Addis Ababa University, School of Graduate Studies

Studies on the Interaction of Arthropods, Fungi and Mycotoxin on stored Maize Grain in Ethiopia

By

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Dedication

To my wife Tigist Belay

To the late Hilemariam Kebede and Kebede Angessa

To the late Belay Woldemedihin

Abbreviations

ACN: Acetonitrill

AF: Aflatoxin

ANOVA: Analysis of variance

Bt: *Bacillus thuringiensis*

CSA: Central Stastical Agency

DON: Deoxynivalenol

ELEM: Equine Leukoencephalomalacia

ESI: Electro-Spray Ionization

FAO: Food and Agriculture Organization of the United Nations

FB: Fumonisin B

GDP: Gross Domestic Product

IAOM: International Association of Operative Millers Food Protection Committee

IAR: Institute for Agricultural Research

IARC: International Agency for Research on Cancer

LC/MS/MS: Liquid Chromatography Tandem-Mass Spectrometry

MEA with NaCl: Malt Extract Agar with sodium chloride.

MeOH: Methanole

OTA: Ochratoxin-A

PDA: Potato Dextrose Agar

RH: Relative humidity

SE: Standard Error

SPSS: Statistical Package for the Social Sciences

ZEN: Zearalenone.

Studies on the Interaction of Arthropods, Fungi and Mycotoxin on Stored Maize Grain in Ethiopia.

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Abstract

In Ethiopia, maize (*Zea mays* L.) is the most important cereal crop produced and consumed by the majority of the population. High productivity and adaptability to a wide range of environment made the maize crop one of the national commodity crops to meet the food self-sufficiency program of the country. However, currently food safety issue related to postharvest arthropod infestation and associated problems put this crop under pressure. These studies were designed to know the species composition, frequency of occurrence, and abundance of stored maize grain inhabiting arthropods with particular reference to mold feeder arthropods (Sap beetles, Staphylinids and others) for designing management options for the pest and to understand the effects of their damage on potentially mycotoxin producing fungal infection and Aflatoxin contamination of stored maize grain. The studies were conducted in purposively selected Woreda of Amhara and Oromia region, Ethiopia, from August 2013 to August 2017. Random sampling method was employed for Arthropods survey. Completely Randomized Design was used for sample collection from selected Woreda of the two regions to determine the effects of insect and mold damaged maize cob on status of sap beetles and other mold feeder insects, corn ear rot types, development of detection methods for sap beetles and Staphylinids and best detection time, and the effects of arthropods on potentially mycotoxin producing fungal infection and Aflatoxin contamination. LC/MS/MS method was used to detect aflatoxin contamination in stored maize grain. It was known from the results obtained that 81 arthropods; belonging to class Insecta (87.65%), Arachnida (8.64%), and Crustaceans (3.70%) were identified. In all selected Woreda from both regions and in both years, *Sitophilus* spp., and *Sitotroga cerealella* from grain feeders; *Nitidulidae* spp., *Mycetophagidae* spp., and *Drosophillidae* spp., from mold feeders; *Lepinotus* from psocids; *Tyrophagus putrescentiae* from mites; *Staphylinidae* spp., and *Dactylosternum abdominal* from natural enemies associated with mold feeder arthropods and parasitic Hymenoptera from natural enemies associated with grain and grain product feeders were found to be the most frequently and abundantly encountered arthropods. In the current study, 27 species were recorded for the first time as occurring in Ethiopia. In the study on status of sap beetles and other mold feeder arthropods on pre-harvest maize cob damage types, 19 species of mold feeder arthropod occurred in association with insect and mold damaged cobs. However, only *Carpophilus* sp., *Brachypeplus* sp., *Litargus balteatus* LeConte, *Carpophilus dimidiatus* (F.), *Carpophilus hemipterus* (L.), and *Entomobrya* spp., appeared in more than 30% of insect and mold damaged cobs. Occurrence of corn ear rot types per 120 mold damaged cobs varied significantly

(P<0.05). In both regions (years), abundance of selected sap beetles and other mold feeder insects were significantly higher on insect and mold damaged cob than undamaged cob (P<0.05). Moreover, in both regions (years), the mean number of selected sap beetles and other mold feeder insect per insect and mold damaged cobs were significantly (P<0.05) higher for *Carpophilus hemipterus* (L.) and *Litargus balteatus* LeConte than *Carpophillus dimidiatus, Typhae stercorea* and *Brachypeplus* spp. More than four species were detected from each of the sap beetles and staphylinids using fermented maize cob and maize cob treated with fruit juice, in the study on how to detect these insect in stored maize grain ecosystem. *Carpophillus hemipterus* (100%, 42.11%) and *Brachypeplus* spp., (100%, 34.75%) were found to be the two most frequently and abundantly detected sap beetles during this experiment. Among the four identified genera of staphylinids, *Atheta* spp., were found to be the most frequently and abundantly detected genera (92.5%, 15.64%). Total mean number of *Carpophilus hemipterus* and *Brachypeplus* spp., per trap captured indicated that fermented maize cob and fermented banana juice mixed with fermented pineapple juice were significantly (P<0.05) more effective than fermented banana and pineapple juice in detecting these insects. However, no significant difference (P>0.05) was observed among these attractant in detecting staphylinids. Significantly (P<0.05), more *Carpophillus hemipterus* was detected in the first two weeks. However, significantly (P<0.05) more *Brachypeplus* spp., and Staphylinids were detected in the last two weeks. The result obtained from the effects of insects on potentially mycotoxin producing fungal infection and aflatoxin contamination in stored maize indicated that the incidence of potentially toxigenic fungi and aflatoxin contamination were significantly (P<0.05) higher on insect damaged maize kernels than undamaged maize kernels and mold feeder arthropods than grain feeder arthropods (P<0.05). The mean number of arthropods, grain temperature and moisture content and number of arthropod and mold damaged maize kernels were significantly (P<0.05) higher on untreated seeds than Malathion treated seeds. Pearson correlation showed significant (P<0.05) positive correlation between arthropod infestation and grain temperature and moisture content and number of arthropod and mold damaged maize kernels in stored maize grain. The present study concluded that the role of arthropod infestation in stored maize grain ecosystem. The role of arthropod in stored maize ecosystem include, exposing maize kernels to potentially mycotoxin producing fungal infection, providing suitable condition for the growth of fungi and vectoring fungal spores in stored maize. The present study also concluded that condition of stored grain could determine species composition of arthropods in stored maize grain ecosystem.

Key words: Arthropods, mold feeders, grain feeders, fungi, Aflatoxin, corn ear rot, Damage

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Chapter-1

General introduction

In Ethiopia, maize (*Zea mays* L.) is the most important cereal crop produced and consumed by the majority of the population. Its importance is mainly due to its high productivity and adaptability to a wide range of environments. Its high productivity and adaptability to a wide range of environments made it one of the national commodity crops to meet the food self-sufficiency program of the country. However, this crop is susceptible to arthropods attack during store. Arthropods in stored grain ecosystem can be classified in to granivorous, mold feeder and natural enemies. Many species of arthropods have been recorded in stored grain in Ethiopia (Mekuria, 1995: Abraham, 1997; Emana and Assefa, 1998). Abraham (2008) reported that over 100 species of arthropods are associated to stored products in Ethiopia.

The infestation of some of the arthropods associated with stored grain begins in the field. However, infestation from the field may be influenced by insect and mold damaged grain. Sap beetles and other mold feeder arthropods in stored grain are highly attracted to insect and mold damaged cobs. Dowd (1995) reported high infestation of sap beetles on maize ears damaged by birds or caterpillars or those ears that have poor husk coverage. Ako *et al*. (2003) in their studies "The effect of *Fusarium verticillioides* on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stems and cobs of maize in West Africa" also showed higher immature survival and adult fecundity of *Mussidia nigrivenella* (Ragonot) and *Carpophilus dimidiatus* (Fabricius) on *Fusarium verticillioides* infected maize plants. Sap beetles are attracted to maize cobs damaged by other insects. They appear to be attracted as well to volatile compounds produced by *Fusarium verticillioides* which caused fusarium ear rot. Sétamou *et al*. (1998) showed that the damage from *Mussidia nigrivenella* predisposes maize to pre- and post-harvest infestation of storage beetles.

The role of arthropods in stored grain ecosystem might be of energy transformer, grainvorous, , fungivorous, predacious or parasitic. On the other hand, their effects on stored grain may result in grain damage; by their feeding activities and exposing the grain to infection of potentially toxigenic fungi (Avantaggio *et al*., 2002), contamination of stored grain by allergens (Hage-Hamsten and Johansson, 1998), by vectoring mycotoxin producing fungi (Lussenhop and

Wicklow, 1990) and providing suitable condition for the growth of fungi through the release of heat and water from their metabolic activities (Dix and All, 1987).

It is well known that stored grains are infested by many species of arthropods with different role and effects. However, this information is lacking in Ethiopia. According to Hagstrum and Subramanyam (2009) over 1900 species of arthropods are known to occur in stored grain or to be associated with grain-based foods. The infestation of sap beetles and other mold feeder arthropod in stored grain ecosystem usually begins in the field and this infestation is usually influenced by insect and mold damaged commodities. However, this information is not available in Ethiopia. Moreover, sap beetles are attracted by volatiles dispersed from fermented and decomposed fruit juice and plant materials (Bartelt *et al*., 1990, 1992, 1993; Rondon *et al*, 2004; Hossain *et al*., 2012) and hence their management geared to trap baited with fermented plant materials. However, this information is also lacking in Ethiopia. Apart from these, no information is available on the role of arthropods in toxigenic fungal infection and mycotoxin contamination in Ethiopian stored grain ecosystem. Therefore, the objectives of this study were intended:

1.1 Objective:

1.1.1 General objective

- To understand the quality of the grain and the interaction of arthropods, fungi and Aflatoxin on stored maize grain in Ethiopia.

Chapter-2

2. Literature review

2.1 Overview of maize production and storage in Ethiopia

In Ethiopia cereals are considered as a major food crops both in terms of area coverage and volume of production. They are grown in all regions of Ethiopia. Out of the total grain crop area of, 79.69% (8.7million hectares) was under cereals. Among these cereals, the share of maize was about 23.42% (about 2.6 million hectares) of area coverage (CSA, 2012). From production point of view maize, wheat, teff and sorghum accounted for 23.24% (3.75 million tons), 14.36% (23.1 million quintals), 18.57% (2.99 million tons) and 16.52% (2.66 million tons) of the grain production, respectively. The survey conducted in the year 2011/12 on "Meher season post-harvest crop production" indicated that the total land areas of about 12,086,603.89 hectares were covered by grain crops. Out of the total grain crop areas, cereals accounted for about 79.34% (9,588,923.71 hectares). Of this maize accounted for 17% (about 2,054,723.69 hectares) and gave 6069413 tons of grain yields (CSA, 2012).

Farmers in Ethiopia use cribs or platforms for storing maize in cob form. Outdoor containers made from mud plaster, or baskets, with thatched roof and usually rise off the ground on stones or a wooden platform *(gotera);* underground pits and mud-plaster bins kept inside the house *(gota)* are used for storing maize grain in different parts of Ethiopia. Small quantities of grains are also found in a variety of small containers (sacks, bins, tins, boxes) kept inside the house. Farmers in the central, northern, southern and western parts of Ethiopia usually store their grain in the above-ground bin locally known as gotera (Gilman, 1968). However, underground pit was restricted to the eastern part of Amhara Region (N & S Wollo, Oromyia and N Shoa Zones) (Boxall, 1974; Gilman & Boxall, 1974; Niles, 1976; Lynch et al., 1986). Generally, the majority of Ethiopian farmers (93.3%) use traditional storage containers that expose their stored grains to storage pests attack and/or other factors. These traditional storage containers are conducive to hold large amount of grain residues which can be used as a source of storage insect pests' infestation.

2.2 Arthropods associated with stored maize in Ethiopia

Stored maize suffers heavy losses in terms of quantity and quality from several insect pests. Insects from the Orders Coleoptera and Lepidoptera were found to be the most important stored maize grain insect pests in Ethiopia (Emana, 1993b; Firdissa and Abraham, 1993b, 1999). Emana (1993a) on his "Studies on the distribution and control of Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) in Sidama administrative region" recorded eight insect species belonging to four orders and seven families associated with stored maize grain in Sidama Administrative Region namely: *Sitotroga cerealella* (Olivier), *Sitophilus zeamais* (Motschulsky), *Ephestia cautella* (Walker*), Tribolium castaneum (*Herbst*), Tribolium confusum* Jaluelin de val*, Plodia interpunctella (*Hubner*)*, *Rhizopertha dominica* (F.) and *Liposcelis* sp. Moreover, Abraham (1997) reported about 37 species of arthropod pests associated with maize grain in storage around Bako area, western Ethiopia. Seventeen arthropod species were reported from Jimma zone by Waktole and Amsalu (2012).

In Ethiopia, insect pests of stored maize grain caused losses ranging from 20%-30% (Abraham, 1991; Emana, 1993b). Emana (1993a) reported 30% to 90% losses of maize grain stored in Sidama administrative zone, southern Ethiopia. Currently, there are different management options that have been recommended to reduce this loss. These include early harvest, removal of infested grains, drying seeds thoroughly before storage, admixing grains with ash and botanicals and use of insecticides (Emana, 1999; Waktole; 2014).

2.3 Mold feeder arthropods in stored grain

In stored grain ecosystem, fungi which grow on stored grain are among the first level consumer in the energy flow. Arthropods which feed on fungi grown on stored grain are called mycophagous (mold feeder) arthropods. Mold feeder arthropod in stored grain include *Carpophilus* spp., *Brachypeplus* sp., *Ahasverus advena* (Waltl), *Litargus balteatus* LeConte, *Typhae stercorea* (L.), *Cryptophagus* spp., *Tyrophagus putrescentiae* (Schrank) and others (Magro *et al*., 1999). Fungus–feeding insects such as *Ahasverus advena*, *Coninomus* spp., *Cryptophagus* spp., *Typhae stercorea* and *Litargus balteatus* were identified from stored rice in Portugal from which several fungi were isolated, mainly *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. and *Trichothecium* sp. A large number of fungus feeders and *Aspergillus niger* van Tieghem,

Aspergillus flavus Link, *Aspergillus candidus* Link and *Penicillium islandicum* were identified simultaneously from paddy samples taken near the probe traps (Carvalho *et al*., 2004).

Carpophillus spp., were found to be the largest group of mold feeder insect in association with stored grain in Ethiopia. Surveys conducted on farm-stored maize in western and northwestern Ethiopia (Gojam, Wellega, Gambella, Illubabor, Jimma, Assosa and Shewa) during 1989 and 1993 showed *Carpophillus* spp., among the most common and dominant pests (Adhanom and Abraham, 1985; Abraham, 1991; 1993a). Mekuria (1995) also reported *Carpophillus* spp., among the most widely spread storage pest of maize in some maize growing areas of southwestern Ethiopia during the 1992 and 1993 seasons. Several other authors across the world reported many mold feeder arthropods in association with stored grain (Walker and Boxall, 1974; Pellitteri and Boush, 1983; Arbogast and Throne, 1997; Magro *et al*., 1999; Hagstrum and Subramanyam, 2009). Mold feeder arthropods are usually involved in vectoring mycotoxigenic species of *Aspergillus* and *Fusarium* to stored product (Lussenhop and Wicklow, 1990; Dowd, 1991, 1995).

2.4 Detection of arthropods associated with stored grain

Detection of granivorous and mold feeder arthropod infestation is essential for quality assurance and to ensure prolonged shelf-life of stored commodities. Several methods have been developed to detect arthropod infestation in association with stored grain. Visual inspection, sampling and sieving methods, Berlese funnel method, X-ray technique (radiography), Uric acid analysis, Carbon-dioxide analysis, specific gravity methods (floatation methods), acoustic detection and attractant traps are among the most important methods used to detect arthropods in stored grain. Incubation of highly infested stored grain sample for 120 days at 21±3 °C and 60 % RH was also used to detect hidden infestation of arthropods especially mold feeder ones (Pellitteri and Boush, 1983).

Arthropods are attracted by volatiles coming from stored food commodities. Sap beetles are among the most important stored grain arthropods attracted by volatiles emanating from fermenting plant material. Several authors reported the role of fermenting plant materials in attracting sap beetles (Warner, 1961; Smilanick *et al*., 1978; Bartelt *et a*l., 1994; Bartelt and Weisleder, 1996; Hossain *et al*., 2012). On the other hand, the mold which was grown on stored grain is responsible for attracting mold feeder arthropods. A large number of fungus feeders and

Aspergillus niger van Tieghem, *Aspergillus flavus* Link, *Aspergillus candidus* Link and *Penicillium islandicum* Sopp were identified simultaneously from paddy samples taken near the probe traps from Portugal (Carvalho *et al*., 2004).

2.5 Field infestation of sap beetles and other mold feeder insects in stored maize and factors associated to their infestation.

Sap beetles (Coleoptera: Nitidulidae) are among the most important stored grain insects reported from more than 200 species of plants (Arbogast and Throne, 1997; Magro *et al*., 1999; Hagstrum and Subramanyam, 2009). Most species of these beetles begin their infestation from the field especially when the plant is damaged by insect and mold.The damage on maize cob caused by *Mussidia nigrivenella* Ragonot resulted in the infestation of *S. zeamais* and *Carpophilus* sp., (Sétamou, 1996). Sétamou *et al*. (1998) demonstrated that damage by *M. nigrivenella* predisposes maize to pre- and post-harvest infestation of storage beetles and to infection of *A. flavus* Link which in turn leads to aflatoxin contamination. Bartelt and Wicklow (1999) and Munkvold (2003a) reported that maize plants infected with *Fusarium verticillioide* (Sacc.) release volatile chemical that attract sap beetles. Nout and Bartelt (1998) also suggested that a likely factor contributing to the initial attraction of sap beetles to damaged plant is that yeast present on damaged site proliferate and emit attractive volatiles.

Generally speaking, Sap beetles are attracted by volatiles dispersed from fermented and decomposed fruit juice and plant material (Bartelt *et al*., 1990, 1992, 1993; Rondon *et al*, 2004; Hossain *et al*., 2012). Field infestation of other mold feeder insects in stored maize were also reported in association with damaged maize cob. Mycetophagidae were among the pre-harvest mold feeder insect recoded in association with wounded ear (Rodriguez-Del-Bosque, *et al*., 2007).

2.6 Corn ear rot and associated mycotoxin

Mycotoxin problems associated with corn arise from diseases on ears of corn caused by *Fusarium*, *Aspergillus* and *Penicillium* species (Creppy, 2002). *Fusarium* ear rot (pink ear rot) and Gibberella ear rot (red ear rot) are the two most common corn ear rot diseases caused by *Fusarium* species. Fusarium ear rot is caused by *Fusarium verticillioides* (Munkvold and Desjardin, 1997), *Fusarium subglutinans*, and *Fusarium proliferatum* while Gibberella ear rot is caused by *Fusarium graminearum* (Chulze, 2010) and less importantly *Fusarium culmorum*. The symptom of Gibberella ear rot usually appears at the tip of the ear, with a reddish mold eventually covering the ear extensively, while the symptom of Fusarium ear rot produces a white to pink or salmon-colored mold, beginning anywhere on the ear or scattered throughout. Deoxynivalenol (DON) and zearalenone (ZEA) are considered as the two most common mycotoxin associated with Gibberella ear rot, while fumonisin (FUM) is the most common mycotoxin associated with Fusarium ear rot.

Aspergillus and *Penicillium* species are also other corn ear rot fungus, which causes *Aspergillus* and *Penicillium* ear rot, respectively. However, both these species are more often considered as 'storage fungi'. Thus, Aspergillus and Penicillium species are known to form mycotoxin in stored grain. Aflatoxin and ochratoxin A are the two most common mycotoxin associated with *Aspergillus* and *Penicillium* species. *Aspergillus flavus* and *A. parasiticus* are the two most common toxigenic fungi associated with aflatoxin contamination, while *Aspergillus ochraceus* and *Penicillium verrucosum* are the two most common toxigenic fungi associated with ochratoxin A contamination. Different authors in Ethiopia reported these fungi in association with maize. Amare (2010) isolated different fungi which include *Aspergillus*: *A. flavus; Fusarium*: *F. graminearum* and *F. verticillioides* and *Penicillium* spp., from maize collected from Adama, Ambo and Dire dawa. Dabassa (2014) also isolated *Aspergilus, Penicillium and Fusarium species* from maize collected from Jimma. Apart from these, Chali Ofgea (2015) isolated the genera *Aspergillus, Penicillium*. *Fusarium and Alternaria* from maize collected from Shashemene and Arsi Negelle. Moreover, maize is among the agricultural commodities from which aflatoxin was detected (Habtamu and Kelbessa, 2001)

2.6.1 Aflatoxin

Aflatoxins are one group of toxic secondary metabolites produced by certain species of the genus *Aspergillus*, mainly by *A. flavus* and *A. parasiticus*. Among, more than 18 structurally related aflatoxins; the four major types of aflatoxins namely AFB1, AFB2, AG1 and AFG2 are produced by these fungi. *A. flavus* produces aflatoxin B1 and B2, while *A. parasiticus* produces all the four common types of aflatoxins (B1, B2, G1 and G2). In addition to the four common types of aflatoxins, aflatoxin-M1 and aflatoxin-M2 are produced as a metabolic product of aflatoxin B1 and B2, respectively (Bhat and Vasanthi, 2003). For example, lactating animals including humans are able to metabolise aflatoxin B1 and B2 to aflatoxin-M1 and aflatoxin-M2, respectively in their milk (Sadeghi *et al*., 2009). The importance of aflatoxin was first recognized in 1960 when 100,000 Turkeys and other poultry in the UK died in a single event due to "Turkey 'X' Disease". Later on, the cause of the disease was examined and shown due to toxins in peanut meal infected with *A. flavus* and the toxins were named “aflatoxins” (Blount, 1961).

2.6.2 Fumonisins

Fumonisins are one of the most important mycotoxins found in agricultural commodities especially in maize and maize based Products. *F. verticillioides* and *Fusarium proliferatum* (Matsushima) Nirenberg are the two most common fungi producing these toxins. Among 28 fumonisin analogues, which are in fumonisins A, B, C, H and P series, fumonisin B (FB) comprising of FB1, FB2, and FB3 are found to be toxicologically important. Studies conducted on the proportion of fumonisin in maize indicated that FB1 accounts for 70%-80% of the total fumonisins (Gelderblom *et al*., 1988; Leslie *et al*., 1992), whlile FB2 accounts for 15%-25% and FB3 usually makes up 3%-8% (Branham and Plattner, 1993). The other analogues may occur in naturally contaminated maize at levels below 5% of the total fumonisins present (Musser *et al*., 1996). The toxicity of fumonisin has been associated with equine leukoencephalomalacia (ELEM) and pulmonary edema in swine and esophageal cancer in humans in Asia (Ross *et al*., 1992; Marasas *et al*., 2004). Moreover, it affects brain, liver, kidney, pancreas, testes, thymus, gastrointestinal tract, and blood cells.

2.6.3 Ochratoxin

Ochratoxin are another group of economically important mycotoxin produced mainly by *Aspergillus ochraceus* and *Penicillium verrucosum* (Ahmed and Jutta, 2015). The family of these groups of mycotoxin includes ochratoxin-A, B (dechlorinated OTA) and C (ethylated OTA) which differ slightly from each other in chemical structures. Among these types of ochratoxin, ochratoxin A is found to be the most prevalent toxin and is classified as a group 2b potential human carcinogen by the International Agency for Research on Cancer (IARC, 1993; Pfohl-Leszkowicz and Manderville, 2012; Heussner and Bingle, 2015). The kidney is the major organ affected by ochratoxin-A (Ringot *et al.,* 2006).

2.6.4 Deoxynivalenol (DON)

It is one of the most agriculturally important mycotoxin produced by molds of the genus *Fusarium*. *F.graminearum* and *F. culmorum* are the two major toxigenic fungi producing this mycotoxin. This mycotoxin also called vomitoxin because it has an emetic effect (feed refusal and vomiting) in animal (swine) following the consumption of food contaminated with high concentration of deoxynivalenol (DON) (Atanassov *et al*., 1994; Snijders and Krechting, 1992). Maize, barley, wheat and oats are the major source of deoxynivalenol as they are usually infected by fusarium fungi (gibberella ear rot). Symptoms of DON exposure in humans include diarrhea, lethargy, intestinal hemorrhage, and increased susceptibility to other diseases (Bonnet *et al*., 2012).

2.6.5 Zearalenone (ZEN)

Zearalenone is also another type of toxic secondary metabolites produced by species of the genus Fusarium i.e. *F. graminearum, F. culmorum* and *F. crookwellense*. This mycotoxin may have estrogenic properties. Thus, zearalenone can negatively affect breeding, fecundity, and hormonal balances in animals (Frizzell *et al.*, 2011, Woloshuk *et al.*, 2010). Zearalenone has been found in a wide range of food including wheat, barley, maize, rice, oats, sorghum and some legumes.

2.7 The role of arthropods in toxigenic fungal infection and mycotoxin contamination

Arthropods are among those factors associated with toxigenic fungal infection and mycotoxin contamination. The role of arthropods in toxigenic fungal infection and mycotoxin contamination include vectoring fungal spore to a desirable location, exposing the host plant through damaging

a host derived barrier to the fungus and producing metabolic heat and water to create favorable condition for the growth of fungi (Setamou *et al*., 1998; Hell *et al*., 2003; Dowd, 1998).

2.7.1 Exposing host plant to infection.

Fungi and bacteria that can be present on the surface of seeds cannot penetrate in to the internal part of seeds. The protective properties of the grain coating membrane made them resistant to fungal and bacterial infection under normal condition. Therefore, fungi and bacteria are mostly located on the seed coat, and embryo infection is uncommon without grain coating membrane damage. Arthropods are among those factors (harvesting, threshing, bird and rodent damage etc.) disturbing the natural condition of seeds by causing damage (Setamou *et al*., 1998).

Insect damage on agricultural commodities provides sites for fungi to enter the susceptible parts of seed (embryo and endosperm). Several authors reported the role of insect damage in toxigenic fungal infection and mycotoxin contamination in plant (Setamou *et al*., 1998; Hell *et al*., 2003; Dowd, 1998). Williams *et al*. (2002) conducted research on "Southwestern corn borer damage and aflatoxin accumulation in conventional and transgenic corn hybrids". On this research, both non Bt and Bt hybrids treated with southwestern corn borer, southwestern corn borer and *Aspergillus flavus* inoculums and *A. flavus* inoculums only. Finally they found high aflatoxin accumulation in non Bt hybrids than Bt hybrids both in southwestern corn borer and southwestern corn borer together with *A. flavus* inoculum treated samples. They also observed high larval establishment in non Bt hybrids than Bt hybrids. Moreover, Saladini *et al.* (2008) evaluated "Impact of insecticide treatments on *Ostrinia nubilalis* (Hu bner) (Lepidoptera: Crambidae) and their influence on mycotoxin contamination of maize kernels" and found reduction of ear damage and fumonisin contamination by 44.1% and 68%, respectively in insecticide treated samples.

In Ethiopia, research on the role of insect damage in toxigenic fungal infection and mycotoxin contamination has not been conducted. However, those arthropods associated with agricultural commodities damage, toxigenic fungal infection and mycotoxin contamination in other countries are also found in Ethiopia (Abraham, 1997; Emana and Assefa, 1998). The probability of these insects in facilitating toxigenic fungal infection and mycotxin contamination in Ethiopia is also expected to be high. Bennin, where the role of insect damage in aflatoxin contamination have

been clearly observed, may be used as a bench mark to observe the role of these insects in mycotoxigenic fungal infection and aflatoxin contamination on Ethiopian stored maize grain (Hell *et al*., 2000). The climate of Benin is fully found in Ethiopia. Climate is a primary factor for both insect and disease outbreak. All elements in disease triangle such as favourable environment (insect damage, drought, mechanical damage, traditional storage system and tropical climate nature), pathogen (aflatoxin, fumonisin and ochratoxin producing strain) and primary host (maize and groundnut) for toxigenic fungal infection are found in Ethiopia. Therefore, like in Benin, in Ethiopia also research has to be conducted on this area in order to understand the interaction of these disease triangle elements.

2.7.2 Vectoring Fungal Spore

For infection of agricultural commodities (plant) to occur, either the pathogen or plant must move and create a contact. However, the absence of natural motility both in plants and plant pathogen (fungal spore) made them to require moveable vectors for their success. Therefore, arthropods are among moveable vectors to do this role. Arthropod vectors vectoring fungal spores from origin of inoclum to plant include insects and mites. Several studies indicated the involvement of arthropods in carrying fungal spores on their bodies and in their gut. Dromph (2003) in his studies "Collembolans as Vectors of entomopathogenic fungi" showed that fungal spores can be spread by adhering to the body of insects that move through contaminated commodities or by the ingestion and excretion of fungal propagules. Deacon (1997) also mentioned in his book "modern mycology" the role of insects and mites in dispersing fungal spores. Moreover, Smalley (1989) showed the presence of *Aspergillus niger, A. glaucus, A. candidus, Penicillium islandicum, P. citrinum, Paecilomyces, Acremonium, Epicoccum, Fusarium semitectum*, and yeasts fungal spore on the body of maize weevil. This person also noted that the body of maize weevil is dominantly loaded with *A. flavus* and *F. moniliforme*. Moreover, Lussenhop and Wicklow (1991) on their papers "Nitidulid beetles as a source of *A. flavus* infective inoculum" indicated the role of Nitidulidae (*e.g. Carpophilus lugubris* Murrey and *C. freemani* Dobson) in vectoring *A. flavus* on maize.The Nitidulidae (*e.g. Carpophilus lugubris* Murrey and *C. freemani*) consumed *A. flavus* spores, without detrimental effect to themselves (Wicklow, 1988).

In Ethiopia, information on the role of arthropods in vectoring toxigenic fungal spore is very scanty. Emana Getu *et al*. (2001) reported on the role of insect in carrying toxigenic fungal spore on their bodies. Emana Getu *et al*. (2001) on their research "Ecological management of cereal stems borers in Ethiopia" isolated three toxigenic fungi from the bodies of *Chilo partellus* (Swinhoe) and *Busseola fusca* (Fuller) (Table 2.1). Among the three isolated toxigenic fungi *Aspergillus flavus* was found to be the most abundant. The role of arthropods in vectoring toxigenic fungal spores in Ethiopian agricultural commodities is expected to be high. Because, those insect associated with vectoring toxigenic fungal spore and mycotoxin production in other countries also found in Ethiopia. The presence of these insects in Ethiopia probably provides a clue to suitability of Ethiopian condition, not only for infestation of these insects but also for infection of toxigenic fungi and mycotoxin contamination associated with these insects. For instance *Carpophilus hemipterus, Cryptophagus* spp., and *Typhaea stercorea* primarily feed on moldy grain. Although their status was not confirmed, *Carpophilus hemipterus, Cryptophagus* spp., and *Typhaea stercorea* are found in Ethiopia.

Table 2.1 Potentially toxigenic fungi isolated from stem borers in Ethiopia.

Pathogen	**Host insect**	**Abundance**	
Aspergillus flavus	*Chilo partellus, Busseola fusca*	+++**	Aflatoxin
Beauveria bassiana	*Busseola fusca*	++**	
Metarrizhum anisopliae	*Chilo partellus, Sesamia calamistis*	+**	Destruxin

Source: Emana Getu *et al*., 2001

** + = recorded from one sample (larva); ++ = recorded from 2-5 larvae; +++ = recorded from > 5 larvae

2.7.3 Metabolic activities

A stored grain ecosystem consists of abiotic factors such as dockage, intergranular air, water vapor, temperature, and the storage structure and biotic factors which include insects, mites, rodent, fungi and the grains themselves. These components interact and affect each other to cause or to prevent damage to stored grains. About 37 species of arthropods were recorded in stored maize in Ethiopia (Abraham, 1997; Emana and Assefa, 1998). The metabolic activities of these arthropods may result in release of water, carbon dioxide, heat and energy, providing

favourable condition for the growth of storage fungi. Therefore, in stored grain ecosystem, infection of storage fungi usually follows infestation of arthropods.

Several report demonstrated the role of insect metabolic activities in toxigenic fungal infection and mycotoxin contamination in agricultural commodities. Beti *et al.* (1995) in their research "Effects of maize weevils (Coleoptera: Curculionidae) on production of aflatoxin B1 by *Aspergillus flavus* in stored corn" observed that moisture content increased from 15% to 20 % after 30 days in maize infested with *S. zeamais* and significantly more aflatoxin B1 was found in maize weevils, *S. zeamais* infested maize, than in mechanically damaged and controlled maize that had been inoculated with *A. flavus.* Moreover, Magan *et al*. (2003) on their review "Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain" wrote that the role of storage insect pests in the production of metabolic heat which generates water via condensation on surfaces due to temperature differentials and develop classic hot spots which can quickly result in heating and complete spoilage. In Ethiopia, information on the role of metabolic activities of stored agricultural commodities insect pests in providing suitable condition for infection of toxigenic fungi and mycotoxin contamination is scanty. However, Aberra Geyid and Admassu Maru (1987) showed the influence of temperature, moisture content and relative humidity on aflatoxin formation in stored maize, sorghum and teff from Ethiopia.

2.8 Effects of mycotoxin on human and animal health

Consumption of plant derived foods that are contaminated with mycotoxin by human and animal may result in mycotoxicosis. Human and animal food can be infected and contaminated with the three most important genera of mycotoxigenic fungi (*Aspergillus, Fusarium* and *Penicillium*) and their respective mycotoxin (Creppy, 2002). The most important mycotoxins associated with these genera include aflatoxin, ochratoxin, fumonisin and trichothecenes. The disease caused by exposure to these and other mycotoxin is called mycotoxicosis. Mycotoxicosis can occur either acutely or chronically exposure to mycotoxin contaminated food. Acute aflatoxicosis was recognized for the first time in Britain in 1960. Over 100,000 Turkeys died after they fed on peanut contaminated with aflatoxin (Blount, 1961). Studies conducted in Keneya on the effects of acute aflatoxin exposure on humans indicated that up to 60% death. On the other hand, chronic effects of mycotoxin in human populations include impaired growth and development,

immune dysfunction and the disease consequences, carcinogenicity, mutagenicity and teratogenicity.

Studies conducted in Ethiopia indicated the occurrence of acute mycotoxicosis called gangrenous ergotism. The outbreak of this mycotoxicosis occurred in the Wollo Administrative Region and resulted in 93 cases and 47 deaths (Demeke *et al*., 1979; King, 1979). Moreover, Urga *et al*. (2002) reported gangrenous ergot outbreak in Arsi Zone, Ethiopia. Apart from these, high prevalence of liver disease in Ethiopia has been reported by Coady (1965) and has been reviewed by Edemarim (1977). Coady (1965) suggested mycotoxin contamination as a probable reason for high prevalence of liver disease in Ethiopia by mentioning Oettle (1965). Oettle (1965) has presented evidence to indicate that a high incidence of primary carcinoma is associated with a greater moldiness of food stuffs. He maintains that areas of high liver cancer incidence in Africa are all areas of high humidity. A high humidity is necessary for mold growth.

Chapter-3

Arthropods associated with stored maize grain in selected woreda of Amhara and Oromia regions, Ethiopia

3.1 Introduction

Agriculture is considered to be the dominant sector in Ethiopia's economy. It accounts for about 51 % of the GDP (2009). Among agricultural commodities, cereals play a central role and accounting for about 60 % of rural employment, 80 % of total cultivated land, more than 40 % of a typical house hold's food expenditure, and more than 60 % of total caloric intake. According to Alemayehu (2012), maize accounts for the largest share in total production and the total number of farm holdings involved in cereal production. Agricultural Sample Survey conducted by Central Statistical Agency, indicated that maize production was 3.75 million tonnes, which is 25 % higher than teff and 41 % higher than sorghum (CSA, 2008).

Maize (*Zea mays* L.) is the most important food crop grown and stored by farmers in the Amhara and the Oromia region, Ethiopia. The production and productivity of maize in these regions has been increased significantly starting from the release of high yielding varieties by the Ethiopian Institute of Agricultural Research (Bako Agricultural Research Center). However, increasing food production and productivity by itself is nothing without considering post-harvest losses. The survey conducted on "Global Food Losses and Food Waste: Extent Causes and Prevention" showed that about 1.3 billion tons of food are globally wasted or lost per year (Gustavasson, *et al*., 2011). For these post-harvest losses, arthropods are considered as the major causes. The role of arthropods in stored grain ecosystem might be of energy transformer, grainvores, fungivores, predacious or parasitic. On the other hand, their effects on stored grain may result in grain damage; by their feeding activities and exposing the grain to infection of potentially toxigenic fungi (Avantaggio *et al*., 2002), Contamination of stored grain by allergens (Hage-Hamsten and Johansson, 1998), by vectoring mycotoxin producing fungi (Lussenhop and Wicklow, 1990) and providing suitable condition for the growth of fungi by releasing of metabolic heat and water (Dix and All, 1987).

It is well known that stored grain may be infested with many species of arthropods with different role and succession time. This information is lacking in Ethiopia. Many species of arthropods

have been recorded in stored maize grain in Ethiopia (Mekuria, 1995: Abraham, 1997; Emana and Assefa, 1998). Abraham (2008) on the "Proceedings of the 14th annual conference of the plant protection society of Ethiopia (PPSE)" reported over 100 species of arthropods in association with all stored products in Ethiopia. However, this number of species is far below the number of species reported in the available literature. Even among these 100 species, certain species were recorded as "uncommon" and some of them recorded as "uncertain". According to Hagstrum and Subramanyam (2009) over 1900 species of arthropods are known to occur in stored grain or to be associated with grain-based foods. National investigations of stored grain arthropods in China revealed 270 species of arthropods in association with all stored product in China. It is very important to study arthropod communities in stored maize ecosystem in order to understand their role and succession time. In turn, understanding of the role and succession time of each arthropod is very important in order to dictate the condition of stored grain and to formulate a control measure. Therefore, this study was initiated with the following objectives:

- To survey grain and grain product feeder arthropods species composition and abundance in association with stored maize in selected woreda of Amhara and Oromia regions;
- To survey fungus feeder arthropods species composition and abundance in association with stored maize in selected woreda of Amhara and Oromia regions;
- To survey natural enemies associated with grain and grain product feeder arthropods in association with stored maize in selected woreda of Amhara and Oromia regions;
- To survey natural enemies associated with fungus feeder arthropods in association with stored maize in selected woreda of Amhara and Oromia regions.

3.2 Materials and Methods

Description of the study area

This study was conducted in selected Woreda (Districts) of Oromia and Amhara Region (Figure 3.1). In the year 2012 Oromia and Amhara Regional States contributed about eighty percent of maize produced in Ethiopia (CSA, 2011/2012*)*.

Oromia

Five Woreda (Districts) were used for data collection from Oromia Regional States of Ethiopia. They were Bako-Tibe from West Shoa Zone, Sibu-Sire from East Wollega and Kersa, Omo-Nada and Sekoru from Jimma Zone. All these selected Woreda (Districts) were among major maize producing Districts in Ethiopia (CSA, 2011/2012*)*. Bako-Tibe (09°07'N and 037°03'E) and Sibu-Sire (09°02'N and 036°52'E) are located in West Shoa and East Wollega Administrative zone of Oromia Region, respectively. Bako-Tibe characterized by its altitude ranging from 1631 to 1657 m.a.s.l, mean annual rainfall 1281 mm and average annual temperature 19.7 °C, while Sibu-Sire received mean annual rain fall 1295 mm, its altitude ranging from 1845 to 1896 m.a.s.l and average annual temperature 19.2 °C. Jimma Zone (7°40'N and 36°10'E) includes Kersa, Omo-Nada and Sekoru Districts and has an elevation ranging from 880-3360 m.a.s.l. This Zone received average annual rainfall and temperature 1624 mm and 20 °C, respectively. Generally, the agro-ecology of the study areas is ranged from lowlands up to intermediate.

Amhara

Five Districts were used for data collection from Amhara Regional States of Ethiopia. They were Dembecha, Bure, South Achefer, Mecha (Merawi) and Bahirdar Zuria. All of them were from west Gojam Zone. West Gojam zone (011°10'N and 037°15'E) has elevation varies from 1500 to 3500 m.a.s.l. Most of the Districts (75%) from this zone have ambient temperatures ranging from15°C to 20 °C. About 17% of the Districts have 20 °C -27 °C. The annual rain fall ranging from 1272 mm at Bahirdar Zuria to 1792 mm at Mecha. Differently, the agro-ecology of the study areas is ranged from lowlands up to highlands.

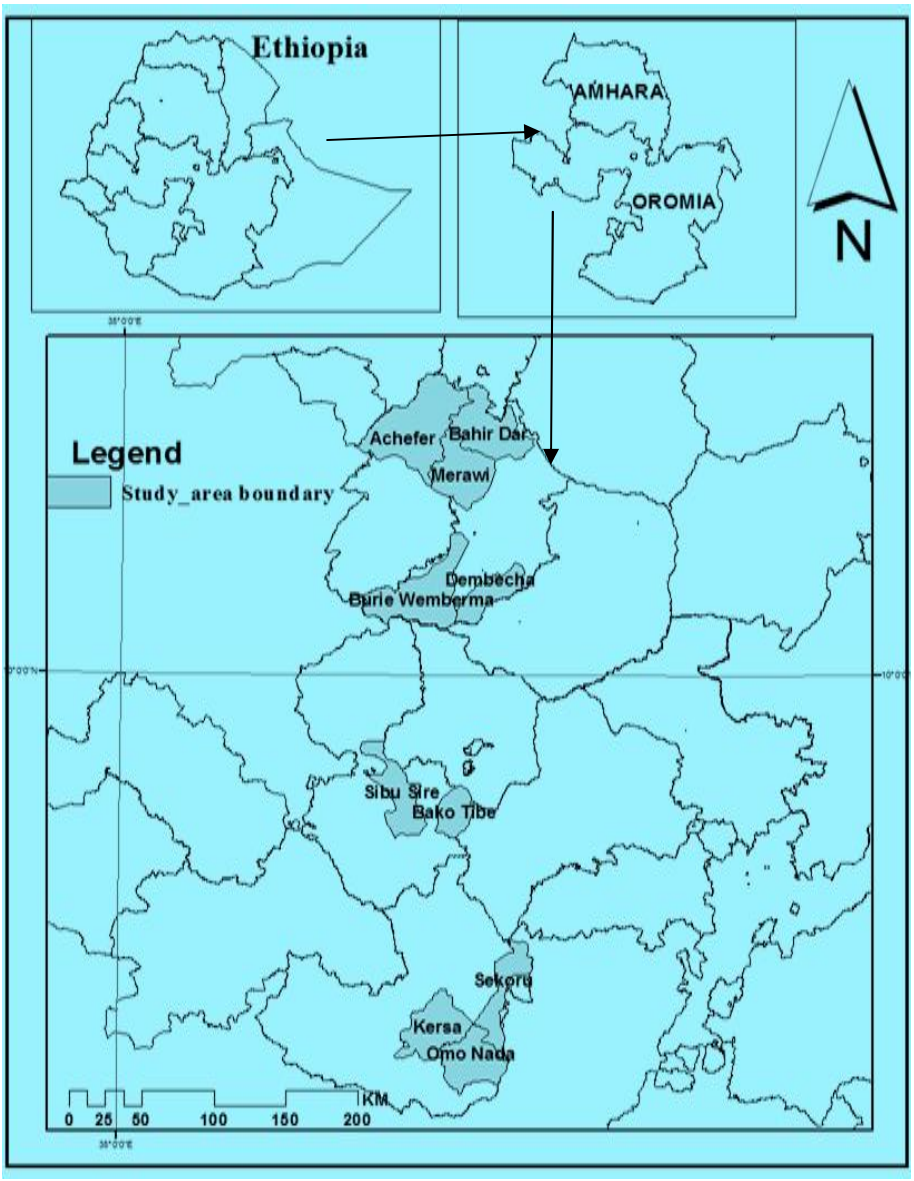


Figure 3.1 Map of the survey Districts in Oromia and Amhara Regional States in Ethiopia

Study design and Sampling

This survey was conducted during August 2014 / 2015 and 2015 / 2016 in 10 major purposively selected maize growing Woredas' of Oromia and Amhara regions in August when the infestation of arthropods are more likely. Maize samples were collected from store in each representative Woredas (Districts) of the region, such that in **Oromia**: in the Districts of Bako Tibe, Sibu-Sire, Kersa, Omo-Nada and Sekoru, while in **Amhara**: in the Districts of Dembecha, Bure, South-Achefer, Mecha and Bhirdar Zuria**.** Fifty randomly selected storage structures were visited for each of the selected woredas in each year. One kilogram of composite sample (from the top, middle, bottom and sides of storage containers) was collcted from each storage structure. Finally the samples collected from fifty storage structures were thoroughly mixed and 10 sub-samples were prepared having 1kg each as a final sample (Fandohan *et al*., 2005*,* 2006). Purposive sampling was used to select study Woredas (Districts), while random sampling was used to collect maize samples from selected Woredas (Districts).

Entomological data for grainvorous arthropods and associated natural enemies

Maize samples collected during August in both years were used to evaluate granivorous arthropods. Arthropods infestation was evaluated by sieving (1 mm and 2 mm mesh sieve) each maize sample. All arthropods found were collected, counted (per 100 g of grain) and identified using keys prepared by NRI (1991), Weidner and Rack (1984) and Rees (2004) and photographed to compare identified arthropods with available photos of the arthropods on different publications/literatures.

Entomological data for fungivorous arthropod and associated natural enemies

Maize samples collected in August were incubated for 120 days at 21±3 °C and 65%-70% relative humidity (RH). Mold feeder arthropods are usually attracted to high moisture content of stored grain which are produced by metabolic activities of arthropods. About 500 g samples were used from each collected sample for this purpose. Preliminary laboratory investigation showed that this condition was enough to monitor mold feeder arthropods in stored grain. Finally, all arthropods found were collected, counted and identified using keys of NRI (1991), Weidner and

Rack (1984), and Rees (2004) and photographed to compare identified arthropods with available photos of the arthropods on different publications/literatures.

Isolation of mites and psocids

Isolation of mites and psocids were conducted using a modified Tullgren Berlese funnel methods. This method is based on repellency against heat. Therefore, about 50 g of sub-sample was placed on a 12 cm diameter sieve with 1mm and 0.5 mm mesh size that was placed approximately 7 cm below a light 60 watts light bulb. A 12 cm diameter of size with 75% alcohol placed below the sieve was used as mites and psocids collector. Mites and psocids in stored maize sample on the sieve were displaced from the grain to petri dishes which contain 75% alcohol after three days exposure to 60 watts light bulb (Plate 3.1).

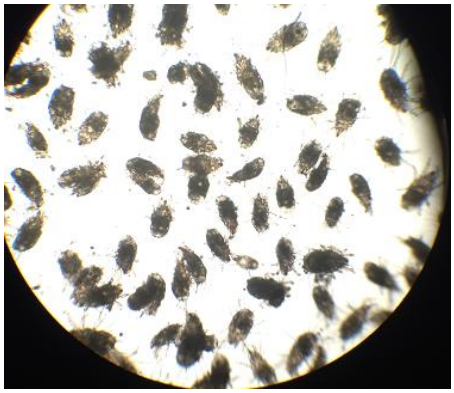


Plate 3.1: Mites collected from stored maize after three days exposure to 60 watts light bulb.

Statistical analysis

The data collected from this study was summarized by the Microsoft Excel pakage 2013 and analyzed using the Statistical Program for Social Sciences (SPSS) version 16. Descriptive statistics (mean and percentage) were performed to determine frequency of occurrence and abundance of grain product feeders, mold feeders and natural enemies associated with grain and grain product feeders and mold feeders arthropod.

3.3 Results

Species composition and frequency

A total of 81 arthropod species belonging to three Classes of Phylum Arthropoda, which include Class Insecta, Class Arachnida and Class Crustacean were identified during the two year survey (Table 3.1 and Plate 3.2-3.17). Insects, arachnids and crustaceans accounted for 87.65%, 8.64% and 3.70 %, respectively. Out of 81 identified species of arthropods, 27 species are being recorded for the first time from Ethiopia (Table 3.14).

Insects

A total of 71 insect species were recorded from 6 orders and 30 families during the two years survey. From the recorded insect species Coleopterans, Psocopterans, Dipterans and Hemipterans accounted for 61.73%, 9.88%, 6.17% and 2.47 %, respectively, of the recorded arthropods. Lepidopteran and Hymenopterans consisted of 3.70% each.

Coleoptera

A total of 50 species of Coleopterans were isolated, out of these *Sitophilus zeamais* (Herbest) (100%) was found to be the most frequently occuring species followed by *Sitophilus oryzae* (Linnaeus) (76.5%), *Cryptolestes ferrugienus* (Stephens) (71%), *Cryptolestes* sp. (70%), *Carpophilus hemipterus* (Linnaeus) (62%), *Cryptolestes pusillus* (Schon.) (61%), *Tribolium confusum* (Jacquelin du val) (59%), *Litargus balteatus* (LeConte) (56.5%), *Philonthus cruentatus* (Gmelin) (55%), *Carpophilus dimidiatus* (Fabricius) (53.5%) and *Typhae stercorea* (Linnaeus) (51.5%). Three other economically important *Tribolium species* such as *Tribolium castaneum* (Herbst), *Tribolium destructor* (Uyttenboogaart) and unidentified *Tribolium* species were also recorded with the frequency of 40.5%, 33% and 28.5%, respectively.

Moreover, two devastating insect pests from the family Bostrichidae, namely *Rhyzopertha dominica* (Fabricius) and *Prostephanus truncatus* (Horn) were also recorded in the sample from Bako-Tibae and Sibu sirae and Sekoru during 2014/15 and 2015/16, respectively. However, both of them encountered in less than 8% of the samples i.e, *Rhyzopertha dominica* (Fabricius) (7%) and *Prostephanus truncatus* (Horn) (2%). Both specimens recorded for *Prostephanus truncatus* (Horn) were dead. Both *Rhyzopertha dominica* (Fabricius) and *Prostephanus truncatus*

(Horn) were not recorded in all selected Woreda of Amhara regions and Omo-Nada and Kersa Woreda of Oromia regional state. *Callosobruchus chinensis* (Linnaeus) was also other economically important stored product pests recorded in 9% of the sample during 2014/15 only.

Mold feeder arthropods [*Carpophilus* sp., *Brachypeplus* sp., *Carpophilus freemani* Dobson, *Ahasverus advena* (Waltl), *Mycetophagus* sp., *Scaphisoma* sp., *Corticaria* sp., *Cartodere* (*Aridius*) *nodifer* (Westwood), *Melanophthalma* sp., *Cryptophagus* sp. and *Cryptophagus cellaris* (Scopoli)], secondary pests of stored cereals grains [*Gnatocerus cornutus* (Fabricius), *Palorus ratzeburgii* (Wissmann), *Palorus subdepressus* (Wollaston) and *Palorus* sp] and natural enemies [family staphylinidae (*Aleochara sp.*, *Anotylus* sp., *Philonthus* sp1., *Philonthus* sp2) and Hydrophilidae (*Dactylosternum abdominal* (Fabricius))] which were recorded in 25-42.5% of the samples. Apart from these, thirteen species of coleopteran such as *Phenolia picta* (MacLeay) (5.5%), *Silvanus planatus* (Germar) (8.5%), *Oryzaephilus surinamensis* (Linnaeus) (4.5%), *Stenoscelis brevis* (Boh.) (3%), *Cryptophillus integer* (Heer) (8%), *Murmidius ovalis* (Beck) (8.5%), *Scymnus* sp. (12.5%), *Anthicus floralis* (Linnaeus) (3.5%), *Dermestus* sp. (0.5%), *Pseudeurostus hilleri* (Reitter) (7.5%), *Stenus cicindeloides* (Schaller) (7.5%), *Scydmaenus tarsatus* (Muller) (5.5%) and *Micrambe bimaculata* (Panzer) (9.5%) were also recorded.

Psocoptera

From the order Psocoptera, eight species were recorded under five families, among them, *Lepinotus patruelis* (Pearman) (60%) was found to be the most frequently encountered, followed by *Liposcelis bostrychophilus* (Badonnel) (52.5%), *Liposcelis* sp1 (47.5%), *Liposcelis* sp**2 (**33%), *Ectopsocus* sp. (32%), *Dorypteryx domestica* (Smithers) (14%), *Lachesilla michiliensis* (Garcia Aldrete) (8.5%) and *Lachesilla* sp., (8%).

Dipterans and Lepidopteran

Five species of Dipterans were identified from two families (Drosophilidae and Sciaridae) during the two year survey. Among these five species *Sciaridae* spp., was found to be the most frequently encountered species in 47.5% of the samples, followed by *Drosophila busckii* (Coquillett) (44.5 %), *Drosophila melanogaster* (Meigen) (33%), *Drosophila* spp., (17.5). On the other hand, three species of Lepidopteran also were isolated from three families Gelechiidae, Pyralidae and Tineidae. Of which Angoumois grain moth, *Sitotroga cerealella* (Olivier) and Indian meal moth,

Plodia interpuntella (Oliver) were encountered in 72% and 39.5% of the samples, respectivey. *Opogona dimidiatella* zeller was the third species of Lepidoptera recorded from the family Tineidae. It was recorded for the first time in Ethiopia. However, it was recorded only in 6% of the samples.

Hymenopterans and Hemipterans

Three species of Hymenopterans and two species of Hemipterans were found in association with *Sitophilus zeamais* (Herbest), *Cryptolestes* spp., *Sitophilus oryzae* (L.), *Tribolium* spp*., Plodia interpuntella* (Oliver) and *Sitotroga cerealella* (Olivier). Out of the three species of Hymenopterans *Anisopteromalus calandrae* (Howard) was found to be the most frequently encountered species consisting of 58.5%, followed by *Theocolax elegans* (Westwood) (41.5%) and *Monomorium* sp. (15%). The two species of Hemipterans, *Xylocoris* sp., and *Xylocoris galactinus* (Fieber) were isolated from 26% and 30.5% of the samples, respectively,

Arachnids and crustaceans

Arachnids and crustaceans accounted for 8.64% and 3.70% of the recorded arthropods, respectively. Seven species of arachnids were recorded under four families, including Acaridae, Oribatida, Ascidae and Withiidae. From Acaridae the two most frequently encountered mites were *Tyrophagus putrescentiae* (Schrank) (56%) and *Acari siro* L (52.5%). Oribatida and Ascidae each of them consisted of one species such that *Oribatida* sp. (9%) and *Blattisocius* sp. (42.5%), respectively, while Withiidae made up three unidentified species which include *Withiidae* sp1., (27%), *Withiidae* sp2., (26%) and *Withiidae* sp3., (6%). On the other hand three species of Crustaceans were recorded under three families which include Armadillidae, Porcellionidae and Oniscidae and each of them represented by one species. *Armadillidiidae* sp., *Porcellionidae* sp., and *Oniscidae* sp*.,* were isolated from 12.5%, 12.5% and 20.5% of the samples, respectively

Table 3.1: Arthropods recorded during 2014/15-2015/16 on stored maize grain

Order	Family	Scientific name	Common name	Samples with Arthropods (%)	
				2014/15	2015/16
Coleoptera	Nitidulidae (6)	*Carpophilus* sp.	a sap beetle	42	34
		Brachypeplus sp.	a sap beetle	38	44
		Phenolia picta (MacLeay)	a sap beetle	9	2
		Carpophilus dimidiatus (F.)	a sap beetle	54	53
		Carpophilus freeman Dobson	Corn sap beetle	38	43
		Carpophilus hemipterus (L.)	Dried fruit beetle	59	65
	Cucujidae	*Cryptolestes ferrugienus* (Stephens)	Rusty grain beetle	69	73
		Cryptolestes pusillus (Schon.)	Flat grain beetle	67	55
		Silvanus planatus (Germar)		11	6
		Cryptolestes sp.	Grain beetle	71	69
		Oryzaephilus surinamensis (L.)	Saw-toothed grain beetle	7	2
		Ahasverus advena (Waltl)	Foreign grain beetle	25	32
	Tenebrionidae	*Tribolium castaneum* (Herbst)	Red flour beetle	37	44
		Tribolium confusum J. de val	Confused flour beetle	62	56
		Tribolium destructor (U.)	Flour beetle	15	11
		Tribolium sp.	Flour beetle	14	18
		Gnatocerus cornutus (F.)	broad horned flour beetle	21	9
		Palorus ratzeburgii (Wissmann)	Small-eyed flour beetle	19	10
		Palorus subdepressus (Wollast.)	Depressed flour beetle	15	21
		Palorus sp.	Flour beetle	7	13
	Curculionidae	*Sitophilus oryzae* (L.)	Rice weevil	72	81
		Sitophilus zeamais (Herbest)	Maize weevil	100	100
		Stenoscelis brevis (Boh.)	Broad-nosed Bark Weevil	6	0
	Mycetophagidae	*Typhae stercorea* (L.)	Hairy fungus beetle	45	58
		Litargus balteatus LeConte.	Hairy fungus beetle	52	61

		Mycetophagus sp.	A fungus beetle	43	42

Table-1 Continued

Order	Family	Scientific name	Common name	Samples with Arthropods (%)	
				2014/15	2015/16
Coleoptera	Languridae	*Cryptophillus integer* (Heer)	Fungus beetle	11	5
	Hydrophilidae	*Dactylosternum abdominale*	Beetle (water scavenger)	38	14
	Murmidiidae	*Murmidius ovalis*	Minute beetles	6	11
	Coccinellidae	*Scymnus* spp.	Lady beetles	20	5
	Bruchidae	*Callosobruchus chinensis*	Adzuki bean weevil.	9	0
	Anthicidae	*Anthicus floralis* (L.)	Narrownecked grain beetle	5	2
	Bostrichidae	*Rhizoperta dominca*	Lesser grain borer	7	0
		Prostephanus truncatus	Larger grain borer	0	2
	Dermestidae	*Dermestus* sp.	Skin beetles	1	0
	Ptinidae	*Pseudeurostus hilleri*		1	14
	Staphylionidae	*Aleochara* sp.	Rove beetle	27	47
		Anotylus sp.	Rove beetle	12	18
		Philonthus cruentatus	Rove beetle	51	59
		Philonthus sp1.	Rove beetle	29	22
		Philonthus sp2.	Rove beetle	17	9
		Stenus cicindeloides	Rove beetle	11	4
		Scydmaenus tarsatus	Ant like stone beetle	7	3
		Scaphisoma sp.	Shining fungus beetle	27	34
	Latridiidae	*Corticaria* sp.	Minute brown scavenger beetle.	19	42
		Cartodere (Aridius) nodifer	Swollen fungus beetle	23	28

		Melanophthalma sp.	Minute brown scavenger beetle	7	22
	Cryptophagidae	*Cryptophagus* sp.	Sillken fungus beetle	25	32
		Cryptophagus cellaris	Cellar fungus beetle	13	25
		Micrambe bimaculata	Silken fungus beetle	5	14

Table-1 Continued

Order	Family	Scientific name	Common name	Samples with Arthropods (%)	
				2014/15	2015/16
Psocoptera	Liposcelididae	*Liposcelis* sp1.	Psocid	42	53
		Liposcelis bostrychophilus	Psocid	54	51
		Liposcelis sp**2.**	Psocid	28	38
	Lachesillidae	*Lachesilla michiliensis*.	Psocid	11	6
		Lachesilla sp.	Psocid	7	9
	Ectopsocidae	*Ectopsocus* sp.	Psocid	43	21
	Psyllipsocidae	*Dorypteryx domestica*	Psocid	5	23
	Trogiidae	*Lepinotus patruelis*	Psocid	59	61
Diptera	Drosophilidae	*Drosophila* sp1.	Small fruit flies	11	23
		Drosophila sp2.	Small fruit flies	9	27
		Drosophila melanogaster	Small fruit flies	17	49
		Drosophila busckii	Small fruit flies	42	47
	Sciaridae	*Sciaridae* sp.	Fungus gnat	51	44
Hymenoptera	Petromalidae	*Anisopteromalus calandrae*	A pteromalid wasp	53	64
	Petromalidae	*Theocolax elegans*	A petromalid wasp	44	39
	Formicidae	*Monomorium* sp.	Ants	17	13
Lepidoptera	Pyralidae	*Plodia interpuntella* (Oliver)	Indian meal moth	46	33
	Gelechiidae	*Sitotroga cereallela* (Oliver)	Angoumois grain moth	78	66

	Tineidae	*Opogona dimidiatella*	Fungus Moth	5	7

Table-1 Continued

Order	Family	Scientific name	Common name	Samples with Arthropods (%)	
				2014/15	2014/15
Hemiptera	Anthocoridae	*Xylocoris galactinus* (Fieber)		23	38
		Xylocoris sp.	Warehouse pirate bug	37	15
Arachnida	Acaridae	*Acari siro* L.	Grain mites	53	52
		Tyrophagus putrescentiae (Schrank)	Fungus mite	59	53
	Oribatida	*Oribatida* sp.		7	11
	Ascidae	*Blattisocius* sp		38	47
Pseudoscorpion	Withiidae	*Withiidae* sp1.	Pseudoscorpion	21	33
		Withiidae sp2.	Pseudoscorpion	28	17
		Withiidae sp3.	Pseudoscorpion	7	5
Isopoda	Armadillidae	*Armadillidiidae* sp.	Pillbugs	7	18
	Porcellionidae	*Porcellionidae* sp.	Sowbugs	6	19
	Oniscidae	*Oniscidae* sp.	Sowbugs	13	28

Abundance of selected groups of arthropods

Granivorous arthropods

Based on the mean number of species per 100 g of grain, in all cases (in all selected woreda from both regions and in both years) *Sitophilus* spp., were the most abundant granivorous arthropod with the mean number per 100 g of maize grain ranging from 60.50±9.70 in 2014/15 at South Achefer in Amhara region to 440.60±25.05 in 2014/15 at Omo-Nada in Oromia region followed by *Sitotroga cerealella* ranging from 13.60±2.68 in 2014/15 at Sibu-Sirae in Oromia region to 44.90±1.48 in 2015/16 at Sekoru in Oromia region. In most cases (in all selected woreda in oromia region during 2014/15, only in Bako-Tibae during 2015/16 and in all selected woreda in Amhara Region in both year) *Cryptolestes* spp., with the mean number per 100 g of grain ranging from 2.60±0.52 in 2014/15 at South Achefer to 41.20±1.98 2014/15 at Omo-Nada in Oromia region, were more abundant than *Tribolium* spp., ranging from 1.80±0.63 in 2014/15 at South Achefer in Amhara region to 37.70±1.18 in 2015/16 at Sekoru in Oromia region (Tables 3.2 and 3.3).

Table 3.2: Mean number of the most important grain and grain product feeders per 100 g of maize grain from selected Woreda of Oromia region during 2014/15 and 2015/16.

Granivores arthropod	Mean number of granivorves arthropod per/100 g of grain				
	Bako-Tibae	**Sibu-Sirae**	**Kerssa**	**Omo-Nada**	**Sekoru**
2014/15 (mean ± standard error)					
Sitophilus spp.	87.20±6.30	76.00±7.39	289.70±24.76	440.60±25.05	390.00±21.92
Cryptolestes spp.	11.30±1.89	5.40±1.08	21.70±2.44	41.20±1.98	35.60±3.13
Tribolium spp.	4.50±1.14	2.30±1.02	17.40±1.72	26.00±3.17	16.90±4.24
Rhyzopertha dominica	1.00±0.21	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Sitotroga cerealella	14.70±2.05	13.60±2.68	38.10±1.93	35.60±2.16	24.30±3.06
2015/16 (mean ± standard error)					
Sitophilus spp.	87.50±4.19	73.90±5.34	233.50±26.18	433.20±12.47	432.00±8.82
Cryptolestes spp.	13.80±1.26	8.80±0.49	18.70±0.78	23.60±1.25	30.70±1.09
Tribolium spp.	9.20±1.03	9.00±0.82	24.60±1.85	24.00±2.40	37.70±1.18
Rhyzopertha dominica	0.80±0.20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Sitotroga cerealella	20.00±1.17	18.20±0.95	36.30±1.96	41.50±1.64	44.90±1.48

Table 3.3: Mean number of the most important grain and grain product feeders per 100 g of maize grain from selected Woreda of Amhara region during 2014/15 and 2015/16.

Granivores arthropod	Mean number of arthropod per/100 g of grain				
	Dembecha	**South Achefer**	**Burae**	**Mecha**	**Bahirdar Zuria**
2014/15 (mean ± standard error)					
Sitophilus spp.	120.60±13.30	60.50±9.70	248.50±30.82	81.40±7.73	221.00±16.02
Cryptolestes spp.	13.40±1.43	2.60±0.52	27.30±0.82	3.20±0.84	25.50±3.91
Tribolium spp.	5.60±1.36	1.80±0.63	20.80±2.07	2.50±0.50	18.70±2.41
Rhyzopertha dominica	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Sitotroga cerealella	31.30±0.89	25.90±1.57	38.40±1.32	30.10±3.61	39.80±1.71
2015/16 (mean ± error)					
Sitophilus spp.	103.50±2.32	72.00±2.62	311.90±17.45	85.50±2.43	315.50±17.51
Cryptolestes spp.	16.40±1.46	7.20±0.84	30.50±2.03	11.70±0.87	39.10±1.44
Tribolium spp.	7.80±0.94	3.70±0.42	30.20±1.75	4.10±0.51	27.20±1.76
Rhyzopertha dominica	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Sitotroga cerealella	29.60±1.09	25.90±1.46	35.90±1.92	31.30±1.51	42.10±1.92

Fungivorous arthropod

Based on the mean number of species per 100 g of moldy grain, in all cases Nitidulid beetles (with the mean number per 100 g of moldy grain ranging from 11.70±0.47 in 2015/16 at South Achefer to 32.70±0.86 in 2014/15 at Bahirdar Zuria in Amhara region) and *Drosophilla* spp., (with the mean number per 100 g of moldy grain ranging from 22.90±0.23 in 2014/15 at Dembecha to 30.00±0.26 in 2014/15 at Mecha in Amhara region) were the most abundant fungivorous arthropod followed by *Mycetophagidae* spp., ranging from 5.10±0.31 in 2014/15 at Burae to 22.90±0.43 in 2014/15 at Bahirdar Zuria in Amhara region. The mean number of *Lathriididae* spp., per 100 g of moldy grain ranged from 0.00±0.00 in 2015/16 at Bako-Tibae in Oromia region to 4.90±0.18 in 2014/15 at Bahirdar Zuria in Amhara region. Similarly, the mean number of *Cryptophagidae* spp., per 100 g of moldy grain was ranged from 0.00±0.00 in 2015/16 at Sibu-Sirae and Kerssa in Oromia region to 2.60±0.64 in 2014/15 at Bahirdar Zuria in Amhara region (Tables 3.4 and 3.5).

Table 3.4: Mean number of mold feeder arthropods per 100 g moldy grain sampled from selected Woreda of Oromia region during 2014/15 and 2015/16.

Fungivores arthropod	Mean number of arthropod per/100 gm of moldy grain				
	Bako-Tibae	**Sibu-Sirae**	**Kerssa**	**Omo-Nada**	**Sekoru**
2014/15 (mean ± standard error)					
Nitidulidae spp.	13.30±0.54	11.50±0.45	16.40±0.52	20.60±0.62	23.00±1.15
Mycetophagidae spp.	7.9±0.23	2.90±0.24	5.90±0.53	8.20±0.25	10.10±0.28
Lathriididae spp.	0.60±0.15	0.30±0.15	0.80±0.29	1.00±0.26	1.70±0.40
Cryptophagidae spp.	0.90±0.23	0.00±0.00	0.00±0.00	1.40±0.37	2.30±0.54
Drosophilla spp.	8.80±0.39	8.80±0.42	11.70±0.30	14.20±0.36	14.25±0.51
2015/16 (mean ± standard error)					
Nitidulidae spp.	11.80±0.73	11.90±0.31	15.20±0.71	21.20±0.44	24.80±0.47
Mycetophagidae spp.	4.20±0.63	3.60±0.16	6.70±0.34	8.30±0.37	8.80±0.59
Lathriididae spp.	0.00±0.00	0.50±0.17	1.00±0.33	1.60±0.34	2.10±0.46
Cryptophagidae spp	0.90±0.17	0.00±0.00	1.20±0.79	1.30±0.31	1.90±0.35
Drosophilla spp.	8.70±0.47	8.70±0.37	12.70±0.37	14.40±0.45	14.50±1.08

Table 3.5: Mean number of mold feeder arthropods per 100 g moldy grain from selected Woreda of Amhara region during 2014/15 and 2015/16.

Fungivores arthropod	Mean number of arthropod per/100 g of moldy grain				
	Dembecha	**South Achefer**	**Burae**	**Mecha**	**Bahirdar Zuria**
2014/15 (mean ± standard error)					
Nitidulidae spp.	18.90±0.82	15.10±0.38	22.50±0.96	29.40±0.88	32.70±0.86
Mycetophagidae spp.	13.30±0.43	11.20±0.33	5.10±0.31	14.80±0.33	22.90±0.43
Lathriididae spp.	3.70±0.54	1.90±0.23	2.50±0.40	4.10±0.24	4.90±0.18
Cryptophagidae spp.	0.70±0.26	1.80±0.39	2.30±0.26	2.10±0.50	2.60±0.64
Drosophilla spp.	22.90±0.23	24.50±0.43	25.90±0.28	30.00±0.26	25.60±0.34
2015/16 (mean ± standard error)					
Nitidulidae spp.	21.80±1.09	11.70±0.47	28.60±1.39	25.40±1.01	30.80±0.39
Mycetophagidae spp.	8.10±0.72	9.40±0.56	9.60±0.48	14.70±0.82	17.20±0.74
Lathriididae spp.	1.30±0.26	0.80±0.25	3.00±0.26	3.60±0.40	3.60±0.31
Cryptophagidae spp.	0.52±0.17	1.20±0.29	1.20±0.30	1.10±0.28	2.60±0.64
Drosophilla spp.	23.70±0.37	24.50±1.18	27.20±0.47	28.40±0.22	26.00±0.33

Psocids and mites

The mean number of psocids and mites per 50 g of moldy stored grain varied from one species to other. *Liposcelis* spp., *Lachesilla* spp., *Ectopsocus* sp., *Dorypteryx domestica* and *Lepinotus* spp., were found to be the most commonly encountered psocids during the two year survey. Among these *Lepinotus* spp., (with the mean number per 50 g of moldy grain ranging from 21.50±0.27 in 2015/16 at South Achefer to 32.80±0.47 in 2014/15 at Bahirdar Zuria in Amhara region) were found to be the most abundanant followed by *Liposcelis* spp., ranging from 19.30±0.30 at Dembecha to 30.80±0.33 at Bahirdar Zuria in 2014/15 in Amhara region. On the other hand, *Tyrophagus putrescentiae* and *Acarus siro* were the two species of mites encountered in moldy stored grain. *Tyrophagus putrescentiae* with the mean number per 50 g of moldy grain ranging from 19.00±0.82 at Kersa to 124.90±1.43 in 2014/15 at Omo Nada in Oromia region was the most abundant mite encountered in moldy grains. Moreover, other mites were also encountered ranging from 10.90±0.48 at Kersa in 2014/15 in Oromia region to 67.40±1.49 in 2015/16 at Bahirdar Zuria in Amhara region (Tables 3.6, 3.7, 3.8 and 3.9).

Natural enemies

Parasitic Hymenoptera (with the mean number per 100 g of grain ranging from 1.63±0.25 in 2015/16 at Mecha in Amhara region to 7.20±0.66 in 2014/15 at Sibu-Sirae in Oromia region) were the most abundant natural enemies in association with granivorous arthropods. Parasitic Hemiptera and Pseudoscorpion were also recorded with the mean number per 100 g of grain ranging from 0.76±0.16 in 2015/16 at South Achefer in Amhara region to 1.30±0.37 in 2015/16 at Omo-Nada in Oromia region and 0.00±0.00 in 2014/15 at Bako-Tibae in Oromia region to 1.10±0.30 in 2014/15 at Mecha in Amhara region, respectively. On the other hand, *Staphylinidae* spp., with the mean number per 100 g of moldy grain ranging from 8.60±1.01 in 2015/16 at Bako-Tibae in Oromia region to 19.50±0.48 in 2014/15 at Bahirdar Zuria were the most abundant followed by *Dactylosternum abdominale* ranging from 4.50±0.45 in 2014/15 at Bako-Tibae to 11.90±1.37 in 2014/15 at Sekoru in Oromia region and *Scynmus* sp., ranging from 0.00±0.00 in 2014/15 at Sekoru to 1.70±1.05 in 2014/15 at Omo-Nada in Oromia region in (Tables 3.10, 3.11, 3.12 and 3.13).

Table 3.6: Mean number of the most important Psocids per 50 g of moldy grain from selected Woreda of Oromia a region during 2014/15 and 2015/16.

Psocids	Selected Woreda from Oromia region				
	Bako-Tibae	Sibu-Sirae	Kerssa	Omo-Nada	Sekoru
2014/15 (mean ± standard error)					
Liposcelis spp.	23.70±0.21	18.90±0.71	18.20±0.93	24.50±0.91	25.00±0.33
Lachesilla spp.	0.80±0.13	1.00±0.21	0.00±0.00	0.80±0.20	0.00±0.00
Ectopsocus sp.	1.80±0.29	1.10±0.18	2.90±0.23	4.40±0.16	3.90±0.35
Dorypteryx domestica	0.00±0.00	1.20±0.29	1.20±0.25	0.00±0.00	0.80±0.13
Lepinotus spp.	26.70±0.37	26.80±0.29	29.20±0.33	29.60±0.43	28.80±0.38
2015/16 (mean ± standard error)					
Liposcelis spp.	20.70±0.63	25.60±0.45	27.80±0.28	25.20±0.25	24.80±0.92
Lachesilla spp.	0.00±0.00	0. 70±0.15	0.00±0.00	1.10±0.28	1.10±0.28
Ectopsocus sp.	1.90±0.28	0.90±0.23	1.90±0.35	3.80±0.55	2.60±0.27
Dorypteryx domestica	0.60±0.16	0.00±0.00	0.10±0.01	0.00±0.00	1.00±0.21
Lepinotus spp.	25.00±0.26	27.60±0.37	28.10±0.36	28.20±0.39	29.10±0.48

Table 3.7: Mean number of the most important Psocids per 50 g of moldy grain from selected Woreda of Amhara region during 2014/15 and 2015/16

Psocids	Selected Woreda from Amhara region				
	Dembecha	South Achefer	Burae	Mecha	Bahirdar Zuria
2014/15 (mean ± standard error)					
Liposcelis spp.	19.30±0.30	19.70±0.63	19.90±0.35	29.10±0.71	30.80±0.33
Lachesilla spp.	0.70±0.15	0.00±0.00	0.00±0.00	0.90±0.10	0.00±0.00
Ectopsocus sp.	1.7±0.26	2.40±0.27	1.10±0.10	2.10±0. 23	3.10±0.23
Dorypteryx domestica	0.00±0.00	0.80±0.20	0.00±0.00	1.10±0.23	0.00±0.00
Lepinotus spp.,	24.30±0.36	22.00±0.26	30.80±0.42	32.30±0.45	32.80±0.47
2015/16 (mean ± standard error)					
Liposcelis spp.	20.50±0.34	20.40±0.40	18.60±0.16	27.00±1.03	28.50±0.75
Lachesilla spp.	1.00±0.29	0.90±0.18	0.00±0.00	1.00±0.21	0.00±0.00
Ectopsocus sp.	2.00±0.21	2.00±0.26	1.30±0.15	2.10±0.28	2.70±0.26
Dorypteryx domestica	1.10±0.23	0.90±0.17	0.00±0.00	0.00±0.00	0.00±0.00
Lepinotus spp.	23.20±0.20	21.50±0.27	29.80±0.33	30.50±0.37	31.80±0.29

Table 3.8: Mean number of the most important mites per 50 g of moldy grain from selected Woreda of Oromia region during 2014/15 and 2015/16.

Mites	Selected Woreda from Oromia region				
	Bako-Tibae	Sibu-Sirae	Kerssa	Omo-Nada	Sekoru
2014/15 (mean ± standard error)					
Acarus siro	1.20±0.20	0.80±0.13	1.80±0.25	1.30±0.26	3.40±0.45
Tyrophagus putrescentiae	34.10±1.46	41.20±0.55	19.00±0.82	124.90±1.43	63.80±1.73
Others	19.80±0.86	19.30±0.62	10.90±0.48	25.90±0.57	30.70±0.83
2015/16 (mean ± standard error)					
Acarus siro	1.60±0.34	2.40±0.54	1.90±0.23	1.00±0.26	4.40±0.50
Tyrophagus putrescentiae	40.10±0.48	49.40±0.56	25.20±0.68	88.60±1.61	134.80±1.69
Others	27.70±0.90	22.90±0.84	16.90±0.50	13.30±0.68	39.20±0.76

Table 3.9: Mean number of the most important mites per 50 g of moldy grain from selected Woreda of Amhara region during 2014/15 and 2015/16.

Mites	Selected Woreda of Amhara region				
	Dembecha	South Achefer	Burae	Mecha	Bahirdar Zuria
2014/15 (mean ± standard error)					
Acarus siro	1.10±0.28	1.40±0.22	4.00±0.26	2.00±0.33	2.90±0.28
Tyrophagus putrescentiae	38.40±0.97	42.90±0.86	22.70±0.52	70.30±0.56	87.10±2.23
Others	21.80±0.76	21.70±0.67	9.80±0.44	39.90±1.18	69.30±1.19
2015/16 (mean ± standard error)					
Acarus siro	1.50±0.27	0.00±0.00	5.10±0.38	2.10±0.23	6.10±0.64
Tyrophagus putrescentiae	43.60±0.82	50.70±0.83	29.50±1.04	77.10±2.02	102.10±2.28
Others	26.70±0.92	20.60±0.65	18.60±0.58	58.80±1.63	67.40±1.49

Table 3.10: Mean number of the most important natural enemies per 100 g of moldy grain from selected Woreda of Oromia region during 2014/15 and 2015/16.

Natural enemies1	Selected Woreda from Oromia region				
	Bako-Tibae	Sibu-Sirae	Kerssa	Omo-Nada	Sekoru
2014/15 (mean ± standard error)					
Staphylinidae spp.	9.70±0.78	11.40±1.02	8.70±1.16	18.80±2.49	17.10±2.33
Scynmus sp.	0.70±0.13	1.30±0.07	1.70±0.67	1.70±1.05	0.00±0.00
Dactylosternum abdominale	4.50±0.45	8.20±0.25	6.40±1.07	10.20±1.32	11.90±1.37
2015/16 (mean ± standard error)					
Staphylinidae spp.	8.60±1.01	12.90±0.86	11.20±1.55	20.80±2.34	19.20±1.75
Scynmus sp.	1.50±0.15	1.70±0.82	1.20±0.63	1.00±0.67	1.10±0.99
Dactylosternum abdominale	5.50±0.60	6.10±0.50	7.40±1.17	10.70±2.36	8.90±1.45

Table 3.11: Mean number of the most important natural enemies per 100 g of moldy grain from selected Woreda of Amhara region during 2014/15 and 2015/16.

Natural enemies	Selected Woreda from Amhara region				
	Dembecha	South Achefer	Burae	Mecha	Bahirdar Zuria
2014/15 (mean ± standard error)					
Staphylinidae spp.	11.20±0.53	15.20±0.98	12.80±0.89	17.90±1.02	19.50±0.48
Scynmus sp.	1.10±0.23	0.00±0.00	1.30±0.26	1.00±0.15	0.00±0.00
Dactylosternum abdominale	7.30±0.50	5.50±0.54	8.20±0.42	9.90±0.60	11.30±0.63
2015/16 (mean ± standard error)					
Staphylinidae spp.	10.00±0.37	10.70±0.45	14.30±0.91	16.80±1.17	16.20±0.77
Scynmus sp.	0.90±0.10	1.10±0.28	0.60±0.16	0.70±0.15	1.00±0.30
Dactylosternum abdominale	8.10±0.38	7.70±0.63	6.00±0.56	8.40±0.31	9.30±0.54

Table3.12: Mean number of the most important natural enemies per 100 g of grain from selected Woreda of Oromia region during 2014/15 and 2015/16.

Natural enemies2	Selected Woreda from Oromia region				
	Bako-Tibae	Sibu-Sirae	Kerssa	Omo-Nada	Sekoru
2014/15 (mean ± standard error)					
Parasitic Hymenoptera	5.20±0.73	7.20±0.66	3.20±0.42	3.30±0.40	2.90±0.23
Parasitic Hemiptera	1.10±0.10	1.00±0.26	1.20±0.33	1.30±0.37	1.00±0.30
Pseudoscorpion	0.00±0.00	0.60±0.16	0.70±0.21	0.90±0.18	0.70±0.15
2015/16(mean ± standard error)					
Parasitic Hymenoptera	2.20±0.29	1.80±0.39	2.00±0.26	3.20±0.42	3.00±0.42
Parasitic Hemiptera	0.70±0.15	0.90±0.28	1.00±0.21	1.10±0.23	0.00±0.00
Pseudoscorpion	0.80±0.20	0.80±0.29	0.00±0.00	0.80±0.13	0.80±0.25

Table 3.13: Mean number of the most important natural enemies per 100 g of grain from selected Woreda of Amhara region during 2014/15 and 2015/16.

Natural enemies	Selected Woreda from Amhara region				
	Dembecha	South Achefer	Burae	Mecha	Bahirdar Zuria
2014/15 (mean ± standard error)					
Parasitic Hymenoptera	2.12±0.44	2.01±0.42	1.82±0.30	1.77±0.30	1.71±0.22
Parasitic Hemiptera	0.91±0.26	0.86±0.25	0.96±0.27	1.16±0.26	1.17±0.36
Pseudoscorpion	0.91±0.26	0.70±0.00	0.86±0.25	1.10±0.30	1.09±0.35
2015/16 (mean ± standard error)					
Parasitic Hymenoptera	2.00±0.31	1.85±0.29	2.00±0.52	1.63±0.25	1.77±0.41
Parasitic Hemiptera	0.86±0.25	0.76±0.16	0.96±0.27	1.16±0.26	1.22±0.00
Pseudoscorpion	0.95±0.33	0.70±0.00	0.70±0.00	1.01±0.27	0.91±0.27

Table 3.14 Arthropods recorded for the first time in association with stored maize in Ethiopia

Order	Family	Scientific name	Common name	Number of sample with arthropods (N=100)	
				2014/15	2015/16
Coleoptera	Nitidulidae	*Phenolia picta* (MacLeay)	Sap beetles	9	2
	Cucujidae	*Silvanus planatus*		11	6
	Curculionidae	*Stenoscelis brevis* (Boh.)	Broad-nosed Bark Weevil	6	0
	Languridae	*Cryptophillus integer* (Heer)		11	5
	Hydrophilidae	*Dactylosternum abdominale*	Beetle (water scavenger)	38	14
	Anthicidae	*Anthicus floralis* (L.)		5	2
	Staphylionidae	*Aleochara* sp.	Rove beetle	27	47
		Anotylus sp.	Rove beetle	12	18
		Philonthus cruentatus	Rove beetle	51	59
		Stenus cicindeloides	Rove beetle	11	4
		Scydmaenus tarsatus	Ant like stone beetle	7	3
		Scaphisoma sp.	Shining fungus beetle	27	34
	Latridiidae	*Cartodere (Aridius) nodifer*		23	28
		Melanophthalma sp.		7	22
	Cryptophagidae	*Cryptophagus cellaris*		13	25
		Micrambe bimaculata		5	14
Psocoptera	Lachesillidae	*Lachesilla michiliensis*	Psocid	11	6
	Ectopsocidae	*Ectopsocus* sp.	Psocid	43	21
	Psyllipsocidae	*Dorypteryx domestica*	Psocid	5	23
	Trogiidae	*Lepinotus patruelis*	Psocid	59	61

Diptera	Drosophillidae	*Drosophila busckii*	Small fruit flies	42	47
Lepidoptera	Tineidae	*Opogona dimidiatella*	Fungus Moth	5	7
Hemiptera	Anthocoridae	*Xylocoris galactinus* (Fieber)		23	38
Arachnida	Ascidae	*Blattisocius* sp.		38	47
Isopoda	Armadillidae	*Armadillidiidae* sp.	Pillbugs	7	18
	Porcellionidae	*Porcellionidae* sp.	Sowbugs	6	19
	Oniscidae	*Oniscidae* sp.	Sowbugs	13	28

A

B

C

D

E

F

Plate 3.2: Coleoptera: Nitidulidae in stored maize from Ethiopia (A, *Carpophilus* sp.; B, *Brachypeplus* sp.*;* C. *Phenolia picta;* D, *Carpophilus dimidiatus;* E, *Carpophilus freemani;* F, *Carpophilus hemipterus*) (Size=2.5×1.20 inch).

A

B

C

D.

E.

F.

Plate 3.3: Coleoptera: Cucujidae in stored maize from Ethiopia (A, *Cryptolestes ferrugienus*.; B, *Cryptolestes pusillus.;* C. *Cryptolestes* sp.*;* D, *Oryzaephilus surinamensis.;* E, *Ahasverus advena;* F, *Silvanus planatus*) (Size=2.5×1.20 inch).

A

B

C

D

E

F

Plate 3.4: Coleoptera: Tenebrionidae in stored maize from Ethiopia (A, *Tribolium castaneum.*; B, Antennae of *Tribolium castaneum.;* C. *Tribolium* sp.*;* D, *Tribolium confusum.;* E, Antennae of *Tribolium confusum.;* F, *Tribolium destructor*.) (Size=2.5×1.20 inch).

G

H

I

J

K

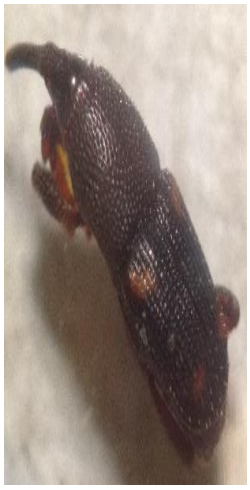
L

Plate 3.4: Coleoptera: Tenebrionidae in stored maize from Ethiopia (G, *Palorus subdepressus.*; H, *Palorus ratzeburgii.;* I. *Gnatocerus cornutus* (female)*.;* J, *Gnatocerus cornutus* (Male).; K, *Palorus* species-1.; L, Pronotum and head of *Palorus* species-1) (Size=2.5×1.20 inch).

A

B

C



D

E

F

Plate 3.5: Coleoptera: Curculionidae in stored maize from Ethiopia (A, *Sitophilus oryzae.*; B, Aedeagus of *Sitophilus oryzae.;* C. *Stenoscelis brevis.;* D, *Sitophilus zeamais.;* E, Aedeagus of *Sitophilus zeamais.;* F, Head and pronotum of *Stenoscelis brevis*.)

A

B

C

D

E

Plate 3.6: Coleoptera, A, *Typhae stercorea.*; B, *Litargus balteatus.;* C. *Mycetophagus sp*., (Mycetophagidae). D, *Cryptophillus integer* (Languriidae**)**; E, *Callosobruchus chinensis (*Bruchidae) (Size 2.2×1.25 inch).




A B C D




E F G H

Plate 3.7: Coleoptera: Staphylionidae in Stored Maize from Ethiopia (A, *Anotylus* sp.; B, *Aleochara* sp..; C. *Stenus cicindeloides*..; D, *Philonthus cruentatus*.; E, *Philonthus* sp1.; F, *Philonthus* sp2.; G, *Scydmaenus tarsatus*.; H, *Scaphisoma* sp.) (Size: 2×0.98 inch).

A

B

C

D.

E.

F.

Plate 3.8: Coleoptera: Latridiidae (A, B, C) [A, *Corticaria* sp.; B, *Melanophthalma* sp.*;* C. *Cartodere (Aridius) nodifer*] and Cryptophagidae (D, E, F) [D, *Cryptophagus* sp.; E, *Cryptophagus cellaris*; F. *Micrambe bimaculata*] (Size: 2×1. 20 inches).

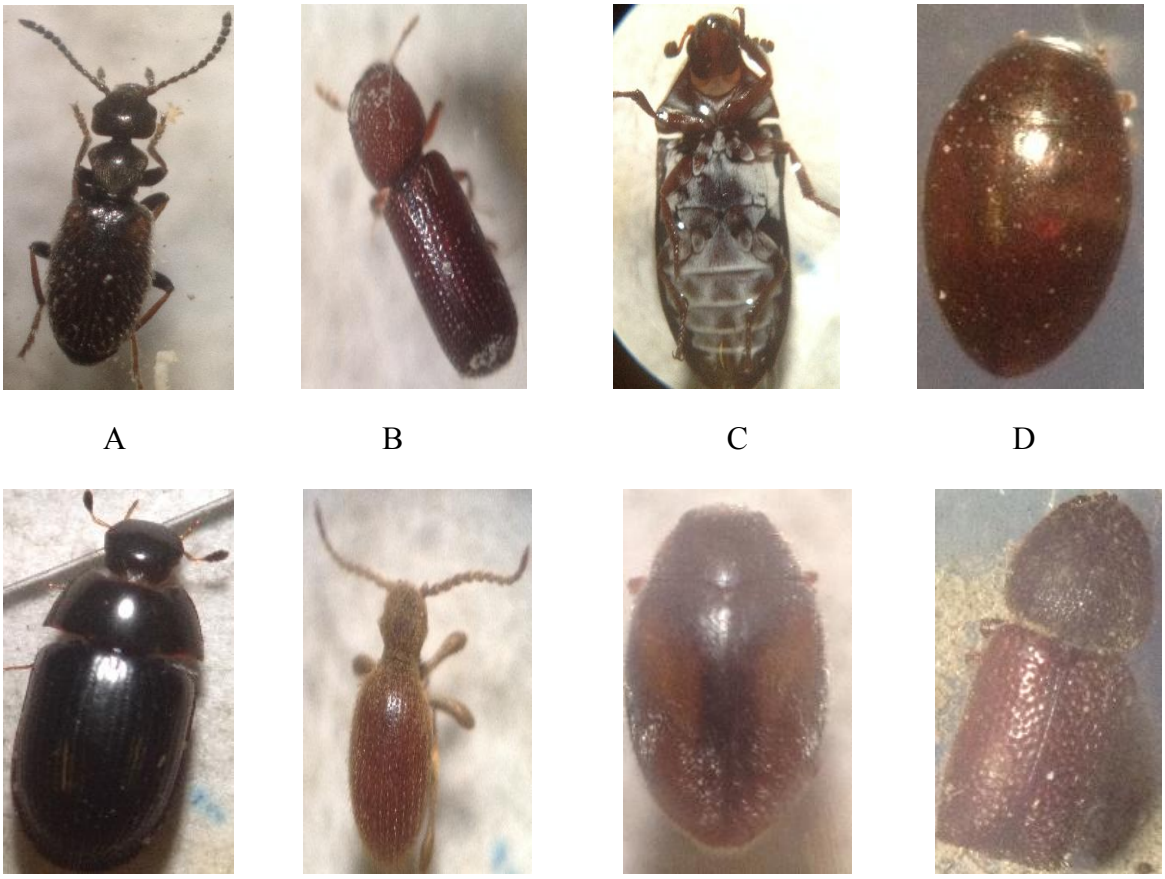


Plate 3.9: Other Coleoptera in stored maize from Ethiopia (A, *Anthicus floralis* (Anthicidae); B, *Rhizoperta dominca* (Bostrichidae).; C. *Dermestus* sp. (Dermestidae).; D, *Murmidius ovalis*; E, *Dactylosternum abdominale* (Hydrophilidae).; F, *Pseudeurostus hilleri* (Ptinidae); G, *Scymnus interruptus* (Coccinellidae); H, *Prostephanus truncatus*) (Size: 2×1. 20 inches).

A B C D

E F G H

Plate 3.10: Psocoptera (Booklice) in stored maize from Ethiopia. (A, *Liposcelis decolor*; B, *Liposcelis bostrychophilus;* C. *Lachesilla* sp.*;* D, *Lachesilla michiliensis*; E, *Liposcelis entomophila*; F, *Ectopsocus briggsi***;** G**,** *Dorypteryx domestica;* H**,** *Lepinotus patruelis*) (Size: 2×1. 20 inches).

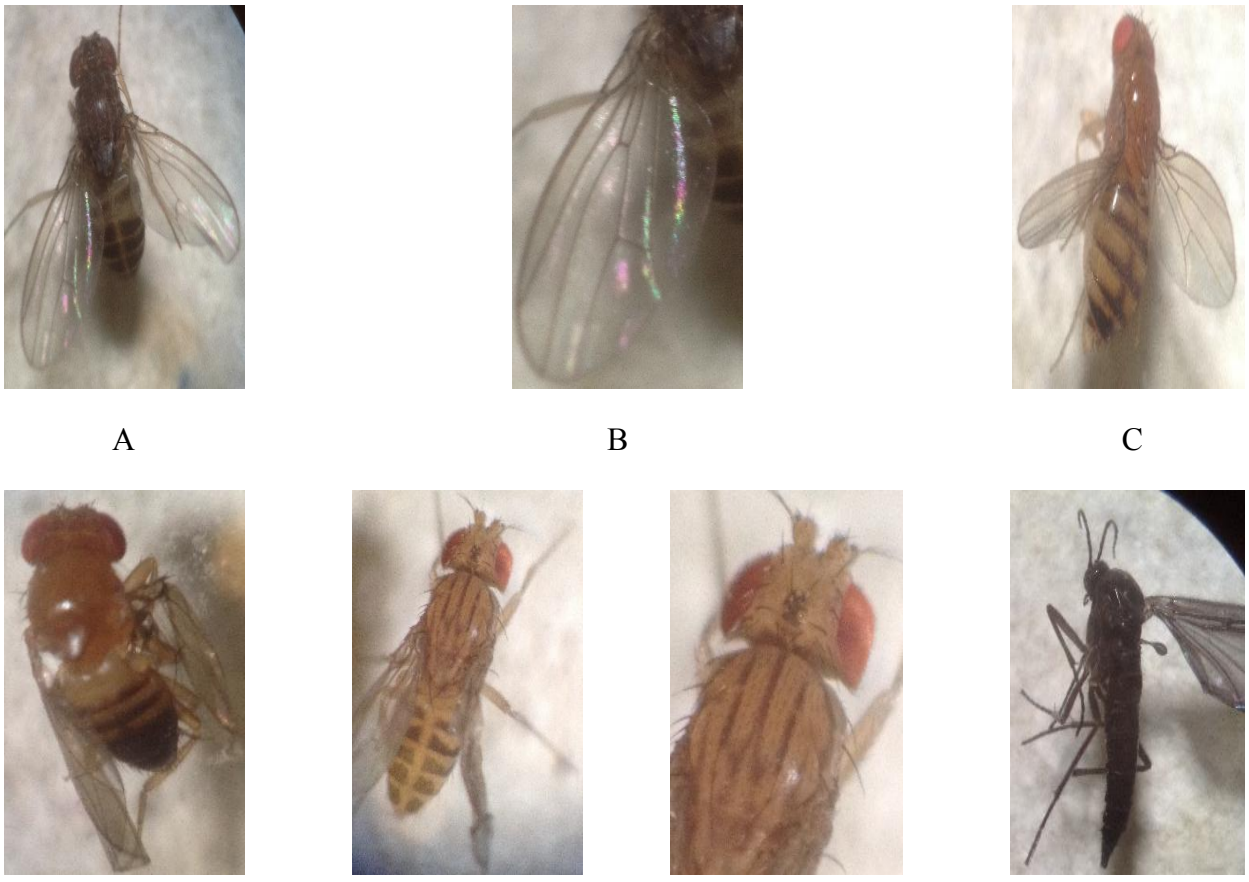


Plate 3.11: Diptera in stored maize from Ethiopia. (A, *Drosophila* sp1.; B, *Drosophila* sp. (wing venation)*.;* C. *Drosophila* sp2*.;* D, *Drosophila melanogaster*; E, *Drosophila busckii*; F, *Drosophila busckii* (pronotal band)*.;* ***G, Sciaridae*** sp.) (Size: 2×1. 20 inches).

A B

Plate 3.12: Hemiptera in stored maize from Ethiopia. (A, *Xylocoris galactinus* B, *Xylocoris* sp.) (Size: 2×1. 20 inches).

A B C

Plate 3.13: Hymenoptera in stored maize from Ethiopia. (A, *Anisopteromalus calandrae.*; B, *Theocolax elegans.;* C, *Monomorium sp.*) (Size: 2×1. 20 inches).

A. **B.** **C.**

Plate 3.14: Lepidoptera in stored maize from Ethiopia. (A, *Opogona dimidiatella.*; B, *Sitotroga cereallela.;* C, *Plodia interpuntella.*) (Size: 2.5×1. 20 inches).

A. **B.** **C.** **D.**

Plate 3.15: Mites in stored maize from Ethiopia. (A, *Tyrophagus putrescentiae.*; B, *Acarus siro.;* C, *Blattisocius* sp. D, Unidentified mite species) (Size: 2.5×1. 20 inches).



A. **B.** **C.**

Plate 3.16: Pseudoscorpions in stored maize from Ethiopia. (*Withiidae* sp1.; B, *Withiidae* sp2.; C, *Withiidae* sp3) (Size: 2.5×1. 20 inches).



A. **B.** **C.**

Plate 3.17: Isopoda in stored maize from Ethiopia. (A, *Armadillidiidae* spp.; B, *Porcellionidae* spp.; C, *Oniscidae* sp.) (Size: 2.5×1. 20 inches).

3.4 Discussions

Stored maize grain houses a large number of arthropods and in this study 81 arthropods species, belonging to the class Insecta, Crustaceans, and Arachnida were recorded from stored maize grain. The majority of arthropods that were encountered in this survey were insects (87.65%), of which Coleopterans, Psocopterans, Dipterans, Hymenopterans, Lepidopteran and Hemipterans shared (61.73%), (9.88%), (6.17%), (3.70%), (3.70%) and (2.47%) of the total, respectively. On the other hand, Arachnids and Crustaceans consisted of 8.64% and 3.70 % of arthropods, respectively. The present finding is in contrary to the previous studies in Ethiopia as none of the studies recorded such many arthropods in stored maize grain in particular and stored cereals in general (Abraham; 1996, 1997; Emana, 1993; Emana and Assefa, 1998; McFarlane, 1969a, 1969b; Walker and Boxall, 1974). This variation may possibly be explained by variation in detection methods, sampling period, storage condition and change in climate. Of the 81 arthropod species, 27 were recorded for the first time from Ethiopia. Change in storage condition due to incubation of highly infested stored grain for four month at 21±3 °C and 65%-70% relative humidity (RH) might have resulted in the occurrence of these 27 species for the first time (Table 3.14).

Of all the granivorous arthropods, *Sitophilus* spp., were found to be the most frequently and abundantly encountered arthropods followed by *S. cerealella*, *Cryptolestes* spp., (in most case) and *Tribolium* spp. These findings are in agreement with those of Adhanom and Abraham (1985), Mekuria (1995) and Abraham (1991; 1993a) who recorded *Sitophilus* spp., *S. cereallela*, *Cryptolestes* spp., and *Tribolium* spp., among the most common and dominant pests. Although, their frequency and abundance were very low, two devastating insect pests from the family Bostrichidae, namely *R. dominica* (Fab.) and *P. turncatus* were also recorded. Abraham (1996) reported *R. dominica* as "uncommon" pest in association with stored maize. However, according to Abraham (1991; 1996; 1997; 2003) *P. turncatus* has not been recorded in Ethiopia and thus in the present finding this insect may appeared to be new record. Apart from these, *C. chinensis* was also recorded in 9% of the sample during 2013/14 only. IAR (1990) reported *C. chinensis* as a key pest of haricot bean and cowpea in storage. This insect was recorded in stored maize probably due to cross infestation from haricot bean and cowpea in the nearby storage or residual infestation from previously stored haricot bean and cowpea grain.

Different species of mold feeder insects were also encountered in the present study. *Carpophilus* spp. (Nitidulidae), *L. balteatus* and *T. stercorea* were the most frequently and abundantly encountered fungus beetle. *Drosophilla* spp., and *Sciaridae* sp., were also the two most commonly encountered Dipterans in association with moldy grain. On average members of the family Lathridiidae and Cryptophagidae also occurred in 23.5% and 19 % of the sample, respectively. Adhanom and Abraham (1985), Mekuria (1995) and Abraham (1991; 1993a) reported *Carpophilus* spp., among the most widely spread storage pest of maize growing areas of southwestern Ethiopia during the 1992 and 1993 seasons. Pellitteri and Boush (1983) reported *C. hemipterus* as the most widely distributed sap beetles. They also noted this insect as the most economically important storage pest under the family Nitidulidae. Similarly, in the present study, *C. hemipterus* was found to be encountered in 62% of the samples. Abraham (1996) was recorded *Drosophilla* spp as a "common" arthropod in association with stored maize. Magro *et al*. (1999) reported Cryptophagidae and Lathridiidae families as exclusively fungus-feeders in stored rice. The present finding of Mycetophagidae differed somewhat from those of earlier studies. Previously Mycetophagidae such as *T. stercorea* and *L. balteatus* were recorded as "uncommon" and "minor" fungus feeder insects respectively (Walker and Boxall, 1974), while in the present study both *T. stercorea* (51.5%) and *L. balteatus* (56.5%) were recorded as a common fungus feeder arthropod. This is possibly due to variation of storage condition.

In the order Psocoptera, eight species were recorded under five families and among them *L.patruelis* (60%) was found to be the most frequently encountered psocid followed by *L. bostrychophilus* (52.5%), *Liposcelis* sp1., (47.5%), *Liposcelis* sp2**.,** **(**33%), *Ectopsocus* sp., (32%), *D. domestica* (14%), *L. michiliensis* (8.5%) and *Lachesilla* sp., (8%). *Lepinotus* and *Liposcelis* spp., were found to be the two most abundant psocids encountered in association with moldy grain. The results reported by previous authors (Garcia Aldrete and Gutierrez; Diaz, 1995 and Turner and Ali, 1996) are also similar to the present findings. Moreover, Sinha (1988) was reported Liposcelid specia (e.g. *L. bostrychophila*) and *L. reticulates* as the two widely distributed psocids in stored grain. On the other hand, Rees (2004) reported *Lachesilla quercus* Kolbe as harmful pests in coastal grain handling facilities in Australia. Further, Sinha and Srivastava (1970) in their study "Cellulose digestion in *Liposcelis entomophilus* End. (Psocoptera, Liposcelidae)" identified *L. entomophilus* in packaged rice and moldy rice stems in the fields. According to, Kalinovic and Rozman (2000) Psocoptera were mostly found on moldy basement walls feeding on fungi and bacteria.

Other insects were also recorded among Coleopterans: mold feeder insects [*Brachypeplus* sp., *A. advena*, *Scaphisoma* sp., *P. picta*, *S. brevis*, *A. floralis*, *P. hilleri, C. integer* and *M. ovalis*]; secondary pests [(*G. cornutus*, *P. ratzeburgii*, *P. subdepressus*, *Palorus* sp., *S. planatus*, and *O. surinamensis*]. *O. dimidiatella* and *P. interpunctella* were also the two most common Lepidopterans which were encountered in association with moldy grains and insect damaged stored grains, respectively. Except *Scaphisoma* sp., and *S. brevis* all these insects were recorded in stored grain ecosystem (Pellitteri and Boush, 1983; Arbogast and Throne, 1997; Hagstrum and Subramanyam, 2009).

In addition to insects in the present study, Arachnida and Isopoda accounted for 8.64% and 3.70 % of the recorded arthropods, respectively. *T. putrescentiae* and *A. siro* were the most frequently encountered mites in association with moldy grains. *T. putrescentiae* was found to be the most abundant of all identified mite species. Moreover, three unidentified species of Arachnids were also recorded under the family of Withiidae. Three unidentified species of Isopoda were also recorded under three families (Armadillidae, Porcellionidae and Oniscidae). Trematerra and Fiorilli (1999) reported Arachnida (mites and pseudoscorpions), Isopoda (*Porcellio scaber* (Latreille) and Insecta in association with feed-mill in Central Italy. Thind and Clarke (2001) reported *T. putrescentiae* and *A. siro* among the most common species encountered in cereal based food destined for human consumption. According to Hagstrum *et al*. (2013) *T. putrescentiae* and *A. siro* were the two leading mites encountered in 142 and 88 stored agricultural commodities, respectively. *T. putrescentiae* is widely distributed in stored products in tropical and subtropical regions (Hughes, 1976).

Members of two parasitic insect orders associated with granivorous arthropods, Hymenopteran and Hemiptera, were encountered in 38.33% and 28.25% of the samples, respectively. Moreover, members of three unidentified predacious Pseudoscorpions from the family Withiidae associated with Psocids in stored grain appeared in 18.5% of the samples. Generally, Hymenopterans were the most frequently and abundantly encountered parasitic insect order in association with granivorous insect. On the other hand, members of three predacious beetle families associated with fungivorus arthropod, the Staphylinidae, Hydrophilidae and Coccinelidae, were also encountered in a number of samples. Staphylinidae (8.60±3.20-19.50±1.51) was found to be the most abundant family encountered in association with fungivorous arthropod. According to Arbogast and Throne (1997), Hymenopterans and Hemipterans were among the insects found in maize storage in South Carolina farms during the 1985-1986 storage seasons. The finding of the three unidentified Whithidae family was in agreement

with Abraham (1996) who found *Withius somalicus* (Beier) in association with stored maize in western Ethiopia. Members of Staphylinidae and Coccinelidae (*Scynmus* sp.) were among arthropods recorded by Pellitteri and Boush (1983) in association with feed mills in Southern Wiscosin. *D. abdominale* was found to be among the two species of Hydrophilidae reported in stored product (Hagstrum and Subramanyam, 2009).

3.5 Conclusion

A complex species of arthropods such as grain and grain product feeders, mold feeders and natural enemies associated with grain and grain product feeders and mold feeder arthropods colonized stored maize in Ethiopia. The findings of the present study was include the occurrence of high frequency of fungus feeding insects such as *C. hemipterus*, *C. dimidiatus*, *L. balteatus*, *T. stercorea* and *T. putrescentiae* in association with stored maize. The infestation of these insect may indicated the presence of potentially toxigenic fungi and mycotoxin contamination in stored maize grain in Ethiopia. Further, they may indicate the presence of allergens that cause occupational allergies.

Chapter-4

Status of sap beetles and other mold feeder arthropods on insect and mold damaged pre-harvest maize cob in Ethiopia

4.1 Introduction

Nitidulid beetles (sap beetles) are worldwide pests of a wide variety of agricultural commodities including flowers, fruits, sap, fungi, decaying and fermenting plant tissues, dead animal tissue and grain, both before and after harvest (Hinton, 1945). In addition to their direct effect, these beetles are implicated as vectors of *Ceratocystis fagacearum* (Bretz) Hunt and mycotoxin producing fungi which caused oak wilt and mycotoxin contamination in oak and maize, respectively. Dowd (1995) in his review "sap beetles and mycotoxins in maize" reported the role of sap beetles in vectoring mycotoxigenic species of *Aspergillus* and *Fusarium* to maize. Moreover, he showed high infestation of these beetles in ears damaged by birds or caterpillars or those ears that have poor husk coverage. On the other hand, Ako *et al*. (2003) in their studies "The effect of *Fusarium verticillioides* (Sacc.) Nirenberg on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stem and cobs of maize in West Africa" showed higher immature survival and adult fecundity of *Eldana saccharina* (Walker), *M. nigrivenella* and *C. dimidiatus* on *F. verticillioides* infected maize plants. Alcohols, esters and aldehydes produced by *F. verticillioides* were responsible for attracting nitidulid beetles (Bartelt and Wicklow, 1999)

In addition to Nitidulid beetles, other mold feeders also play a great role in toxigenic fungal infection and mycotoxin contamination in agricultural commodities. Tsai *et al*. (2007) conducted studies on "Effect of three stored-grain fungi on the development of *T. stercorea*" and found the shortest developmental time and females laid more eggs on pure cultures of *Aspergillus flavus* Link compared to *Eurotium rubrum* König, and *Penicillium purpurogenum* Stoll. Apart from this, Tsai *et al*. (2007) showed hairy fungus beetles can complete their life cycle, while feeding on a fungal culture producing high levels of aflatoxin. Mold feeder insects including sap beetles have the potential of obtaining corn ear rot fungal spores from infected plant materials and transport them to damaged kernels in the field, as well as stored grain ecosystem. Dowd (1998) and Gilbertson *et al*. (1986) reported the involvement of European corn borers (*Ostrinia*

nubilalis), sap beetles [*Carpophilus* spp. and *Glischrochilus quadrisignatus* (Say)], western flower thrips [*Frankliniella occidentalis* (Pergande)], and corn rootworm beetles (*Diabrotica* spp.) in dispersing *F. verticillioides* fungal spore.

Sap beetles are attracted to maize cob damaged by other insects. Tamaki *et al*. (1982) and Attwater and Busch (1983) reported sap beetles as "secondary invaders of ears damaged by insects such as the corn earworm *Heliothis zea* (Boddie) or the European corn borer *O. nubilalis*". They appear to be attracted as well to volatile compounds produced by *F. verticillioides* which cause fusarium ear rot. Munkvold (2003a) also reported that sap beetles potentially acquire Fusarium spores from infected plant material and carry them to damaged kernels that are prone to infection. Moreover, Sétamou *et al*. (1998) demonstrated that the damage from *M. nigrivenella* predisposes maize to pre- and post-harvest infestation of storage beetles and to infection of *A. flavus* and subsequent aflatoxin contamination. In Ethiopia, no information was available on those pre-harvest sap beetles and other mold feeder insect abundance associated with insect and mold damaged maize ears. To devise effective management practices against these insects, it is very important to know the abundance and source of infestation of these insects. Therefore the objectives of this study were to,

- identify corn ear rot type associated with pre-harvest moldy maize cob/ears.
- identify sap beetles and other mold feeder arthropods associated with maize ear damaged by insect and molds,
- evaluate the effects of pre-harvest insect and mold damaged maize ears/cobs on abundance of selected mold feeder insects.

4.2 Materials and Methods

Sampling

Sampling was carried-out during dough-hard stage at the moisture content ranging from 20 % to 25%. The study was conducted in selected Districts of Oromia and Amhara regions. The Districts in Oromia include Bako-Tibe, Sibu-Sire, Kersa, Omo-nada and Sekoru while in Amhara the study Districts were Dembecha, Bure, South-Achefer, Mecha and Bhirdar Zuria. The study was conducted in 2015/16 in Oromia and 2016/17 in Amhara. Six maize cobs/Ears were randomly harvested from each selected Districts and mixed together in order to prepare thirty regional composite samples for each of healthy (asymptomatic), insect damaged only, maize ears simultaneously damaged by insect and mold and mold damaged only (symptomatic) maize ears (Plate 4.1). Ears were transported to the laboratory and inspected for the presence of sap beetles and other mold feeder's insect. Finally insects were identified using identification keys (Chinery, 1993).

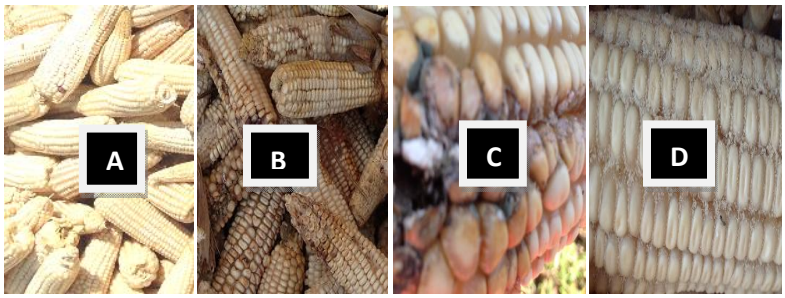


Plate 4.1 Maize cob (ear) damage types (A. Undamaged maize cob; B. Mold damaged; C. Simultaneously damaged by insect (Stalk borer) and mold; D. Insect damaged (Weevil) (Size: 1.5×1 inch).

Entomological data collection

In laboratory, each maize cob (ear) was examined. Sap beetles and other mold feeder arthropods were removed, identified, counted and categorized under order, family, genus or species and were pinned and preserved dry or in 75% alcohol. Data were collected for each of identified sap beetles and other mold feeders' arthropod species associated to insect and mold damaged cobs and number of selected sap beetles and other mold feeder's arthropods per damage type. All the

samples were incubated at the temperature ranging from 25 °C to 30 °C and relative humidity 65% for two months in order to observe any hidden infestation.

Types of corn ear rot

One hundred and twenty mold damaged cobs were randomly assessed from each selected Districts of the two regions. Ten fields were randomly selected from each woreda and 12 mold damaged cobs randomly harvested from each field (12×10=120). Then, the cobs harvested from each region, mixed and composited, consisted of 600 mold damaged maize cobs. Types of maize ear rots were identified and differentiated using an illustrated Compendium of maize disease compiled by warren (2000). Finally, the data from the two year composite samples were pooled together in order to compute the number of each corn ear rot types per 1200 mold damaged maize cobs.

Statistical analysis

The data obtained on numbers of selected sap beetles and other mold feeder arthropods per cobs in different treatment (damage types such as maize ear damaged by insect only, insect and mold simultaneously, mold only and undamaged maize ears) and corn ear rot types were analyzed using the SPSS computer software (1989). Univariate and One-way analysis of variance (ANOVA) was done to compare the effects of treatments, which were laid down in completely randomized design (CRD). Comparison of mean number of selected sap beetles and other mold feeder arthropod per treatment and corn ear rot types were done using Tukey's Studentized range tests at 5% level of significance. Number of samples (insect and mold damaged maize cobs) with identified sap beetles and other mold feeder arthropods (frequency of occurrence) were expressed using percentage.

4.3 Results

Occurrence of insects

A total of nineteen mold feeder insects in 8 families were identified in association with insect and mold damaged cobs. However, only *Carpophilus* sp., *Brachypeplus* sp., *Litargus balteatus* LeConte. *Carpophilus dimidiatus* (F.), *Carpophilus hemipterus* (L.), and *Entomobrya* spp., were recorded in more than 30% of the samples. From the Nitidulidae family, *Carpophilus hemipterus* (L.) (56.67%), *Carpophilus* sp. (42.5%), *Carpophilus dimidiatus* (F.) (38.34%) and *Brachypeplus* sp. (37.5%) were the most frequently occurring species. *L. balteatus* and *Typhae stercorea* (L.) with the proportion of 33.33 % and 28.33 %, respectively were the most frequently occurring insect species from the Mycetophagidae family (Table 4.1).

Table 4.1 Mold feeder arthropods associated with insect and mold damaged maize cobs at pre-harvest 2015/16-2016/17.

Order	Family		Common name	Number of sample with insect in % (N=60)		
				2015/16	2016/17	Average
Coleoptera	Nitidulidae (4)	*Carpophilus* sp.	A sap beetle	43.33	41.67	42.5
		Brachypeplus sp.	A sap beetle	40	35	37.5
		Carpophilus dimidiatus	A sap beetle	36.67	40	38.34
		Carpophilus hemipterus	Dried fruit beetle	63.33	50	56.67
	Mycetophagidae	*Typhae stercorea*	Hairy fungus beetle	35	21.66	28.33
		Litargus balteatus	Hairy fungus beetle.	43.33	23.33	33.33
	Latridiidae	*Cartodere (Aridius) nodifer*	Swollen fungus beetle.	3.33	11.67	7.5
		Corticaria sp.	Minute brown scavenger beetle.	23.33	20	21.67
	Cryptophagidae	*Cryptophagus* spp. (3)	Sillken fungus beetle	11.67	15	13.34
	Monotomidae	*Rhizopghagus* sp.	Small flattened bark beetles.	15	13.33	14.17
	Scydmaenidae	*Scaphisoma* sp.	Shining fungus beetle	6.67	8.33	7.5
Collembolla	Entomobryidae	*Hypogastrura* sp.	Spring tail	15	5.56	10.28
	Hypogastruidae	*Entomobrya* spp. (5)	Spring tail	48.33	43.33	45.83

Occurrence of corn ear rot

Data from two years identified *Giberella, Fusarium, Aspergillus, Penicillium*, *Stenocarpella* and other unidentified ear rot from 1200 mold damaged cobs. Significantly high incidence of *Gibberella* and *Fusarium* ear rots ($p<0.05$) were observed, followed by *Penicillium* and *Stenocarpella* ear rot. *Aspergillus* ear rot together with other unidentified ear rots ($p<0.05$) were found to be the least abundant (Table 4.2; Plate 4.2).

Table 4.2: Mean number of corn ear rot type per 120 mold damaged maize cobs.

Types of corn ear rot	Mean number of corn ear rot/120 mold damaged cobs
Aspergillus ear rot	10.20 ± 0.96^{c}
Fusarium ear rot	31.60±1.97a
Giberella ear rot	35.80 ± 1.05^{a}
Penicillium ear rot	18.60 ± 1.30^{b}
Diplodia ear rot	17.40 ± 1.79^{b}
Others	6.40 ± 0.88^{c}
F=69.66, p=0.000 (F-value and P-value from ANOVA table)	

Mean number corn ear types followed by different letters were significantly different ($p<0.05$) (Tukey Studentized Range Test).

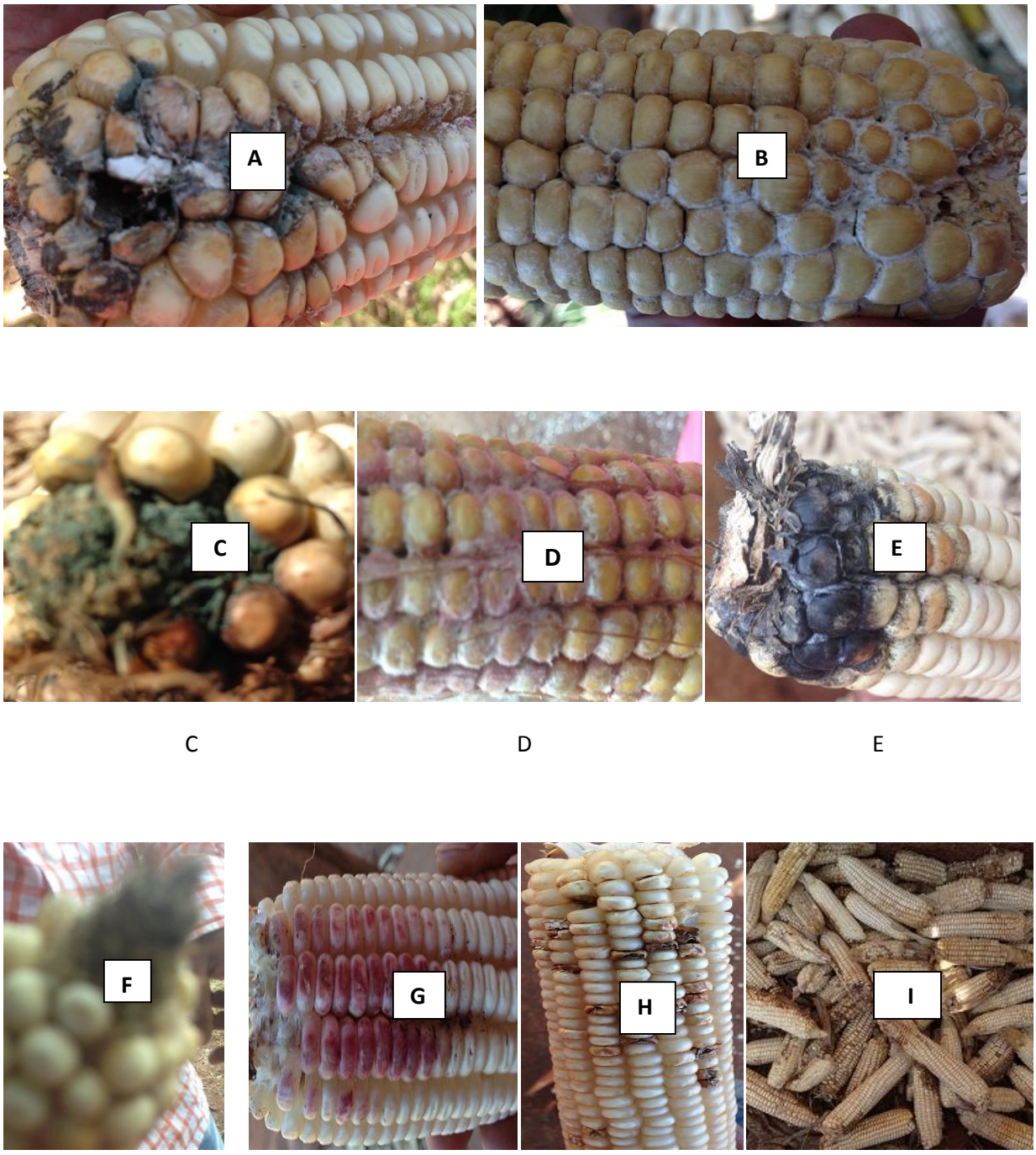


Plate 4.1 Major corn ear rot associated with pre-harvest maize cob in Ethiopia, A., *Fusarium* and *Penicillium* ear rot.; B., *Diplodia* ear rot; C., *Penicillium* ear rot. D. *Giberella* ear rot; E., Black ear rot; F, *Aspergillus* ear rot; G, *Giberella* ear rot; H, *Fusarium* ear rot; I, Moldy cob.

Effects of insect and mold damage to maize ears on abundance of selected mold feeder insects

Comparison of damage type in relation to selected arthropods

In both years (regions) and conditions (before and after incubation), the mean number of sap beetles *C. hemipterus*, *C. dimidiatus* and *Brachypeplus* sp., and Mycetophagids *T. stercorea* and *L. balteatus* per cob were significantly ($p<0.05$) higher on maize ears damaged by insect only, mold only and insect and mold simultaneously than undamaged cobs (Table 4.3). In all cases, the mean numbers of *C. hemipterus* and *L. balteatus* were found to be significantly ($p<0.05$) higher on maize cob damaged by insect and mold simultaneously than maize cob damaged by insect and mold individually. Except in the case of Amhara region 2016/17 befoe incubation, the mean number of *Brachypeplus* sp., per cob were found to be significantly ($p<0.05$) higher on maize cob damaged by insect and mold simultaneously and mold alone than insect alone (Table 4.4).

Comparison of selected arthropods in relation to damage type.

In both years (regions) and conditions (before and after incubation), except at Amhara region during 2016/17 before incubation on cob damaged by mold only, the mean number of selected sap beetles and other mold feeder arthropods per cob damaged by insect only, mold only and insect and mold simultaneously were significantly ($p<0.05$) higher for *C. hemipterus* followed by *L. balteatus* than *Brachypeplus* sp., *C. dimidiatus* and *T. stercorea*. No significant difference ($p>0.05$) was observed between *C. hemipterus* and *L. balteatus* during 2016/17 (before incubation) survey at Amhara region on the cob damaged by mold only. On the other hand, compared with *C. dimidiatus* and *T. stercorea*, except at Amhara region during 2016/17 survey the mean number of *Brachypeplus* sp., per cob damaged by insect and mold simultaneously and mold only ((before incubation) was found to be significantly ($p>0.05$) higher. No significant ($p>0.05$) difference was observed during 2016/17 survey at Amhara region before incubation between the mean number of *Brachypeplus* sp., and *C. dimidiatus* per cob damaged by insect only, mold only and insect and mold simultaneously (Table 4.3 and 4.4).

Table 4.3: Mean number of selected sap beetles and other mold feeder insects per maize cobs damage types (Oromia and Amhara region before incubation).

2015/16 (Oromia)					
Maize cob damaged type	Mean number of insect per/cob±Standard error				
	C. hemipterus	*C. dimidiatus*	*T. stercorea*	*L. balteatus*	*Brachypeplus* sp.
Insect damaged	3.20±0.21^{bC}	1.47±0.14^{bA}	1.10±0.16^{bA}	2.37±0.15^{bB}	1.27±0.12^{bA}
Insect damaged + Mold damaged	5.13±0.26^{cD}	1.40±0.20^{bA}	1.20±0.18^{bA}	3.67±0.19^{cC}	2.23±0.14^{dB}
Mold damaged	3.20±0.18^{bD}	1.00±0.13^{bA}	1.13±0.13^{bAB}	2.20±0.11^{bC}	1.67±0.11^{cBC}
Undamaged	0.00±0.00^{aA}	0.03±0.03^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}
F -value	125.92,	22.46,	17.63,	133.19,	78.39,
P-value	0.000	0.000	0.000	0.000	0.000
2016/17 (Amhara)					
Maize cob damaged type	Mean number of insect per/cob±Standard error				
	C. hemipterus	*C. dimidiatus*	*T. stercorea*	*L. balteatus*	*Brachypeplus* sp.
Insect damaged	2.47±0.68^{bD}	1.13± 0.57^{bB}	0.83±0.37^{bA}	1.87±0.63^{bC}	1.33±0.48^{bB}
Insect damaged + Mold damaged	4.73± 1.01^{cD}	1. 23±0.73^{bcAB}	0.90±0.48^{bA}	2.97±0.81^{cC}	1.60±0.93^{bB}
Mold damaged	2.23±1.07^{bC}	1.47±0.51^{cB}	0.90±0.54^{bA}	1.83±0.15^{bBC}	1.68±1.15^{bB}
Undamaged	0.60±0.49^{aB}	. 0.00±0.00^{aA}	0.00±0.00^{aA}	0.57±0.50^{aB}	0.00±0.00^{aA}
F -value	119.87,	46.08,	34.43,	44.09,	30.02,
P-value	0.000	0.000	0.000	0.000	0.000

Means followed by the same lower case letter within a column and upper case letter with in row are not significantly different at 5% level (Tukey Studentized Range Test).

Table 4.4: Mean number of selected sap beetles and other mold feeder insects per maize cobs damage types (Oromia and Amhara region after incubation).

2015/16 (Oromia)					
Maize cob damaged type	Mean number of insect per/cob±Standard error				
	C.hemipterus	*C.dimidiatus*	*T.stercorea*	*L.balteatus*	*Brachypeplus* sp.
Insect damaged	2.73±0.78^{bC}	1.10±0.76^{bA}	0.87±0.86^{bA}	2.10±0.55^{bB}	1.20±0.66^{bA}
Insect damaged + Mold damaged	3.87±1.20^{cD}	1.40±1.07^{bA}	1.20±0.96^{bA}	3.17±0.75^{cC}	2.13±0.78^{dB}
Mold damaged	2.80±0.61^{bD}	1.00±0.74^{bA}	1.13±0.73^{bA}	2.20±0.61^{bC}	1.63±0.61^{cB}
Undamaged	0.00±0.00^{aA}	0.03±0.18^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}
F -value	135.17,	18.22,	173.68,	70.11,	78.39,
P-value	0.000	0.000	0.000	0.000	0.000
2016/17 (Amhara)					
Maize cob damaged type	Mean number of insect per/cob±Standard error				
	C.hemipterus	*C.dimidiatus*	*T.stercorea*	*L.balteatus*	*Brachypeplus* sp.
Insect damaged	3.17±1.49^{bC}	1.27±0.78^{bA}	1.20±0.76^{bA}	2.50±0.97^{bB}	1.70±0.60^{bA}
Insect damaged + Mold damaged	5.47±1.81^{cD}	2.13±0.94^{cAB}	1.53±0.77^{bcA}	4.20±1.40^{cC}	2.63±0.61^{cB}
Mold damaged	3.36±1.03^{bC}	1.80±0.89^{cA}	1.80±0.71^{cA}	2.67±0.96^{bB}	2.53±0.82^{cB}
Undamaged	0.60±0.50^{aAC}	0.26±0.49^{aB}	0.03±0.18^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}
F -value	69.89,	32.15,	42.16,	94.79,	126.85,
P-value	0.000	0.000	0.000	0.000	0.000

Means followed by the same lower case letter within a column and upper case letter with in row are not significantly different at 5% level (Tukey Studentized Range Test).

4.4 Discussions

The results obtained from the two years surveys showed the occurrence of 19 species of mold feeder insects on maize cob damaged by insects and mold *viz., Carpophilus* sp. *Brachypeplus* sp., *Carpophilus dimidiatus* (F.), *Carpophilus hemipterus* (L.), *Typhae stercorea* (L.), *Litargus balteatus* LeConte, *Cartodere (Aridius) nodifer, Corticaria* sp*., Cryptophagus* spp (3), *Rhizopghagus* sp, *Scaphisoma* sp, *Hypogastrura* sp1and *Entomobrya spp* (5). In agreement with this, Rodriguez-Del-Bosque, *et al*., 2007 in their work on "Effect of ear wounding and cultural practice on abundance of *Carpophilus freemani* (Coleopteran: Nitidulidae) and other Microcoleopteran in Maize in Northeastern Mexico" recorded fourteen species of arthropods (Nitidulidae-four species, Cucujidae, Curculionidae, Mycetophagidae, Tenebrionidae each of them with two species and Anthribidae and Bostrichidae each of them with one species. Tamaki *et al*. (1982) and Attwater and Busch (1983) also reported that sap beetles as "secondary invaders of ears damaged by insects such as the corn earworm *Heliothis zea* (Boddie) or the European corn borer *Ostrinia nubilalis* (Hubner)". Magaro *et al*. (2006) reported fungus feeders such as Cryptophagidae, Lathridiidae and Mycetophagidae families in association with stored product. A pre-harvest commodity damaged by mold is possibly the main source for infestation of these mold feeder families in stored products. Carvalho *et al*. (2004) observed a large number of fungus-feeders insect in paddy samples in association with *Aspergillus niger* van Tieghem, *A. flavus*, *A. candidus* and *Penicillium islandicum* (Sopp). The original source of fungi in stored product is field. Therefore, harvesting mold damaged maize cob together with undamaged maize cob may be used as a source of infestation of fungus feeder arthropods in stored maize.

More than five types of corn ear rots were also identified from the present study namely: *Giberella* ear rots*, Fusarium* ear rot*, Aspergillus* ear rot*, Penicillium* ear rot*, Stenocarpella* ear rot and other unidentified ear rots. *Fusarium* ear rot, *Giberella* ear rot and *Diplodia* ear rot were identified from Zeway, Arsi Negele, Hawassa, Areka, Billito/Siraro, Ejaji, Shallo and Wondotika area, Ethiopia (Tewabech *et al*., 2001). Similarly, Mukanga *et al*. (2010) reported eight different types of ear rot diseases in association with pre-harvest maize cob in South and central Zambia. From the present study significantly high incidence of *Gibberella* and *Fusarium* ear rots ($p<0.05$) were observed, followed by *Penicillium* and *Stenocarpella* ear rot. Xiang *et al*. (2010)

reported *Fusarium* ear rot and *Gibberella* ear rot among the most predominant types of maize ear rot disease in the world. Mukanga *et al*. (2010) also found high incidence of *Fusarium* ear rot in association with pre-harvest maize cob in South and Central Zambia. The result from the present study is also in agreement with report from tropical regions of Africa (MacDonald and Chapman, 1997; Kapindu *et al*., 1999; Bigirwa *et al*., 2006). Apart from these, Marin *et al*. (1995) reported that the species of the genus *Aspergillus* mainly that in section *Flavi, Nigri* and *Curcumdati*, species of *Penicillium* spp., and species of *Fusarium* such as *F. verticilliodes*, *F. proliferatum* and *F. graminearum* were the major fungi colonizing maize and maize kernel.

The present studies also showed that, in both selected regions (years) and condition the mean number of sap beetles such as *C. hemipterus*, *C. dimidiatus* and *Brachypeplus* sp., and Mycetophagids such as *T. stercorea* and *L. balteatus* per cobs were significantly higher on maize damaged by insects only, mold only and insect and mold simultaneously than undamaged cobs. Sap beetles and other mold feeder arthropods were usually attracted to maize cob damaged by insects and molds. Sétamou (1996) reported the infestation by *S. zeamais* and *Carpophilus* sp., were followed by *M nigrivenella* damage on maize cobs. This finding was also supported by McMillan (1987) who observed that Nitidulid beetles such as *Carpophilus* spp., were attracted to damaged maize cobs. The possible reason behind this was volatile compounds produced by *F. verticilloides* that infected following the area of insect damaged cob (Bartelt and Wicklow, 1999). *C. dimidiatus* was attracted to *Fusarium* infected grain and feeding on the surface of moldy grain (Dobie *et al*., 1991). The field experiment on "The effect of endophytic *F. verticilloides* on infestation of two maize varieties by Lepidopterous stem borers and coleopteran grain feeder" also showed higher infestation of Lepidoptera on *F. verticilloides* infected plants than the control (Schulthess *et al*, 2002). Cardwell *et al*. (2000) also observed that increased populations of both Lepidopteran stem borers and Coleopteran beetles' on maize infected with *F. verticillioides* compared to uninfected plots. Moreover, Dunkel (1988) noted that, some fungi attract post-harvest insect pests and are favorable for their development.

In this study, in most case *C. hemipterus* was found to be the most abundant and followed by *L. balteatus*. Moreover, except at Amhara region during 2016/17 survey, *Brachypeplus* sp., was more abundant than *C. dimidiatus* and *T. stercorea* on the cob damaged by insect and mold simultaneously and mold only. In Israel, *C. hemipterus* is among the ten species of Carpophilinae

subfamily which are pests of economic importance in date plantations (Blumberg *et al*, 1993; Blumberg, 2008). In Libya, it is considered among the most abundant Nitidulids in date palm groves (Najla *et al*, 2005). Apart from these, in Egypt 89 % of untreated dates were infested by *C. hemipterus*. *C. hemipterus* preferred moist zone for their reproduction where the growth of mold was very high (Amos and Waterhouse, 1967). This was the first report showed that the association between *C. hemipterus* and pre-harvest maize cob damaged by insect and mold in Ethiopia. Dowd (1998) reported that *C. hemipterus* was among the most important insects in promoting mycotoxin problem in the United States maize.

Litargus balteatus was also another mold feeder insect found commonly in association with pre-harvest maize cob damaged by insect and mold. It was among the most important mold feeder insect on which (on the external parts and in the excrement) the conidia and ascospores of *Ceretocystis alba* were observed (Devay *et al*., 1968). This mold feeder insect was identified from stored rice where *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. and *Trichothecium* sp. were isolated (Magaro, 2004). Field infestation was possibly the main source for infestation of this insect under storage condition. .*Brachypeplus* sp., was also significantly attracted to pre-harvest maize cob damaged by insect and mold simultaneously and mold only. Heath *et al*. (2009) in their study "Insect Associates of *Ceratocystis albifundus* and patterns of association in a Native Savanna Ecosystem in South Africa" identified *Brachypeplus* sp., as one of the most important mold feeder insect from which *C. albifundus* was isolated. In Ethiopia, both *L. balteatus* and *Brachypeplus* sp., were recorded as "minor" mold feeder insects in association with stored product (Walker. and Boxall, 1974). However, previously both of them were not identified in pre-harvest maize cob damaged by insect and mold in Ethiopia.

4.5 Conclusions

A total of nineteen mold feeder insects in 8 families were identified in association with insect and mold damaged maize cobs. According to the present study, the mean number of selected sap beetles and other mold feeder arthropods per cobs were significantly higher on insect and mold damaged maize cobs than undamaged maize cobs. Moreover, in this study, in most cases *C. hemipterus* was found to be the most abundant and followed by *L. balteatus* in association with insect and mold damaged cobs. More than five types of corn ear rots were also identified from the present studies which include *Giberella* ear rot*, Fusarium* ear rot*, Aspergillus* ear rot*, Penicillium* ear rot*, Stenocarpella* ear rot and other unidentified ear rot. Significantly high incidence of *Gibberella and Fusarium* ear rots ($p<0.05$) were observed in association with these pre-harvest mold damaged cobs. Generally, this study confirmed that insect and mold damaged pre-harvest maize cob were associated with mold feeder arthropods and potentially mycotoxin producing fungi in stored maize grain ecosystem.

Harvesting maize cob damaged by insect and mold may led to an increase in risk for infestation of mold feeder insect, infection of potentially toxigenic fungi and mycotoxin contamination in stored grain ecosystem. Therefore, the present study demonstrated the role of sanitation, in integrated pest management in stored grain ecosystem. The present study also showed that, mold feeder insect including sap beetles may have a potential to obtain mycotoxin producing fungal spore on the course of their transportation from the field to stored grain ecosystem.

Chapter-5

Development of Detection Techniques to Sap Beetles and Staphylinids in Stored Maize Grain in Ethiopia

5.1 Introduction

Sap beetles are considered as insect pests of date palms, figs, stone fruits, strawberries, maize and many small fruits and vegetables. Severe damage to maize by *Glischrochilus* sp., was reported. Moreover, these beetles have been implicated as a vector of potentially toxigenic fungi which could produce mycotoxin. Windels *et al*., (1976), reported the roles of *Glischrochilus quadrisignatus* (Say) in the epidemiology of *Giberella* ear rot. According to Windels *et al*. (1976), the same *Fusarium* spp., were isolated from buried ears, sap beetles larva and adults, ears in the field, and harvested ears. Wicklow *et al*., (1998) found the involvement of *Carpophilus lugubris* (Nitulides beetles) in vectoring *A. flavus* fungal spores and aflatoxin contamination. Apart from these, sap beetle infestation may speed up spoilage of agricultural commodities by enhancing natural fermentation of commodities (Lussenhop and Wicklow, 1990 and Dowd, 1991, 1995). *Brachypeples* spp., *C. hemipterus*, *C. dimidiatus*, *Carpophillus ligneous* (Murray), *Carpophillus obsoletus* (Erichson) are the most common sap beetles in association with stored agricultural commodities.

Staphylinids are among the most important natural enemies associated with sap beetles. Miller and Williams (1983) noted that Adults and immature stages of the Staphylinid predator *Atheta coriaria* (Kraatz) consumed eggs and early instars of several nitidulids, including *Carpophillus hemipterus*, *Carpophillus lugubris*, *Carpophillus freeman*, *Urophorous humeralis*. Several authors reported Staphylinids in association with stored products (Walker and Boxall, 1974; Pellitteri and Boush, 1983; Hagstrum and Subramanyam, 2009). In Ethiopia, *Brachypeples* spp., and *C. hemipterus* were reported as a "minor" and "uncommon" sap beetles, respectively while *Paedrus duplex* (Eppels) was reported as a "common" staphylinidae in association with stored products.

Sap beetles are attracted by volatiles dispersed from fermented and decomposed fruit juice and plant materials (Bartelt *et al*., 1990, 1992, 1993; Rondon *et al*, 2004; Hossain *et al*., 2012). However, this information was limited only to field condition. No information was available on

how to detect sap beetles and its associated natural enemies Staphylindids in stored grain ecosystem. It is very important to develop detection methods for these insects in order to know the association of these insects, mycotoxin producing fungi and mycotoxin contamination, and to implement an integrated pest management strategy against sap beetles in stored grain ecosystem. Accordingly, the present study aimed,

- To evaluate, the potential of fermented cob, fermented banana, fermented pineapple juice and a mixture of fermented banana and pineapple juice to detect sap beetles and Staphylinids in stored maize.
- To evaluate the effects of maize cob fermentation on detection of sap beetles and Staphylinids in stored maize grain.

5.2 Materials and methods

Study site

The study was conducted at Addis Ababa University in the Insectary of Insect Science Stream in 2016/17 and 2017/18. The Insectary was adjusted to temperature ranging from 22-25 °C and relative humidity 65%-70%.

Experimental materials, treatments and design

This experiment was designed to develop detection methods for sap beetles and saphylindis associated with stored maize in Ethiopia. Detection methods were developed using fermented cobs and fermented fruit juice. Fermented cobs were prepared by soaking the cob in one liter of water for three consecutive days every week for one month, each day for ten seconds. Fermented banana and pineapple juice were prepared by using 20 g yeast, 4500 mL of fruit juice which was composed of one part fruit juice and two parts water (1500 mL fruit juice + 3000 mL water). Finally, three weevil damaged cobs were treated with prepared fermented fruit juice for three consecutive days every week for one month (1000 mL of fermented fruit juice was used for each week) and placed in 5 liter plastic container and covered with fine mesh. At the same time three fermented cobs were placed in the same type of plastic container and covered with fine mesh. The treatments were arranged as FC= for fermented cobs, FB= for cob treated with fermented banana juice, FP= for cob treated with fermented pineapple juice, FBFP= for cob treated with equal mixture of fermented banana and pineapple juice and C= control sample without fermented cob and fruit juice treatment. Each treatment was replicated ten (10) times (5) times in the first year (2016/17) (5) times in the second year (2017/18) and inspected weekly for one month by treating the cobs with newly prepared fruit juice during each inspection time. Similarly treatments were arranged as FC= for fermented maize cobs and UFC= for unfermented maize cobs with replication of forty (40) times. Completely randomized design was used for these arrangements.

Statistical analysis

The data obtained on number of sap beetles and Staphylinids per trap for *C. hemipterus*, *Brachypeplus* spp., and *Staphylinids* per trap were analyzed using the SPSS computer software (1989). All data were log transformed to stabilize variances. One-way analysis of variance

(ANOVA) was done to compare the performance of each developed detection methods, which were laid down in a completely randomized design (CRD). On the other hand, Kruskal-Wallis H-test was conducted to compare detected *C. hemipterus*, *Brachypeplus* spp., and *Staphylinids* per trap in relation to weeks. Comparisons of mean number of detected *Carpophillus hemipterus*, *Brachypeplus* spp., and *Staphylinids* per trap were done using Tukey's Studentized range tests at 5% level of significance. Comparison of mean number of detected *C. hemipterus*, *Brachypeplus* spp., and *staphylinids* per trap in relation to weeks were done using independent paired-wise tests (Appendix 4.1). The difference in mean number of detected sap beetles and *Staphylinids* between fermented and unfermented maize cobs was done using Mann-Whitney U-tests. The frequency and relative abundance of each sap beetles and staphylinids per trap was done using the following formula (Marasas, 1988).

Frequency= (Number of trap with sap beetles and staphylinids genera or species ÷ total number of trap) ×100

Relative abundance= (Number of individual sap beetles and staphylinids genera or species ÷ total number of genera or species) ×100

5.3 Results

Species composition

More than four species were detected from each of sap beetles and Staphylinids from fermented maize cob and maize cob treated with fruit juice. *Carpophillus hemipterus* with 100% frequency of occurrence and 42.11% relative abundance and *Brachypeplus* spp., with 100% frequency of occurrence and 34.75% relative abundance were found to be the most frequently and abundantly detected sap beetles during the experiment. Other *Carpophillus* spp., were also detected in frequency of occurrence and relative abundance of 82.5% and 22.67%, respectively. *Phenolia picta* (MacLeay) was found to be the least frequently and abundantly detected sap beetles with frequency of occurrence 12.5% and relative abundance of 0.47%. On the other hand, unidentified staphylinids species were detected in frequency of 100% and relative abundance of 58.80%. Among the four identified genera of staphylinids, *Atheta* spp., were found to be the most frequently and abundantly detected genera with frequency of 92.5% and relative abundance of 15.64% and followed by *Philonthus* spp with frequency occurrence of 90% and relative abundance of 10.53%, *Aleochara* spp., with frequency of 80% and relative abundance of 10.02% and *Anolytus* spp., frequency occurrence of 20% and relative abundance of 5.51% (Table 5.1).

Table 5.1: Frequency and abundance of sap beetles and staphylinids detected using fermented cob and fruit juice.

Sap beetles	Number of traps with sap beetles (N=40)	Percentage of traps with sap beetles (frequency= %)	Number of individual	Relative abundance (%)
Phenolia picta (MacLeay)	5	12.5	7	0.47
Carpophilus spp.	33	82.5	336	22.67
Brachypeplus spp.	40	100	515	34.75
Carpophilus hemipterus (L.)	40	100	624	42.11
Total			1482	
Staphylinids	Number of traps with staphylinids (N=40)	Percentage of traps with staphylinids (frequency= %)	Number of individual	Relative abundance (%)
Philonthus spp	36	90	417	10.53
Aleochara spp.	32	80	397	10.02

Anolytus spp.	8	20	198	5.51
Atheta spp.	37	92.5	619	15.64
others	40	100	2328	58.80
Total			3959	

Comparison of detection methods

Carpophilus hemipterus

In all cases (week1-week4), fermented cob and fermented banana mixed with fermented pineapple juice detected significantly more number of *C. hemipterus* than fermented pineapple juice and fermented banana ($p<0.001$). Fermented banana detected significantly more number of *C. hemipterus* than fermented pineapple juice ($p<0.001$) at week1 and week2. No significant difference was observed between the number of *C. hemipterus* detected by fermented pineapple juice and fermented banana at week3 and week4 ($p>0.05$). Overall, significantly more number of *C. hemipterus* was captured by using fermented cob and fermented banana juice mixed with fermented pineapple juice than fermented banana and fermented pineapple juice ($p<0.001$) and fermented banana than fermented pineapple juice ($p<0.001$) (Table 5.2).

Brachypeplus spp., and *Staphylinids*

At week3 and week4, fermented cob and fermented banana mixed with fermented pineapple juice detected significantly more numbers of *Brachypeplus* spp than fermented pineapple juice and fermented banana ($p<0.001$). At week2, fermented cob detected significantly more number of *Brachypeplus* spp than fermented pineapple juice, fermented banana and fermented banana mixed with fermented pineapple juice ($p<0.001$). At week1, no significant difference was observed among detection methods ($p>0.05$). Overall, more *Brachypeplus* spp., was captured by using fermented cob and fermented banana juice mixed with fermented pineapple juice than fermented banana and fermented pineapple juice ($p<0.001$) (Table 5.3). However, in all case, no significant difference was observed among the detection methods during the detection of Staphylinids ($p>0.05$) (Table 5.4).

Table 5.2: Mean number of *Carpophilus hemipterus* per trap captured by using (FC= fermented cob, FB=fermented banana, FPA=fermented pineapple juice and FB+FPA=fermented banana juice mixed with fermented pineapple juice).

Detection methods	**Weeks of detection**				
	Week-1	Week-2	Week-3	Week-4	Total
FC	9.00 ± 0.45^{b}	5.40 ± 0.40^{c}	3.00 ± 0.33^{c}	2.00 ± 0.26^{b}	19.40 ± 0.92^{c}
FB	10.20 ± 0.36^{b}	3.30 ± 0.37^{b}	0.50 ± 0.17^{a}	0.60 ± 0.22^{a}	14.60 ± 0.79^{b}
FPA	6.50 ± 0.48^{a}	1.20 ± 0.20^{a}	1.00 ± 0.26^{ab}	0.80 ± 0.20^{a}	9.50 ± 0.86^{a}
FB+FPA	10.40 ± 0.56^{b}	4.60 ± 0.40^{bc}	1.80 ± 0.20^{b}	2.10 ± 0.23^{b}	18.90 ± 0.73^{c}
F	14.75	27.20	19.37	11.73	30.72
P	0.000	0.000	0.000	0.000	0.000

Means followed by the same letter (s) in a column are not significantly different at $p<0.001$ (Tukey Studentized Range Test).

Table 5.3: Mean number of *Brachypeplus* spp., per trap captured by using (FC= fermented cob, FB=fermented banana, FPA=fermented pineapple juice and FB+FPA=fermented banana juice mixed with fermented pineapple juice).

Detection methods	**Weeks of detection**				
	Week-1	Week-2	Week-3	Week-4	Total
FC	1.8 ± 0.25^{a}	3.5 ± 0.27^{b}	7.5 ± 0.43^{b}	3.9 ± 0.31^{ab}	16.70 ± 0.47^{b}
FB	1.4 ± 0.22^{a}	0.70 ± 0.15^{a}	5.4 ± 0.31^{a}	3.2 ± 0.33^{a}	10.70 ± 0.60^{a}
FPA	1.6 ± 0.27^{a}	0.50 ± 0.17^{a}	4.2 ± 0.33^{a}	2.7 ± 0.58^{a}	9.00 ± 0.56^{a}
FB+FPA	1.2 ± 0.13^{a}	0.60 ± 0.16^{a}	8.1 ± 0.38^{b}	5.2 ± 0.42^{b}	15.10 ± 0.35^{b}
F	1.333	56.24	25.06	6.60	51.80
P	0.279^{ns}	0.000	0.000	0.000	0.000

Means followed by the same letter (s) in a column are not significantly different at $p<0.001$ (Tukey Studentized Range Test).

^{ns}= Non significant

Table 5.4: Mean number of *Staphylinids* per trap captured by using (FC= fermented cob, FB=fermented banana, FPA=fermented pineapple juice and FB+FPA=fermented banana juice mixed with fermented pineapple juice).

Detection methods	**Weeks of detection**				
	Week-1	Week-2	Week-3	Week-4	Total
FC	17.60±0.77	16.10±0.87	25.40±0.75	35.80±0.80	94.90±1.86
FB	18.90±1.03	16.20±0.65	26.60±0.78	34.20±0.92	95.90±1.77
FPA	18.40±0.52	17.90±0.78	28.00±0.75	36.90±0.79	101.20±2.00
FB+FPA	17.70±0.80	17.40±0.83	26.20±0.65	35.20±0.83	96.50±1.42
F	0.59	1.28	2.21	1.83	2.48
P	0.629^{ns}	0.297^{ns}	0.103^{ns}	0.160^{ns}	0.077^{ns}

Tukey Studentized Range Test **(^{ns}= Non significant)**

Comparison of detection time

The detection methods showed significant variation among the weeks in the number of *Carpophilus hemipterus* (H (3) =106.78, p=0.000), *Brachypeplus* spp. (H (3) =104.78, p=0.000) and staphylinds (H (3) =134.39, p=0.000) per trap captured. Dunn's pair-wise tests (Appendix1) showed that more *carpophilus hemipterus* was captured at week-1 than week-2 ($p<0.001$), week-3 ($p<0.001$) and week-4 ($p<0.001$); at week-2 than week-3 ($p<0.05$) and week-4 ($p<0.001$). However, no significant difference was observed between week-3 and week-4 ($p>0.05$). Overall, more *Carpophillus hemipterus* was captured in the first two weeks. Differently, more number of *Brachypeplus* spp and staphylinds were captured at week-4 than week-3 ($p<0.05$), week-2 ($p<0.001$) and week-1 ($p<0.001$) and at week-3 than week1 ($p<0.001$) and week-2 ($p<0.001$). Unlike *C. hemipterus* in the case of *Brachypeplus* spp., and Staphylinds more insects were captured at week-3 and at week-4 (Table 5.5).

Table-5.5: P values for comparison of *Carpophilus hemipterus*, *Brachypeplus* spp., and *Staphylinds* captured during the four weeks inspection time.

Variable	*Carpophilus hemipterus*			
	P values for multiple comparison (two-sided comparison) kruskal-wallis test			
	Week-1	Week-2	Week-3	Week-4
Week-1		0.000***	0.000***	0.000***
Week-2	0.000***		0.002***	0.000***
Week-3	0.000***	0.002***		1.000^{NS}
Week-4	0.000***	0.000***	1.000^{NS}	
Brachypeplus spp.				
Variable	P values for multiple comparison (two-sided comparison) kruskal-wallis test			
	Week-1	Week-2	Week-3	Week-4
Week-1		1.000^{NS}	0.000***	0.000***
Week-2	1.000^{NS}		0.000***	0.000***
Week-3	0.000***	0.000***		0.004***
Week-4	0.000***	0.000***	0.004***	
Staphylinds				
Variable	P values for multiple comparison (two-sided comparison) kruskal-wallis test			
	Week-1	Week-2	Week-3	Week-4
Week-1		1.000^{NS}	0.000***	0.000***
Week-2	1.000^{NS}		0.000***	0.000***
Week-3	0.000***	0.000***		0.001***
Week-4	0.000***	0.000***	0.001***	

Pairs with $p<0.05$ was considered statistically significant (***) while $p>0.05$ was considered statistically non-significant (NS) (Dunn's pair-wise tests).

Comparison between fermented and unfermented maize cobs

Mann–Whitney (U-test) indicated that mean rank of sap beetles (U=4, z=-7.75, p<0.05, r= -0.87) and Staphylinids (U=1, z=-7.78, p<0.05, r= -0.87) per cob were found to be significantly higher on fermented cobs than unfermented cobs (Table 5.6) indicating that more number of these insects were detected by fermented cob (attracted to fermented cobs).

Table 5.6 Mean and sum rank of fermented and unfermented maize cobs

	Status of cob	N	Mean Rank	Sum Rank
Sap beetles	Unfermented cobs	40	20.60	824.00
	Fermented cobs	40	60.40	2416.00
	Total	80		
Staphylinids	Unfermented cobs	40	20.52	821.00
	Fermented cobs	40	60.48	2419.00
	Total	80		

Status of maize cobs with **high Mean and Sum Rank** found to be detecting significantly more number of Sap beetles and Staphylinids than status of maize cobs with **low Mean and Sum Rank**.

5.4 Discussions

The present study showed that more than four species of Sap beetles and Staphylinids were detected using fermented cob, fermented banana juice, fermented pine apple juice and fermented pine apple juice mixed with fermented banana juice. More numbers of Sap beetles and Staphylinids were detected using fermented maize cobs than unfermented maize cobs. Fermented and decomposed materials have long been known to be attractive to Nitidulid beetles (Rondon *et al*, 2004). Parson (1943) and Hayashi (1978) found sap beetles in association with fungi, decaying and fermenting plant tissues. Several authors have been advocated fermenting food baits and plant material to detect sap beetles (Smilanick *et al*., 1978; Bartelt *et al*., 1994; Bartelt and Weisleder, 1996; James *et al*., 1998; Hossain *et al*., 2012). The present study was also supported by Miller and Williams (1983) who reported adults and immature stages of staphylinid predator *Atheta coriaria* in association with eggs and early instars of several Nitidulids, including *C. hemipterus*, *C. lugubris*, *C. freemani* , *U. humeralis* and *Epuraea luteola*. This predator produced a generation in 13 days at 26.7 °C and consuming 20 *C. hemipterus* eggs per day.

Overall, significantly more number of *Carpophilus hemipterus* was captured by using fermented cob and fermented banana juice mixed with fermented pineapple juice($F=30.72$; $p<0.001$) than fermented banana and fermented pineapple juice, and fermented banana ($F=30.72$; $p<0.001$) than fermented pineapple juice. Moreover, more *Brachypeplus* spp., was captured by using fermented cob and fermented banana juice mixed with fermented pineapple juice ($F=51.80$; $p<0.001$) than fermented banana and fermented pineapple juice. This variation in attractiveness of fermented cob and fermented fruit juice to *C. hemipterus* and *Brachypeplus* spp., is possibly due to variation in the amount and kind of volatile compound produced during fermentation and kind of yeast species associated with fermented plant materials.

Variation in the amount and kind of volatile compound produced during fermentation can have profound effects on the population of sap beetles attracted to fermented plant material. A screening of microbial cultures on corn medium with glucose for their attractiveness to sap beetles such as *Carpophilus humeralis* showed that a wide range of attractiveness among microbial cultures, ranging from none to very attractive. The ability of yeast species to assimilate and/or ferment the carbohydrates present on sweet corn determined volatile production and

attractiveness to sap beetles. For instance, *Candida Shehatae* (ferments glucose and maltose) considerably produced more attractive volatiles than *Candida guilliermondii,* which only ferments glucose. Moreover, several authors in Australia showed that the native species of sap beetles were readily attracted to fermenting peach and apple juice, which have been used in traps for population monitoring (James *et al*., 1998; Hossain *et al*., 1999; Mansfield and Hossain, 2004).

Generally, more *C. hemipterus* was captured in the first two weeks. This is possibly due to reduction of volatile compound over time which attracts *Carpophillus hemipterus* in the last two weeks. Fornari *et al*. (2013) on their study" Evaluation of damage, food attractants and population dynamics of strawberry sap beetle" showed that strawberry fruits lost its attractiveness, gradually diminishing the number of trapped insects, compared with 14 days after exposure. They observed that there was no capture of adult beetles by the 28-day-old juice. Differently, more *Brachypeplus* spp., and Staphylinds were captured at week-4 than week-3 ($p<0.05$), week-2 ($p<0.001$) and week-1 ($p<0.001$) and at week-3 than week1 ($p<0.001$) and week-2 ($p<0.001$). Unlike *C. hemipterus* in the case of *Brachypeplus* spp., and Staphylinds, more insects were captured at week-3 and week-4. This is possibly due to repeated treatment of maize cob with water and fermented fruit juice may lead to decaying of maize cob or growth of mold on maize cob which could attract *Brachypeplus* spp. In agreement with this although the species of sap beetle was not specified, Parson (1943) and Hayashi (1978) have been found sap beetles in association with fungi, decaying and fermenting plant tissues. Cline *et al*. (2013) and Cline *et al*. (2014) also noted that species in the genus *Brachypeplus* are known to occur in association with palm vegetative or reproductive structures, specifically within the subcortical confines of dead and/or decaying plants. Moreover, they observed that the larva of *Brachypeplus glaber* LeConte specialized on *Fusarium solani* and *Penicillium* species while the adult on *Fusarium oxysoproum, F. verticillioides*, and *Cladosporium*. More Staphylinds were also captured at week-3 and week-4 in association with *Brachypeplus* spp.

5.5 Conclusions

The result obtained from this study confirmed that among the developed detection methods, fermented cobs and fruit juice mixture were more effective than individual fermented fruit juice in detecting sap beetles and associated Staphylinds in stored maze in Ethiopia. More than four species were detected from each of sap beetles and Staphylinids from fermented maize cob and maize cob treated with fruit juice. The present study also showed that *C. hemipterus* was more associated with fermentation of maize cob while *Brachypeplus* spp., and Staphylinds were more associated with decomposition of maize cob and growth of fungi on maize cob. Moreover, the present study showed the correct time of fermenting maize cob and maize cob treated with fermented fruit juice to detect sap beetles (*C. hemipterus* and *Brachypeplus* spp.) and Staphylinids. It is very important to detect sap beetles and associated staphylinids in order to implement an integrated pest management program. The present study is also very important in order to predict the condition of stored grain ecosystem. The flying activities of *C. hemipterus*, *Brachypeplus* spp., and Staphylinds around the storage structure might be showed fermented and decomposed maize cob or grain some where in the storage. Thus, the infestation of stored grain by *C. hemipterus* and *Brachypeplus* spp., might be an indication of poor condition of stored grain ecosystem. To my knowledge, detection of sap beetles and associated staphylinids at the same time in stored grain ecosystem have not previously been documented. Therefore, this study is valuable for providing baseline information on how to detect sap beetles and their associated staphylinids in stored grain ecosystem.

Chapter-6

Effects of arthropods on potentially toxigenic fungal infection and aflatoxin contamination of stored maize grain in Ethiopia

6.1 Introduction

The infection and contamination of food and feeds by potentially toxigenic fungi and their respective mycotoxin, are a serious health and economic problem throughout the world. For instance, Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma and chronic hepatitis. Maize is among the most important cereal crops commonly infected and contaminated by potentially toxigenic fungi and mycotoxin, respectively. Arthropods play a major role in mycotoxigenic fungal infection and mycotoxin contamination of stored grain. The effects of arthropods in stored grain may include grain-damage; by their feeding activities and exposing the grain to infection of potentially toxigenic fungi (Avantaggio *et al*., 2002), by vectoring mycotoxin producing fungal spore (Lussenhop and Wicklow, 1990) and providing suitable condition for the growth of fungi through their metabolic activities (Dix and All, 1987).

Insect damage on agricultural commodities provides sites for fungi to enter the susceptible parts of seed (embryo and endosperm). Several authors noted the role of insect damage in toxigenic fungal infection and mycotoxin contamination in plant (Dowd, 1998; Setamou *et al*., 1998; Hell *et al*., 2003). According to Tuite *et al.* (1985), Barry *et al.* (1992) and Wicklow (1988) feeding by insects breaks the pericarp and rendering grain more vulnerable to invasion by storage fungi. Sétamou *et al.* (1998) reported the role of *M. nigrivenella* damage in predisposing of maize to pre- and postharvest infestations by beetle pests, *A. flavus* infections and subsequent aflatoxin contamination.

Moreover, arthropods can disseminate spores of potentially toxigenic fungi in the field and stored products (McMillian, 1987). The maize weevil, *S. zeamais* Motschulsky (Coleoptera: Curcilionidae) is among those insects which disperse *Aspergillus* spores as it has been isolated from their bodies. Lynch & Wilson (1991) in their study "Effect of harvest date on termite damage, yield, and aflatoxin contamination in groundnut in Burkina Faso " reported that insects could act as vectors by transporting fungal spores on their bodies, and contaminating grain as

they moved about. Smalley (1989) also showed the presence of *Aspergillus niger, A. glaucus, A. candidus, Penicillium islandicum, P. citrinum, Paecilomyces, Acremonium, Epicoccum, Fusarium semitectum*, and yeasts fungal spore on the body of maize weevil. However, according to Smalley (1989) the body of maize weevil is dominantly loaded with *A. flavus* and *F. moniliforme*. Apart from these Lussenhop & Wicklow (1991) in their review "Nitidulid beetles as a source of *Aspergillus flavus* infective inoculum" indicated the role of Nitidulidae (*e.g. Carpophilus lugubris* Murrey and *C. freemani*) in vectoring *A. flavus* in maize. Arthropods associated with agricultural commodities damage, toxigenic fungal infection and mycotoxin contamination in other countries were also found in Ethiopia (Abraham, 1997; Emana and Assefa, 1998). However, there is no information on the role of arthropods in toxigenic fungal infection and mycotoxin contamination in Ethiopia. Hence, the current experiment was initiated with the following objectives:

1. To determine the effect of arthropod on temperature and moisture content of stored maize grain;
2. To determine the effect of arthropod damage on potentially toxigenic fungal infection of stored maize kernels;
3. To determine the occurrence of potentially toxigenic fungi on insect bodies;
4. To determine the effect of arthropod damage on mycotoxin contamination of stored maize.

6.2 Materials and Methods

Sampling

Fifty samples of stored maize were collected during February 2017 from Jimma Zone (Omo-Nada and Kersa) from each of Malathion treated and untreated grain. Each sample consisted of 0.50 kg. Then the samples were stored for six months under laboratory conditions with temperature ranging from 23 °C to 25 °C and relative humidity ranging from 65% to 70%. After six months, 500 composite maize kernels were collected from each of insect damaged and undamaged maize kernels for final mycological analysis and 9 composite samples each of them with 100 g from each treatment for Aflatoxin analysis.

Grain temperature, moisture, arthropod infestation, and damage evaluation

Grain temperature and moisture content, arthropod infestation and arthropod and mold damaged maize kernels were measured after six month of incubation period. Grain moisture content and temperature were determined by using oven dry method and digital probe grain/food thermometer, respectively. Arthropod infestation was determined by taking 100 g of maize kernels from each representative sample of treated and untreated seeds. Two hundred maize kernels were randomly taken from each treatment (treated and untreated maize kernels) to determine percent damaged maize kernels. Maize kernels containing holes were separated from sound maize kernels and percent damaged maize kernels were calculated by the following formula:

Maize Kernels damage (%) = Number of damaged maize kernels ÷ Total number of maize kernels×100

Isolation and identification of potentially toxigenic fungi from maize

Mycological analysis was performed using direct plating method. For this, 500 maize kernels were collected randomly from each of Malathion treated (undamaged maize kernels) and untreated seeds (arthropod damaged maize kernel). Maize kernels with borer symptom (circular and irregular hole) considered as insect damaged maize kernels (Plate 6.1). All collected kernels of maize were surface sanitized in a 5% aqueous solution of sodium hypochlorite (NaOCl) for 1

min, rinsed twice with sterile distilled water, and then dried with sterile paper towels. Five hundred seeds were then plated (5 seeds per plate in 50 replicates) for each treatment on malt extract agar (MEA) with NaCl and potato- dextrose agar (PDA). The plates were incubated in the dark at 26 °C ±3 for 5 to 7 days, and the fungal colonies that developed from the kernels were identified and counted. The colonies that developed were streaked for isolation and maintained on potato-dextrose agar (PDA) slants. The isolated fungi of the genus *Penicillium* and *Aspergillus* were identified using the taxonomic key of Pitt and Hocking (1997), based on morphological and biochemical characteristics. *Penicillium* spp., are Charcterized by blue mold and branched condiophore, with Metullae and stregmata on which condia are born in chains. On the other hand, *Aspergillus* spp., differs in colour from green, dusty (*Aspergillus fumigates*), black (*Aspergillus niger*) depending on spp (Appendix 2). In Aspergillus the condiophore is straight ending in a large vesicle from which primary and secondary sterigmata/phialids arise bearing condia in chains. For the species belonging to *Fusarium* genus, in addition to the Pitt and Hocking key, the key of Samson *et al*. (1995) and the *Fusarium* genus atlas (Gerlach and Nirenberg, 1982) was also used. For conducting these identification procedures in rigorous conditions of comparison, the pure cultures were grown on potato dextrose agar (PDA) medium (Pitt and Hocking, 1997). Finally **frequency**, **relative density** and also **incidence** of fungi genera and species were estimated by the following formulas (Marasas, 1988).

- **Fr (%) = (ns/N) × 100**
- **RD (%) = (ni/Ni) × 100**
- **In (%) = (ng/Ng) ×100**

Where, Fr is frequency , ns is the number of fungi genera or species in samples, N is the number of samples, RD is Relative Density , ni is the number of isolated fungi genera or species and Ni is the number of all fungi, In, incidence, ng, number of infected grain, Ng, total number of grain.

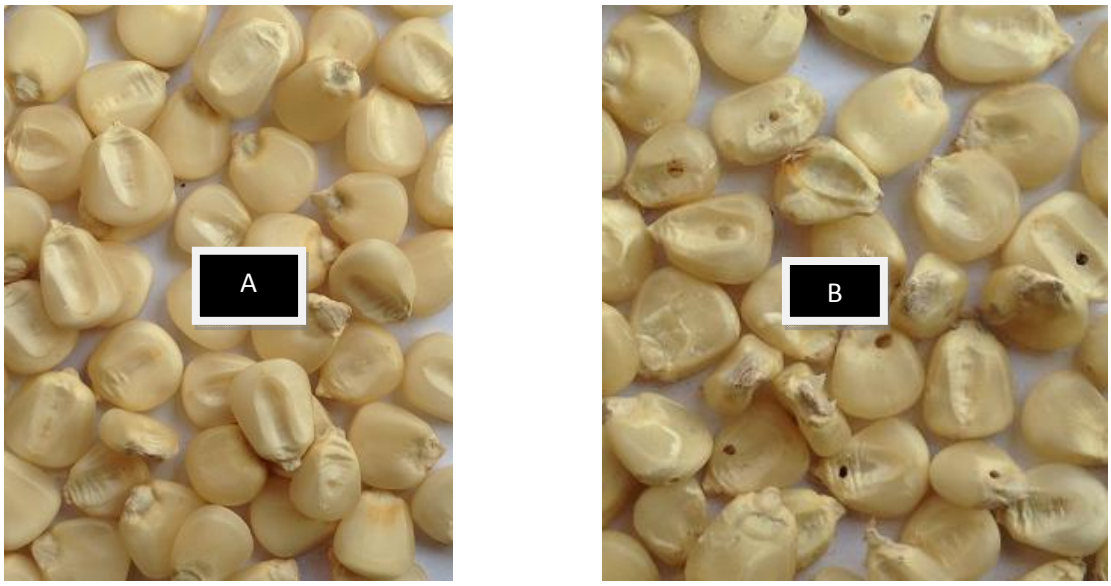


Plate 6.1: Malathion treated seeds (undamaged maize kernels) (A) and untreated seeds (insect damaged maize kernels) (B).

Isolation and identification of potentially toxigenic fungi from insect

One hundred live adult of each test insect (*Sitophilus* spp., *Lepinotus* spp., *Carpophillus hemipterus*, *Brachypeplus* spp., *Litargus balteatus*) (Plate 6.2: A-E) were sterilized in 2% sodium hypochlorite solution for 3 min and then rinsed in sterile water three times according to the method described by Sinha and Sinha (1990). The insects were placed on filter paper and allowed to dry. After drying, ten insects of each species were transferred to petri dish with five replications (10×5-Replication) containing sterilized PDA and MEA with NaCl containing amoxicillin to inhibit bacterial growth and incubated at 30 °C for 4–7 days.

A. B. C.

D. E

Plate 6.2: *Sitophilus* sp. (A), *Lepinotus* sp. (B), *Carpophilus hemipterus* (C), *Litargus balteatus* (D) *Brachypeplus* sp. (E).

Aflatoxn analysis

Sampling

A total of 50 samples were collected from each of insect damaged and undamaged maize kernel. Finally the samples were composited and 9 sub-samples (nine unit of samples) were taken from each of damaged and undamaged maize kernel samples for final Aflatoxin analysis.

Preparation of mobile phase

A mobile phase was prepared using 250 ml ACN (25%), 150 ml. MeOH (15%), 1 ml formic acid (0.1%) and 599 ml de-ionized water (60%). Each component of mobile was mixed and sonicated in HPLC reservoir for 30 min. Then, the mixture was filtered using 0.45 micro-meter filter paper in the filtration apparatus. Similarly, equal volume mixture was prepared without formic acid for standard solution preparations.

Preparation of working standard solution

Preparation of working standard solution was performed by accurately weighing 10 mg of each Aflatoxin (AFB_1, AFB_2, AFG_1 and AFG_2) and transferring in to four different 100 ml volumetric flasks and dissolved using mobile phase for the preparation of 100 ppm individual AFs., with series 23 dilutions various concentration (10 ppm and 1 ppm) of individual standards. Aflatoxin were prepared from 1 ppm standard solutions of individual Aflatoxis mixed 50 ppb/ µg/kg solution were prepared and finally 0.1, 0.5, 1, 2, 5, 8, 10 and 15 ppb/ µg/kg were prepared from 50 ppb/ µg/kg by mobile phase without formic acid for calibration curve.

Sample Preparation and extraction

The collected samples (both damaged and undamaged maize kernels) were mixed for homogenization. After homogenization the samples were grounded using grinding device. The grinder was cleaned using acetone before and after grinding in order to prevent Aflatoxin cross-contamination. About 5 gm of each maize flour sample was prepared for sample extraction and 15 mL of ACN: H_2O (84:16) was used as extraction solvent and each were blend in vortex mixture (Karl Hecht KG D97647 sand heim, Germany) at 800 rmp for 1 min and then shacked for 60 min on auto mechanical shaker. Centrifugations were done for 10 min at 3000 rpm and then quantitatively the supernatant were transferred to 50 ml round bottom flask. The extractions were performed two times with extraction solvent in order to enhance the extracts of analyte from the sample. Then, the extracts were evaporated using rotary evaporator at (40 °C, 772 mbar) and then reconstituted using 10 mLof mobile phase (60 % H_2O: 25 % ACN: 15% MeOH). The solutions was filtrated using 0.45 micro-meter syringe filter paper followed by 0.20 micro-meter syringe filter paper and the filterates were transferred in an auto sampler vial for LC-MS/MS analysis without further pre-treatment

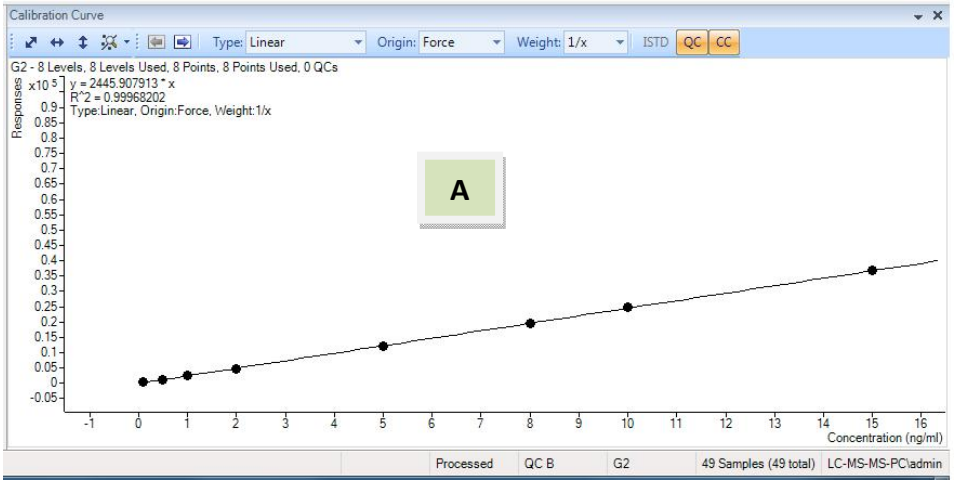
LC-MS/MS measuring conditions

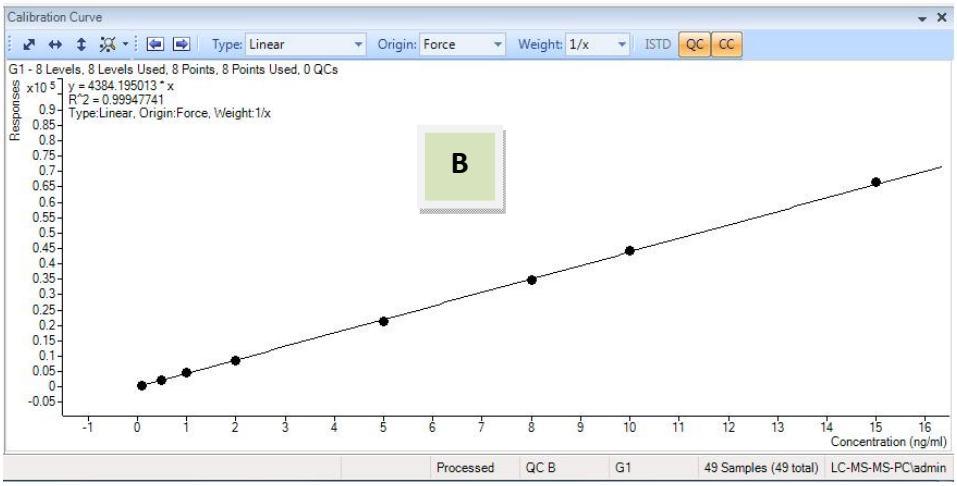
The detection and quantification of Aflatoxins were performed with LC-MS/MS by injecting 1 mL of the sample on a reversed-phase, eclipsed plus C-18 column (4.6×15 mm), 3.5 micro-meter particle size used as the stationary phase. The column was eluted using a gradient flow (0.5 mL/min) of the mobile phase (60 % H_2O: 25 % ACN: 15% MeOH containing 0.1 % formic acid) and the injection volume was 10 mL used for in LC-MS/MS analysis. The parameter of ESI operation conditions was gas flow rate 0.5 mL/min, nebulizer 40 psi and positive ion source (mass spectrometry detection was carried out on positive ionization mode, because this mode gives sharp and sensitive signals). The temperatures of column, sheath gas and dry gas were 35 °C, 350 °C and 350 °C, respectively. The LC-MS/MS parameters for analytes were cell accelerator voltage (7V), ionization mode (+ve), fragmentor (130V), dwell (100 ms). The LC system was coupled to a triple-qudrupoles mass spectrometer equipped with electro spray ionization (ESI) probe. The two most abundant product ions per analyte were chosen for quantitative and confirmation purpose. Two product ions were monitored for each Aflatoxins

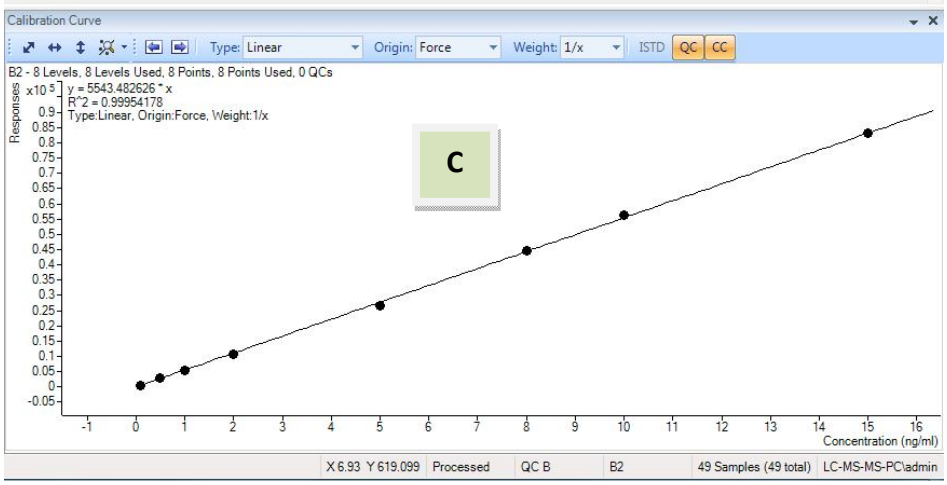
and LC-MS/MS quantitative analysis was carried out using MRM mode. The use of precursor and product ions in MS analysis allowed for sensitive detection and confirmation of all Aflatoxins.

Calibration curve

Linearity of LC-MS system was done by injecting different concentrations of reference standards. The system was calibrated by using the working solutions of Aflatoxins in the range of 0.1-15 ppb/ µg/kg in a mobile phase of ACN: H_2O: MeOH. The calibration curve, chromatogram, mass spectra and resultant mass peak were performed with origin 8 and Agilent mass hunter workstation software-data Acquisition for 6460 Series triple quadrupole. The calibration standard for each concentration was constructed peak area integration of the AFs versus the concentration of the standard. The calibration curve for Aflatoxins (B1, B2, G1 and G2) in LC-MS/MS method as shown from Figure 6.1(A-D). The analyzed working solution gives excellent values of regression coefficient for Aflatoxins. Regression coefficient (R2) values were >0.999 which was considered as evidence of an acceptance fit of the data to the regression line. Higher regression coefficient shows high correlation between responses and concentration of aflatoxins.







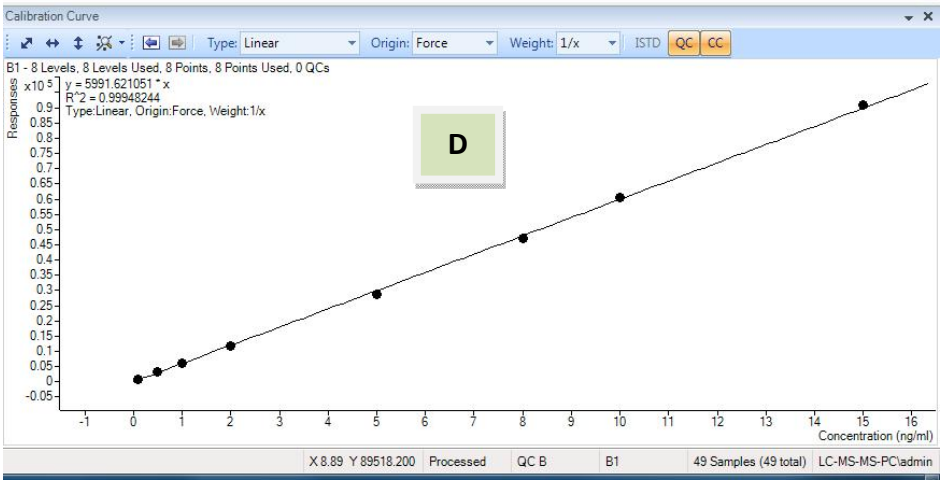


Figure 6.1(A-D): Calibration curve for Aflatoxin G2 (A), G1 (B), B2(C) and B1 (D).

Data analysis

The difference in maize grain temperature, moisture content, arthropod infestation, number of arthropods and mold damaged maize kerenels, number of colony forming units of each species, total number of colony forming units, colony diameter, number of maize kernels infected with potentially toxigenic fungi, and level of Aflatoxin in maize between insect damaged (untreated) and undamaged (treated) maize kernels were compared by independent sample T-test or Mann–Whitney *U*-test depending on the distribution of data (SPSS). P-value < 0.05 was considered statistically significant. For insects, the difference in number of infected insect, total number of colony forming units per plate, number of colony forming units of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and other species, per plate among selected insects were compared by one way of analysis of variance (ANOVA) using SPSS. Comparison of mean number of infected insects, total colony forming units, colony forming units of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and other species, among selected insect between insect damaged (untreated) and undamaged (treated) maize kernels were done using Tukey's Studentized range tests at 5% level of significance. P-value < 0.05 was considered statistically significant. Pearson correlations were computed to determine the relationships among mean number of arthropods, arthropod and mold damaged maize kernels and maize grain temperature and moisture content.

6.3 Results

Grain temperature, moisture, arthropod infestation, and damage evaluation

The mean value of moisture content [t (98) = 9.18, p=0.000] and temperature [t (98) =11.82, p=0.000] were found to be significantly higher on untreated seeds than Malathion treated seeds. Similarly, the mean number of arthropods per 100 g of grain [t (98) = 16.06, p=0.000], arthropod damaged maize kernels per 200 maize kernels [t (98) = 33.43, p=0.000] and mold damaged maize kernels per 200 maize kernels [t (98) = 0.50, p=0.000] were significantly higher on untreated seeds than Malathion treated seeds (Table 6.1). There was also statistically significant (p<.05) positive correlation between mean number of arthropods per 100 g of maize grain, mean number of arthropod and mold damaged maize kernels per 200 maize kernels and maize grain temperature and moisture content as shown in the table below (Table 6.2).

Table 6.1 Mean value of maize grain temperature, moisture content, mean number of arthropod and mean number of arthropod and mold damaged maize kernels in treated and untreated seeds.

No	Parameters	Mean ± standard error	F-value	*t*-value	df	p-value
1	Moisture content (%)					
	Untreated seeds	15.48±0.28[a]	1.60	9.18	98	0.000***
	Treated seeds	12.17±0.23[b]				
2	Temperature (^{O} C)					
	Untreated seeds	25.59±0.37[a]	3.32	11.82	98	0.000***
	Treated seeds	19.94±0.30[b]				
3	Number of arthropods (per 100g grain)					
	Untreated seeds	45.86±1.52[a]	0.54	16.06	98	0.000***
	Treated seeds	9.18±1.70[b]				
4	Arthropod damaged maize kernels (%)					
	Untreated seeds	76.14±1.76[a]	0.48	33.43	98	0.000***
	Treated seeds	13.60±0.96[b]				
5	Mold damaged maize kernels (%)					
	Untreated seeds	48.02±1.40[a]	0.50	34.56	98	0.000***
	Treated seeds	8.88±0.29[b]				

Means followed by the different letter within a column are significantly different at 5% of probability level.

Table 6.2 Coefficients of simple correlation (r) and p-value among the variables analyzed to evaluate association of arthropods infestation with arthropod and mold damaged maize kernels and maize grain temperature and moisture content.

		Moisture C	Temperature	Arthropod No	ADMK	MDMK
Moisture C	Pearson Correlation	1				
	Sig. (2-tailed)	-				
	N	100				
Temperature	Pearson Correlation	0.555**	1			
	Sig. (2-tailed)	0.000	-			
	N	100	100			
Arthropod No	Pearson Correlation	0.594**	0.637**	1		
	Sig. (2-tailed)	0.000	0.000	-		
	N	100	100	100		
ADMK	Pearson Correlation	0.644**	0.716**	0.808**	1	
	Sig. (2-tailed)	0.000	0.000	0.000	-	
	N	100	100	100	100	
MDMK	Pearson Correlation	0.597**	0.659**	0.807**	0.888**	1
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	
	N	100	100	100	100	100

**. Correlation is significant at the 0.01 level (2-tailed).

ADMK=Arthropod damaged maize kernels; MDMK=Mold damaged maize kernels; Moisture C=Moisture content.

Effect of arthropod damage to maize kernels on infection of potentially toxigenic fungi

Colony forming units per plate of each species of fungi

This study showed that maize kernels from both insect damaged and undamaged composite samples were infected by more than seven species of potentially toxigenic fungi which include *A. flavus*, *A. niger*, *A. parasiticus*, *A. ochraceus*, *A. fumigatus*, *Fusarium* spp., *Penicillium* spp., and other species (Plate 6.3). Overall, on PDA the colony forming units of *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *A. ochraceus*, *A. fumigatus*, *Penicillium* spp., and others per plate were found to be significantly higher on untreated (arthropod damaged) maize kernel than treated (undamaged) maize kernel ($p<0.05$). However, no significant difference was observed between the colony forming units of *Fusarium* spp., per plate on arthropod damaged and undamaged maize kernel ($p>0.05$). Similarly, on MEA (with NaCl) the colony forming units of *A. flavus*, *A. niger*, *A. parasiticus*, *A. ochraceus*, *A. fumigates*, *Penicillium* spp., and other species per plate were found to be significantly higher on untreated (arthropod damaged) maize kernel than treated (undamaged) maize kernel ($p<0.05$). However, the incidence of *Fusarium* spp., were significantly higher on undamaged maize kernel than damaged maize kernel ($p<0.05$). (Figure 6.2 A and B).

A.

B.

C.

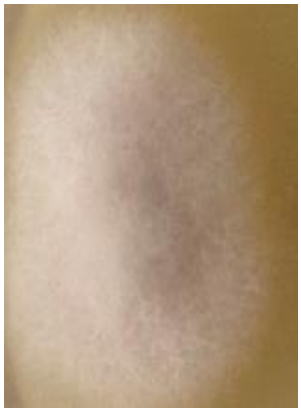
D.

E.

F.

G.

H.



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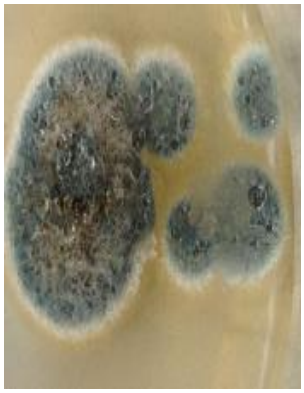

J.

K.

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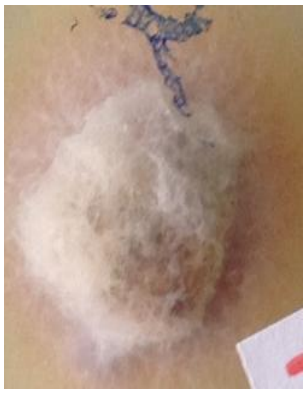
M.

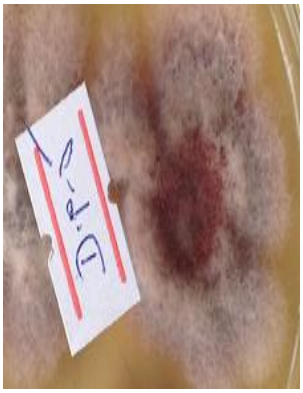


N.

O.

P.

Q.



R.

Plate 6.3: Front view of *Aspergillus niger* on MEA (A) and PDA (B), *Aspergillus flavus* on MEA (C) and PDA (D), *Aspergillus parasiticus* on PDA (E) and MEA (F), *Penicillium* sp. on PDA (G) and MEA (H), *Aspergillus fumigates* on PDA (J), *Aspergillus candidus* on PDA (K) and MEA (I), *Trichoderma* sp. on MEA (L) and PDA (M), *Fusarium* sp. on MEA (N) and PDA (R), *Aspergillus ochraceus* on PDA (O) and MEA (P), *Aspergillus* sp., on PDA (Q) (Size:2×1.55 inch).

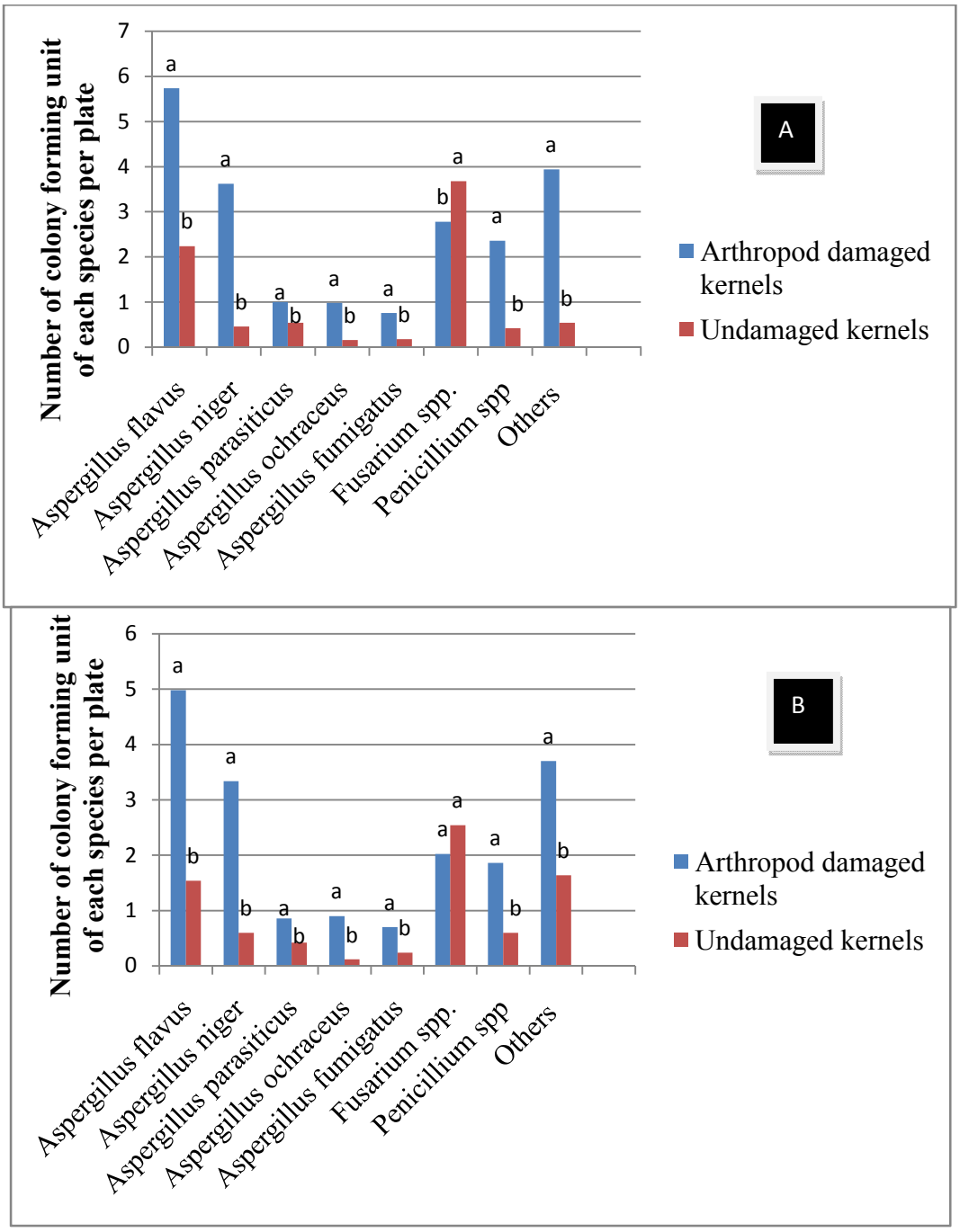


Figure-6.2: Colony forming unit of each fungal species on arthropod damaged and undamaged maize kernels directly plating on PDA (A) and MEA with NaCl on the (B) (with in each species bars followed by differerent lower cases are significantly different at 5% of probability level of significance) (t-test).

Frequency and relative density of potentially toxigenic fungi

In the present study the highest relative density were recorded for *Aspergillus flavus* (27.12%), others (20.15%) and *Aspergillus niger* (18.19%) on PDA agar plating methods in treatment of arthropods damaged maize kernels and *Fusarium* spp. (32.99%), others (21.30%) and *Aspergillus flavus* (20.00%) in treatment of undamaged maize kernels. Similarly the highest relative density were recorded for *Aspergillus flavus* (27.10%), other (18.60%) and *Aspergillus niger* (18.19%) on ME with NaCl agar plating methods in treatment of arthropods damaged maize kernel and *Fusarium* spp., (44.76%), *Aspergillus flavus* (27.26%) and others (6.57%) in the treatment of undamaged maize kernels.

The frequency of occurrence on PDA plating method for *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus fumigates*, *Fusarium* spp., *Penicillium* spp and others were found to be 96, 92, 64, 72, 56, 86, 90 and 94%, respectively in treatment of arthropods damaged maize kernels and 78, 48, 40, 10, 24, 86, 34 and 76% respectively in treatment of undamaged maize kernels. On the other hand the frequency of occurrence on MEA with NaCl plating methods for *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus fumigates*, *Fusarium* spp., *Penicillium* spp and others were found to be 100, 96, 70, 70, 60, 98, 98 and 100%, respectively in treatment of arthropods damaged maize kernels and 92, 30, 48, 10, 18, 100, 30 and 42% in treatment of undamaged maize kernels. In general the frequency of occurrence was significantly ($p=0.013$ for PDA plating method and $p=0.008$ for MEA with NaCl plating method) higher on arthropods damaged maize kernels than undamaged maize kernels (Table 6.3 and 6.4).

Table-6.3: Effects of arthropod damage on frequency and relative density of potentially toxigenic fungi on maize kernels directly plating on PDA.

Species	Media and Treatment1-PDA			
	Untreated		Treated	
	Frequency (%)	Relative density	Frequency (%)	Relative density
Aspergillus flavus	96	27.12	78	20.00
Aspergillus niger	92	18.19	48	7.80
Aspergillus parasiticus	64	4.68	40	5.45
Aspergillus ochraceus	72	4.90	10	1.56
Aspergillus fumigatus	56	3.81	24	3.12
Fusarium spp.	86	11.00	86	32.99
Penicillium spp	90	10.13	34	7.80
Others	94	20.15	76	21.30

Table-6.4: Effects of arthropod damage on frequency and relative density of potentially toxigenic fungi on maize kernels directly plating on MEA with NaCl.

Species	Media and Treatment2-MEA			
	Untreated		Treated	
	Frequency	Relative density	Frequency	Relative density
Aspergillus flavus	100	27.10	92	27.26
Aspergillus niger	96	17.09	30	5.60
Aspergillus parasiticus	70	4.72	48	6.56
Aspergillus ochraceus	70	4.62	10	1.95
Aspergillus fumigatus	60	3.59	18	2.19
Fusarium spp.	98	13.13	100	44.76
Penicillium spp	98	11.14	30	5.10
Others	100	18.60	42	6.57

Total number of colony forming unit per plate, colony diameter and number of kernel infected with mold.

The total number of colony forming units were found to be significantly (t (98) = -15.14, p=0.000 for PDA plating and t (98) = -18.84, p=0.000 for MEA with NaCl plating methods) higher for arthropod damaged maize kernels than undamaged maize kernels. Similarly, the average colony diameters of fungi were found to be significantly (t (98) = 16.20, p=0.000 for PDA plating and t (98) = 16.19, p=0.000 for MEA with NaCl plating methods) bigger on arthropod damaged maize kernels than undamaged maize kernels. Moreover, the numbers of infected maize kernels were also significantly (t (98) = 21.81, p=0.000 for PDA plating and t (98) = 11.27, p=0.000 for MEA with NaCl plating methods) larger on arthropod damaged maize kernel than undamaged maize kernels (Table 6.5).

Table-6.5: Number of infected seeds (N=500), colony diameter (in cm) and total number of colony forming unit on arthropod damaged and undamaged maize kernels.

Treatment	Number of infected seeds on		Colony diameter on		Total number of colony forming unit On	
	PDA	MEA with NaCl	PDA	MEA with NaCl	PDA	MEA with NaCl
Untreated (arthropod damaged)	4.40±0.10	3.70±0.11	3.84±0.09	3.95±0.91	18.36±0.53	21.18±0.62
Treated (undamaged)	1.32±0.09	1.70±0.14	1.74±0.08	1.83±0.03	7.70±0.46	8.22±0.29
t-value	21.80	11.27	16.20	16.19	-15.14	-18.84
df	98	98	98	98	98	98
p	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **

*** = Significantly different at 5% of probability level (t-test).

A.

B.

C.

D.

Plate 6.4: Mixed colony forming units of fungi on insect damaged maize kernels directly plating on PDA (A&C) and MEA with NaCl (B&D) and undamaged maize kernels directly plating on PDA (E&G) and MEA with NaCl (F&H) (Size: 3.00×3.00 inch).

E.

F.

G.

H.

Plate 6.4: Mixed colony forming units of fungi on insect damaged maize kernels directly plating on PDA (A&C) and MEA with NaCl (B&D) and undamaged maize kernels directly plating on PDA (E&G) and MEA with NaCl (F&H) (Size: 3.00×3.00 inch).

Occurrence of potentially toxigenic fungi in insect body

On Potato dextrose Agar (PDA), the number of infected insects per plate were be significantly ($p<0.05$) higher for *C. hemipterus*, *Brachypeplus* spp., and *L. balteatus* than *Sitophillus* and *Lepinotus* spp. Similarly, the total number of colony forming units were significantly ($p<0.05$) higher for *C. hemipterus*, *Brachypeplus* spp., and *L. balteatus* than *Sitophillus* and *Lepinotus* spp. The mean number of colony forming units of *Aspergillus* spp. were found to be significantly ($p<0.05$) higher on *C. hemipterus* and *L. balteatus* than *Brachypeplu*s and *Lepinotus* spp. The incidence of *Fusarium* spp., were found in decreasing order *Brachypeplus* spp> *Litargus balteatus*> *C. hemipterus*= *Lepinotus* spp.> *Sitophillus* spp. On the other hand, the incidence of *Penicillium* spp, were significantly higher on *C. hemipterus* than *Brachypeplus* spp., *L. balteatus* and *Lepinotus* spp.; *Brachypeplus* spp., *Litargus balteatus* and *Lepinotus* spp., than *Sitophillus* spp. Moreover, significantly ($p<0.05$) more numbers of other fungal species were carried by *C. hemipterus* and *Brachypeplus* spp., than *L balteatus* and *Lepinotus* spp.; *L balteatus* and *Lepinotus* spp., than *Sitophillus* spp., (Table 6.6).

On Malt extract Agar with sodium chloride (MEA with NaCl), the number of infected insect and total number of colony forming units per plate were significantly ($p<0.05$) higher on *C. hemipterus*, *Brachypeplus* spp., and *L. balteatus* than *Sitophillus* and *Lepinotus* spp. and *Lepinotus* spp. than *Sitophillus* spp. The mean number of *Aspergillus* spp. colony forming unit per plate were found to be significantly ($p<0.05$) higher on *C. hemipterus* and *L. balteatus* than *Brachypeplus* spp., *Sitophillus* spp. and *Lepinotus* spp; *Brachypeplus* spp. than *Sitophillus* spp. and *Lepinotus* spp. and *Lepinotus* spp. than *Sitophillus* spp. On the other hand, the mean number of colony forming unit of *Fusarium* spp. per plate were found to be significantly ($p<0.05$) higher on *Brachypeplus* spp. than *C. hemipterus* and *L. balteatus*, *Sitophillus* spp. and *Lepinotus* spp followed by in decreasing order *C. hemipterus* and *L. balteatus* > *Lepinotus* spp> *Sitophillus* spp. The mean number of colony forming units of *Penicillium* spp were observed ($p<0.05$) in decreasing order *C. hemipterus*= *L. balteatus*≥ *Brachypeplus* spp.≥ *Lepinotus* spp.> *Sitophillus* spp. Moreover, other species of fungi were observed in decreasing order of occurrence *C. hemipterus*≥ *Brachypeplus* spp.≥ *L. balteatus*> *Lepinotus* spp> *Sitophillus* spp. On both, media (PDA and MEA with NaCl), overall incidence of *Aspergillus*, *Fusarium, Penicillium* and other

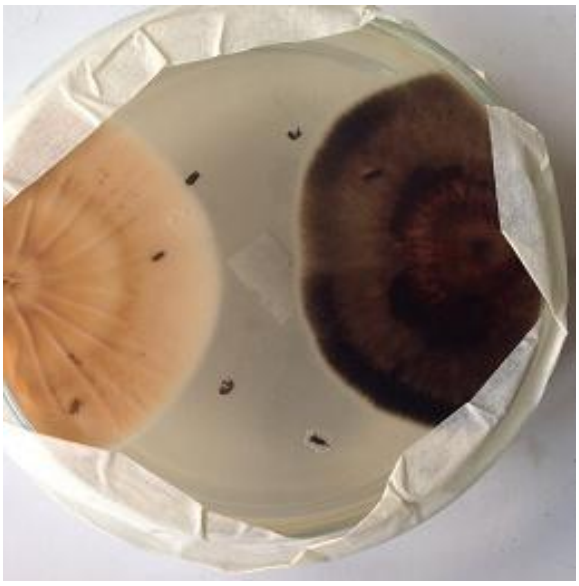
species were significantly ($p<0.05$) higher on mold feeder insect (*C. hemipterus*, *Brachypeplus* spp., *L. balteatus* and *Lepinotus* spp.) than grain feeder insect (*Sitophillus* spp.).

Table-6.6: Incidence of potentially toxigenic fungi on selected insect body directly plated on Potato Dextrose Agar (A) and Malt Extract Agar with NaCl (B).

Potato Dextrose Agar (PDA) (A)						
Insect	Parameter					
	Number of infected insect	Number of colony/plate	*Aspergillus* spp.	*Fusarium* spp.	*Penicillium* spp	Other spp.
C. hemipterus	9.40±0.24^{a}	59.60±1.86^{a}	22.20±1.16^{a}	14.20±0.37^{c}	13.00±0.63^{a}	10.20±0.37^{a}
Brachypeplus spp	9.00±0.32^{a}	57.00±1.64^{a}	13.80±0.66^{b}	21.80±0.58^{a}	10.60±0.51^{b}	10.80±0.38^{a}
Litargus balteatus	9.20±0.37^{a}	60.00±1.34^{a}	25.80±1.24^{a}	18.20±0.57^{b}	8.40±0.40^{b}	7.60±0.24^{b}
Sitophillus spp.	5.20±0.20^{b}	24.00±1.34^{c}	8.00±0.45^{c}	6.00±0.71^{d}	5.60±0.51^{d}	4.40±0.40^{c}
Lepinotus spp.	6.60±0.51^{b}	40.00±2.50^{b}	12.20±0.58^{b}	12.40±0.75^{c}	8.20±0.58^{bc}	7.60±0.81^{b}
F-value	29.43	77.49	70.26	95.56	27.28	27.89
p-value	0.000	0.000	0.000	0.000	0.000	0.000
Malt Extract Agar with NaCl (MEA with NaCl) (B)						
Insect	Parameter					
	Number of infected insect	Number of colony/plate	*Aspergillus* spp.	*Fusarium* spp.	*Penicillium* spp	Other spp.
C. hemipterus	9.0±0.55^{a}	66.60±1.57^{a}	26.80±1.98^{a}	16.00±0.71^{b}	12.00±0.45^{a}	11.80±0.37^{a}
Brachypeplus spp	9.6±0.24^{a}	65.40±1.66^{a}	19.20±1.24^{b}	27.00±0.89^{a}	9.40±0.40^{ab}	9.80±0.66^{ab}
Litargus balteatus	10±0.00^{a}	64.40±1.91^{a}	26.40±1.03^{a}	17.20±0.58^{b}	12.80±0.86^{a}	8.00±0.45^{b}
Sitophillus spp.	4.8±0.37^{c}	21.00±1.79^{c}	8.80±0.73^{c}	5.60±0.68^{d}	4.60±1.08^{c}	2.00±0.54^{d}
Lepinotus spp.	7.6±0.51^{b}	40.20±1.88^{b}	15.00±1.30^{b}	11.60±0.51^{c}	8.20±0.66^{b}	5.40±0.25^{c}
F-value	29.21	131.69	33.51	131.68	19.82	64.30
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Means followed by different letters were significantly different ($p<0.05$) (Tukey Studentized Range Test).

A.



B.

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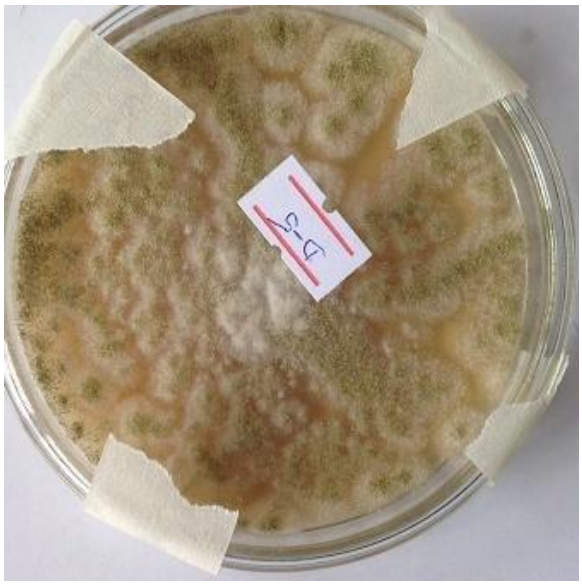


E.

F.

G.

H.

Plate 6.5: Examples of Mixed colony forming units of fungi on *Sitophillus* spp directly plating on PDA (A-front view &B-reverse view); *C. hemipterus* directly plating on PDA (C-front view &D-reverse view); pure culture of *A. flavus* (E) and *A. niger* (F) isolated from *C. hemipterus* and Conidiophores and conidia of *A. flavus*, (G) and *A. niger* (H) grown on dead *L. balteatus* (Size: 3.00×3.00 inch).

Effects of insect damage on aflatoxin contamination of stored maize

Aflatoxin was detected in all samples taken from both insect damaged and undamaged maize kernels except that of Aflatoxin-G_2 and B_2 which were not detected in four and two samples of undamaged maize kernels, respectively. The total Aflatoxin content in insect damaged maize kernel ranged from 4.29-6.17 µg /kg. Differently the total Aflatoxin content in undamaged maize kernels ranged from 0.34-0.74 µg/kg. The contents of Aflatoxin-B_1 in insect damaged and undamaged maize kernels ranged from 4.00-5.86 µg/kg and 0.31-0.66 µg/kg, respectively, while the contents of Aflatoxin-B_2 in insect damaged and undamaged maize kernels ranged from 0.05-0.09 µg/kg and 0.00-0.03 µg/kg, respectively. Aflatoxin-$G_1$was detected ranging from 0.13-0.18 µg/kg in insect damaged maize kernels and 0.01-0.07 µg/kg in undamaged maize kernels, while Aflatoxin-G_2 was detected ranging from 0.06-0.10 µg/kg in insect damaged maize kernels and 0.00-0.03 µg/kg in undamaged maize kernels (Table 6.7). Aflatoxin B1 was found to be the most predominant Aflatoxin composing Total Aflatoxin and followed by Aflatoxin G_1. In this study 93% and 3% of total aflatoxin in insect damaged maize kernels and 87% and 9% of total aflatoxin in undamaged maize kernels were Aflatoxin B1 and Aflatoxin G1, respectively (Figure 6.3 and 6.5 A-E).

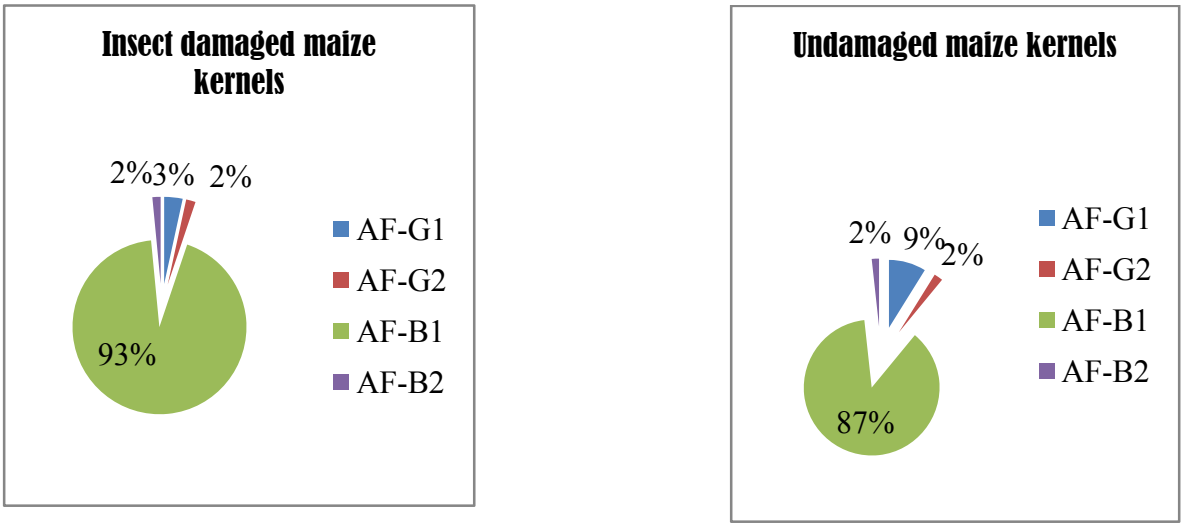


Figure 6.3: Contribution of each Aflatoxin (Aflatoxin G1, G2, B1 and B2) in composing Total Aflatoxin (insect damaged maize kernels (left) and undamaged maize kernels (right).

Table 6.7: Aflatoxin content in insect damaged and undamaged maize kernels (Malathion treated) stored for six month under laboratory condition.

Treatments	Aflatoxin (µg/kg)				
	G_1	G_2	B_2	B_1	Total
Damaged-1	0.1318	0.0788	0.06	4.56	4.8306
Damaged-2	0.1257	0.0859	0.08	4.7	4.9916
Damaged-3	0.1488	0.0889	0.05	4.0	4.2877
Damaged-4	0.16	0.0889	0.07	4.88	5.1989
Damaged-5	0.1492	0.0559	0.07	4.99	5.2651
Damaged-6	0.17	0.10	0.09	4.55	4.91
Damaged-7	0.16	0.07	0.08	4.03	4.34
Damaged-8	0.18	0.06	0.07	5.86	6.17
Damaged-9	0.14	0.07	0.06	4.68	4.95
Undamaged-1	0.0724	0.00	0.01	0.52	0.6024
Undamaged-2	0.05	0.00	0.01	0.32	0.38
Undamaged-3	0.0236	0.00	0.01	0.31	0.3436
Undamaged-4	0.0211	0.01	0.0	0.33	0.3611
Undamaged-5	0.01	0.00	0.01	0.44	0.46
Undamaged-6	0.05	0.02	0.02	0.59	0.68
Undamaged-7	0.04	0.01	0.00	0.6	0.65
Undamaged-8	0.06	0.03	0.03	0.55	0.67
Undamaged-9	0.04	0.02	0.02	0.66	0.74

Comparison of the means of Aflatoxin G1, G2, B1, B2 and total Aflatoxin level in insect damaged and undamaged maize kernels.

Independent sample t-test showed that significant difference in level of Aflatoxin B1, B2, G1, G2 and Total Aflatoxin between insect damaged and undamaged maize kernels. Aflatoxin B1 (t = 22.23, p = 0.000), B2 (t = 11.09, p=0.000), G1 (t = 12.50, p = 0.000), G2 (t = 10.99, p = 0.000 and Total Aflatoxin (t = 23.17, p = 0.000) were found to be significantly higher in insect damaged maize kernels than undamaged maize kernels (Figure 6.4).

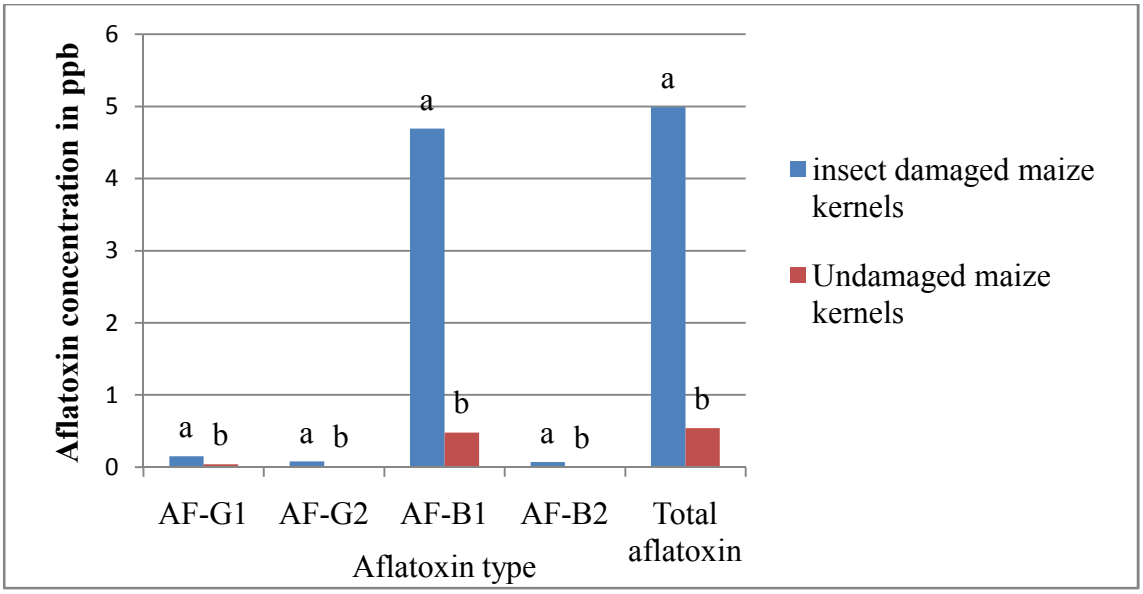
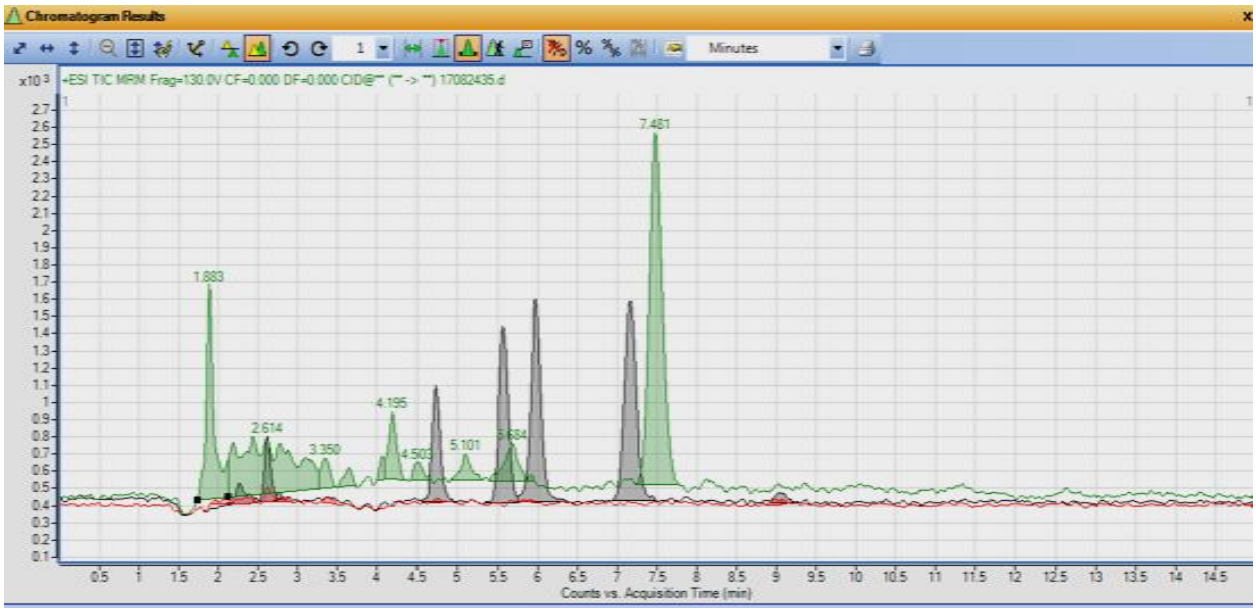
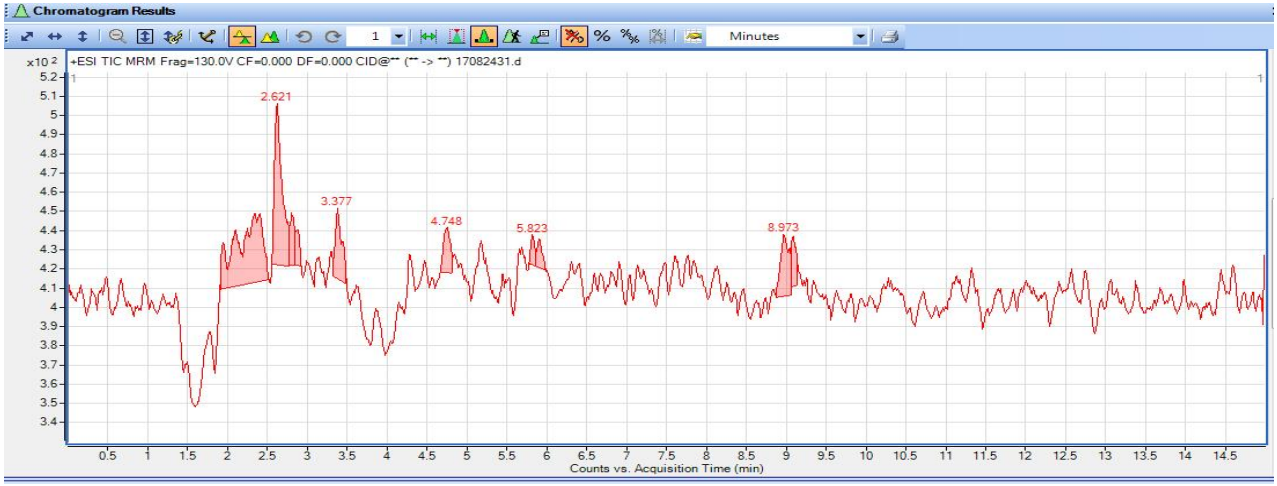


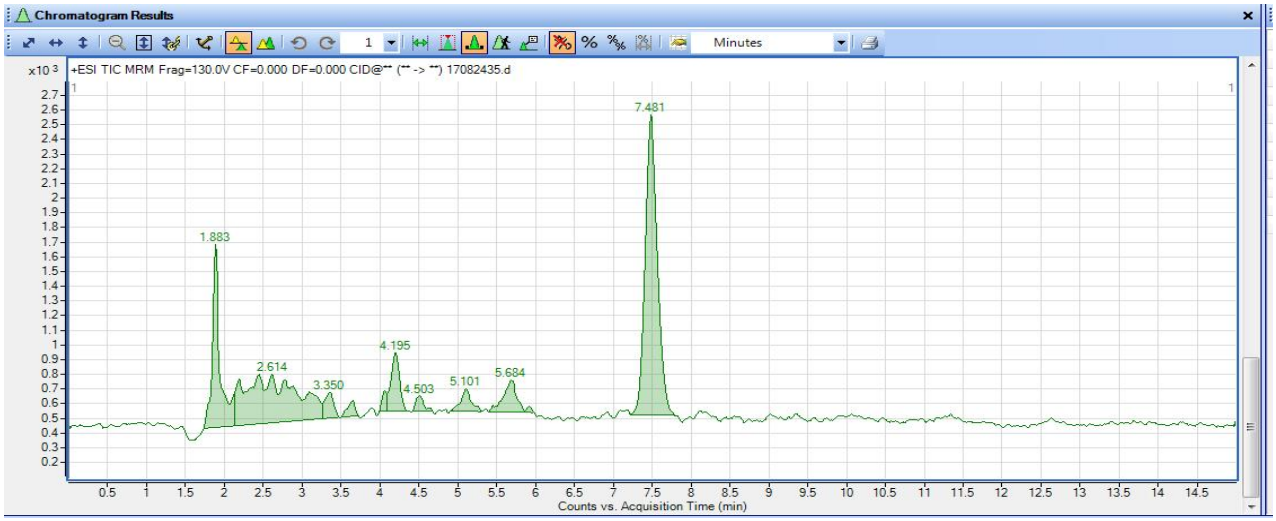
Figure 6.4: Comparison of means of Aflatoxin between insect damaged and undamaged maize kernels (bars followed by different small letters are considered significantly different at 5% of probability level) (T-test).



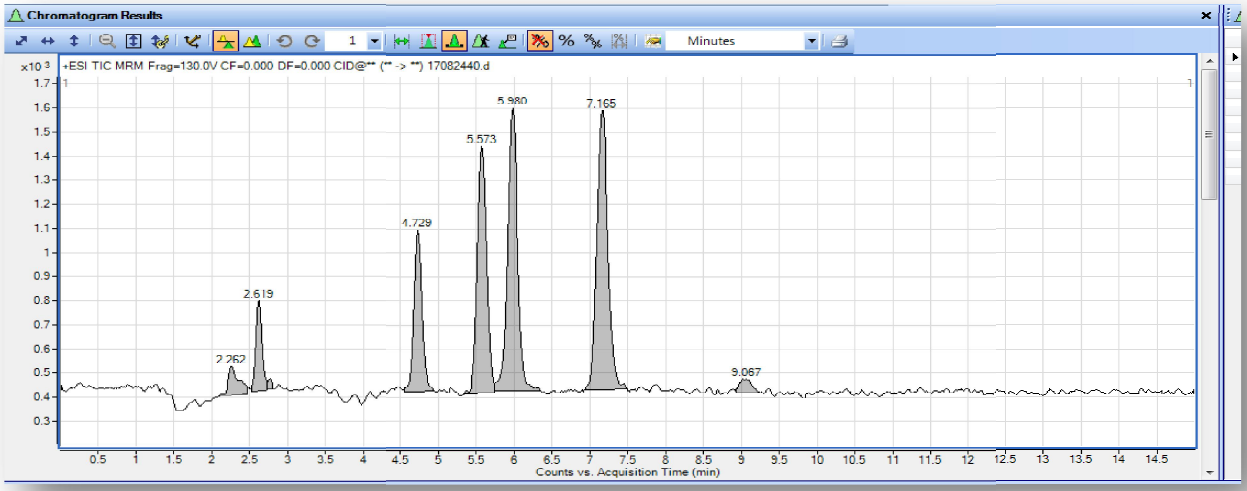
A. Undamaged Maize Kernels, Damaged Maize Kernels and Undamaged maize kernels spiked with Aflatoxin Standards (AF-G2, G1, B2 and B1 from the left to right).



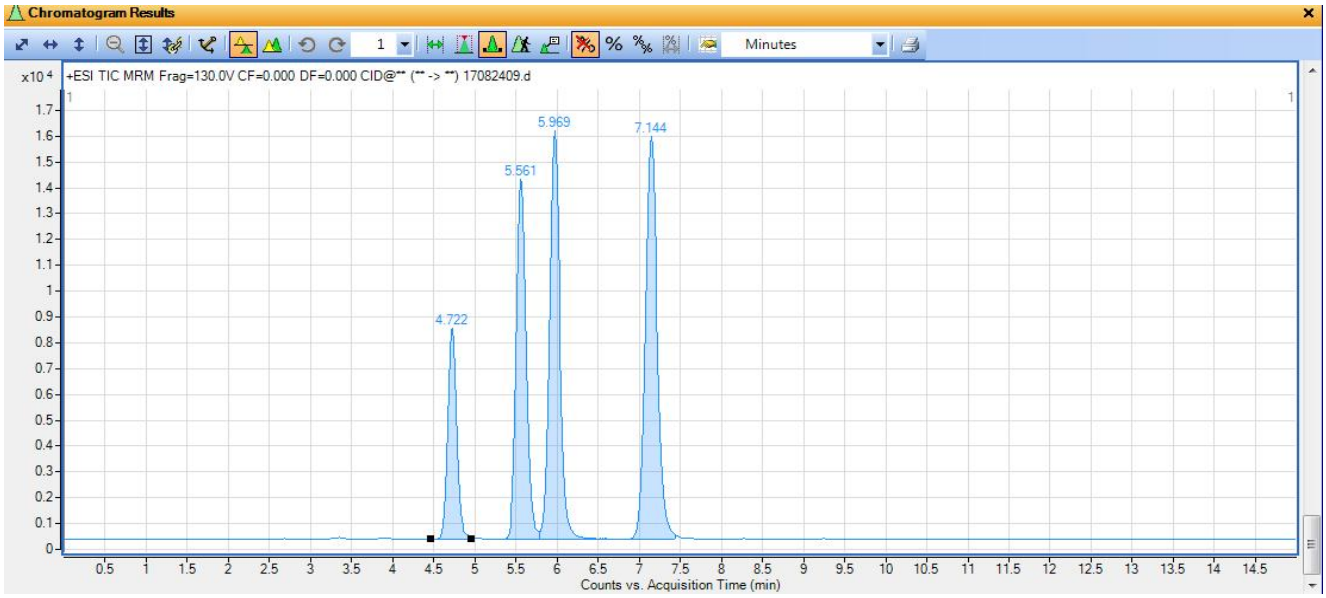
B. **Undamaged Maize Kernels**



C. **Damaged Maize Kernels**



D. Undamaged maize kernels spiked with Aflatoxin Standards (1ppb)(AF-G2, G1, B2 and B1 from the left to right or retention time from 4.729-7.165).



E. Pure Aflatoxin Mix Standard (15 ppb)-AF-G2, AF-G1, AF-B2 and AF-B1 with retention time 4.772, 5.561, 5.969 and 7.144, respectively.

Figure 6.5 Chromatogram results of undamaged maize kernels (B), damaged maize kernels (C), undamaged maize kernels spiked with 1 ppb Aflatoxin mix standard (D), combination of undamaged maize kernels, damaged maize kernels and spiked sample (A) and pure Aflatoxin mix standards (15 ppb)(E).

6.4 Discussions

This study showed significantly higher grain temperature, moisture content; mean number of arthropods and mean number of arthropod and mold damaged maize kernels in untreated seeds than Malathion treated seeds. The result obtained from Pearson correlation underlined high infestation of arthropod caused high grain temperature and moisture content and high mean number of arthropod and mold of damaged maize kernels in untreated seeds. The quality factors of stored corn such as increasing percentage of insect and mold damaged maize kernels, the elevation of seed moisture content and the proliferation of molds increased with increasing of *Sitophilus zeamais* infestation (Caneppele *et al*., 2003). In the present study, the mean value of moisture content in untreated seed was 15.48%. This moisture was considered as unsafe for the quality of stored maize (Obeng-Ofori *et al.,* 2008). FAO (2011) reported stored maize with high moisture content provide suitable condition for the growth and development of fungi and other organisms like *Aspergillus, Penicillium* and *Fusarium,* and their respective mycotoxin. Moreover, positive correlation was observed between arthropod infestation and mold damaged maize kernels. This finding is in agreement with the finding of Dunkel (1988), who confirmed the association between arthropods and fungi provide suitable condition for the development of these organisms.

The data from the present study showed the effects of arthropod damage to maize kernels on potentially mycotoxin producing fungal infection and Aflatoxin contamination. The infection of fungi and Aflatoxin contamination in the present study were significantly higher on arthropod damaged maize kernels than undamaged maize kernels. Insects usually damage maize kernels where natural infection of potentially mycotoxin producing fungi occurs. The present finding is in agreement with the findings of the previous authors (Avantaggio *et al*., 2002; Munkvold and Hellminch, 2000; St. Leger *et al*., 2000). Xu *et al*. (2003) observed positive relation between corn ear worm damage and grain mold incidence on maize. Dowd *et al*. (1998) also noted the involvement of arthropods in facilitating the establishment of mycotoxigenic fungi on nuts, bolls, pods and ears of crops. According to Setamou (1999) the percentage of grain infected by *A. flavus* and samples contaminated with Aflatoxin increased with increasing lepidopteran cob borer *M. nigrivenella* Ragonot damage. Moreover, field trial conducted by Folcher *et al*. (2009) showed that the insecticide deltamethrine against the two main maize borers *O. nubilalis* and

Sesamia nonagrioides Lefebvre [Lepidoptera: Noctuidae] was more effective than the fungicide tebuconazole in reducing mycotoxin level in maize.

This study also showed that maize kernels from both insect damaged and undamaged composite samples were infected by more than seven species of potentially mycotoxin producing fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus fumigatus*, *Fusarium* spp., *Penicillium* spp., and other species. This finding was supported by the findings of previous authors from Ethiopia (Chali Ofgea, 2015; Dabassa, 2014; Wubet and Abate, 1998; Amare, 2002 and Mashilla, 2004). The frequency occurrences of these fungi were higher in insect damaged maize kernels than undamaged maize kernels. Moreover, in most cases, the relative density was also higher in insect damaged maize kernels than undamaged maize kernels. *A. flavus* and *A. niger* from *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp and other unidentified species were found to be the most frequently encountered fungi in association with insect damaged maize kernels. On the other hand, *Fusarium* spp., and *A. flavus* were found to be the two most frequently encountered fungi in association with undamaged maize kernels. This confirms report from Kenya which have shown high occurrence of *A. flavus*, *A. niger*, *Fusarium* spp., and *Penicillium* spp., in maize seeds from selected Districts of Kenya (Wagara *et al*., 2014). Moreover, *A. flavus* (66.7%), *F. verticillioides* (76.9%) and *Fusarium proliferatum* (64.1%) were among the most frequently encountered fungi on maize samples from Nigeria (Egbuta *et al*., 2015). Apart from these *A. flavus* and *A. niger* constituted more than 50% of the total fungi isolated from Egyptian maize samples (Nooh *et al*., 2014).

Moreover, all test insects in the present study carried *Aspergillus*, *Fusarium*, *Penicillium* and other spp., of fungal spore on their bodies. However, the incidences of these fungi significantly varied among test insects. The number of infected insects and total number of colony forming unit per plate were significantly higher on *C. hemipterus*, *Brachypeplus* spp., and *L. balteatus* than *Sitophillus* and *Lepinotus* spp and *Lepinotus* spp., than *Sitophillus* spp. The incidence of *Aspergillus* spp., were significantly higher on *C. hemipterus* and *Litargus balteatus* than other insect where as the incidence *Fusarium* and *Penicillium* spp., were significantly higher on *Brachypeplus* spp., and *C. hemipterus*, respectively than other insects. This finding confirms the role of insect in vectoring fungal spore by carrying on their bodies and contaminating grain as

they move about (McMillian, 1987; Dowd, 1991; Lynch & Wilson, 1991; Lamboni and Hell, 2009 and Drakulic *et al*., 2017).

According to the present study the Nitidulid beetles such as *C. hemipterus* and *Brachypeplus* spp., were highly loaded with potentially mycotoxin producing fungi. This is in agreement with Dowd (1991) who reported Nitidulids (sap beetles) as a known carrier of toxigenic fungi including *A. flavus.* Moreover, these beetles carried *F. verticillioides* and *F. graminearum* spores on their body (Munkvold, 2003b). Apart from these, the present study shows that high incidence of *Fusarium* spp., on *Brachypeplus* spp. This finding is supported by Cline *et al*. (2014) who isolated *F. solani, F. oxysporium* and *F. verticilloids* from the gut of *Brachypeplus* spp. The high incidence of potentially mycotoxin producing fungi on the bodies of *C. hemipterus*, *Brachypeplus* spp., *L. balteatus*, *Lepinotus* spp than *Sitophillus* spp., possibly explained by their ecology and feeding behavior. *C. hemipterus*, *Brachypeplus* spp., *L. balteatus* and *Lepinotus* spp., were classified as mold feeder arthropods while *Sitophillus* spp., were classified as grain feeder arthropods. Moreover, *C. hemipterus*, *Brachypeplus* spp., *L. balteatus* and *Lepinotus* spp., preferred high moisture content of grain than *Sitophillus* spp., which provide suitable condition for the growth of fungi (IAOM, 2016 and Rees, 2004).

All composite samples from both insect damaged and undamaged maize kernels contaminated with Aflatoxin. Of the four main types of Aflatoxin, AF-B_1 and AF-G_1 occurred most frequently and in the largest amount (AF-B1, 4.00-5.86 µg /kg in insect damaged maize kernels and 0.31-0.66 µg /kg in undamaged maize kernels; AF-G_1, 0.13-0.18 µg /kg in insect damaged maize kernels and 0.01-0.07 µg /kg in undamaged maize kernels). The prevalence of both Aflatoxin B1 and G_1 in the current study was 100 %. These findings are in close agreement with that of Shah *et al*. (2010). who observed Aflatoxin B1 in 77.78 and 88.89% of the maize sample from Upper and Lower Swat of Pakistan region, respectively. Moreover, Logrieco *et al*. (2003) noted that AF-B1 and AF-G1 as the two most commonly encountered Aflatoxin (in frequency and amount) in association with most plant products. Aflatoxin contamination in the present study is significantly higher in insect damaged maize kernels than undamaged maize kernels. This finding confirms the findings of several authors (Setamou *et al*., 1998; Hell *et al*., 2000; Kimanya *et al*., 2008; Folcher *et al*., 2009).

6.5 Conclusion

The present study confirms the involvement of insect in infection of potentially mycotoxin producing fungi and Aflatoxin contamination in stored maize. The feeding activity of insect may disturb the natural barrier of maize kernels and provide portal entry for the entrance of potentially mycotoxin producing fungal spore including Aflatoxin producing fungal spore. Moreover, as the test insect in the present study indicated, insects carry a huge amount of potentially mycotoxin producing fungal spores on their bodies. This is also plays a big role in facilitating mycotoxin contamination in stored grain. Generally, chromatograms of insect damaged kernels in the present study confirm such a situation.

Chapter 7

General Conclusion and Recommendations

7.1 Conclusion

A total of 81 species of arthropods were recorded during the two year survey in maize samples immediately after sampling and after 120 days of incubation period at 21 ± 3 ^{o} C and 65-70% relative humidity (RH). Grain and grain product feeder arthropods and their associated natural enemy, and mold feeder arthropods and their associated natural enemies were the first and the second, respectively to be detected in stored maize. This is possibly indicating the role of stored maize grain condition in succession of these arthropods. This identification assists in predicting the condition of the grain. The condition of the grain has epidemiological significance to public health. High infestation of *S. zeamais* weevils were observed in the present study. This might be associated with high incidence of potentially mycotoxin producing fungi and mycotoxin contamination. For instance, in acute doses, Aflatoxin causes hemorrhage, acute liver damage, edema, and death in humans. Moreover, in the present study, *Tyrophagus putrescentiae* was identified as the most frequently and abundantly encountered mite species. This mite was associated with occupational allergy and asthma in workers who handle grains in elevators.

The present study also shows that pre-harvest maize cob damage types were found to be the main factors in determining species composition and abundance of sap beetles and other mold feeder arthropods in stored maize. Pre-harvest insect and mold damaged cob were identified as the main host for these arthropods. Therefore, the result obtained from this study underlined the role of sanitation in managing these arthropods and corn ear rot fungi in stored maize.

It is very important to detect sap beetles and associated Staphylinids in order to understand the role of these insect in stored maize grain ecosystem. Accordingly, the present study investigated how to detect these insects and found that the role of fermentation and decomposition of the maize cob by water and fermented fruit juice in detecting (attracting) these insect. The result obtained from this study also shows that *Carpophillus hemipterus* was more of associated with fermentation of cobs while *Brachypeplus* spp. and staphylinds were more of associated with decomposition of cob.

The present study confirms the involvement of insect in the incidence of potentially mycotoxin producing fungi and Aflatoxin contamination in stored maize. The feeding activity of insect may disturb the natural barrier of maize kernels and provide portal entry for the entrance of potentially mycotoxin producing fungal spore including Aflatoxin producing fungal spore. Moreover, as the test insect in the present study indicated, insects carry a huge amount of potentially mycotoxin producing fungal spore on their bodies. This also plays a big role in facilitating mycotoxin contamination in stored grain. Apart from these, the result obtained from this study shows that the incidence of potentially mycotoxin producing fungi significantly higher on mold feeder arthropods (*Carpophillus hemipterus*, *Brachypeplus* spp., *Litargus balteatus* and *Lepinotus* spp.) than grain feeder arthropods (*Sitophillus* spp.). This is indicating that the role of mold feeder arthropods in stored maize ecosystem.

7.2 Recommendations

- Field experiments should be conducted to determine the effects of *Tyrophagus putrescentiae* on occupational allergy and asthma in workers who handle grains in elevators.
- The present study observed two dead specimens of *Prostephanus truncatus* (larger grain borer). Thus further study should be conducted to confirm this.
- More complete studies should also be carried out to determine the association among grainivorous arthropods, fungivorous arthropods, mycotoxin producing fungi and mycotoxin contamination in stored maize ecosystem.
- More studies should be conducted to determine the effects of pre-harvest maize cob sanitation on incidence of sap beetles and other mold feeder arthropods in stored maize.
- Field experiments should be conducted to determine the efficacy of fermented cob and fermented fruit juice to attract sap beetles and Staphylinids in large granary.
- More, studies should be conducted to determine the association between sap beetles and staphylinids.
- Studies should be conducted to determine effects of grainivorous arthropod management on incidence of mold feeder arthropods, mycotoxin producing fungi and mycotoxin contamination.

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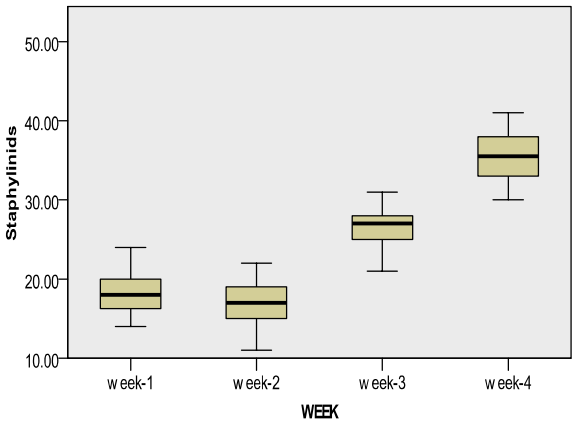
Appendix 1

Independent-samples Kruskal-Wallis test with pair-wise comparison to compare best week to detect Staphylinids.

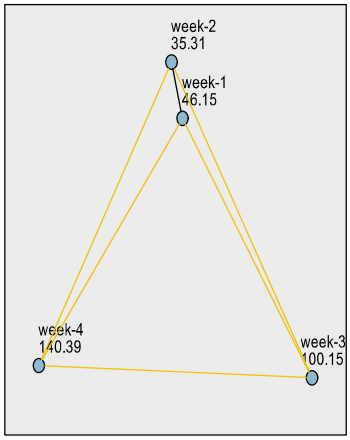
Independent-Samples Kruskal-Wallis Test



Total N	160
Test Statistic	134.393
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Pairwise Comparisons of WEEK



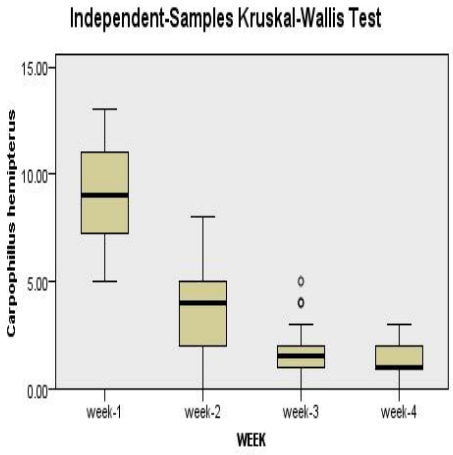
Each node shows the sample average rank of WEEK.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
week-2-week-1	10.838	10.347	1.047	.295	1.000
week-2-week-3	-64.838	10.347	-6.266	.000	.000
week-2-week-4	-105.075	10.347	-10.155	.000	.000
week-1-week-3	-54.000	10.347	-5.219	.000	.000
week-1-week-4	-94.238	10.347	-9.107	.000	.000
week-3-week-4	-40.238	10.347	-3.889	.000	.001

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

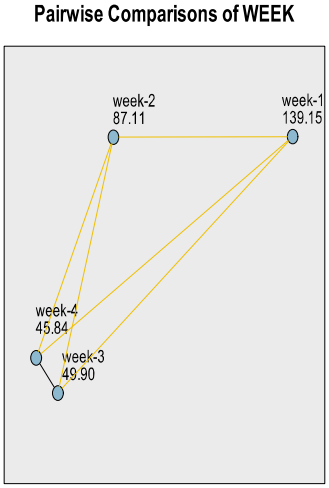
Appendix-1

Independent-samples Kruskal-Wallis test with pair-wise comparison to compare best week to detect *Carpophillus hemipterus*.



Total N	160
Test Statistic	106.777
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.



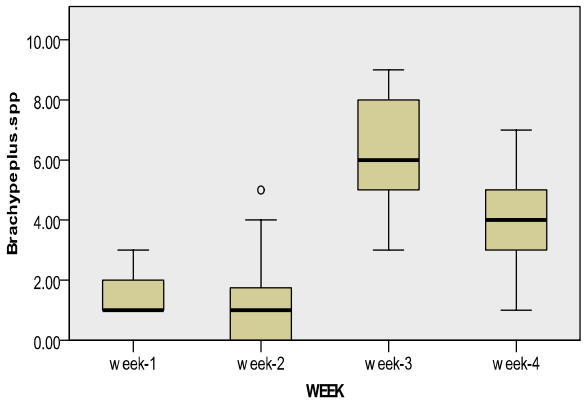
Each node shows the sample average rank of WEEK.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
week-4-week-3	4.062	10.261	.396	.692	1.000
week-4-week-2	41.275	10.261	4.022	.000	.000
week-4-week-1	93.312	10.261	9.094	.000	.000
week-3-week-2	37.212	10.261	3.627	.000	.002
week-3-week-1	89.250	10.261	8.698	.000	.000
week-2-week-1	52.038	10.261	5.071	.000	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Appendix 5.4 Independent-samples Kruskal-Wallis test with pair-wise comparison to compare best week to detect *Brachypeplus* spp.

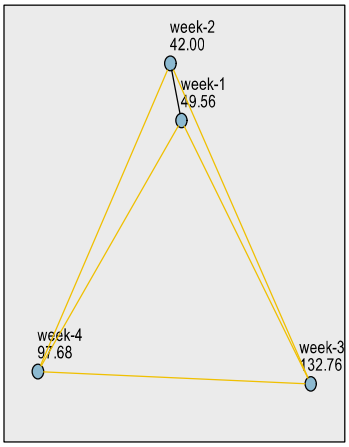
Independent-Samples Kruskal-Wallis Test



Total N	160
Test Statistic	104.781
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Pairwise Comparisons of WEEK



Each node shows the sample average rank of WEEK.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
week-2-week-1	7.562	10.214	.740	.459	1.000
week-2-week-4	-55.675	10.214	-5.451	.000	.000
week-2-week-3	-90.762	10.214	-8.886	.000	.000
week-1-week-4	-48.112	10.214	-4.710	.000	.000
week-1-week-3	-83.200	10.214	-8.146	.000	.000
week-4-week-3	35.088	10.214	3.435	.001	.004

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Appendix 2

Characterstics	*A.flavus*	*A.parasiticus*
Condiophore arrengement	Consistently biseriate	Mostly uniserate, sometimes mixed
Condia	Almost smooth to slightly roughened	Distinctly verruculose
Colony color	Yellow green	Ivy green
Colony surface	Irregular, some aerial hyphae	Compact, velvetly
Chemical analysis	$AFB_{1+}AFB_2$ and cyclopizanoic acid (proteolytic)	$AFB_{1+}AFB_2$ + AFG_1+AFG_2+AFM_1 (Lipolytic)

Declaration

I, Girma Kebede Angessa, declare that this thesis is my original work, has not been presented for a degree in any other University, and that all sources of material used for the thesis have been duly acknowledged.

Name: _____

Signature: _____

Date: _____

Place: _____

Approved by: Advisor: Professor, Emana Getu

Signature: _____

Date: _____