plants or starvation and interference with respiration due to suffocation of maize weevils. This result thus, suggests the potency of both the leaf powder and solvent extracts of the tested plants in protecting maize grains against weevils. Toxicity caused by crude extracts of the two botanicals tested against maize weevils in the current study, is in accordance to the finding of pervious researchers (Jembere *et al*., 2005; Zewde and Jembere, 2010; Gebre-Selase and Emana, 2009; Emana, 2014, Bulto *et al*., 2017).

To the best of our knowledge, however, no report exists in the literature regarding the bio-assayed toxic potency of *C. aurea* against *S. zeamais* and other storage insect pests in Ethiopia, except only one by earlier researcher (Bulto *et al*., 2017) in which its leaf powder was shown to be potent against maize weevil. Besides, no reports exist in literature regarding the effectiveness leaf powder and solvent extracts *M. ferruginea* against maize weevils, in particular and storage pests, in general, except the effectiveness of its seed and root extracts.

In the present study, the efficacy of the tested botanicals in weevil's morality was also varied ($P < 0.05$) significantly with the type of solvent used for extraction; the highest being in ethanol exacts, followed by extracts in acetone and distilled water. This result agrees with finding of Jembere (2002), in which the water extracts of *M. ferruginea* was indicated to be the 3rd effective, 3 days after post treatment exposure, following acetone and ethanol extracts against maize weevils. Fredrick (2012) also shown that the deleterious effects of the plant extracts against the maize weevil varied with the type of solvent extract applied.

The aforementioned highest efficacy botanicals solvent extracts in ethanol might also be probably due to its broad solubility properties of organic compounds of the tested botanicals. In accordance with this finding, Amoh (2010) shown that ethanol is an accepted solvent for contact application, because of its broad solubility properties and low toxicity, among the various solvents (water, ethanol, acetone, petroleum ether and others) that have been used in the preparation of plants extracts for testing their toxicity to insect pests. Koffi *et al*. (2010) also revealed that ethanol extracts were found to be more effective than aqueous extracts of the same plant in general, as a result of higher solubility of organic compounds in ethanol.

In the present study, the efficacy of the tested botanicals was also varied among solvent extracts and leaf powder treatments, i.e. solvent extracts of leaves applied at a rate of 15% induced significantly higher weevils mortality at 2 days after treatment application than leaf powder of the two tested botanicals applied at the same rate, which caused significantly higher morality at 4 days of treatment application. This might be probably because the powder formulations caused insect mortality due to their physical actions; through blockage of the spiracles, abrasion of insect cuticle and lead to water loss than direct toxicity (Ashamo and Akinnawonu, 2012). Fredrick (2012) also indicated that mortality induced by contact of botanicals powder was not immediate, but generally increased with increase in time of exposure.

One of the reason for the aforementioned slow acting problem posed by leaf powder of plants against adult weevils, but great inhibition of progeny production in the present study, might probably be related to the feeding behavior (being internal) the insect that enable it to spend most of its life feeding inside the grain, while enabling its developmental stages to spend all of their life feeding inside the treated grain. Thus, the higher protection of the botanicals against F1 progeny emergence, percent damage and weight loss of stored maize by weevils in this study could probably be related to the various effect (toxicity, feeding and oviposition deterrence) of the plants phytochemicals to adult rather than the direct toxicity to the immature stages. Similarly, Haman (2016) indicated that the active ingredients of the botanicals could not penetrate in to the grains, since powders can only coat the outer part of the grain. Qwarse (2015) also shown that the reduction of F1 progeny emergence of weevils in treated grains might be due to factors such as increased parental adult mortality of the *Phytolacca dodecandra* leaf and root extracts tested.

5.5 Conclusion

In the current investigation, among the different solvent extracts of *C. aurea* and *M. ferruginea* bio-assayed in filter paper, the polar solvent extracts of were found to be effective. Besides, among different polar extracts screened from filter paper bioassay and compared against maize weevils in treated maize grain, ethanol extracts of the two tested plants were the first effective, and acetone extracts were the second effective and water extracts were the third effective. This confirmed the possibility using these solvents in the preparation of plant extracts for testing toxicity, screening phytochemicals and other possible bioassays against maize weevils. Besides, the effectiveness of water extracts also assured that the water extracts of the tested plants leaves could be possibility used by farmers for managing weevils under traditional storage condition, since the preparation of crude extract by water is cheap and easy.

The current study also confirmed all the treatments of leaf powder and solvent extracts of the tested botanicals applied at all rates (10% and 15% of solvent extracts, and 5%, 10 % and 15% of powder) induced significantly higher protection of maize grains against maize weevils. It also justified that the higher protection of maize grain by the tested botanicals against maize weevils was the result of different phytochemical effects of the plants leaves in parental adults than immature stages.

Almost all of the aforementioned facts confirmed that both the leaf powder and the solvent extracts of *C. aurea* and *M. ferruginea* were potent in protecting maize grains against maize weevils attack at all rates (10% and 15% of solvent extracts and 5%, 10% and 15% of powder). This in turn justifies the presence of possibility to exploit the potential of *C. aurea* and *M. ferruginea* leaf crude extracts in the management of *S. zeamais* under farmer's traditional storage conditions. Thus, the leaf powder and solvent extracts can be recommended for managing maize weevils on stored maize under farmer's storage conditions in Ethiopia. However, the effect of the these crude extracts on human being, natural enemies and cost effectiveness under farmer's storage conditions need further study before wide implementation of the outcomes this study.

Chapter 6. Efficacy of Two Locally Available Inert Dusts against *S. zeamais* under Laboratory Condition

6.1 Introduction

In Ethiopia, maize is one of the major cereal crops grown for its food, feed, firewood and construction purposes (Sori, 2014). Of the cereal crops, maize ranks second to tef in area coverage and first in total production in Ethiopia. Currently, it also ranks first in total production and productivity among all the cereals grown in southern Ethiopia (Gemu *et al*., 2013). However, its production and yield are highly affected by an array of biotic and abiotic stresses (Tefera *et al*., 2011). Among biotic constraints, insect pests are the main factor responsible for causing high losses (Adams and Schulter, 1978) and the most important of these pests in the field and storage are Lepidopterous stem borers and Coleopterous weevils, respectively (Emana and Abate, 1999; Demissie *et al*., 2008). To control these pests, the majority of smallholder farmers in Africa use synthetic pesticides. However, environmental, health and other concerns (development of resistant strains of insect pests, toxicity to beneficial organisms, and the like) put the use of pesticides under question mark (Harish *et al*., 2013).

Thus, there is a need for development of locally available, safe and ecologically sound control options such as inert dusts as benign component of IPM. Besides, it was reported that inert dusts such as clay powders, sands, wood ashes, silicates, lime and dianamite have been used traditionally by farmers to protect stored grain from insect pests in developing countries (De Lima, 1987; Tadesse, 2008).

The main merits of using inert dusts were reported to be their low mammalian toxicity, long-term protection, easier application and maintenance of grain quality (Korunic *et al*., 1996) and their ease of availability. Most of the early formulations however were not widely accepted by the grain industries in developed countries for a variety of reasons, including the high rates required for mortality, variation in toxicity among target species, damage to grain handling equipment and health problems with worker exposure to dusts (Arthur, 1997). Nevertheless, more recently, materials including diatomaceous earths and silica aerogels have been developed and used increasingly in commercial storage in the developed world, replacing conventional chemicals (Golob, 1997). That is, Silica-based inert materials such as silica aerogels and diatomaceous earths have been proven very effective in smaller quantities and such information is essential in order to establish a sustainable management strategy against insect pests of stored maize grain (Demissie *et al*., 2008). However, synthetic silicates, which are manufactured for industrial uses, have very high silicon dioxide content, and are very expensive and therefore, inappropriate for use as grain protectants at small scale farmers level (Golob *et al*., 2002). Thus, identifying useful locally available, cheap, ecologically sound and safe inert materials or dusts against storage pests of stored maize grain such as *S. zeamais* is very essential and will aid in development of sustainable management strategies.

From all the aforementioned facts and the attempts that have been made to divert attention away from reliance on a single control options to find natural, cheaper and safe materials for the control of storage pests, the present study was initiated with the following objective: to evaluate the bio-efficacy of two colored locally available inert dusts against the most economically important insect pest of stored maize grain, maize weevil under laboratory condition.

6.2 Materials and Methods

The study period and insect culture were as shown in chapter 5

Inert Dusts: Two different colored locally available inert dusts (white and red) collected from the study area (Plate 8) were sieved to remove larger particles. These inert dusts were then applied at the rates of 1.25%, 2.5%, 5% and 10% (w/w), which were equivalent to 1.25 g/100 g, 2.5 g/100 g, 5 g/100 g and 10 g/100 g of maize grains following similar procedures by Tadesse (2003) and Demissie *et al*. (2008).

Treatment Application: Following the methods used by Tadesse (2003) and Demissie *et al*. (2008), the aforementioned four different rates of each inert material were weighed and added to each 1 L glass jar containing 100 g of disinfested maize grains and shaken well to get a uniform coating. Treated and untreated controls were included. In the treated control, Malathion 5% dust at the recommended rate of 0.05% (w/w) was used. In the untreated control, neither the inert dusts, nor Malathion 5% dust was used. After treatment, twenty pairs of three to seven day old, unsexed *S. zeamais* were introduced to the treated and untreated seed in the glass jars (Demissie *et al*., 2008). Then, the jars were covered with nylon mesh and held in place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. The experiments were laid down in a Completely Randomized Design (CRD) in three replications. All treatments were maintained under the same laboratory conditions indicated in chapter 5. Data were collected on:

a) Adult Mortality: Data on adult mortality were recorded after 1, 2, 3, 4, 7 and 14 days after treatment application, while the dead and alive adults were removed from the jars after the last count at 21st day as described by Tadesse (2003) and Demissie *et al*. (2008).

b) F1 progeny emerged, **Grain Damage and Weight Loss:** These were determined as indicated in chapter 5.

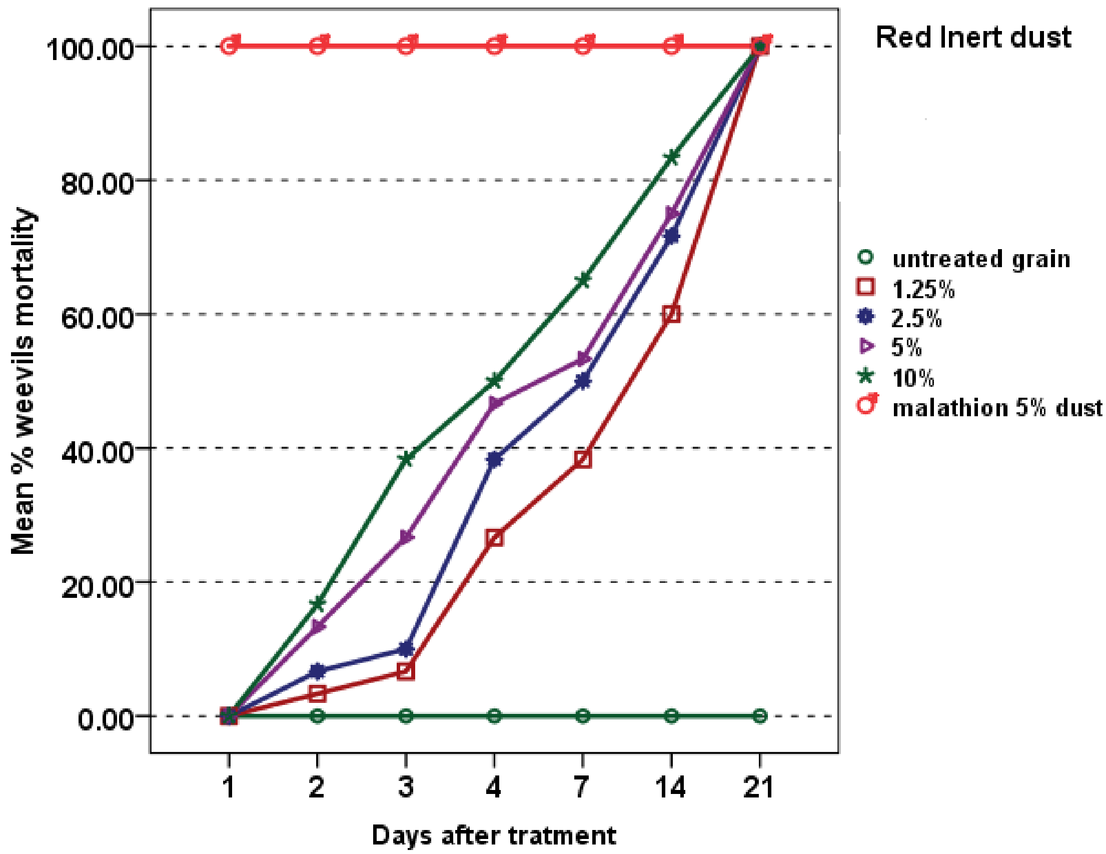
Data analysis: was done as indicated chapter 5.

6.3 Results

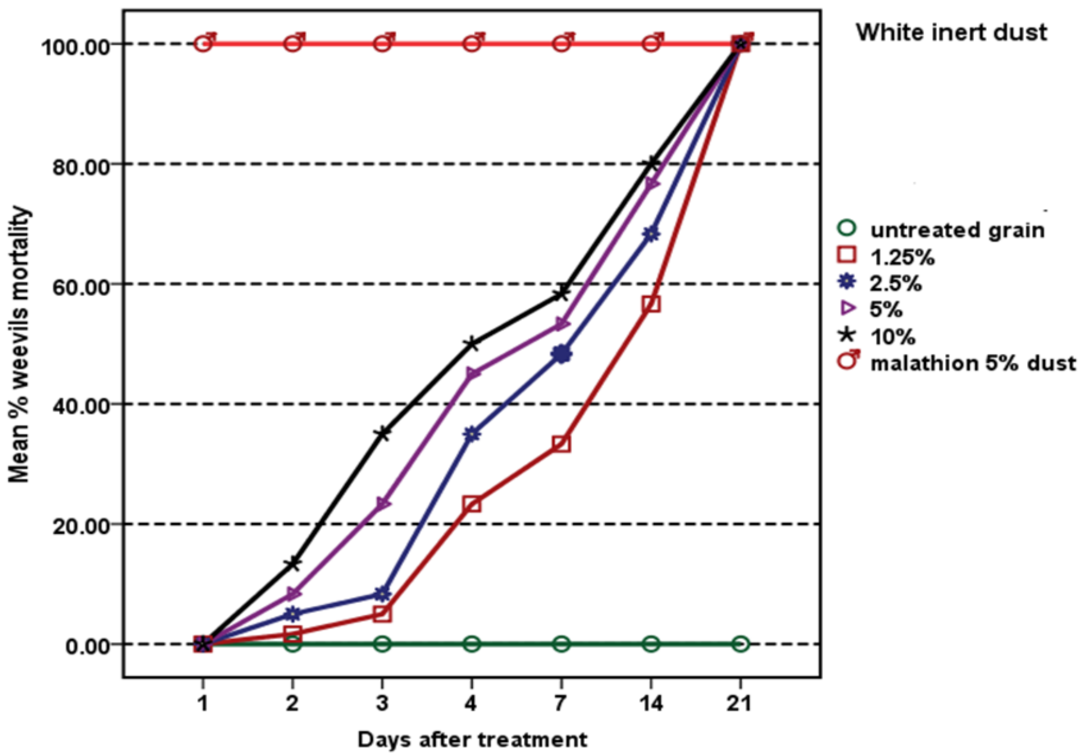
The Effect of Inert Dusts on the Mortality of Parental Maize Weevil Adults

Adult mortality among the treatments were not significant up to four days from the date of treatment application. Weevils mortality among all treatments the tested inert dusts applied at lower rates (1.25% and 2.5%) were not also significant up to 7 days of treatment application. However, mean percent mortality of the parent adult weevil in the treatments and the untreated check were significantly different ($P<0.05$) from each other at two days after treatment application (Figure 6.1).

Significantly ($P < 0.05$) higher (≥ 65%) maize weevils mortality were recorded from all treatments of the tested inert dusts applied at the dosages of 2.5%, 5% and 10% at 14 days after treatment application. Besides, 100% weevil's mortality was induced by all treatments of the tested inert dusts applied at all doses at 21 days after treatment application, which was similar to that of the positive control. Furthermore, significant death of weevils was found to be induced gradually (at 14 days after treatment application) by both of the tested inert dusts applied at the lower rates (1.25 and 2.5%), in comparisons to relatively higher dosages (5 and 10 %), in which it occurred at 7 days after treatment application. In general, the weevil's mortality was found to be increased as both the rates as well as days after treatment application were increased (Figure 6.1).



a)



b)

Figure 6. 1 Mean % mortality (mean ± SE) of parental maize weevil adults exposed to grains admixed with different rate of two colored inert dusts: a) red and b) white color

The Effect of Inert Dusts on Emergence of F1 Progeny, Percentage Protection, grain damage and Weight Loss

Significantly ($P < 0.05$) lower F1 progeny emergence, percent grain damage and weight loss were recorded from all treatments the tested inert dusts applied at all rates as compared to negative control. Significantly ($P < 0.05$) higher protection of maize grain against weevils was recorded from all treatments of the tested inert dusts applied at higher dosages (5% and 10%) than the lower rates (1.25 and 2.5%) in terms of the number of F_1 progeny emergence (≥ 52.64% inhibition), percent grain damage (≤ 5.67) and weight loss (≤ 1.12) (Table 6.1).

The correlation among the treatments of the two inert dusts applied at different dosages and the efficacy parameters measured (the number of F1 progeny emerged, percentage grain damage and weight loss) was found to be highly significant and strongly negative (r was in the range between -0.965 and -0.866). However, the correlation between the number of F1 progeny produced, and percent grain damage ($r \geq 0.947$) and weight loss ($r \geq 0.920$) of all treatments of inert dusts at all rates was highly significant and strongly positive (Table 6.2).

Table 6. 1 Mean number of F1 progeny, percentage protection and weight loss caused by *S. zeamais* in maize grains treated with inert dusts

Treatments	Dosage(g/100g)	Mean number of F1 progeny	Percentage protection	Mean % grain damage	Mean % weight loss
Red inert dust	1.25	18.00±0.33d	43.16	6.67±0.33d	2.00±0.03c
	2.5	15.00±1.20c	52.64	6.33±1.00c	1.60±0.03bc
	5	13.33±0.33b	57.91	5.00±0.58b	1.03±0.02b
	10	10.00±0.33a	68.42	2.00±0.58a	0.66±0.00a
White inert dust	1.25	20.00±0.58d	36.85	7.33±0.33d	2.33±0.01c
	2.5	16.67±1.00c	47.33	6.67±0.67c	2.10±0.03c
	5	15.00±0.58c	52.64	5.67±1.20c	1.12±0.02b
	10	11.67±0.00a	63.15	2.67±0.88ab	0.77±0.00a
Untreated grain (control)	0	31.67±0.33e	0.00	11.67±0.33e	5.33±0.01d
Malathion 5% (control)	0.05	00±0.00a	100.00	0.00±0.00a	0.00±0.00a

Means followed by the same letter in a row are not statistically significantly different at $P < 0.05$ using Tukey's studentized (THSD) test.

Table 6. 2 Correlation among efficacy determining parameters of inert dusts

Efficacy parameters	Rate of INDS	F1 RIND	F1 WIND	GD RIND	GD WIND	WL RIND	WL WIND
Rate of INDS	1						
F1RIND	-.943**	1					
F1WIND	-.949**	-	1				
GDRIND	-.965**	.967**	-	1			
GDWIND	-.934**	-	.947**	-	1		
WLRIND	-.866**	.920**	-	.910**	-	1	
WLWIND	-.912**	-	.932**	-	.866**	-	1

INDS = inert dusts, F1RIND = F1 of red inert dust, F1WIND = F1 of white inert dust, GDR = grain damage of red inert dust, GDW = grain damage of white inert dust, WLR = weight loss of red inert dust and WLW= weight loss of white inert dust.

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed) with hyphen (-) represent no association and those without asterisk are non-significant.

6.4 Discussion

The current study shown that the treatments of local inert dusts applied at the rates of 5% and above (10%) were more effective in terms of parental weevil's mortality than lower doses. This finding is in line with finding of Al-Iraqi and Al-Naqib (2006), in which the mortality of the four species of beetles (*Tribolium confusum, Trogoderma granarium, Oryzaephilus surinamensis* and *Rhizopertha dominica*) due to treatments of four local rocky dusts namely, ninivite, kaolinite, montmorillonite and bentonite was shown to be increased with increased concentration used. Similarly, Tadesse (2005) and Ibrahim (2017) also indicated that filter cake or Melkabam (by-products of Aluminum sulfate factory) applied at dosages of 0.5% (w/w) and above (5%) caused higher level of mortality of maize weevils. Demissie (2015) also explained that inert materials involving clays have been effective on stored product insects at high rates (>10 g /kg of grain) and suggests as they might be viable protectants of grain in underdeveloped countries.

The present study also indicted that the treatments of local inert dusts applied at the rates of 5% and 10% induced significantly higher protection of maize grains against maize weevils, in terms of the number of F1 progeny emerged, percent grain damage and weight loss. This was probably because inert dusts filled the interstitial space between grains mass; prevent weevil's movement, mating and reproduction. Besides, it has also revealed that all treatments of local inert dusts induced higher protection of maize grains against maize weevils than untreated check, as they induced dehydration of insects through dissection and abrasion of exoskeleton. Similarly, Demissie *et al.* (2008) shown that in addition to causing adult mortality, the different inert dusts either completely hindered or significantly reduced progeny emergence, after testing the efficacy different inert dusts against the maize weevil.

Significantly higher mortality of weevils were recorded from all treatments of the tested inert dusts applied at the rates of 5% and above (10%), following 14 and 21 days after treatment in the present study could probably be related to their slow acting and non-toxic mode of action. Similarly, Abd EL-Aziz (2011) indicated that inert dusts are chemically unreactive and thus, used for managing storage insect pests by killing physically than chemical means. McLaughlin (1994) also shown that the action of caused by inert dust is progressive, and extended post treatment exposures for longer period against insect pests in treated grains significantly decrease the rates required to kill the population of insects. Chakraverty *et al*. (2003) also explained that inert dusts act slowly and take twenty or more days to cause insect mortality.

6.5 Conclusion

The present study has indicated that the treatments of local inert dusts applied at the rates of 5% and 10% induced significantly higher protection of maize grains against maize weevils. This fact also revealed that the local inert dusts tested at the rates of 5% and above (10%) were potent in preventing maize grains against maize weevils. Thus, these inert dusts could be used in the management of maize weevils as safe, ecologically sound and cheap management alternative to synthetic chemicals under subsistence farmer's storage conditions in Ethiopia. However, the efficacy these inert dusts against weevils under subsistence farmer's storage condition need further study before wide implementation of the outcomes this study.

Findings of this study have revealed that the two local inert dusts applied at the rates of 5% and above (10%) induced significantly higher mortality, following 14 days and 100% mortality, following 21 days of treatment application and thus, these inert dusts were concluded as slow acting substances.

Chapter 7. Maize Varietal Screening against *S. zeamais* Under Laboratory Condition

7.1 Introduction

Maize (*Zea mays* L.) is one of the most important crop in the world (Nand, 2015). Besides, among cereals, it is the most important basic crop, providing food and income to millions of resource-poor smallholders in Eastern and Southern Africa countries, in general and Ethiopia, in particular (Tefera *et al*., 2011). Of the cereal crops, it ranks second to teff in area coverage and first in total production nationally. Currently, it also ranks first in total production and productivity among all cereals grown in southern Ethiopia (Gemu *et al*., 2013). However, in Ethiopia among other things, food security has been greatly threatened by excessive post-harvest losses of grains like maize caused by storage insect pests, predominantly by the maize weevil and Angoumois grain moth (Worku *et al*., 2012).). Thus, different management strategies have been used to control maize weevil, in particular and storage pests, in general. Among these strategies, synthetic chemical insecticides have been most commonly used by resource poor farmers, who lack technical knowledge on the safe handling and use them in many parts Africa, including Ethiopia (Mvumi *et al*., 1995; Mvumi and Stathers, 2003). But, environmental pollution, high cost of application, direct toxicity to users, adverse effects on beneficial and non-target organisms and increased risk to workers safety (Ofuya and Longe, 2009) have been associated as the negative attributes related to their repeated use. This has directed to the search and development of alternative management strategies such as the use of resistant maize varieties against *S. zeamais*.

The use of resistant varieties is the cheapest, effective and ecologically safe method of protecting grains such as maize against insect pests in Africa, since there is no special technology which has to be adopted by the farmer (Helbig, 1997). Their use also requires little or no scientific knowledge by the farmers (Ahmed and Yusuf, 2007). Because of these and the aforementioned several reasons, it is very crucial to screen some currently available maize varieties in Ethiopia for resistance against storage insect pest's, in general and maize weevil, in particular. The present study, therefore, was designed with the following objectives:

1) To evaluate some currently available varieties of maize in Ethiopia against the most economically important insect pest of stored maize, *S. zeamais* under laboratory coondition

2) To determine the possibility of use of the resistant varieties as source of resistance in breeding programs

7.2 Materials and Methods

The study was conducted between 1 October - 30 June of the year 2016/2017.

Insects Culture: Culture of experimental insects was done as indicated in chapter 5.

Collection of Maize Varieties: A total of twenty one (21) currently available maize grain varieties including sixteen hybrids (BH-660, BH-540, BH-543, BHQPY-545, BH-661, BH-546, BH-547, SPRH-1, AMH-760Q, AMH-851(Jibat), AMH-853, AMH-854, MH-138Q, OHL HUV, Shone and Limu) and five open pollinated varieties (Melkasa-2, Melkasa-4, Melkasa-6Q, Gibe-2 and Morka) were collected from Bako Agricultural Research Center, Western Ethiopia (Plate 4 b). These varieties were used for screening of resistance varieties against the experimental insects (maize weevils) in Insect Science Laboratory of Addis Ababa University.

All of the aforementioned varieties used for the experiment were F2 because they represents the generation that is stored by farmers and vulnerable to *S. zeamais*. The varieties are also under production in various parts of Ethiopia presently. As described by earlier researchers (Abebe *et al*., 2009; Keba and Sori, 2013; Demissie *et al*., 2015), grains of each variety were disinfested by preserving them in a deep freezer at -20 ± 2 °C for two weeks to avoid any field infestation by insect pests. Then, the varieties were also conditioned or acclimatized for additional two weeks to the laboratory conditions prior to starting the experiment. The moisture content of the seeds was adjusted to 12 - 13% through sun drying.

Evaluation of Maize grain Varieties for Resistance against Maize Weevils: About 100 g seeds from each of the maize grain varieties were placed in a 1 L glass jar (Plate 9). The no choice experimental method, in which predetermined test insects were introduced in each jar was used for this study as adopted by Abebe *et al.* (2009). Accordingly, thirty newly emerged three to seven days old non-sexed maize weevil adults were introduced in each jars containing 100 g of each variety and were kept for fourteen days for oviposition (Derera *et al*., 2001). Grains of each variety without the test insects were maintained under similar conditions and used as a control. Then after, the jars were covered with nylon mesh and held in a place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. All treatments were set up in a completely randomized design with three replications and conducted in a laboratory at 25 - 30 °C and 65 - 70% RH. This has helped in maintaining uniform grain temperature and moisture content among all samples and to enhance oviposition, following similar procedures by pervious researchers (Abebe *et al*., 2009; Tefera *et al*., 2013; Demissie *et al*., 2015). Mortality was assessed thirteen days after introduction of weevils.

Data Collection: Following similar procedures by Abebe *et al*. (2009), Tefera *et al*. (2013), Keba and Sori (2013) and Demissie *et al*. (2015), data was recorded on the number of parental insects alive and dead taken after 13 days the oviposition period. Besides, the numbers of the test insects that emerged from each variety were counted every two days, starting from the onset first F1 emergence until no new F1 insects will emerge for about 56 days. The total numbers of F1 insect's progenies that emerged per genotype were obtained by summing-up each of the test insects F1 progenies recorded per genotype. The median development time (MDT) for each of variety was also computed as the days from the middle of the oviposition period to fifty percent of the F1 progenies development as described by Dobie (1974). Furthermore, fifty-eight days after introduction of the insects, 30 seeds were randomly taken from each jar. Then, the number of damaged and undamaged grains were counted, and percent grain damage and weight loss were determined as indicated in chapter 5.

Dobie's Index of Susceptibility (DIS): This was calculated using the method of Dobie (1974), which is based on the number of F1 progeny and the length of median developmental time.

DIS = 100x [(Natural log F1) / (MDT)], where DIS = Dobie's index of susceptibility, F1 = the total number of first generation emerging adults and MDT = the median developmental period in days. The Dobie Index of susceptibility, ranging from 0 to 11, was used to classify the maize grain varieties into susceptibility groups (Dobie, 1974): where 0 to 4 were classified as resistant; 4.1 to 7.0 were classified as moderately resistant; 7.1 to 10.0 were classified as susceptible and > 10 were classified as highly susceptible.

Selection Index (SI): In addition to DIS, the maize grain varieties were also categorized into resistant and susceptible categories based on the selection indices adapted from the method of Tefera *et al*. (2011) and Mwololo (2013) with slight modification, to see whether the response classes of the varieties varies from those that were categorized by Dobie index of susceptibility. In selection index the susceptibility parameters (parental adults mortality, F1 progeny emerged, weight of undamaged grain, weight of damaged grains, percent grain damage and percent weight loss) were integrated and thus, a selection index was calculated by summing the ratios between values and overall mean and dividing it by 5 (number of parameters) as described by Tefera *et al*. (2011). Varieties with selection index < 0.8 were classified as resistant, those in between 0.8 to 0.99 were classified as moderately resistant, those in between 1.00 to 1.39 were classified as moderately susceptible and those with > 1.40 were classified as susceptible as adopted by previous researcher, Mwololo (2013).

Data Analysis: Data entry and analysis was done using Microsoft Excel 2010 and SPSS Version 16. Data on response of different varieties of maize grain on parental weevil's mortality, F1 progeny emergence, percent grain damage and weight loss were analyzed by one-way analysis of variance (ANOVA). Significant differences between means were separated using Tukey's studentized (THSD) test at 5% confidence interval. Standard errors (± se) are given following means in Tables. Correlation between the treatments (the different varieties) and the efficacy measuring parameters like weight loss and others were determined using Pearson's correlation as indicated in chapter 5.

7.3 Results

Response of different varieties of maize grain (hybrids and OPVs) to maize weevil

Statistically significant differences ($P < 0.05$) were observed among the maize grain varieties tested in terms of F1 progeny emergence, median developmental time, percent grain damage, percent weight loss, and index of susceptibility as well as selection index as shown in Tables 7.1 and 7.2.

Parental weevil's mortality was not as such significantly ($P > 0.05$) different among most of the maize varieties infested or tested in comparison to the rest parameters of susceptibility measured. However, adult weevils that feed on six out of twenty one varieties; Melkasa-6Q, MH-138Q, SPRH, BHPY-545, AMH-760Q and Jibat (AMH-851), respectively, followed by 5 out of 21; BH-661, AMH-853, OHL HUV, Melkasa-2 and BH-540, respectively suffered with higher mortality (that ranges between 13.67 & 16.33%), as compared with the rest of ten varieties which had lower mortality (< 13.67%). Besides, significantly ($p < 0.05$) lower maize weevils progenies emergence, percent grain damage and weight loss were recorded in six out of twenty one varieties; Melkasa-6Q, MH-138Q, SPRH, BHQPY-545, AMH-760Q and Jibat (AMH-851), respectively, followed by five out of twenty one; BH-661, AMH-853, OHL HUV, Melkasa-2 and BH-540, respectively. However, significantly ($p < 0.05$) higher maize weevil's progenies emergence, percent grain damage and weight loss were recorded in the rest of ten varieties; Melkasa-4, BH-543, BH-546, BH-547, Morka (imp. USB), AMH-854, Gibe-2, Limu, BH-660 and Shone varieties (Table 7.1).

Furthermore, relatively longer median developmental time ranging from 44.33 to 53.67 were required by weevils reared in six out of twenty one varieties; Melkasa-6Q, MH-138Q, SPRH, BHQPY-545, AMH-760Q and Jibat (AMH-851), respectively, followed by five out of twenty one; BH-661, AMH-853, OHL HUV, Melkasa-2 and BH-540, respectively, as compared in the rest of ten varieties in which relatively shorter median developmental time (< 44.33) were required. Moreover, the index of susceptibility was also found to be lower (in the rage between 2.50 & 3.21) for six out of twenty one varieties; Melkasa-6Q, MH-138Q, SPRH, BHQPY-545, AMH-760Q and Jibat (AMH-851), respectively, followed by five out of twenty one; BH-661, AMH-853, OHL HUV, Melkasa-2 and BH-540, respectively, as compared to the rest in which it was relatively higher (in the rage of 4.06 - 5.10) (Table 7.1).

Thus, based on susceptibility index, out of twenty one tested varieties, six varieties including Melkasa-6Q, MH-138Q, SPRH, BHQPY-545, AMH-760Q and Jibat (AMH-851) were categorized as resistant varieties, whereas the rest fifteen varieties (Melkasa-2, BH-540, BH-661, AMH-853, OHL HUV, Melkasa-4, BH-543, BH-546, BH-547, Morka (imp. USB), AMH-854, Gibe-2, Limu, BH-660 and Shone) were rated as moderately susceptible varieties (Table 7.1). However, the same varieties were rated as resistant, moderately resistant, moderately susceptible and susceptible categories based on selection index adapted from method of earlier scientists, as indicated in materials and methods section. This selection index was derived from F1 progeny emerged, weight of undamaged, weight of damaged grains, percentage grain damage and weight loss. Based on it, six; Melkasa-6Q, MH-138Q, SPRH, BHQPY- 545, AMH-760Q and Jibat (AMH-851) were rated as resistant, five; BH-661, AMH-853, OHL HUV, Melkasa-2 and BH-540 were rated as moderately resistant varieties, eight; Melkasa-4, BH-543, BH-546, BH-547, Morka (imp. USB), AMH-854, Gibe-2 and Limu were rated as moderately susceptible and two; BH-660 and Shone were rated as susceptible varieties (Table 7.2).

The correlation among the susceptibility parameters of different maize grain varieties were highly significant (Tables 7.3 and 7.4). Index of susceptibility had strong positive association with susceptibility parameters like the number of F1 progeny, percentage grain damage and weight loss. However, it had strong negative relationship with percentage parental adult weevil's mortality and median developmental time. The correlation was also strongly positive between the number of F1 progeny emerged, and the percentage grain damage and weight loss, while it was strongly negative between the median development time, and percent grain damage and weight loss. Furthermore, the correlation was strongly negative between the parental adult mortality, and the percentage grain damage and weight loss (Table 7.3). Similarly, selection index had strong positive association with parameters like weight of damaged grains, the number of F1 progeny emerged, percent grain damage and weight loss, while it had strong negative relationship with weight of undamaged grains (Table 7.4).

Table 7. 1 Response of different varieties of maize grain against maize weevils based on DIS

Variety	Parental adults mortality	F1 progeny emerged	Median development time	% Grain damage	% weight loss	DIS (Dobie's Index of susceptibility)	Resistance category
Melkasa-2 (OP)	13.67±0.33ab	64.33±0.33cd	44.67±0.33abc	8.33±0.24cdef	5.99±0.24cdef	4.05±0.04d	MR
Melkasa-4 (OP)	12.33±0.33abc	68.33±0.33def	43.33±0.33ab	9.67±0.24efgh	6.95±0.24efgh	4.23±0.04def	MR
Melkasa-6Q (OP)	16.33±0.33e	22.00±0.58a	55.67±0.88gd	3.67±0.24a	2.64±0.24a	2.46±0.16a	R
BH-540 (H)	11.67±0.33a	66.67±0.33cde	43.00±0.58ab	12.33±0.41hij	6.47±0.41defg	4.21±0.13def	MR
BH-543 (H)	11.33±0.33a	75.33±0.33gh	42.33±0.33ab	9.00±0.24defg	8.86±0.24ghij	4.43±0.03def	MR
BHQPY-545 (H)	14.67±0.33ab	26.67±0.33ab	48.33±0.33abcd	12.33±0.24hij	4.79±0.24bcd	2.95±0.06bc	R
BH-546 (H)	12.00±0.58ab	73.00±0.58fgh	42.33±0.88ab	6.67±0.24bcd	8.86±0.241hij	4.40±0.09def	MR
BH-547 (H)	11.67±0.33ab	77.67±0.33hi	43.00±0.58ab	13.67±0.24hijk	9.82±0.24ijk	4.39±0.06def	MR
BH-660 (H)	11.00±0.58a	128.67±0.33k	41.33±0.33a	21.33±0.41l	15.33±0.96l	5.10±0.04g	MR
BH-661 (H)	13.67±0.33ab	63.00±0.58c	44.00 ±0.33abc	4.67±0.24ab	5.27±0.24bcde	4.10±0.06d	MR
MH-138Q (H)	15.33±0.33b	25.00±0.58ab	51.00±0.33cd	7.33±0.24bcde	3.35±0.24ab	2.78±0.21ab	R
Morka (imp. USB) (OP)	11.67±0.33ab	80.67±0.33ij	42.00±0.58a	15.00±0.24ij	10.78±0.41jk	4.54±0.06ef	MR
AMH-851 (Jibat) (H)	13.67±0.33ab	29.67±0.88b	45.67±0.33abc	6.67±0.24bcd	4.79±0.24bcd	3.22±0.05b	R
AMH-853 (H)	12.67±1.20ab	64.33±0.88cd	44.00±0.58abc	8.33±0.96cdef	5.98±0.63cdef	4.11±0.05de	MR
AMH-854 (H)	11.67±0.33a	74.33±0.33gh	42.33±0.33ab	11.67±0.24ghi	8.38±0.24ghi	4.42±0.03def	MR
AMH-760Q (H)	13.67±1.20ab	28.33±0.88b	46.67±0.58de	4.67±0.24ab	4.07±0.24abc	3.11±0.08bc	R
SPRH (H)	15.00±0.58b	26.33±0.33ab	49.67±0.33abcd	5.67±0.33abc	3.35±0.24ab	2.86±0.02abc	R
OHL HUV (H)	13.33±0.33ab	63.33±0.33c	44.00±0.58abc	8.33±0.33cdef	5.99±0.24cdef	4.10±0.05d	MR
Gibe-2 (OP)	12.33±0.33abc	68.67±0.33def	43.00±0.58ab	10.33±0.33fgh	7.42±0.24fgh	4.27±0.06def	MR
Limu (P3812W) (H)	11.67±0.33ab	71.00±0.58efg	42.67±0.33ab	11.00±0.58fghe	7.9±0.41fghi	4.34±0.04def	MR
Shone (PHB-30) (H)	11.67±0.33ab	82.67±0.33j	42.00±0.58a	16.00±0.33j	11.5±0.41k	4.57±0.06e	MR

Means followed by the same letter within the column are not significantly different at $P < 0.05$ using Tukey's studentized (THSD) test.

MR = moderately resistant; *R = resistant*; *S = susceptible*; *OP = open pollinated varieties*; *H = hybrids.*

Table 7. 2 Response of different varieties of maize grain against maize weevils based on SI

Variety	Weight of undamaged	Weight of damaged	Percent grain damage	Percent weight loss	F1 emerged	Selection index	Resistanc e category
Melkasa-2 (OP)	3.68	0.13	8.33	5.03	65.33	0.94	MR
Melkasa-4 (OP)	3.61	0.17	9.67	5.40	66.67	1.0	MS
Melkasa-6Q (OP)	3.85	0.04	3.67	2.70	22.00	0.52	R
BH-540 (H)	3.64	0.13	9.00	5.80	68.33	0.98	MR
BH-543 (H)	3.51	0.22	12.33	6.80	75.33	1.21	MS
BHQPY-545 (H)	3.73	0.14	6.67	3.12	27.67	0.72	R
BH-546 (H)	3.51	0.22	12.33	6.93	73.00	1.20	MS
BH-547 (H)	3.45	0.26	13.67	7.10	77.67	1.30	MS
BH-660 (H)	3.15	0.52	21.33	8.33	128.67	1.93	S
BH-661 (H)	3.71	0.11	7.33	4.60	63.00	0.87	MR
MH-138Q (H)	3.81	0.07	4.67	2.87	25.00	0.59	R
Morka (imp. USB) (OP)	3.40	0.32	15.00	7.10	80.67	1.39	MS
AMH-851 (Jibat) (H)	3.73	0.13	6.67	3.37	29.67	0.73	R
AMH-853 (H)	3.67	0.14	8.33	4.93	64.33	0.93	MR
AMH-854 (H)	3.53	0.20	11.67	6.80	74.33	1.16	MS
AMH-760Q (H)	3.77	0.10	5.67	3.23	28.33	0.66	R
SPRH (H)	3.81	0.07	4.67	2.91	26.33	0.60	R
OHL HUV (H)	3.67	0.15	8.33	4.60	63.33	0.93	MR
Gibe-2 (OP)	3.59	0.19	10.33	5.57	68.67	1.10	MS
Limu (P3812W) (H)	3.56	0.20	11.00	5.90	71.00	1.12	MS
Shone (PHB-30) (H)	3.36	0.34	16.00	7.40	82.67	1.45	S

MR = moderately resistant; *R = resistant*; *S = susceptible*; *OP = open pollinated varieties*; *H = hybrids.*

Table 7. 3 Correlation among susceptibility parameters of different maize grain varieties of DIS

Susceptibility Parameters	SI	PAM	F1	MDT	GD	WL
SI	1					
PAM	-.804**	1				
F1	.948**	-.696**	1			
MDT	-.881**	.610**	-.726**	1		
GD	.853**	-.694**	.918**	-.674**	1	
WL	.908**	-.786**	.893**	-.768**	.886**	1

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 and those without asterisk are non-significant.

SI = selection index, PAM = parental adults mortality, MDT = median developmental time, GD = percent grain damage and WL = weight loss

Table 7. 4 Correlation among susceptibility parameters of different maize grain varieties of SI

Susceptibility Parameters	SI	U	D	F1	GD	WL
SI	1					
U	-.992**	1				
D	.955**	-.971**	1			
F1	.951**	-.922**	.842**	1		
GD	.982**	-.977**	.936**	.918**	1	
WL	.925**	-.904**	.816**	.893**	.886**	1

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed) and those without asterisk are non-significant.

U = weight of damaged grain, D = weight of undamaged grain, F1 progeny emerged, GD = percent grain damage and WL = weight loss

7.4 Discussion

The current study has indicated the existence of considerable variation among the tested maize grain varieties (open pollinated and hybrids) in terms number of F1 progeny emergence, median developmental time, percent grain damage, percent weight loss, the susceptibility index and the selection index. These variations in turn suggests the presence heritable variation among different maize grain varieties tested in terms of possession of resistant factors (qualities) that conifer resistance to them against weevils. Similarly, resistance of maize grain to its post-harvest insect pests was indicated to be an inherent characteristic or trait, which demonstrates itself as antixenosis and antibiosis (Kumar *et al*., 2006; Tefera *et al*., 2011; Wanja *et al*., 2015).

Out of twenty-one maize grain varieties tested for resistance against weevils in the current study, only six were resistant, and the remaing were moderately resistant, moderately susceptible and susceptible varieties. Among the resistant varieties, most (four out of six) were quality protein maize grain varieties. The fact that these four varieties were resistant against maize weevil might be probably due to the effect of protein constituents such as tryptophan and lysine content (biochemical effect) which probably might adversely affect weevils feeding or preference as a host &/or growth and development. In line with this finding, different types of resistance factors to insect pests attack have been also reported and indicated as they include both morphological and biochemical traits, and work individually or collectively (Wanja *et al*., 2015). Similarly, Arnason *et al*. (2004) also indicated that protein content was negatively correlated with the susceptibility of maize cultivars to *S. zeamais*.

In the present study, the parental weevil's mortality was not as such significantly different among most of the varieties infested, in comparison to the rest of the susceptibility parameters measured. This finding suggests that parental adult mortality was not as such a good parameter for screening resistant varieties against maize weevils, in comparison to the other parameters tested. This might be probably because of this parameter has been measured after the short duration of oviposition. Similarly, Tadesse (1991) indicated that, as this parameter might not be a good indicator of susceptibility, since adult weevils were found to survive without food for more than ten days in a laboratory test. Dobie (1974) also shown that there was no evidence for a variation among the varieties in their effects upon the mortality of parental *S. zeamais* adults, because of their low overall mortality. Similar finding was also reported by previous researchers (Abebe *et al*., 2009; Tefera *et al*., 2013).

The current study also indicated that almost all of the five parameters measured (F1 progeny, median developmental time, grain damage, grain weight loss and the susceptibility index were significantly varied among the tested varieties in contrast to parental weevil’s mortality. It has also revealed that all the five parameters measured were strongly correlated. It has also shown that selection index which had integrated more parameters was better than Dobie’s Index of susceptibility, in which only two parameters were considered. All these results suggests that as all of the aforementioned parameters were relatively better parameters for screening of varieties of grains against maize weevil. This finding is in accordance findings of Tefera *et al*. (2011) and Mwololo (2013), in which F1 progeny emergence, percentage grain damage, percentage weight loss and flour weight were shown as they can be integrated to calculate a susceptibility index.

In the present study again, significantly lower numbers of F1 progeny emergence, lower percent grain damage and weight loss, as well as requirement of longer developmental time of F1 progeny were observed in six of resistant varieties than in the rest of fifteen varieties, in which the inverse were true. Similarly, Tadesse (1991) also indicated that the extent of damage during storage depends on the number of F1 progeny emerged in each generation and the duration required for each generation.

7.5 Conclusion

Six verities; Melkasa-6Q, MH-138Q, SPRH, BHQPY-545, AMH-760Q and Jibat (AMH-851) out of twenty-one maize grain varieties in the present study possess traits for resistance against maize weevils. These varieties could be stored relatively for longer periods under subsistence farmer's storage conditions. Hence, these resistance varieties could be implemented and adopted as a cheep, ecologically sound and effective management method to reduce loss by *S. zeamais* under subsistence farmers storage conditions at national level, in general. Besides, the resistant variety screened in the current study could be also used as a source of resistance in breeding programs to diversify the basis of resistance against maize weevils. However, these resistance varieties should be exploited after repeated and refined tests to screen out the best materials or factors that conifer resistance to them for practical application in breeding programs to develop resistant varieties. From the present study, it is also possible to conclude that all the parameters tested rather than parental weevils mortality were relatively better methods for screening of maize grains against maize weevils. Thus, these parameters can be integrated to calculate selection index, based on which varieties were well categorized in to various response classes of susceptibility than susceptibility index by Dobie's, which consider only two parameters.

Chapter 8. Integration of Botanicals and Inert Dusts with Resistant Varieties for the Management of *S. zeamais* under Laboratory Condition

8.1 Introduction

In Africa, small-scale farmers produce the bulk of grain and food security of these farmers depends on not only to their success to grow, but also to their success to store their staple food that they need for their families (Blum and Bekele, 2002). Accordingly, in developing countries like Ethiopia, lack of appropriate grain storage technologies leads up to 20-30% post-harvest losses, particularly due to post-harvest insect pests such as maize weevils (Tefera and Abass, 2012). In addition, estimations based on some limited observations indicated that grain losses in maize due to storage insect pests alone are about 30-100% (Tadesse, 2003; Demissie, 2006). Furthermore, storage insect pests are reported to be the most detrimental of even among all factors (biotic and abiotic factors) that cause about 43% of the total physical and nutritional loss of grains such as maize occurring in the developing world (Chomchalow, 2003). As a result, post-harvest losses resulting from insects pests remain a huge challenge (Tefera *et al*., 2010).

Thus, losing crops to insect pests constitutes a great constraint to the realization of food security of farmers not only in Sub-Saharan Africa (SSA), including Ethiopia, but also worldwide (Obeng-Ofori, 2008). Accordingly, post-harvest losses reduction of food grains like maize due to storage insect pests is very crucial to ensure food security and to feed the ever-increasing population of the SSA countries (Dejene, 2004). However, less attention and resources have been allocated for this purpose over the past decades. These and other factors mentioned above, along with the current concerns of synthetic insecticides and the desire for residue free grains by consumers, indicate the presence of great demand for searching and developing of alternative management options such as an integrated pest management (IPM). It has been reported that IPM give priorities to non-chemical methods such as botanicals, inert dusts and varietal resistance among others. Thus, the current investigation was initiated with the following objectives:

1) To study the effect of integration of *Calpurnia aurea* and *Millettia ferruginea*, and red and white locally available inert dusts with three resistant varieties of maize; Melkasa-6Q, MH-138Q and SPRH at selected doses against *S. zeamais* under laboratory conditions

2) To compare the efficacy of integration the aforementioned tactics with unitary tactics (the two botanicals & the two inert dusts) at selected rates against *S. zeamais* under laboratory conditions

8.2 Materials and methods

The study period: The study was conducted in the same period indicated in chapter 5.

Insect's culture: This study was done as indicated chapter 5.

Integration of botanicals and inert dusts with resistant varieties against *S. zeamais*: Following the methods adopted by Gebre-Selase and Emana (2009) and Kidane and Jembere (2010), 100 g of disinfected the top three resistant varieties of maize grains among screened varieties of chapter seven were introduced into 1 L glass jars (Plate 9). These were treated differently with 0.625%, 1.25% and 2.5% doses (rates) of the leaf powders of the test plants (Plate 5). Each plastic jars containing a total of 100 g of grains treated with respective concentrations of the plants leaves powder was also be admixed with 0.625%, 1.25% and 2.5% rates each of the two colored inert dusts (red and white) (Plate 8), following similar procedures adapted by Ibrahim (2017), and Ibrahim and Sisay (2012).

Untreated grains of the aforementioned three varieties were kept under similar conditions and served as a negative control, while Malathion (5%) dust at recommended dose of 0.05% (w/w) was served as positive standard check. The jars were then covered with nylon mesh and held in a place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. The corresponding glass jars were shaken well for five minutes in order to have a uniform mixture of treatments with the grains and were kept in the laboratory at 27 ± 5 ^oC and 55 - 70% RH (Jembere *et al*., 1995; Kidane and Jembere, 2010). Twenty randomly picked newly emerged three to seven days old unsexed parental experimental insects were introduced to each treated and untreated jars and were kept for fourteen days of oviposition (Derera *et al*., 2001; Tadesse and Basedow, 2005). Each experiment was arranged in a completely randomized design with three replications. The adult mortality counts were performed after 1, 2, 3, 4, 5, 7 and 12 days after treatment application. All dead and live adults were taken away from the jars after the last count and the jars were be kept under the same laboratory conditions for 56 days for F1 progenies count as indicated in chapter 5.

Data Collection: Data were also collected on number of F1 progeny emerged, number and weight of damaged and undamaged grains. Then, parental adult weevil's mortality, number of F1 progeny emerged, percent grain damage and weight losses were determined as indicated chapter five.

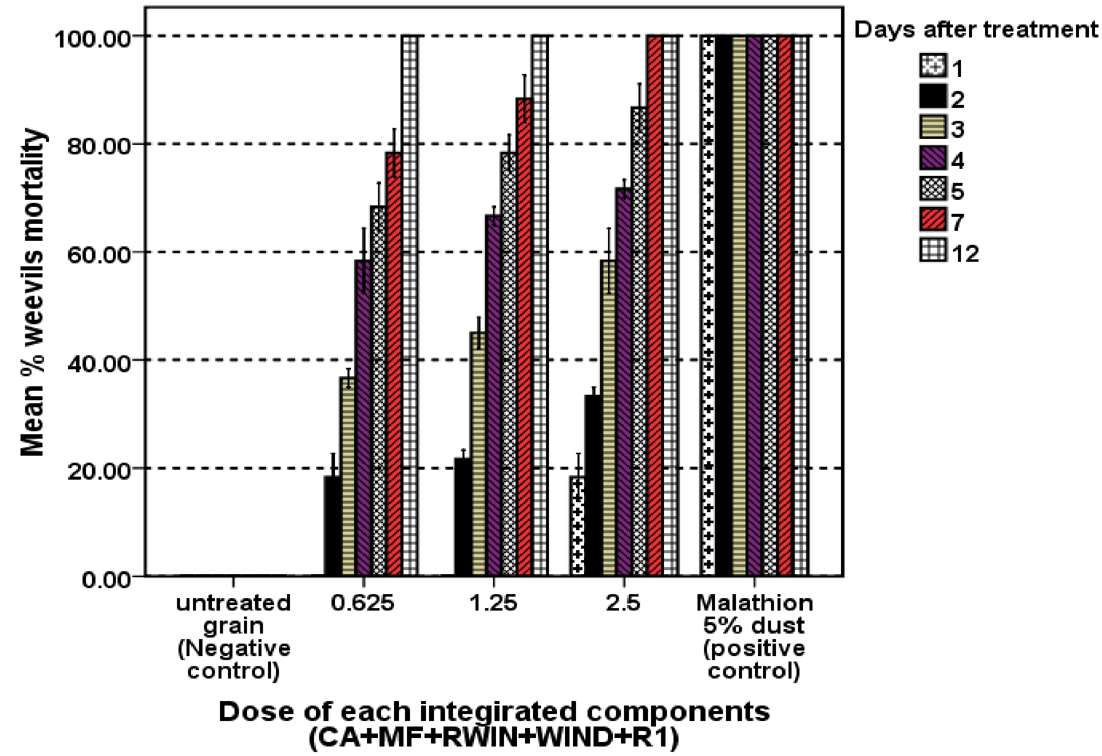
Data Analysis: This was done as indicated in chapter 5. In addition, Univariate analysis was also used to compare the effect of integration treatments with unitary tactics tested against parental weevil's mortality, F1 progeny emergence, and percent grain damage and weight loss.

8.3 Results

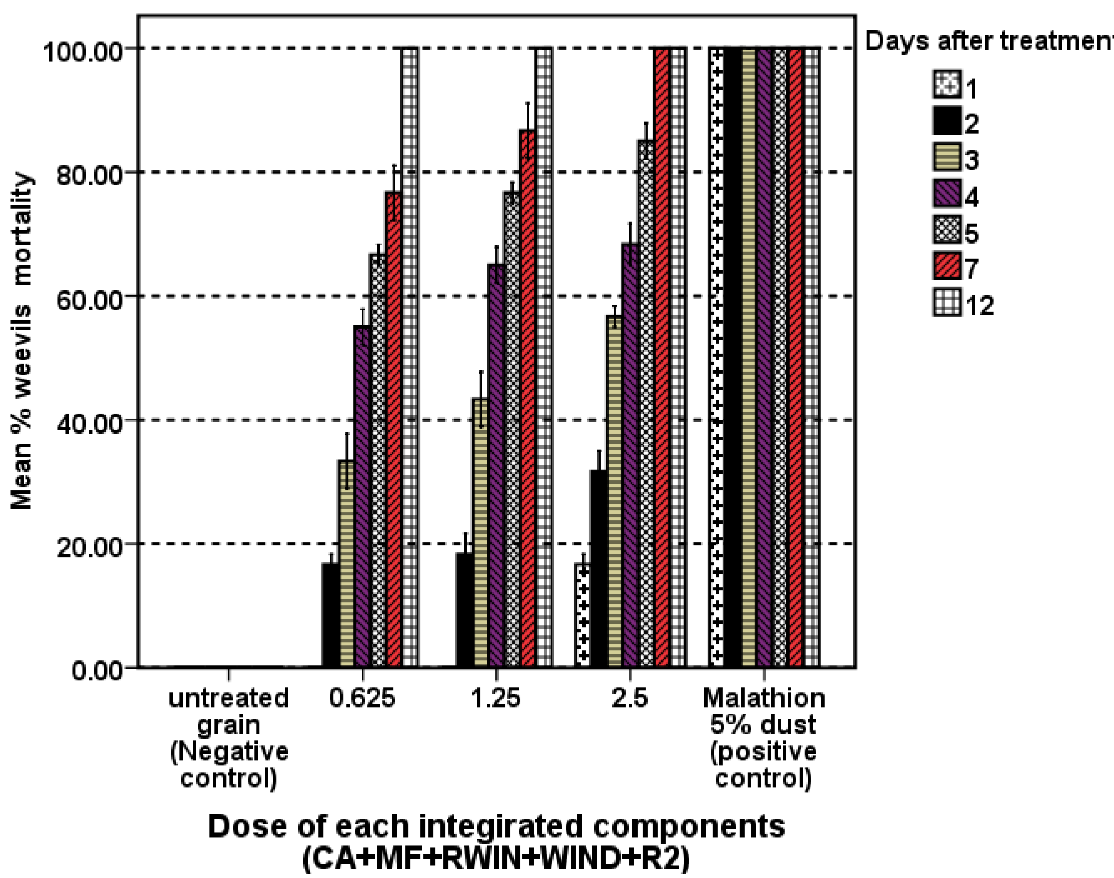
The Effect of Integration of Botanicals, Inert Dusts and Resistant Varieties of maize grains on Maize Weevils Mortality

Integration the botanicals and inert dusts with the resistant varieties had significant ($P< 0.05$) effect on percent mortality of maize weevils, in comparison to the untreated check. Significantly ($P < 0.05$) higher parental weevil's mortality were recorded from all treatments of integration applied all rates at 2 days after treatment application as compared to the untreated check. Mortality effect of the tested integrated treatments was also increased with both increased dosages and days after treatment application (Figure 8.1).

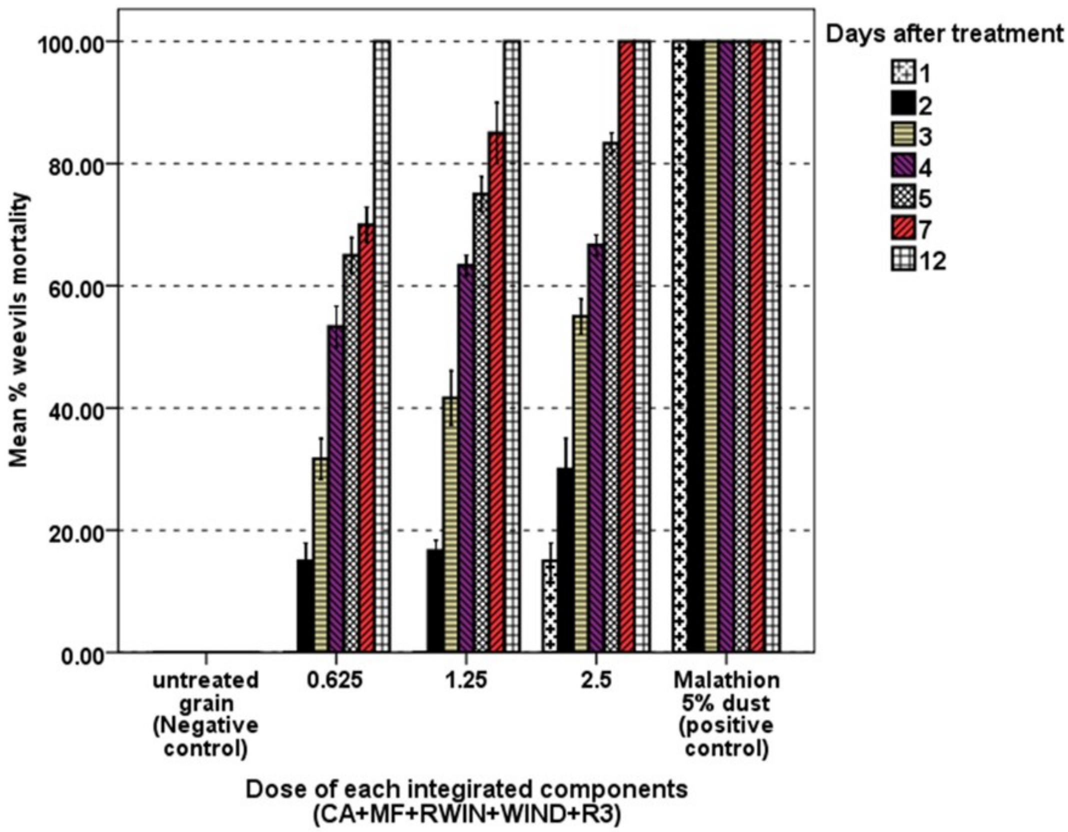
Significant percentage weevil's mortality were not recorded from all treatments of integration applied at all rates prior to four days post treatment exposure. However, significantly ($P < 0.05$) high weevil's mortality (> 53%) were recorded from all treatments of integration applied at all dosages at four days after treatment application. Besides, the weevil's mortality were significantly ($P < 0.05$) higher (> 60 and 65%) in the all treatments of integration applied at all doses at 5 and 7 days after treatment application, respectively. Furthermore, 100% weevils mortality was recorded from all treatments of integration applied at all rates at 12 days of treatment application likewise that of the positive control (Figure 8.1).


Days after treatment
1
2
3
4
5
7
12
Mean % weevils mortality
100.00
80.00
60.00
40.00
20.00
0.00
untreated grain (Negative control)
0.625
1.25
2.5
Malathion 5% dust (positive control)
Dose of each integirated components
(CA+MF+RWIN+WIND+R1)

a)


Days after treatment
1
2
3
4
5
7
12
Mean % weevils mortality
100.00
80.00
60.00
40.00
20.00
0.00
untreated grain (Negative control)
0.625
1.25
2.5
Malathion 5% dust (positive control)
Dose of each integirated components
(CA+MF+RWIN+WIND+R2)

b)



c)

CA = Calpurnia aurea powder, MF = Milittia ferruginea powder, RIN = Red inert dust, WIN = White inert dust, R1= the 1st, R2 = the 2nd, R3 = the 3rd resistant varieties of maize among the screened ones and the top three resistant varieties among screened varieties in chapter 7 were Melkasa-6Q, MH-138Q and SPRH, respectively.

Figure 8. 1 Mean % mortality (mean ± SE) of adult weevils due to integration of botanicals, inert dusts and with the top three resistant varieties: a) Melkasa-6Q, b) MH-138Q and c) SPRH respectively applied at the rates of 0.65%, 1.25% and 2.5% each after days of post treatment exposure

The Effect of Botanicals, Inert Dusts and Resistant Varieties Integration on Maize Weevils F1 Progeny Emergence, Percentage Grain Damage and Weight Loss

Significantly ($P < 0.05$) lower F1 progeny emergence, percent grain damage and weight loss of maize weevils were recorded from all treatments of integration applied at all rates than untreated check. All the parameters measured were also significantly ($P < 0.05$) lower in all treatments of integration applied at the rates of 5% and 10%, as compared to those applied at lower dosage (2.5%). Significantly ($P < 0.05$) higher protection of maize grains against weevils was recorded from all treatments of integration applied at all rates in terms of F1 progeny emergence (≥ 60.31% inhibition), grain damage (≤ 5.67) and weight loss (≤ 1.30) than negative control. But, 100% inhibition of F1 progeny emergence, no percent grain damage and weight loss of maize grains were observed in all of the three treatments of integration applied at dosage of 10 % likewise with that of the positive control (Table 8.1`).

The correlation among the treatments of integration applied at different dosages and the efficacy parameters measured (the number of F1 progeny emerged, percentage grain damage and weight loss) was highly significant and strongly negative (r was in the range between -0.950 and -0.790). However, the correlation was highly significant and strongly positive between the number of F1 progeny produced, and percent grain damage ($r \geq 0.952$) and weight loss ($r \geq 0.963$) of all the three treatments of integration applied at different rates (Table 8.2).

Table 8. 1 The Effect of integration of CA, MF, RIND and WIND with 3 resistant varieties on f1 emergence, % protection, and % grain damage and % weight loss

Treatments	Dosage(g/100g)	Mean number of F1 progeny	Percentage protection	Mean% grain damage	Mean% weight loss
T1 (CA+MF+RID+WID+R1)	0.625 (2.5%)	8.67±1.67b	72.62	4.67±0.88c	0.79±0.27b
	1.25 (5%)	4.67±2.67ab	85.25	2.33±0.33b	0.35±0.27ab
	2.5 (10%)	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
T2 (CA+MF+RE+WID+R2)	0.625 (2.5%)	9.33±2.19b	70.54	5.33±0.33c	0.87±0.01b
	1.25 (5%)	5.33±1.67b	83.17	2.67±0.88b	0.42±0.00ab
	2.5 (10%)	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
T3 (CA+MF+RE+WID+R3)	0.625 (2.5%)	10.67±2.33b	60.31	5.67±0.33c	1.30±0.27b
	1.25 (5%)	5.67±1.73b	82.10	3.33±0.88b	0.46±0.47ab
	2.5 (10%)	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a
Untreated grain	0	31.67±0.88c	0.00	11.67±0.33d	5.39±0.33c
Malathion dust 5%	0.05	0.00±0.00a	100.00	0.00±0.00a	0.00±0.00a

Means followed by the same letter in a row are not statistically significantly different at P < 0.05 using Tukey's studentized (THSD) test.

CA = Calpurnia aurea powder, MF = Millettia ferruginea powder, RIN = Red inert dust, WIN = White inert dust, R1= the 1st, R2 = the 2nd, R3 = the 3rd resistant varieties of maize among the screened ones T1 (CA+MF+RID+WID+R1) = IPM1, T2 (CA+MF+RE+WID+R2) = IPM2, T3 (CA+MF+RE+WID+R3) = IPM3, T=treatment and IPM=integrated components.

Table 8. 2 Correlation among efficacy determining parameters of integrated components

Efficacy parameters	IPM dose	F1IP M1	F1IP M2	F1IP M3	GDIPM 1	GDIP M2	GDIP M3	WLIP M1	WLIPM M2	WLIP M3
IPMC dose	**1**									
F1IPM1	-.850**	1								
F1IPM2	-.868**	-	1							
F1IPM3	-.883**	-	-	1						
GDIPM1	-.905**	.956**	-	-	1					
GDIPM2	-.939**	-	.952**	-	-	1				
GDIPM3	-.950**	-	-	.952**	-	-	1			
WLIPM1	-.790**	.965**	.969**	-	.944**	-	-	1		
WLIPMM2	-.799**	-	.971**	.963**	-	.904**	-	-	1	
WLIPM3	-.838**	.973**	.979**	-	-	-	.918**	-	-	1

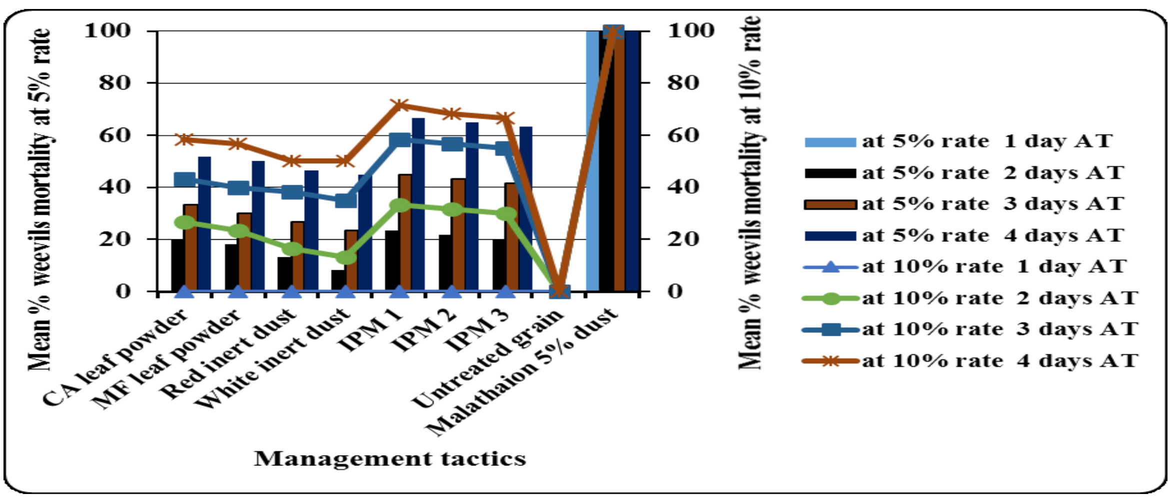
Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed), with hyphen (-) represent no association and those without asterisk are non-significant

CA = Calpurnia aurea powder, MF = Millettia ferruginea powder, RIN = Red inert dust, WIN = White inert dust, R1= the 1st, R2 = the 2nd, R3 = the 3rd resistant varieties of maize among the screened ones T1 (CA+MF+RID+WID+R1) = IPM1, T2 (CA+MF+RE+WID+R2) = IPM2, T3 (CA+MF+RE+WID+R3) = IPM3, T=treatment and IPM=integrated components.

Comparison of Integration with Others in Terms Parental Weevils Mortality

Percentage adult weevil's mortality was found to be increased with both increased dosages (concentration) and days after treatment application for all of treatments of all tested tactics applied at different rates, the highest being in integrated treatments, followed by the two botanicals leaf powders, while the minimum was in treatments of inert dusts at 2 - 4 days after treatment application (Figure 8.2).

All of the treatments of the tested management tactics also induced significantly ($P < 0.05$) higher mortality of weevils (8.33 - 71.67) at the tested rates (5% and 10%) at 2 days after treatment application than untreated check. The maximum of which being in integrated treatments, followed by the *C. aurea* and *M. ferruginea* leaf powder, while relatively the minimum was in red and white inert dusts treatments respectively. Besides, significantly ($P < 0.05$) higher weevil's mortality (45.00 - 71.67) were also observed in all treatments of all tested management tactics applied at the tested doses (5% and 10%) at 4 days after treatment application, followed by at 3 days after treatment application (23.33 - 58.33) than at 2 days after treatment application (8.33 - 33.33). However, no adult weevil's mortality was recorded in all treatments of the tested tactics applied at 5% & 10% rates at 1 day after treatment application (Figure 8.2).



AT = after treatment

CA = Calpurnia aurea powder, MF = Millettia ferruginea powder, RIN = Red inert dust, WIN = White inert dust, R1= the 1st, R2 = the 2nd, R3 = the 3rd resistant varieties of maize among the screened ones in chapter 7, T1 (CA+MF+RID+WID+R1) = IPM1, T2 (CA+MF+RE+WID+R2) = IPM2, T3 (CA+MF+RE+WID+R3) = IPM3, T=treatment, R1, R2 and R3 = Melkasa-6Q, MH-138Q and SPRH, respectively and IPM=integrated components

Figure 8. 2 Comparison of integrated treatments with others in terms of adult weevil's mortality in selected rates

Comparison of Integrated Treatments with Others in Terms of F1 progeny emerged, percent protection, percent grain damage and weight loss

The number F1 progeny emerged, percent grain damage and weight loss were significantly ($P < 0.05$) lower in all treatments of the tested management tactics applied at the tasted rates (5% & 10%) than untreated check. Significantly ($P < 0.05$) higher (≥ 52.64%) inhibition of F1 progeny emergence, higher reduction in grain damage (≤ 5.67) and weight loss (≤ 1.12) of maize grain were recorded from all treatments of integration and other tactics applied at all rates as compared to negative control. The maximums of these inhibitions of F1 progeny emergence, higher reduction in grain damage and weight loss were recorded from integration treatments, followed by the two botanicals leaf powder tested, while inert dusts induced the minimums relatively. But, 100% F1 progeny emergence inhibition, no percent grain damage and weight loss of maize grains were recorded from all treatments of the tested integration tactics applied at dosage of 10% likewise that of Malathion 5% dust (Figure 8.3).

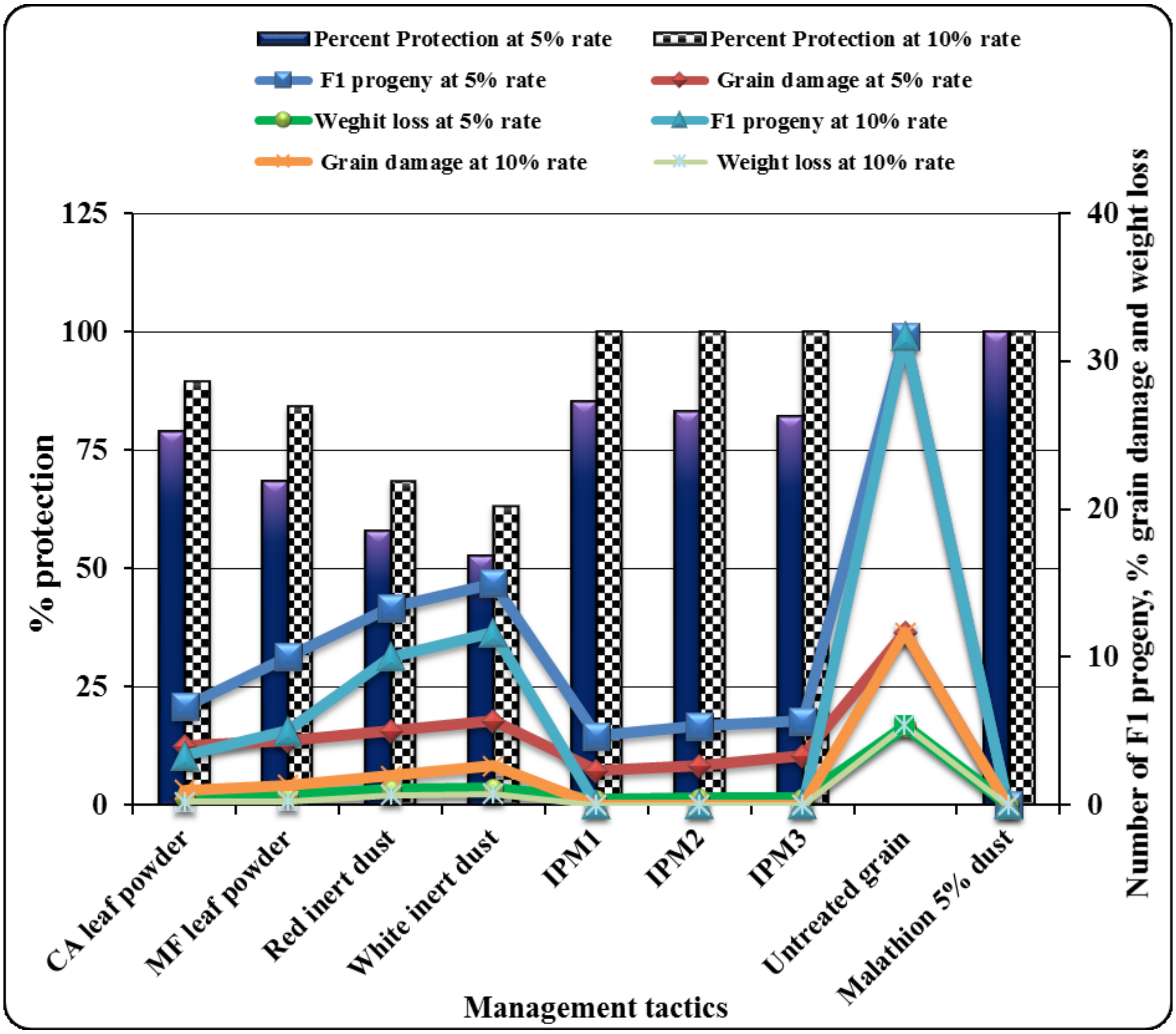


Figure 8. 3 Comparisons of integrated components with others at 5 and 10% dosage in terms of efficacy parameters; F1 progeny emergence, percent protection, and percent grain damage and weight loss

8.4 Discussion

The current study has showed significantly higher mortality of weevils at 7 days after treatment application and 100% mortality at 12 days after treatment application by all treatments of integration applied at all dosages. It has also indicated significantly higher inhibition of F1 progeny emergence, higher reduction in grain damage and weight loss due to all treatments of integration applied at all rates, and hence, the effectiveness of integration in weevils management. This suggests that integrating botanicals and inert dusts with resistant varieties could enhance their potency in managing weevils. This in turn might be probably because of the existence of great possibility of synergism of morality factors in integration than those used alone. This finding is in line with of the finding of preliminary studies conducted by Demissie (2006), in which the combined use of minimum rates of *Chenopodium* plant powder, botanical triplex, silicosec and filter cakes with weevil tolerant varieties had reduced grain damage. Similarly, Ibrahim (2017) also indicated that integrating neem seed and Mexican tea powder provided significant protection to maize from the maize weevil. Katamssadan (2016) also indicated that the powders of *P. glandulosus* and *A. indica* at their different proportions of their binary combinations generally caused significant mortality to adult *C. maculatus* and *S. zeamais*, compared to untreated grain. Ibrahim and Sisay (2012) also revealed that combinations of different rates of Malathion 5% dust and neem seed powder caused higher weevils mortality than the untreated control. Ibrahim (2015) also shown that the rates of mortality of weevils in all of the combinations of Malathion dust and Mexican tea powder was significantly higher than that of untreated check. Ibrahim (2014) also indicated that the combination of neem seed powder and Malathion at the proportions of 40% + 20% and 50% + 10% on maize was additive with respect to the mortality caused to *Sitotroga cerealella*.

Previous researcher (Bayih, 2014) also reported being in direct association of the mortality effects of the integrated treatments with dose applied and days after treatment application against the weevils in the present study.

The present study has also revealed significantly higher mortality of maize weevils at 4 days after treatment application due to all treatments of integration and other tactics tested at 5% and 10% doses, the maximums of which being in integration, followed by botanicals, while relatively the minimums were in treatments of inert dusts. This finding suggests that all the tested control tactics can be used in the management maize weevils. In line with this finding, Bayih (2014) also revealed that the unitary and binary botanical formulations at lower and higher rates were effective against *Z. subfasciatus.*

The present study has also shown that the integration the different management tactics tested induced significantly higher protection of maize grains against weevils than unitary tactics tested for about two months. This might be due the enhanced mortality effect in integration than unitary tactics tested, which in turn might be probably due to the presence of great possibility of synergism of morality factors in integration than those applied unitarily. Similarly, Dobie (1977) shown that for the reason of their synergetic effects, integration of one management method with other sustainable method could provide long lasting solution to losses in storage. Agona and Muyinza (2003) and Bayih (2014) also indicated that combining (integrating) of different botanicals has enhanced their potency in controlling stored grain insect pests than those applied unitarily. Demissie (2006) also revealed that the combined use of minimum rates of *Chenopodium* plant powder, botanical triplex, silicosec and filter cakes with weevil tolerant varieties had reduced grain damage at Bako of Ethiopia. Ibrahim (2017) also indicated that integrating neem seed and mexican tea powder provided significant protection to maize from the maize weevil.

8.5 Conclusion

In the current study, it is possible see significantly higher mortality of weevils at 7 days after treatment application and 100% mortality at 12 days after treatment application due to all treatments of integration applied at all rates. It is also possible see significantly higher inhibition of F1 progeny emergence, higher reduction in grain damage and weight loss due to all treatments integration applied at all dosages and hence, the effectiveness of integration treatments in weevils management. This confirmed that integration of the different tactics (botanicals and inert dusts with resistant varieties) at all tested rates; 2.5% and above (5% and 10%) were potent in preventing maize grains against maize weevils. Thus, integration the aforementioned tactics at all tested rates; 2.5% and above (5 and 10%) could be used in the management of maize weevils as ecologically sound and cheap management alternative to synthetic pesticides under farmer's storage conditions in Ethiopia and elsewhere with similar pest problems.

From the current study, it was possible to concluded that all the management tactics tested at rates of 5% and above (10%) were potent in preventing maize grains against maize weevils, though integration treatments were the most effective among them, followed by botanicals and inert dusts. Thus, all of the management tactics tested applied at the rates of 5% and above (10%) could be used in the management of maize weevils as safe, ecologically sound and cheap management alternative to synthetic chemicals under farmer's storage conditions in Ethiopia. Besides, it also confirmed that integration treatments rather than unitary tactics tested could be used under farmer's storage conditions to achieve better protection of stored maize grain against maize weevils.

Chapter 9. General Conclusion and Recommendations

From the current survey, it is possible to see that storage insect pests were the most economically important post-harvest constraints of storing maize by farmers in the study areas of Hadiya and Silte zones. Varieties of storage insect pests were found to be responsible for the damage of stored grains. Insect pest infestation, the associated grain damage and weight loss of stored maize were common all over the study area of Hadiya and Silte zones. The level of infestation, and amount of damage and weight losses caused were found to be influenced by several factors, including the grain forms stored, storage facilities used, length of storage and pest management practices adopted. Besides, despite of various storage facilities and management practices in use, the knowledge of farmers regarding handling of grains and application of the management tactics was low. Most of the management tactics utilized were inefficient, due to lack of knowledge among farmers to use them appropriately, like for example inappropriate type of control measures at inappropriate time, place, target and rates were used. However, some of the traditional control measures (botanicals & cultural) were appeared to be promising.

Generally, all indigenous practices and measures or methods used by farmers to preserve and prevent their maize grains from insect attack in the present study were inadequate to provide sufficient protection. Thus, the various storage insect pests losses along with unwise (injudicious) use of chemicals were found to be greatly threatened the food security farmers in the study area. Hence, relevant and periodical trainings need to be organized by government and any concerned bodies so as to provide it for extension workers, as well as farmers on appropriate handling practices of grains, type of storage facilities used, form of grain stored, management practices used and others.

The laboratory studies done at Insect since Stream of Addis Ababa University confirmed that the different traditional or bio-rational methods tested were effective in the protection of stored maize grains against maize weevil. Regarding the botanicals bio assayed, all the leaf powder and polar solvent (ethanol, acetone and water) extracts of *C. aurea* and *M. ferruginea* tested at all rates (5%, 10% and 15% of powder and 10 and 15% of solvent extracts) in the present study were found to be potent in protecting maize grains against maize weevils. Thus, the leaf powder and polar solvent extracts of *C. aurea* and *M. ferruginea* are suggested to be used at the rates 5%, 10% and 15% of and 10 and 15%, respectively in managing maize weevils in stored maize grain as ecologically sound and cheap management alternative to synthetic chemicals under subsistence farmer's storage conditions in Ethiopia.

From different inert dusts tested in the present study also, the local inert dusts measured at the rates of 5% and above (10%) were potent in preventing maize grains against maize weevils. Thus, inert dusts are also suggested as they can be used at the rates of 5% and above (10%) in managing maize weevils in stored maize grain as ecologically sound and cheap management alternatives to synthetic chemicals under farmer's storage conditions in Ethiopia.

Six varieties out of twenty-one maize grain varieties tested in the present study possess traits for resistance against maize weevils. These varieties could be stored relatively for longer periods in subsistence farmer's storage conditions. Hence, these resistance varieties are suggested to be used and adopted as a cheap, ecologically sound and effective management method to reduce loss of stored maize grain by *S. zeamais* under farmer's storage condition at national level. The resistance varieties are also suggested to be used as a source of resistance in breeding programs to diversify the basis of resistance against maize weevils. The resistant varieties could also be used in integration with other compatible insect pests control options under subsistence farmer's storage conditions in Ethiopia to get better protection of stored maize grain.

In the current study also, integration of *Calpurnia aurea* and *Millettia ferruginea*, and red and white locally available inert dusts with three resistant varieties of maize; Melkasa-6Q, MH-138Q and SPRH at all rates; 2.5% and above (5% and 10%) were potent in preventing maize grains against maize weevils attack. Thus, integration the aforementioned tactics at all rates; 2.5% and above (5% and 10%) are also suggested to be used in managing maize weevils as safe, ecologically sound and cheap management alternative to synthetic chemicals under farmer's storage conditions in Ethiopia. Besides, integration the aforementioned tactics was found to be the most effective, followed by botanicals and inert dusts at the rates of 5% and 10%. Thus, it is better to use integration of the aforementioned tactics at the rates of 5% and 10% in managing maize weevils as safe, ecologically sound and cheap management alternative to synthetic pesticides than unitary ones under farmer's storage conditions in Ethiopia.

Thus, generally the different insect pest's management experiments carried out in the present study provided useful results regarding their potency in protection of maize grain against maize weevils. However, their evaluation regarding their effect on human being, natural enemies and cost effectiveness under farmer's storage conditions is necessary before wide implementation of the outcomes these studies

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Appendix

Annex 1: Plates


a)

b)

Plate 1 Pictures showing: a) shelling method and b) partial view of samples taken during survey


a)

b)

Plate 2 Some pictures during interview of farmers in the survey sites

Plate 3 Some of storage facilities used by farmers in the survey sites

a)

b)

Plate 4 a) Picture taken during identification of insect pests associated to stored maize and b) Partial view of maize varieties collected from Bako

a)

b)

c)

d)

Plate 5 a) *C. aurea* leaf and b) *M. ferruginea* leaf dried under shade, b) leaf powder of *M. furginea* (upper one) and *C. aurea* (later one) and c) weight measuring through digital balance for both powder treatment and solvent extraction

a)

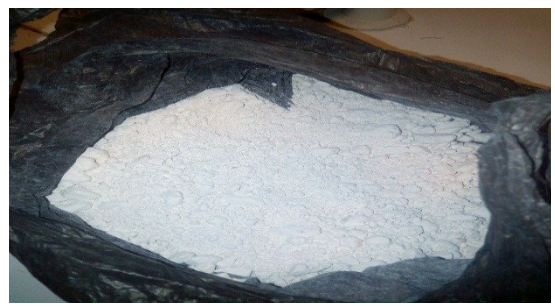
b)

c)

Plate 6 Partial view of a) polar solvent extracts and b) non polar solvent extracts at different rate and c) filter paper bio-assay

Plate 7 Partial view of insect rearing, botanicals powder and solvent extracts, inert dusts, varietal resistance screening and integration setup

a)



b)

Plate 8 Sample pictures of: a) red and b) white locally available inert dusts

Plate 9 Partial view of maize varietal screening set up (upper one)

Annex 2: Sample Tables for Summary of Table of Analysis of Variance (ANOVA) (a-e)

a) Summary table of analysis of variance (ANOVA) of level of infestation of major insect pests, percent grain damage, and weight and germination loss in four different farmer's traditional storage methods tested over 3-9 months period

parameters	Source of error	Sum of Squares	df	Mean Square	F	pr>F
Level of insect infestation	St. periods	82608.33	2	41304.16	160.80	<.0001
	St. methods	48910.71	3	16303.57	634.70	<.0001
	Spp. insects	72291.09	7	10327.30	402.10	<.0001
	St. periods * St. methods * Spp. insects	1990.78	42	47.40	18.45	<.0001
Percent grain damage	St. periods	82608.33	2	41304.16	160.80	<.0001
	St. methods	48910.71	3	16303.57	634.70	<.0001
	St. periods * St. methods	72291.09	7	10327.30	402.10	<.0001
Percent weight loss	St. periods	1990.78	42	47.40	18.45	<.0001
	St. methods	15270.03	2	7635.01	352.43	<.0001
	St. periods * St. methods	1174.57	3	391.52	180.70	<.0001
Percent germination loss	St. periods	292.77	6	48.80	22.52	<.0001
	St. methods	37057.26	2	18528.63	168.14	<.0001
	St. periods * St. methods	1039.10	3	346.37	314.18	<.0001

St = storage, Spp. = species

b) Summary of table of ANOVA of the effects of *C. aurea* and *M. ferruginea* leaf powder on the parental adult weevil's mortality

Source of error	Sum of Squares	df	Mean Square	F	Pr>F
Days after treatment	40685.83	5	8137.17	976.46	<.0001
CA powder	91852.78	4	22963.19	2756	<.0001
days after treatment* CA powder	28023.89	20	1401.19	168.14	<.0001
Days after treatment	41399.17	5	8279.83	993.58	<.0001
MF powder	91435.00	4	22858.75	2743	<.0001
Days after treatment * MF powder	28388.33	20	1419.42	170.33	<.0001

CA = C. aurea, MF = M. ferruginea

c) Summary of ANOVA of the effects of *C. aurea* and *M. ferruginea* leaf powder on F1 progeny emergence, % grain damage and weight loss

Parameters tested	Source of error	Sum of Squares	df	Mean Square	F	Pr>F
F1 of CA powder	Between groups	2133.333	4	533.333	106.667	<.0001
	Within group	50.000	10	5.000		<.0001
	Total	2183.333	14			<.0001
F1 MF powder	Between groups	233.333	10	23.333	233.333	<.0001
	Within group	233.333	10	23.333		<.0001
	Total	2543.333	14			<.0001
% grain damage of CA	Between groups	292.667	4	73.167	57.763	<.0001
	Within group	12.667	10	1.267		<.0001
	Total	305.333	14			<.0001
% grain damage of MF	Between groups	289.733	4	72.433	120.722	<.0001
	Within group	6.000	10	.600		<.0001
	Total	295.733	14			<.0001
% weight loss of CA	Between groups	63.421	4	15.855	873.086	<.0001
	Within group	.182	10	.018		<.0001
	Total	63.603	14			<.0001
% weight loss of MF	Between groups	81.207	4	20.302	101.216	<.0001
	Within group	2.006	10	.201		<.0001
	Total	83.213	14			<.0001

CA = C. aurea, *MF = M. ferruginea, F1 = F1 progeny produced*

d) Summary of ANOVA of the effects of *C. aurea* and *M. ferruginea* solvent extracts on the parental adult weevils mortality

Source of error	Sum of Squares	df	Mean Square	F	Pr>F
CA1 solvent extract	913.00	1	913.01	86.54	<.0001
time	2309.69	3	769.90	72.98	<.0001
CA1 * dose	690.78	4	172.70	16.37	<.0001
CA1 * time	57928.35	12	4827.36	457.57	<.0001
dose * time	22.03	3	7.34	0.70	>.05
CA 1 s. extract * dose * time	94.35	12	7.86	0.75	>.05
Days after treatment	79532.08	4	19883.02	1446	<.0001
CA 2 solvent extract	1080.00	1	1080.00	78.55	<.0001
time	1927.50	3	642.50	46.73	<.0001
CA 2 * dose	747.08	4	186.77	13.58	<.0001
CA 2 * time	58499.58	12	4874.97	354.54	<.0001
dose * time	8.33	3	2.78	.20	>.05
CA 2 s. extract * dose * time	47.92	12	3.99	.29	>.05
Days after treatment	47536.25	4	11884.06	864.30	<.0001
MF1 solvent extract	1050.21	1	1050.20	76.38	<.0001
time	2315.63	3	771.88	56.14	<.0001
MF1 * dose	736.25	4	184.06	13.39	<.0001
MF1 * time	57327.08	12	4777.26	347.44	<.0001
dose * time	43.96	3	14.65	1.07	>.05
MF1 s. extract * dose * time	90.42	12	7.54	.55	>.05
Days after treatment	74150.00	4	18537.50	1171	<.0001
MF2 solvent extract	1050.21	1	1050.21	66.33	<.0001
time	2217.29	3	739.097	46.68	<.0001
MF 2 * dose	750.83	4	187.708	11.86	<.0001
MF2 * time	58948.333	12	4912.361	310.25	<.0001
dose * time	3.958	3	1.319	.08	>.05
MF 2 s. extract * dose * time	57.500	12	4.792	.30	>.05

CA1 = C. aurea 20 g/ 100ml, CA2= C. aurea 30 g/ 100ml, MF1= M. ferruginea 20g/ 100ml, MF2= M. ferruginea 30g/ 100ml

e) Summary of ANOVA of the effects of *C. aurea* and *M. ferruginea* leaf solvent extracts on F1 progeny emergence and % weight loss

Source of error	**Sum of Squares**	**df**	**Mean Square**	**F**	**Pr>F**
F1 CA1 s. extract	4571. 67	4	1142.92	274.30	<.0001
Dose	13.33	1	13.33	3.20	<.0001
F1 CA1 s. extract * dose	11.67	4	2.92	.70	>.05
F1 MF1 s. extract	4280.000	4	1070.000	214.000	<.0001
Dose	30.000	1	30.000	6.000	<.0001
F1 MF1 s. extract * dose	20.000	4	5.000	1.000	>.05
WL CA1 s. extract	31.47	4	7.87	1172	<.0001
Dose	10.05	1	10.05	1497	<.0001
WL CA1 s. extract* dose	31.47	4	7.87	1172	<.0001
WL MF1	31.13	4	7.78	1213	<.0001
Dose	10.53	1	10.53	1640	<.0001
WL MF1 s. extract* dose	31.13	4	7.783	.213E	<.0001

CA1 = C. aurea 20 g/ 100 ml, MF1 = M. ferruginea 20g/ 100ml, s. extract = solvent extract, WL = weight loss, F1 = F1 progeny produced.