

**EVALUATION OF POTENTIAL BOTANICALS AGAINST MAIZE
WEEVIL, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and
ANGOUMOIS GRAIN MOTH, *Sitotroga cerealella* Olivier (Lepidoptera:
Gelechiidae) UNDER LABORATORY CONDITIONS**

M.Sc. THESIS

BY

FEKADU GEMECHU

JULY, 2012

JIMMA UNIVERSITY

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M.Sc. Thesis

By

Fekadu Gemechu

**Submitted to Director for Post Graduate Studies, Jimma University, College of
Agriculture and Veterinary Medicine**

**In Partial Fulfillment of the Requirements for the Degree of Master of Science
in Agricultural Entomology**

JULY, 2012

JIMMA UNIVERSITY

APPROVAL SHEET
School of Graduate Studies

As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by **Fekadu Gemechu**, entitled “**EVALUATION OF POTENTIAL BOTANICALS AGAINST MAIZE WEEVIL, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and ANGOUMOIS GRAIN MOTH, *Sitotroga cereallela* Olivier (Lepidoptera: Gelechiidae) UNDER LABORATORY CONDITIONS**”. I recommend that it be submitted as fulfilling thesis requirement.

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As member of the *board of Examiners* of the *M.Sc. Thesis Open Defense Examination*, we certify that we have read, evaluated the thesis prepared by **Fekadu Gemechu** and examined the candidate. We recommended that the thesis could be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Agricultural Entomology.

Chairperson

Signature

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Signature

External Examiner

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DEDICATION

This thesis is dedicated to God the almighty, who knows the end from the beginning; to my affectionate mother Alemi Gobena who did educate me; and to whole my ever-loving parents for their support; who uplift, encourage, motivate and pray for me to achieve the targets of my life successfully with the help of God.

STATEMENT OF THE AUTHOR

I hereby affirm that the contents of this thesis “**EVALUATION OF POTENTIAL BOTANICALS AGAINST MAIZE WEEVIL, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and ANGOUMOIS GRAIN MOTH, *Sitotroga cereallela* Olivier (Lepidoptera: Gelechiidae) UNDER LABORATORY CONDITIONS**” is the product of my own research and no part has been copied from any published source; except the references, standard mathematical or models/equations/protocols etc and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University and is deposited at the University Library to be available to borrowers under rules of the library. The University may take action if the information provided is found inaccurate at any stage. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College and director of post Graduate Studies when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

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BIOGRAPHICAL SKETCH

The author of this research study, Fekadu Gemechu was born in 1985 at Gida Ayana woreda, East Wollega zone from his father Gemechu Gudeta Jogora and his mother Alemi Gobena Tato. After completion of the primary and secondary school at Gida Ayana junior primary school, high school and preparatory, he joined Bahir Dar University in the year 2003/04, in the field of Biology as major and Chemistry as minor and he graduated with B.Ed. degree on July 9, 2006. After his graduation, he has employed in teaching profession by Oromia Educational Bureau and worked at Limu Gelila High School and Preparatory for three and half years. In February 25, 2010, he joined Jimma University College of Agriculture and Veterinary Medicine, the School of Post Graduate Studies to pursue his M.Sc degree in Agricultural Entomology.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
a. s. l.	Above Sea Level
BCAs	Biological Control Agents
BI	Botanical Insecticides
BSO	Brassica Seed Oil
CL	Confidence Limit
CSO	Cotton Seed Oil
CRD	Completely Randomized Design
CSA	Central Statistical Agency
DE	Diatomaceous Earth
EO	Essential Oils
EPA	Environmental Protection Agency
EARO	Ethiopian Agricultural Research Organization
FAO	Food and Agricultural Organization
FAOSTAT	FAO Statistical Databases
FQPA	Food Quality Protection Act
GLM	General Linear Model
IGR	Insect Growth Regulator
IPM	Integrated Pest Management
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
LC ₅₀	Lethal Concentrations that kills 50 percent of the test animals/insects
LD ₅₀	Lethal Doses that kill 50% of the test insects
LT ₅₀	Lethal Time at which 50% of the test animals dies
MC	Moisture Content
MLT	Median Lethal Time
MSD	Malathion Super Dust
RH	Relative Humidity
TDP	Total Development Period
WHO	World Health Organizations

TABLE OF CONTENTS

CONTENTS	PAGES
APPROVAL SHEET.....	i
DEDICATION.....	ii
STATEMENT OF THE AUTHOR	iii
BIOGRAPHICAL SKETCH	iv
ACKNOWLEDGEMENTS	v
LIST OF ACRONYMS AND ABBREVIATIONS	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF TABLES IN APPENDIX.....	xiv
ABSTRACT	xvi
1.INTRODUCTION	1
2. LITERATURE REVIEW	8
2.1. Post Harvest Insect Pests of Maize	8
2.2. Bio-ecology of <i>Sitophilus zeamais</i> and <i>Sitotroga cereallela</i>	9
2.2.1. General description of <i>Sitophilus zeamais</i>	9
2.2.1.1. Life history of <i>Sitophilus zeamais</i>	10
2.2.1.2. Distribution of <i>Sitophilus zeamais</i>	11
2.2.1.3. Economic importance of <i>Sitophilus zeamais</i>	11
2.2.1.4. Host range of <i>Sitophilus zeamais</i>	12
2.2.2. General description of <i>Sitotroga cereallela</i>	12
2.2.2.1. Life history of <i>Sitotroga cereallela</i>	13
2.2.2.2. Distribution of <i>Sitotroga cereallela</i>	14
2.2.2.3. Economic importance of <i>Sitotroga cereallela</i>	14
2.2.2.4. Host Range of <i>Sitotroga cereallela</i>	15
2.3. Post harvest Insect Pest Control in Maize	16

TABLE OF CONTENTS (CONTINUED)

2.3.1. Cultural control of stored-product insect pests	16
2.3.2. Pheromones	16
2.3.3. Varietals resistance	17
2.3.3.1. Antixenosis	18
2.3.3.2. Antibiosis	18
2.3.3.3. Tolerance	19
2.3.4. Chemical control of stored-product insect pests	19
2.3.5. Biological control of stored-product insect pests	20
2.3.6. Use of botanicals against maize storage insect pests	22
2.4. Classification of Bio-potential Plant Products	23
2.5. Description of the Botanicals Used	24
2.5.1. Neem, <i>Azadirachta indica</i> A. Juss	24
2.5.2. Mexican marigold, <i>Tagetes erecta</i> L.	26
2.5.3. Garlic, <i>Allium sativum</i> L.	26
2.5.4. Lemon grass, <i>Cymbopogon citratus</i> DC Stapf	27
2.5.5. Kelewa/Abayi, <i>Maesa lanceolata</i> Forsskal	27
2.5.6. Kebericho, <i>Echinops kebericho</i> Mesfin	28
2.5.7. Ethiopian mustard, <i>Brassica carinata</i> A. Braun	29
2.5.8. Gime, <i>Chenopodium ambrosoids</i> L. Leaf	30
2.5.9. Cotton, <i>Gossypium hirsutum</i> L. Seed oil	31
2.5.10. Malathion dust (5%) formulation	32
3. MATERIALS AND METHODS	33
3.1. Description of the Study Area	33
3.2. Maize Grain Used for the Experiment	33
3.3. Collection and Preparation of Botanicals and Cooking Oils	33
3.4. Rearing of the Experimental Insects	36
3.5. Experimental Designs and Treatments	36
3.6. Bio-Assay Procedures	37

TABLE OF CONTENTS (CONTINUED)

3.6.1. Adult Mortality Test	37
3.6.2. Progeny Emergence Test	38
3.6.3. Damaged seeds (seeds with holes)	38
3.6.5. Grain Weight Loss	38
3.6.6. Germination Percentage (Viability Index) Test	38
3.7. Bio-assay for the Best Treatments (Cooking Oils)	39
3.8. Data Analysis	39
4. RESULTS AND DISCUSSIONS	40
4.1. Experiment on <i>Sitophilus zeamais</i>	40
4.1.1. Cumulative Toxicity of Botanicals and Cooking Oils	40
4.1.2. <i>Sitophilus zeamais</i> Progeny Emergence	43
4.1.3. Maize grain damage by <i>Sitophilus zeamais</i>	45
4.2. Experiment on <i>Sitotroga cereallela</i>	48
4.2.1. Cumulative Toxicity of Botanicals and Cooking Oils	48
4.2.2. <i>Sitotroga cereallela</i> Progeny Emergence	51
4.2.3. Maize Grain Damage by <i>Sitotroga cereallela</i>	53
4.3. Toxicity of (Cumulative) <i>G. hirsutmn</i> and <i>B. carinata</i> Seed Oils against <i>Sitophilus zeamais</i>	55
4.4. <i>Sitophilus zeamais</i> Progeny Emergence after Grains Treated with <i>G.</i> <i>hirsutmn</i> and <i>B. carinata</i> seed oils	57
4.5. Maize Grain Damage by <i>Sitophilus zeamais</i> after Treated with <i>G. hirsutmn</i> and <i>B. carinata</i> Seed Oils	59
4.6. Toxicity of (Cumulative) <i>G. hirsutmn</i> and <i>B. carinata</i> Seed Oils against <i>Sitotroga cereallela</i>	60
4.7. Progeny Emergence of <i>Sitotroga cereallela</i> after Grains Treated <i>G. hirsutmn</i> and <i>B. carinata</i> Seed Oils	61

TABLE OF CONTENTS (CONTINUED)

**4.8. Maize Grain Damage by *Sitotroga cereallela* after Treated with *G. hirsutmn*
and *B. carinata* Seed Oils63**

5. SUMMARY AND CONCLUSIONS65

6. RECOMMENDATIONS66

REFERENCES68

APPENDIX81

LIST OF TABLES

TABLES	PAGES
Table 1. Description of botanicals and cooking oils used in the experiments	344
Table 2. Percent adult mortality (cumulative) and Median Lethal Time (LT ₅₀) of botanicals and cooking oils against <i>Sitophilus zeamais</i>	455
Table 3. <i>Sitophilus zeamais</i> progeny emergence from maize grains treated with botanicals and cooking oils at different intervals (days).....	466
Table 4. Grain hole number, weight loss and germination percentage of maize grains infested with <i>Sitophilus zeamais</i>	477
Table 5. Pearson correlation coefficients among different variables of maize grains infested by <i>Sitophilus zeamais</i>	488
Table 6. Percent adult mortality (Cumulative) and Median Lethal Time (LT ₅₀) of botanicals and cooking oils against <i>Sitotroga cereallela</i>	511
Table 7. Progeny emergence of <i>Sitotroga cereallela</i> from maize grains treated with botanicals and cooking oils at different time interval (days)	523
Table 8. Grain hole numbers, percent weight loss and percent germination of maize grains infested by <i>Sitotroga cereallela</i>	544
Table 9. Pearson correlation coefficients for maize grains infested by <i>Sitotroga cereallela</i>	55
5	
Table 10. Percent adult mortality (Cumulative) and Median Lethal Time (LT ₅₀) of different concentrations of <i>Gossypium hirsutum</i> and <i>Brassica carinata</i> against <i>Sitophilus</i>	

<i>zeamais</i>	566
.....	566
Table 11: <i>Sitophilus zeamais</i> Progeny emergence from maize grains treated with different concentrations of <i>G. hirsutum</i> and <i>B.carinata</i> seed oils at different time intervals (days)	58
.....	58
9	
Table 12. Hole number counted, percent weight loss and percent germination as infested by <i>Sitophilus zeamais</i>	60
.....	60
Table 13. Adult mortality (Cumulative) and Median Lethal Time (LT50) of <i>Sitotroga cerealella</i> by different concentrations of <i>Gossypium hirsutum</i> and <i>Brassica carinata</i> seed oils	61
.....	61

LIST OF TABLES (CONTINUED)

Table 14. <i>Sitotroga cerealella</i> progeny emergence from maize grains treated with different concentrations of <i>G. hirsutum</i> and <i>Brassica carinata</i> Seed Oils at different time intervals (days)	633
.....	633
Table 15. Table 15. Hole number counted, percent weight loss and percent germination of maize grains treated with different concentrations of <i>G. hirsutum</i> and <i>Brassica carinata</i> Seed Oils as infested by <i>Sitotroga cerealella</i>	644
.....	644

LIST OF FIGURES

FIGURES	PAGES
Figure 1: The processed botanical powders used against <i>S. zeamais</i> and <i>S. cereallela</i> in the experiment	355
Figure 2. Percent adult mortality of <i>Sitophilus zeamais</i> by botanicals and cooking oils at different time intervals (days)	422
Figure 3. Percent adult mortality (Cumulative) of <i>Sitotroga cereallela</i> by botanicals and cooking oils at different time intervals (days)	50
Figure 4. Percent adult mortality (Cumulative) of <i>Sitophilus zeamais</i> by different concentrations of <i>G. hirsutmn</i> and <i>B.carinata</i> seed oils at different time intervals (days)	58

Figure 5. Percent adult mortality (Cumulative) of *Sitotroga cereallela* by different concentrations of *G. hirsutmn* and *B.carinata* seed oils at different time intervals (days)
.....62

2

LIST OF TABLES IN APPENDIX

APPENDIX	PAGES
Annex 1. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 1 day exposures.....	82
Annex 2. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 2 days exposures.....	82
Annex 3. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 3 days exposures.....	83
Annex 4. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 4 days exposures	83
Annex 5. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 5 days exposures	84

Annex 6. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 10 days exposures.....	84
Annex 7. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 15 days exposures.....	85
Annex 8. ANOVA table for showing percent mortality of <i>Sitophilus zeamais</i> at 20 days exposures	85
Annex 9. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 1 day's exposures.....	86
Annex 10. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 2 days exposures.....	86
Annex 11. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 3 days Exposures.....	87
Annex 12. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 4 days exposures.....	87
Annex 13. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 5 days exposures.....	88

LIST OF TABLES IN APPENDICES (CONTINUED)

Annex 14. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 10 days exposures.....	88
Annex 15. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 15 days exposures.....	89
Annex 16. ANOVA table for showing percent mortality of <i>Sitotroga cereallela</i> at 20 days exposures.....	89

Evaluation of Potential Botanicals against Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and Angoumois Grain Moth, *Sitotroga cereallela* Olivier (Lepidoptera: Gelechiidae) Under Laboratory Conditions

ABSTRACT

During storage, maize grains are severely destroyed and lost by insects and other pests. One of the most important causes of grain loss in stored maize is the damage caused by maize weevil, *Sitophilus zeamais* and Angoumois grain moth, *Sitotroga cereallela*. A study was conducted to evaluate selected locally available botanicals namely *A. indica* leaf, bark and kernel, *C. citrates* leaf, *T. erecta* leaf, *A. sativum* stem, *M. lanceolata* seed, *C. ambrosoids* leaf and *E. kebericho* root powder and two purified cooking oils namely, *G. hirsutmn* and *B. carinata* for their effectiveness as grain protectants against maize weevils and Angoumois grain moth at JUCAVM in 2011. The botanicals and cooking oils were compared with untreated control and Malathion super dust (5%) as standard check. The experiment was laid-out in CRD with three replications for each treatment (total of 13x3=39). Different dependent variables such as adult mortality over time, progeny emergency, grain damage in terms of weight loss, number of holes per seed and percent germination were assessed. The results revealed that there was an increase in adult mortality over time, i.e. higher mortality from powders of *C. ambrosoids* at 6.5 LT₅₀, *A. indica* leaf at 6 LT₅₀, bark at 8.4 LT₅₀ and *T. erecta* at 9.2 LT₅₀ scored 70% mortality against *S. zeamais*. Similarly, *A. indica* leaf at 18.8 LT₅₀ and *C.*

ambrosoids at 14.7 LT₅₀ caused 61.1% mortality against *S. cereallela* which is relatively higher than other treatments from the first day to 20 days exposures. Low mortality was recorded from *A. sativum* at 18.01 LT₅₀ with 50%, *C.citratus* at 18.3 LT₅₀, *M. lanceolata* at 16.2 LT₅₀ and *E. kebericho* at 14.5 LT₅₀ caused 55% against *S. zeamais*. In addition, *M. lanceolata* at 219.8 LT₅₀ and *E. kebericho* at 338.1 LT₅₀ with 27.8%, *A. indica* bark at 30.38 LT₅₀ and *C.citratus* at 171 LT₅₀ records 39% mortality against *S. cereallela*. Compared to the untreated control as well as increase in the concentration of cooking oils namely, *G. hirsutmn* scored 100% and *B.carinata* scored 90% against *S.zeamais* and 77.8% against *S.cereallela* over 20 days of exposures. The decrease in progeny emergency and grain damage in terms of grain holes number and weight loss was significantly seen by the grains treated with the two cooking oils. The two cooking oils were seen to be the best and further experiment was done by taking four application rates (0.2ml, 0.3ml, 0.4ml and 0.5ml) out of which maximum mortality and minimum adult progeny was emerged from gains treated with higher concentrations (0.4 and 0.5 ml) agains both test insects. Similar trend was registered from the rest variables. It is recommended that the two cooking oils were found to be the most potent bio-insecticides against maize weevil and grain moth on par with standard check chemical, Malathion.

Key words: Angoumois grain moth, *Sitotroga cerealella*; botanicals; concentrations; cooking oils; exposure time; grain damage; Maize weevil, *Sitophilus zeamais*; mortality

1. INTRODUCTION

Food security always remains the strategic focal point for the astute nations and the only nations with secure food sources can survive honorably in the ever-growing populations of the world. Safety of food grains is one of the most important challenges confronting the grain-handling agencies and stored product entomologists of the world, in their combat against hunger, today. Accordingly, protection of food requires as much attention as is required for its better production. It is supported by the fact that “post-harvest losses are directly proportional to the backwardness of a nation” (Muhammad, 2009).

Cereals are the only source of nutrition for one-third of the world’s population especially in developing and underdeveloped nations of Sub-Saharan Africa and South-east Asia. The three major cereals, rice, wheat and maize constitute about 85% of total global cereals production amounting to about 200 million tones of harvest annually at an average of 10% protein content, out of which a sizeable proportion goes into human consumption. Maize (*Zea mays L.*), which belongs to the grass family Gramineae, is an important cereal crop in the world, especially in Africa serving as source of food and industrial raw materials such as brewery, confectionary, livestock and flour feed mills. Maize is also known to be primary provider of calories supplying 20% of the world’s food calories. It also provides 15% of all food crop protein (Meseret, 2011). Aside from being one of the major sources of food for both human and animals, it is also processed into various food and industrial products including starches, sweeteners, oil, beverage, industrial alcohol and fuel ethanol. Moreover, thousands of foods and other everyday items such as toothpaste, cosmetics, adhesives, shoe polish, ceramics, explosives, construction materials, metal molds, paints, paper goods and textiles contain maize components. In addition, maize seed products are rapidly replacing petroleum in many industrial applications. Polylactide acid (PLA), a biodegradable polymer made from maize is being used successfully in the manufacture of a wide variety of everyday items such as clothing, packaging, carpeting, recreational equipment and food utensils (Parugrug and Roxas, 2008). Its seed grain is consumed in different ways. For example, it could be grilled, boiled, roasted or made into various products (Abdurahman, 2009).

Maize plant is regarded as versatile and with many uses since it can thrive in diverse climates. Although maize is grown at altitudes of 500 to 2400 meter above sea level (Frew and Girma, 2001), it thrives best in relatively wet and intermediate altitudes (Wale, 2006). Out of the 900 million poor consumers for whom maize is the preferred staple, 120-140 million poor families and about one-third of all are with malnourished children. Between now and 2050, the demand for maize in the developing world will double and by 2025 maize will have become the crop with the greatest production globally and in the developing world including Ethiopia (CIMMYT/IITA, 2011). In Ethiopia, maize is second in the area coverage among cereal crops, accounting for about 1.8 million hectares with average yield of 2.2 ton ha⁻¹ (CSA, 2009).

Despite the worldwide increase in the demand, production and land coverage for maize, its production is constrained by various biological, physical and environmental factors. These include the problems of insect attack, storage structures, weeds and pathogen infestation, soil fertility and climate (Odeyemi, 2008). The low productivity of maize in Ethiopia is attributable to many factors: drought, degradation of natural resources, poor state of infrastructure, insufficient technology generation, and lack of credit facilities, poor seed quality and weak extension support (Adugna and Melaku, 2001). The great majority of crop harvest in Ethiopia, as in any developing country, is stored on the farm in small quantities. The protection of farm-stored produce largely depends on the virtue of the traditional storage systems which has two major aspects namely, the storage structures and management practices. The nature of the structures mainly varies with respect to the materials they are made from, the type and amount of produce they can accommodate and their location. The management practices, although influenced by the nature of the storage structures, are largely dependent on various environmental factors and the tradition of the society (Eshetu *et al.*, 2006).

In realizing this, one of the stumbling blocks seems to be the yield losses due to pests. The important constraints of having every day sufficient food is the post harvest preservation of its quality and quantity. During storage, food grains and products are severely destroyed by insects and other pests. The general estimates of yield losses due to insects and diseases range from 5 to 10% in temperate regions and 50 to 100% in tropical regions (Van *et al.*, 2002). According to the study made by Adane (2009), an average grain damage of 29 % was caused by the pests of stored grains and that the moth is the key pest of stored maize, causing 19.7 % damage followed by *S. zeamais*

with 9.6 % damage. Grain weight loss of 12 - 20% caused by the storage pests especially *S. zeamais* and *S. cereallela* is common and even 80% loss may occur in untreated maize grains stored in traditional structures in tropical countries. Weevil damage results directly in lost food (reduced grain weight) and also may reduce future maize production for farmers who plant saved grain as seed, a practice that accounts for about 70% of all maize planted in eastern and southern Africa (Boxall, 2002). In spite of the use of all available means of plant protection, about one-third of the yearly harvest of the world is destroyed by the pests. Losses at times are so severe so as to lead to famine in large areas in many countries of the world. From this, insect pests destroy 14% of all potential food production, despite the yearly application of more than 3000 million kilograms of pesticides worldwide (Pimentel, 2007).

A wide range of insect pests, the commonest among them being under the orders of Coleoptera (beetles) and Lepidoptera (moths), attack stored products of maize (Bekele *et al.*, 1997; Emanu and Asefa, 1998; Ferdu *et al.*, 2001). In Ethiopia around Bako area, the dominant, primary storage pest of maize is *S. zeamais* followed by *S. cereallela* Olivier and rodents but each leave distinctly different patterns of damage. *Sitophilus zeamais* emerge through irregular-shaped exit holes, and under heavy infestation tunneling under the pericarp is usually visible. *Sitotroga cereallela* damage is characterized by the presence of windows in the pericarp beneath which the full-grown larva has excavated its pupation chamber; adult exit holes are circular, usually with a small piece of translucent window attached (Girma *et al.*, 2008). According to the report of Girma *et al.*, (2008), the two key maize grain storage insect pests are the maize weevil and the Angoumois grain moth. Therefore, in order to meet the food demand for the ever increasing world population, it is necessary to address the issue of maize grain loss to insect pest damage.

The damage caused by *S. zeamais* is obvious when the adult makes holes that reaches approximately 1mm in size in the grain and deposit its eggs within the hole. The insect then seals the hole with a gelatinous waxy secretion. The eggs, larval and pupal stages of the insect take place within the grain after which the emerging adult weevil bores its way out, leaving a characteristic emergence hole on the grain (Rees, 2004; Odeyemi, 2008).

The Angoumois grain moth, attack grains in storage and in the field, although most damage occurs in stored grains. Losses due to infestation by the grain moth have been increasing along with the greater yields and amount of cereal grains being stored in farmer households. The *S. cerealella* alone can account for over 40% of the total losses in stored grain in some areas (Abraham, 2000). It causes weight loss to grains by hollowing them out. The weight losses can be as much as 50% for wheat and 24% for maize (Adugna, 2007). An important factor that contributes to this serious loss of grains is the tendency of the larvae to feed inside the grains, which provides the pest additional protection from direct contact with insecticides (Boshra, 2007).

The protection of stored-maize grains against insect attack is essential, especially for countries that have inadequate storage facilities and climatic conditions that favor deterioration of grains. In addition, the priority is given to post-harvest protection studies, particularly in humid tropical climates, where at least half of the food supply may be lost between harvest and consumption (Dubey *et al.*, 2007; Parugrug and Roxas, 2008). To overcome the mentioned problems, different control measures have been developed world-wide including the use of cultural practices (such as sanitation, storing sound seed, dry grain storage), physical control (managing temperature and aeration), regular sampling to take action before the population of the insect exceeds above economic threshold levels, biological controls (by using natural enemies, predators, parasitoids and pathogenic fungi and species specific viruses such as baculo- or polyhedrosis viruses) and the use of the conventional chemical control measures (Christos *et al.*, 2010).

Synthetic chemical insecticides have been used for many years to control stored grain pests. Although synthetic insecticides and fumigants has long been a usual practice, the indiscriminate use of various insecticides from time to time created a number of risks namely, genetic resistance in pest insects, contamination of food products with toxic residues, increased cost of application, handling hazards, ecological disorders, etc. Further, the use of synthetic chemicals has been restricted due to their carcinogenicity, teratogenicity, hormonal imbalance, long degradation period, and their adverse effects on food and side effects on humans (Dubey *et al.*, 2007; Kumar *et al.*, 2007). In addition, the increased public awareness and concern for environmental safety, increased regulatory constraints, locally unavailability to be used and ventilation restrictions are the negative effects of the synthetic chemicals (Brenda *et al.*, 2010). Such alarming issues have

signified the need for some biodegradable and nature-friendly pesticides, in order to replace the undesirable chemicals. Among the alternative strategies, the use of plants or insecticidal allelochemicals appeared to be the right approach against the menace. Aromatic plants and their essential oils are among the most efficient botanical pesticides, their activities are manifold and they can induce fumigant action and topical toxicity, antifeedant or repellent effects and can also inhibit reproduction. Their modes of action depend upon the molecular patterns and are presently regarded as a new class of ecological products for controlling insect pests (Muhammad, 2009).

Recently, in different parts of the world, attention has been paid towards exploration of plant products as novel chemotherapeutants in plant protection. Because of non-phytotoxicity, systemicity, easy biodegradability and stimulatory nature of host metabolism, botanical insecticides possess the potential to be of value in pest management. Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals pose little threat to the environment or to human health (Dubey *et al.*, 2008). In the context of agricultural pest management, botanical insecticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and postharvest protection of food in developing countries (Isman, 2006). The wide-scale commercial use of plant extracts as insecticides began in the 1850s with introduction of nicotine from *Nicotiana tabacum*, rotenone from *Lonchocarpus* sp., derris dust from *Derris elliptica*, pyrethrum from the flower heads of *Chrysanthemum cinerariaefolium*, *Tagetes* sp, *Capsicum* sp, and *Lantana* sp.

Some plant families may accumulate a restricted number of anti-insect chemicals, so called secondary metabolites, whilst others possess a wide variety of different structural compounds. The synthetic pesticide approach had its beginning in the use of botanical materials (Araya, 2007).

Botanicals extracts are generally regarded as safe insecticides, which have a broad spectrum of insecticidal activity, relatively specific mode of action, low mammalian toxicity and non persistence. Moreover, their preparation and application at farm level are more convenient for the farmers. Accordingly, the botanical insecticides are used in the integrated-pest-management programs. Furthermore, the developed nations of the globe are emphasizing upon the adoption of organic farming for the conservation of the world health as well as for the development of

sustainable agriculture. Therefore, it was imperative for us to utilize botanicals for the organic control of stored-food insect pests. The strong activity of botanical oils can make them potential substitutes for the methyl bromide, which has been identified as a major contributor to the ozone depletion (Parugrug and Roxas, 2008).

Many plant materials, added directly to food commodities, may impart a taint to the processed or unprocessed food. The traditional practice of applying few botanical products to stored grains to control storage pests may appear to be acceptable. Desirable characteristics of botanicals for use in pest control whatever the end-use, would probably be that the plant is perennial, easy to grow and not expensive to produce, i.e. requiring little space, labour, water or fertiliser application. Plants should also show no potential to become weeds or the host for plant pathogens themselves and should, if possible, offer complementary economic uses. In addition, the insecticidal product should effectively control the range of pests encountered in local storage situations, be safe to use, pose no environmental hazard, be easy to extract, formulate and use with available skills. For an insecticide to be approved for use in grain-storage, it must fulfill the above ten criteria. The most important criterion is that the botanicals must not affect the quality, flavor, smell or handling of the grains (Muhammad, 2009). Now days, in developing countries including Ethiopia, there is an increasing interest and experience in the use of different types of plant products for the control of stored product insect pests because of drawbacks of conventional control measures (Dawit, 2005; Shaaya and Kostyukovysky, 2006). Therefore, there is a need to evaluate and understand the spectrum and efficacy of novel botanicals against stored-grain insects.

This study was aimed at evaluating the impact of different botanicals against the maize weevils, *S. zeamais* and Angoumois grain moth, *S. cerealella* under laboratory conditions. Hence, *Azadirachta indica* leaf, bark and kernel, *Cymbopogon citratus* leaf, *Tagetes erecta* leaf, *Allium sativum* stem, *Maesa lanceolata* seed, *Chenopodium ambrosoids* leaf, *Echinops kebericho* root in powder formulations and the two cooking oils namely, *Gossypium hirsutum* and *Brassica carinata* seed oils were used for achieving the following objectives.

General objectives:

The general objective of this study was:

⇒ To contribute to the production and productivity increase in maize by generating technologies that can decrease the effects of post harvest insect pests of maize

Specific Objectives:

⇒ To evaluate the deferential toxicity of botanicals and cooking oils against the growth of *S. zeamais* and *S. cereallela*

⇒ To determine the effective dose of the best ranked botanicals/cooking oils against *S. zeamais* and *S. cereallela*

2. LITERATURE REVIEW

In traditional societies, appreciation of trees and other vegetation was based to a large extent on the benefits to human beings including those for pest control. Plant derived chemicals such as pyrethrum, rotenone, and nicotine were used economically for pest control in the west since two to three decades, lost out to synthetic pesticides after the World War II. However, some plant species are still being used in Africa, Indo-Pakistan subcontinent and other Asian countries. Out of 2400 plants reported to have pest control properties, neem (*Azadirachta indica*), turmeric (*Curcuma longa*) and sweet flag (*Acoras calamus*) have been extensively studied during the last two decades (Iqbal, 2005; Javed *et al.*, 2010).

2.1. Post Harvest Insect Pests of Maize

The main agents causing deterioration of stored products are microorganisms, rodents, birds, insects and mites. Among these, insects are the principal pests responsible for losses to food grains. During storage, foods are currently destroyed by insects and other pests (Ngamo *et al.*, 2007). The pests of stored products are the most dangerous of all insects, because they feed on products that have been grown, harvested, sometimes manufactured and stored. Two major groups of insects harbor the most economically important post-harvest products, are included under order coleopterans (beetles) and lepidopteron (moths and butterflies). Several species under coleopteran and Lepidoptera attack crops both in the field and in storage. Stored product insect pests are a problem throughout the world, because they reduce the quantity and quality of grain. The reasons for their widespread presence range from evolutionary adaptations (morphological, physiological and behavioral) to the actions of humans who transport them throughout the world and provide a protected habitat (Pugazhvendan *et al.*, 2009).

The major pests of stored grains for rice and maize include granary weevils (*Sitophilus oryzae*, *S. zeamais*, and *Sitophilus granaries*, respectively), the Angoumois grain moth (*S. cereallela*), the lesser grain borer (*Rhyzopertha dominica*) and several species of pulse beetles (*Callosobruchus chinensis*, *Callosobruchus maculatus*, and *Acanthoscelides obtectus*). *S. zeamais*, *S. oryzae*, *A.*

obtectus, *C. chinensis*, *Zabrotes subfasciatus*, *Tribolium* and *Cryptolestes* species from the order coleopteran and *S. cereallela*, *Ephestia cautella*, *Plodia interpunctella*, *Phthorimae operculella* from order lepidoptera were recorded as major pests of stored grains (Abraham, 1991; 1996; 1997).

Post harvest pests can be primary pests that can be able to attack intact grains, such as the genus *Sitophilus*, those which possess strong mouth parts; while others are secondary pests, attacking already damaged grains or grain products such as the genus *Tribolium*, which have weak mouth parts. Those pests which are associated with stored products exhibit different behaviors of which some are primary and secondary pests which are feeding directly on the product while others are general scavengers, fungus feeders, wood borers or predators of other insects (Addis, 2008).

2.2. Bio-ecology of *Sitophilus zeamais* and *Sitotroga cereallela*

2.2.1. General description of *Sitophilus zeamais*

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is a major pest of stored maize grains in the tropics and temperate regions of the world (Ileke and Oni, 2011). The *S. zeamais* M. is one of the most serious cosmopolitan pest of stored cereal grain, especially of maize, in tropical and sub-tropical regions. In recent years, post-harvest losses to storage insect pests such as the maize weevil; have been recognized as an increasingly important problem in Africa. Cheap and effective methods for reducing *S. zeamais* damage are needed in these countries. A fundamental knowledge of the biology of *S. zeamais* is a prerequisite for devising methods of efficient control. In order to understand the biology of the maize weevil, a sound knowledge about its response to the effects of environmental and biological factors is essential. Insect oviposition behaviour is an important contributor to the fitness of insects because of the consequent effect on the number and quality of offspring. Oviposition behaviour varies according to insect species and strain, population density, environmental conditions, food, age and size of the individual. Despite the importance of *S. zeamais*, there are not any recent quantitative data describing its life history traits over the range of environmental conditions at which it develops. An understanding of the

biology and behaviour of the maize weevil in relation to grain quantity will assist in the development of improved management practices for the control of this pest (Danho *et al.*, 2002).

2.2.1.1. Life history of *Sitophilus zeamais*

The pre-oviposition period is about three days. It remains fecund throughout its lifetime but the effective egg laying period is 50% of the first 5 weeks of its life span. The female lays up to four eggs in a single maize kernel. There are four larval instars all of which remain within the grain. Immediately on hatching, the first instar feeds by burrowing through the tissues of the grain. At the end of the fourth instar the larva uses a mixture of frass and larval secretion to close off the end of the burrow, to form a pupa cell. Under normal developmental conditions, weevil larvae allow their frass to accumulate around them inside the grain in which they are feeding. However, if the carbon dioxide level exceeds 5%, the fourth instar larva makes a small hole in the grain and ejects much of the frass. The larva then assumes a pre-pupal form for a short period before transforming into the pupa. When the adult has developed, it remains inside the grain for several days before emerging, with the time varying with temperature. During this time, its cuticle hardens and matures. Adults emerge and females move to a surface above the food to release sex pheromone. Males are attracted to this pheromone for mating (Mason and Obermeyer, 2004).

The adult maize weevil may remain inside the kernel for some time after eclosion but eventually emerges by chewing its way out. After emergence from the pupae, the adult eats through the outer layer of the grain leaving a roughly circular hole approximately 1.5 mm in diameter. The sex ratio of the newly emerged maize weevil is 1:1 and female weevils live longer than male weevils. Not all excavated holes are used for oviposition; some are abandoned and others are expanded into feeding holes (Campbell, 2002). The cumulative pattern of emergence is clear that 70% of the insects emerged within the first week of initiation of emergence that is 31 - 37 days after ovipositor (Makate, 2010).

2.2.1.2. Distribution of *Sitophilus zeamais*

The maize weevil, *Sitophilus zeamais* Motschulsky is one of the most important cosmopolitan stored product pests in maize including Ethiopia. *Sitophilus zeamais* occurs throughout the warmer, more humid regions of the world, especially where maize is grown. It has also been recorded from Canada, Polynesia, Argentina, Brazil, Burma, Cambodia, Greece, Japan, Morocco, Spain, Syria, Turkey, USA, USSR and Yugoslavia. *Sitophilus zeamais* is widely distributed throughout growing areas of northern Australia (Fikremariam *et al.*, 2009). *Sitophilus zeamais* is a species of weevil that is commonly found in maize crops causing serious losses to resource-poor farmers in the tropics. Infestation by this weevil commences in the field (Girma *et al.*, 2008), but most damage is done during storage.

2.2.1.3. Economic importance of *Sitophilus zeamais*

Post-harvest losses to storage insect pests such as the maize weevil *Sitophilus zeamais* have been recognized as an increasingly important problem in Africa (Fikremariam *et al.*, 2009). Damaged grains have reduced nutritional values, low percent germination and reduced weight and market values. Worldwide seed losses ranging from 20 to 90% have been reported for untreated maize due to the maize weevil, *S. zeamais* (Fikremariam *et al.*, 2009).

Damage caused by *S. zeamais* on stored cereals can be extremely high. It is reported that up to 18.3% weight loss occurred due to *S. zeamais* infestation when single maize kernels were exposed to ovipositing adults and kept at 27 °C and 70% relative humidity for only 37 days. *Sitophilus zeamais* infestation has also resulted in significant reduction in the viability of the grains. These problems have increased as traditional crop varieties have been replaced by improved, high-yielding varieties with shorter growth cycles but which are generally more susceptible to insect damage. In Ethiopia in general, post-harvest losses caused by *S. zeamais* ranging from 20 to 30% are common, and studies in the Bako areas have shown that grain damage levels up to 100% in some samples from farm stores after 6-8 months. Insect contaminants such as excreta (uric acid), exuviate (cast skins), dead bodies, webbing and secretions in food commodities pose a quality-

control problem for food industries. Processing and end-use qualities of food commodities are also affected by insect infestation, as are cash value and marketability of products (Girma *et al.*, 2008).

2.2.1.4. Host range of *Sitophilus zeamais*

Sitophilus zeamais is commonly associated with corn and rice in tropical storage and to a lesser extent in other raw or processed cereals, including wheat, oats, barley, sorghum, rye and buckwheat. The range of moisture contents within which it will breed has been found to be much wider than that of *S. oryzae* and it has been found to attack fruit, such as apples, in storage. *Sitophilus zeamais* commonly infests standing crops prior to harvest, particularly maize, where the moisture contents can exceed 20% (Longstaff, 1981).

2.2.2. General description of *Sitotroga cereallela*

The Angoumois grain moth is one of the serious insect pests of stored grains. Its young larvae bore into grains and feed on the inside contents rendering grains unfit for human consumption. These cereals are vulnerable to this insect attack and can have either one or all deficiencies that include weight loss, reduction in nutritional value, contamination or tanning, rendering the cereal food unfit for human consumption (Saadia, 2011).

Sitotroga cereallela has a wing expanse of 13-19 mm and a length of 6-9 mm. The forewings are clay-yellow and without markings; the hind wings are grey. It is known as a pest of grain cereals, owing to the physical and chemical nature of grains (moisture content, size, texture, color and nutritional content such as fats, protein and carbohydrate) that promote susceptibility to this insect. Carbohydrate and protein contents of grains affect the developmental period, adult weight, fecundity, etc. Grain shelf life can be improved by radiation doses to its eggs (Boshra and Mikhael, 2006).

The Angoumois grain moth is a primary colonizer of stored grain in subtropical and warm temperate climates of the world. Its larvae tunnel inside the kernels, causing substantial damage and making the grain a more suitable place for reproduction of secondary insect pests (Weston and Rattlingourd, 2002). Along with the maize weevil, *S. zeamais*, the larger grain borer, *Prostephanus truncatus* (Horn) and the lesser grain borer, *Rhyzopertha dominica* (Fabricius), are among the most damaging part of insect pests complex in maize stores of small-scale farmers in West Africa (Meikle *et al.*, 2002).

For *Sitotroga cereallela*, the life history data on maize grain are only available from India and USA (Lise, 2004). A comparison of these two studies reveals that great variation exists among different strains with respect to the effect of temperature on important life history parameters of the pest. *S. cereallela* is known as a major pest across the continent and has been recorded in some countries in Africa including Togo, Nigeria, Ghana, Cameroons, Ethiopia and Zimbabwe (Lise, 2004).

2.2.2.1. Life history of *Sitotroga cereallela*

According to the report by Javed *et al.*, (2010), the active period of the adult grain moth is from April to October in Pakistan. In nature, several generations of the grain moth are completed in a year and the life cycle completes in about five weeks at optimal temperatures (30-32⁰C). Ideal temperature for its development is 30 - 32oC at a relative humidity of 75% (Navarajan, 2007; Nadeem, 2010). A population of *S. cereallela* multiplies up to 112.27 numbers in average between two successive generations (Osama and Mohamed, 2012).

The moths mate immediately after emergence. The female lays eggs singly or in clumps on the grain. A female can lay up to 200 eggs during a life span of 5 – 10 days. The larva upon hatching bore into a grain and completes its development entirely within a single grain. At 30°C and 80% r.h. the larval development is completed in about 19 days and the pupal stage lasts about 5 days and the total life cycle is completed in about 28 days (Navarajan, 2007).

The Angoumois grain moth deposits its eggs singly or in groups on or near grain, and newly hatched larvae burrow into the kernels or enter through cracks in the pericarp. Larval-pupal development is completed within the kernel, and pupation occurs in a silk-lined chamber in the burrow. Before pupation, the larva cuts a channel to the outside, leaving only a weakly fastened flap of pericarp through which the adult moth will emerge. The life cycle of this insect varies with temperature, relative humidity, and diet. The total development time of this insect, from egg to adult, was completed in 25 days when reared in sorghum at 30°C and 70% RH. Total development time was 28 days when the insects were reared on corn kernels mixed with some flour at 30 °C and 80% RH and 36 days when the insects were reared in corn at ambient temperature and relative humidity (Joel *et al.*, 2004).

Proportion of egg survivorship varied with temperature and relative humidity; the interaction was not significant. Survivorship was most variable at the transition temperatures of 15 and 35 °C. Duration of the egg stage varied with temperature but not with relative humidity; the interaction was not significant. So, we described duration of egg development as a function of temperature. The shortest incubation periods occurred at 30 and 35°C, and incubation period increased as temperatures decreased below 30 °C (Joel *et al.*, 2004).

2.2.2.2. Distribution of *Sitotroga cereallela*

Sitotroga cereallela is cosmopolitan in distribution and so they appear to live in the temperate and tropical countries (Javed *et al.*, 2010).

2.2.2.3. Economic importance of *Sitotroga cereallela*

Sitotroga cereallela is one of the serious insect pests and most destructive internal feeder of stored grains (Mohamed, 1998). Its young larvae bore into grains and feed on the inside contents rendering grains unfit for human consumption. This species is a serious primary pest that mainly

attacks maize, wheat and sorghum, both in the field and in stores. Infestation with *S. cereallela* starts in the field as females lay their eggs on the developing grains.

In paddy rice, two major insect pests with the most potential economic impact are *S. cereallela* [Oliver] and *Rhyzopertha dominica* [F.]. These storage insect pests represent the major threats as primary grain insects whose larvae feed entirely inside the kernel of the grain and eat from inside becoming more tolerant to fumigation as diffusion of gas into kernels is severely restricted. Infestations with such a primary insect pests are critically more damaging to stored grains than secondary insect pests that eat grains from outside (Manuel *et al.*, 2008).

Larvae start feeding inside the grains, while still in the milk stage, and spend their entire life inside the grain. Thus, it is difficult to detect infestation at this stage. Adults leave a conspicuous emergence hole at one end of the kernel. Stored grains may be completely destroyed. Adults are active fliers, thus, they are able to infest neighboring granaries, which is known as "cross-infestation (Sadia *et al.*, 2011).

The economic losses caused by this insect have been reported to range from 13.1 to 24.0 % (Moore *et al.*, 1966 and Mansoor-ul *et al.*, 2004). This insect alone can account for over 40% of the total losses in stored grain in some areas. An important factor that contributes to this serious loss of grains is the tendency of the larvae to feed inside the grains, which provides the pest additional protection from direct contact with insecticides (Boshra, 2007). Although some chemical controls against stored-product insect pests are currently the most cost-effective and widely-used methods, their poor efficiency shows a major challenge in minimizing the damage brought about by these pests (Feng-Lian *et al.*, 2012).

2.2.2.4. Host Range of *Sitotroga cereallela*

Angoumois grain moth larvae feed on a number of whole kernel grains. It attacks all types of cereal grains, particularly wheat, maize and sorghum, where weight losses can be as much as 50% (Osama and Mohamed, 2012).

2.3. Post harvest Insect Pest Control in Maize

Pest control strategies include: cultural, host plant resistance, chemical, mechanical, genetically and biological control methods and are, in practice for the insect pest management in crops (Nadeem, 2010).

2.3.1. Cultural control of stored-product insect pests

There are so many cultural control methods used by farmers that will reduce infestation of the crop and they may fall into three groups: (1) special practices like the smokes of few plant parts like *Echinops kebericho* root, (2) use of material such as ashes (for its abrasive and lethal effect on the insects cuticle), mineral and oil in which physical barrier effects are responsible for the control of insects, and (3) use of whole or parts of the plants where there may be some natural insecticidal, fungicidal or repellent effect (mainly alcohols, alkaloids and terpenes) (Etana, 2007).

For a better control, the best strategy involve timely harvesting and then proper drying and cleaning of the maize prior to storage. It is also important to prepare all storage structure in advance and containers to be used, by making them ready and safe to receive the new crop. Some recommendations depend upon the type of container structures such as metal, earth ware, washing plastic containers, disinfecting with hot water and drying well before using. The sacks of jute, sisal and nylon should be washed and boiled in hot water to kill off insect pests or their eggs and larvae and then dried prior to use (Danilo, 2003).

2.3.2. Pheromones

Semiochemicals determine insect life situations such as feeding, mating and egg-laying (ovipositing). Semiochemicals are thus potential agents for selective control of pest insects. Biological control with pheromones or kairomones can be used for detection and monitoring of insect populations. Monitoring is important for the efficient use of conventional insecticides.

Mating disruption by use of pheromones is a promising and, in many cases, a successful strategy for control (confusion strategy). The use of Semiochemicals as feeding deterrents is another strategy. The most common strategy for control by the use of Semiochemicals is to attract, trap and kill the pest insects. The olfactory system of insects is very sensitive and limited amounts of Semiochemicals are needed for control. This is demonstrated by the current application of pheromones for control (mating disruption by confusion strategy) of codling moth (*Cydia pomonella*) in apple orchards (Martha, 2010).

The use of pheromones is one of several modern techniques that shows promise in controlling stored-product insects. Stored products therefore present a unique pheromone trapping situation in contrast to the higher insect tolerances that are allowed in forest and field crops. Food-processing and warehouse managers will now be able to use pheromones to trap these elusive insects. The managers view the use of the pheromone traps as part of a progressive pest-suppression program. This insect detection and monitoring program aids sanitation and quality control personnel in evaluating their insect control program (Wendell, 1985).

2.3.3. Varietals resistance

Insect resistance in crop varieties refers to their inherent ability to combat specific insect pests and to achieve better performance over other varieties of the same crop at the same levels of insect populations. Crop varieties differ in their susceptibility to storage insect pests. Traditional varieties are more resistant than new varieties. For example, resistant varieties reduced weight loss from damage by maize weevil by 9.62% compared to 15.79% loss in the checks (Tefera *et al.*, 2010). Resistant varieties function in insect control based on the mechanism of non-preference (antixenosis) and antibiosis, in which biophysical or biochemical factors are involved (Martha, 2010).

It is necessary for factors which influence susceptibility to be elucidated so as to provide information to maize breeders. This will enable them to combine a high degree of resistance with

good grain quality. Obviously the potential yield of any variety is the most important consideration in deciding whether or not to grow it (Makate, 2010).

An increasing number of F1 progeny resulted in an increasing seed damage and seed weight loss. It was found that there is an inverse relationship between susceptibility index and percent mortality and median developmental time; however, the numbers of F1 progeny, percent seed damage and seed weight loss were positively related with the susceptibility index. The use of resistant varieties should be promoted in managing *S. zeamais* in stored maize under subsistence farming conditions in Africa (Fikremariam *et al.*, 2009).

Host plant resistance is a very good method of combating pest in storage. It is perhaps the easiest, most economical and effective means of controlling insect pests on stored grains as there is no special technology which has to be adopted by farmers. Screening of many seed varieties had led to the successful isolation of strains that are resistant to insect pests in some African countries (Martha, 2010).

2.3.3.1. Antixenosis

Oviposition may be affected by small differences in seed coat smoothness and convexity, by plumpness or wrinkling and perhaps by size and hardness of the seed as well as its odor. The cowpea beetle (*Callosobruchus maculatus*) prefers smooth seeded to rough seeded cowpeas. Moreover, it doesn't oviposit on seed hilum, which is spongy in texture deep pit like and rich fibrils. Scanning electron microscopy revealed deep pit in rough coated but not in smooth coated seeds; seeds infested with eggs were less attractive for further oviposition (Donald *et al.*, 2009).

2.3.3.2. Antibiosis

Antibiosis is the mechanism whereby the pests feed but factors in the plant have an adverse effect on them usually expressed as reduced growth, rate of multiplication or on survival. Level of resistance to pests varies among plant varieties (John *et al.*, 1994; Donald *et al.*, 2009).

2.3.3.3. Tolerance

Tolerance is the production of plant biomass in spite of insect attack. Antibiosis and antixenosis are resistance mechanisms that measure the effect of the plant on the insect, whereas tolerance is the effect of the insect on the plant (the less damage, the higher the level of tolerance). Tolerance should be more useful in a pest management program than antibiosis or antixenosis because of compatibility with other control strategies and biotype considerations. Numerous reasons exist for investigating tolerance independently from antibiosis and antixenosis. Since by definition, tolerance is a plant response and does not by itself affect insect behavior, reproduction, growth, or development, this mechanism should not exert selection pressure on the insect population for new biotypes in the way that both antibiosis and antixenosis can (John *et al.*, 1994).

2.3.4. Chemical control of stored-product insect pests

Insect pest control in stored food products relies heavily on the use of synthetic insecticides and fumigants. The components of chemical insecticides can be classified into four chemical types: organochlorines, organophosphates, carbamates and pyrethroids. But, organochlorines are now banned completely because of their risk and hazardous environment due to their persistency. These groups of insecticides have been used for over five decades to control insect pests both at the field and in storage conditions. Insecticidal application is one means of preventing some losses during storage. However, the choice of insecticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food and also the continuous use of chemical insecticides for control of storage insect pests has led to problems such as disturbance of the environment, pest resurgence, pest resistance and lethal effect on non-target organisms in addition to toxicity to the users (Martha, 2010).

Alpha-terthienyl, a naturally occurring secondary plant metabolite is found in abundance in the roots of *Tagetes species* (family Asteraceae). It is activated by ultraviolet light and is toxic to a number of insect species. It generates oxygen radical species and has capacity to inhibit several

enzymes like both in vivo and in vitro. Alpha-terthienyl possesses all the desirable properties of a good insecticide/ pesticide. It is fast acting, non-toxic, economic and a property of degradation makes it more users friendly and safe. Secondary plant metabolites will play an important role in future insecticide development programme (Manish, 2001).

2.3.5. Biological control of stored-product insect pests

There are several ways that natural enemies can be used in biological control. The natural enemies may be predators, parasitoids or pathogens. Predators such as spiders, ladybirds, lacewings or predatory mites, usually feed on a range of different insects. Parasitoids lay eggs on one host insect, and the larvae live and feed on the host, which dies (true parasites do not kill their hosts). The adult parasitoids are typically honey feeders. Pathogens may be bacteria, fungi, viruses, nematodes or protozoa (Christos *et al.*, 2010).

Biological control constitutes the use of predators and parasitoids present in the prevailing environment or their introduction from the other areas into places, where they are not already present. The method of biological control, with predators and parasitoids had the advantage over other methods of pest control and is gaining popularity in integrated pest management of crops over the prevailing methods (Nadeem, 2010).

Biological control is self perpetuating and does not need further expenditures, as the population of biological control agents is once established in a particular area, it stays as such there for a longer period and hence it become an economical method of pest control as compared to others (Flinn and Hagstrum, 2001). In biological control, the conservation, augmentation and redistribution are the three main steps. Effective biological control management requires to introduce more than one species and to maintain the predator-prey relationship. Success in biological control can be achieved by introducing exotic species of parasites and predators, conserving the existing population and by augmenting parasites and predators through the mass rearing and releases (Christopher, 2010).

Biological control as a major component of integrated pest management is perhaps one of the few methods of insect control where costs can be directly offset by increased labor in the form of diligent sanitation, frequent monitoring of the crop or commodity, conscientious cultivation and storage practices, and application of all locally available, compatible control methods (Christopher, 2010).

Classical biological control of field pests in which the natural enemies of imported pests are identified in the pests indigenous to environment and then cultured and released in the adopted habitat has become a common practice, although success varies greatly from case to case. As the name implies, classical biological control was the initial mode of biological control employed in most parts of the world. In contrast to inundative control, it is an attempt to establish a biocontrol agent in the target ecosystem by encouraging it to reproduce and maintain a population alongside the pest, which is why it is also referred to as inoculative biological control (Christopher, 2010).

There is great potential for using biological control to control pests in stored products. Research is continuing to determine the proper prescriptions for use of natural enemies in stored grain. Behavioral, ecological and physiological data are being collected that will facilitate effective deployment of parasitoids and predators. Storage situations other than grain bins, such as feed mills, food warehouses and food factories may be targeted areas for biological control in the future. As part of an IMP system for stored product management, biological control should help reduce the use of pesticides on food and provide for high quality food products (Shadia, 2011).

Biological control is an over-looked component of integrated pest management of stored product pest. Many species of insect natural enemies occurs in stored product ecosystem and these species represent potential biological control agents for the desired pests. The anthocorid bug, *Xylocoris flavipes* Reuter is a cosmopolitan predator of different prey (pests) of stored commodities namely, *Tribolium castaneum*, *T. confusum*, *Crytolestes Pusillus*, *Rhizopertha dominica* and *Trogoderma granarium* (Rahman *et al.*, 2009).

Important natural enemies include parasitoid wasps in the family's Braconidae, Ichneumonidae, pteromalidae and Bethylidae and predatory pirate bugs. A collection of other Predators can be

found in some situations and these include assassin bugs, blister beetles, pseudoscorpions and predatory mites (Shadia, 2009).

Hymenopterous parasitoid of stored product pests, *Anisopteromalus calandrae* (Hymenoptera: *Pteromalidae*), (Howard) the larval ectoparasitoid is classified as *Hymenoptera* (order); *Apocrita* (suborder); *Chalcidoidea* (superfamily); *Pteromalidae* (family); *Pteromalinae* (subfamily); and *Pteromalini* (tribe). The *Pteromalid* parasitoid, *A. calandrae* H. may be an effective biological control agent if it is introduced in sufficient numbers at the beginning of the storage period so as to suppress the initial increase of maize weevil populations. *A. calandrae*, a cosmopolitan parasitoid, attacks several beetle pests of stored products, including the maize weevil, *S. zeamais* (Parichat *et al.*, 2010).

The limited knowledge and practice of biological control of storage pests contrasts sharply with the numerous successful discoveries and applications of biological control of field pests. The reduction in market price/grade of stored commodities infested beyond the accepted and regulated number of insects or insect parts per bushel has historically discouraged the development and application of biological control of stored product protection, even if the entomophages can prevent actual commodity damage (Parichat *et al.*, 2010).

2.3.6. Use of botanicals against maize storage insect pests

Botanical insecticides can be broad spectrum in activity, relatively safe to use, unique in mode of action and easy to process and apply. Tissues of higher plants contain arrays of biochemicals, known as “secondary plant chemicals” (or allelochemicals), which are defensive in function. They include alkaloids, steroids, phenolics, saponins, resins, essential oils, various organic acids and other compounds (Faric, 2006).

Mixing of five botanical products i.e. neem kernel powder, neem leaf powder, eucalyptus leaf powder, *sarifa* leaf powder and *lantana* leaf powder at the rate of 1.0 and 2.0 parts (w/w) per 100 parts of maize and paddy grains proved to be protectants against *S. cerealella* causing adverse effects on development i.e. less per cent of grain damage and less percentage of adult emergence. The germination of treated seeds was not impaired in any case during the exposure period of about

eight months (Yadu *et al.*, 2000). Taponjyou *et al.*, (2002), reported that the toxicity of an essential oil depends on the biological active plant components present in the species and on the treated insects.

Botanical products for the control of storage insect pests can be collected either from the whole plant or from specific parts like seed, leaf and bark by extraction. The most predominant way of using plants in post-harvest protection is the admixture of powders, oils and more purified insecticides including use of essential oils and organic solvent extract of plant parts as fumigants and repellents (Shaaya and Kostyukovsky, 2006). Khan and Marwat (2006), studied the leaves, seeds and bark of neem *Azadirachta indica* and leaves, bark and flowers of kanair (Nerium oleander) in powder form for their repellency against *Rhizopertha dominica* in wheat grains that significantly deterred/repelled the *Rhizopertha dominica* in wheat grains. Plant materials with insecticidal properties provide small-scale farmers with locally available, biodegradable and inexpensive method of pest control for storage. The plants of original insecticides have drawn attention for extensive research, which are now highly encouraged in order to meet the demands of IPM and environmental safety (Mulungu *et al.*, 2007).

Dawit and Bekele (2010), studied continuous application of synthetic insecticides arise development of resistance and pollution of the environment. Laboratory experiments were conducted to test the efficacy of products of orange (*Citrus sinensis*) peels in the control of the stored products beetle *Zabrotes subfasciatus* (L) in stored haricot beans (Latin name). Different levels of the extracts and essential oil of *C. sinensis* was tested. Conventional synthetic insecticide, Pirimiphos-methyl, was used as a standard check. Toxicity potential of different extracts of *C. sinensis* was tested against *Z. subfasciatus*. However, essential oils at highest rate of 750mg applied at 3ml per filter paper gave 100 % mortality after 24 h. Beans treated with 15g of sun dried powder of orange peel and 750mg of essential oil killed 65% and 67% of *Z. subfasciatus* after 96 hours.

2.4. Classification of Bio-potential Plant Products

On the basis of physiological activities on insects, conventionally plant components are classified in to 6 groups, namely repellents, feeding deterrents/ anti-feedants, toxicants, growth retardants,

chemosterilants and attractants (Jacobson, 1982). However, the bio-potential plant products might also be classified as repellants (derive away and make oriented movements away from the source of stimulus), feeding deterrents / anti-feedants (chemicals that inhibit feeding, although do not kill the insect directly), toxicants (directly kill insects), grain protectants (as a kind of natural protectant to protect stored grains), reproduction inhibitors (ground plant parts, extracts, oil and vapours also suppressed fecundity and fertility of many insects), insect growth and development inhibitors (plant extracts shows deleterious on the growth and developments of insects and reduced larval, pupal and adult weight significantly, lengthened the larval and pupal periods and reduced pupal recovery and adult eclosion). The crude extract also retarded development and caused mortality of larvae, cuticle melanization and high mortality in adults (Faric, 2006).

The most common methods comprised by botanicals are the Contact, Fumigation, growth inhibition, antifeedant, repellent and nutritional bioassays (Kim, *et al.*, 2003). The contact and residual toxicity of more than 30 plant extracts were investigated on larvae of Colorado beetle. The botanical extracts from species in *Brassicaceae*, *Asteraceae* and *Cruciaceae* showed appreciable levels of larval toxicity, antifeedant and larvicidal activity of acetone, chloroform, ethyl acetate, hexane and methanol suggest their potential against Lepidopteron (Kamaraj *et al.*, 2008). The presence of certain chemicals in plants prevents insects from feeding which leads to starvation of the insects and in some cases, eventual mortality may occur. Essential oils from some members of the Lamiaceae induced 90% mortality in adult populations of the maize weevil, rice weevil, cowpea weevil and *Sitotroga cereallela* after 24 hours of exposure to a concentration of 1.4-4.5 μ l l⁻¹ (Shaaya *et al.*, 1997).

2.5. Description of the Botanicals Used

2.5.1. Neem, *Azadrachta indica* A. Juss

Two types of botanical insecticides can be obtained from seeds of the Indian neem tree, *Azadrachta indica* (Meliaceae). Neem oil, obtained by cold-pressing seeds, can be effective against soft-bodied insects and mites, but is also useful in the management of phytopathogens. Apart from the physical

effects of neem oil on pests and fungi, disulfides in the oil likely contribute to the bioactivity of this material. More highly valued than neem oil are medium-polarity extracts of the seed residue after removal of the oil, as these extracts contain the complex *triterpene azadirachtin*. Neem seeds actually contain more than a dozen azadirachtin analogs, but the major form is azadirachtin and the remaining minor analogs likely contribute little to overall efficacy of the extract. Seed extracts include considerable quantities of other triterpenoids, notably salannin, nimbin, and derivatives thereof. The role of these other natural substances has been controversial, but most evidence points to azadirachtin as the most important active principle. Neem seeds typically contain 0.2% to 0.6% azadirachtin by weight, so solvent partitions or other chemical processes are required to concentrate this active ingredient to the level of 10% to 50% seen in the technical grade material used to produce commercial products (Isman, 2006).

Azadirachtin has two profound effects on insects. At the physiological level, azadirachtin blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdysis in immature insects. In adult female insects, a similar mechanism of action leads to sterility. In addition, azadirachtin is a potent antifeedant to many insects. The discovery of neem by western science is attributed to Heinrich Schmutterer, who observed that swarming desert locusts in Sudan defoliated almost all local floras except for some introduced neem trees. Indeed, azadirachtin was first isolated based on its exceptional antifeedant activity in the desert locust, and this substance remains the most potent locust antifeedant discovered to date. Unlike pyrethrins, azadirachtin has defied total synthesis to this point (Isman, 2006). Similar to the chinaberry, neem is exotic to Ethiopia and grows very well in warmer areas like Dire Dawa, Werer and Gambella (Eshetu *et al.*, 2006).

Rahman and Faric (2006), studied the bio-efficacy of the extracts, powders, ashes and oils of nishinda, (*Vitex negundo* L.), eucalyptus (*Eucalyptus globulus* Labill.), bankalmi (*Ipomoea sepiaria* K.), 'Neem' (*A. indica* L.), safflower (*Carthamus tinctorius* L.), sesame (*Sesamum indicum* L.) and of 'bablah' (*Acacia arabica* L.) against *C. maculatus* F., fed on *Vigna mungo* seeds, for the oviposition-inhibition, surface-protection, residual-toxicity and direct-toxicity. The results showed that least number of F1 adults, emerged from black gram seeds, treated with 'Neem'

oils, as compared to others. The oil treatment did not, also, show any adverse effect on the germination capacity of seeds, even after three months of the treatment.

2.5.2. Mexican marigold, *Tagetes erecta* L.

There are two basic types of marigold: the large-flowered American (also referred to as African) marigold *Tagetes erecta* and the smaller-flowered French marigold *Tagetes patula*. A less well known species, *Tagetes tenuifolia* has smaller flowers and leaves than most other marigolds. Yellow, orange, golden or bicolored flowers are held either well above the fine-textured, dark green foliage or tucked in with the foliage, depending on the cultivar. They brighten up any sunny area in the landscape and attract attention. As flowers die, they hang on the plants and detract from the appearance of the landscape bed. Cut them off periodically to enhance appearance. Marigolds may be used as a dried flower and are planted 10 to 14 inches apart to form a solid mass of color. Some of the taller selections fall over in heavy rain or in windy weather (Edward, 2007). *Tagetes erecta* is a potential plant whose essential oil from flowers has been effective repellent against insects (Ray *et al.*, 2000).

2.5.3. Garlic, *Allium sativum* L.

In Traditional Chinese Medicine, garlic is known as *dasuan*. It is considered a warm, bitter herb with particular effects on the Large Intestine, Spleen and Stomach meridians. It is used to lower blood pressure, to treat parasitic infections, food poisoning and tumors, and as a mild anticoagulant. It is traditionally contraindicated in patients with a yin deficiency³⁻⁵. Arabian herbalists use garlic to treat abdominal pain, infantile colic, diarrhea, diabetes, eye infections, snake bites, dandruff and tuberculosis⁶. African herbalists use garlic to treat respiratory infections and helminthic infections; many African families use garlic oil drops to treat childhood ear infections. In Ayurvedic medicine, garlic is used to treat respiratory problems, ulcers, colic and flatulence, and garlic oil drops are used to treat earaches. Several folk traditions recommend garlic as an emmenagogue or to induce

abortions. In attempts to reduce the losses caused by the moth *Sitotroga cerealella* and to suppress its populations, the fumigant activities, behavioral influence and ovipositional inhibition of garlic (*Allium sativum*) essential oil and its two major components, diallyl disulfide and diallyl trisulfide, were investigated against the adult grain moth. Their effects on reduction in survival of first instar larvae to adult emergence were also evaluated. Results showed that these three materials (garlic essential oil, diallyl disulfide and diallyl trisulfide) had significant fumigant activity with 50% lethal concentration values at 1.33, 0.99, and 1.02 $\mu\text{L}/\text{L}$ air space, respectively (Feng-Lian, 2012).

2.5.4. Lemon grass, *Cymbopogon citratus* DC Stapf

Lemon Grass is a tall tropical grass belonging to the family Poaceae. It is an aromatic tropical plant with long, slender blades that can grow to a height of 5 ft (1.5 m). It is grown throughout the world. The plant is believed to have a wide range of therapeutic effects including antibacterial, antifungal, and fever-reducing effects and also antimutagenic properties. It contains 75 to 80% citral, an essential oil constituent that attributes to insecticidal, fungicidal and bactericidal activities of the plant (Paranagama *et al.*, 2003). Moreover, powder, solvent extracts and the essential oil of *C. citratus* have been known to have insecticidal activities against *C. maculatus*, *C. chinensis*, *C. rhodesianus*, *Sitophilus zeamais* M. and *S. oryzae* L. (Saljoqi *et al.*, 2006). Essential oil from *Cymbopogon citratus*, are known for their varied pest control properties (Opender *et al.*, 2008).

2.5.5. Kelewa/Abayi, *Maesa lanceolata* Forsskal

Maesa lanceolata Forsskal, (Myrsinaceae) is a plant that is widely used as a medicine in east Africa to treat a variety of ailments, such as sore throat, tapeworms, hepatitis and cholera. In Saudi Arabia a decoction of the heated fresh leaves is used to alleviate rheumatic arthritis, while in Central Africa, it is used against *Entamoeba histolytica* infections. The evidence from many studies indicate that the plant's uses can be extended to protection of other plants against fungal infections.

In view of this finding and the work done by many other researchers, it is proposed that *M. lanceolata* be placed on a high priority list for propagation and conservation (Paul *et al.*, 2003).

According to the survey undergone by Esayas *et al.* (2007) in south western Ethiopia, *M. lanceolata* and *A.sativum* were among the dominant plant parts used as insect pest protectants for sorghum grain in storage. In addition, 30 g/kg dose of *Maesa lanceolata* plant seed powder comes close to the ‘farmers’ dosage’ which greatly reduces the emergence of bruchids and any loss of stored bean stocks becomes insignificant (Munyuli, 2001; Lambert *et al.*, 2008).

2.5.6. Kebericho, *Echinops kebericho* Mesfin

Echinops kebericho Mesfin belongs to the family Asteraceae, commonly known as Kebericho. Taxonomically, the genus *Echinops* comprises 120 species, of which 12 are known to occur in Ethiopia; out of which four species, (*E. kebericho* M., *E. buhaitensis* M., *E. ellenbeckii* O. Hoffm and *E. longisetus* A. Rich) are confined to the Ethiopian highlands. *Echinops kebericho* is so far known from some localities in Ethiopia at altitudes of 2300–2600 m. It is an erect perennial herb or shrub up to 1.2 m high, commonly forms a massive root stock with leafy stems. The leaf lamina is elliptical and is divided into segments that commonly end in spikes. The corolla is white or bright blue. *Echinops kebericho* M. (Orom: QEREBICHO), has been used as a fumigant, particularly after child birth and as a medicinal plant to treat leprosy for centuries. The large tuberous roots are sold either cut up as small pieces or in whole in many open markets in Shewa, Gojjam and Wollega regions (Mesfin and Brook, 2010). *Echinops* species were reported to contain alkaloids, saponins, phytosterols, polyphenols, carotenoids, sesquiterpene (alcohols/lactones), lignans, acetylenic & thiophene compounds and essential oil (Yinebeb, 2008).

It is claimed that the smoke is effective against typhus and fever. People in the central and south-western parts of Ethiopia use the smoke of *E. kebericho* to repel snakes from their vicinity. It is also indicated that the roots are chewed to reduce stomach ache in humans. A decoction of the roots is used to cure intestinal diseases in cattle due to the strong antihelminthic activity of the roots (LD₅₀

= 0.057 mg/ml, as compared to 0.0845 mg/ml of the standard drug niclosamide), which was reported earlier. Infusions of roots of *E. kebericho* are applied for the treatment of migraine, diarrhoea, heart pain and other ailments. *In vitro* studies on other members of the genus showed strong antibacterial, nematicidal and molluscicidal activities (Araya, 2006).

2.5.7. Ethiopian mustard, *Brassica carinata* A. Braun

The genus *Brassica* belongs to the family of Cruciferae and tribe Brassiceae. The genus includes many economically important crops which provide edible roots, stems, leaves, flowers and seeds. Many of the wild forms within the genus *Brassica* have a potential to be used as condiments and oilseeds. The wild forms are useful sources of desirable agronomic and seed quality traits in breeding programs. Seed of gomenzer contains from 37 to 44 % oil depending on the cultivar and environment. Cool temperatures or higher altitudes and well distributed rainfall result in higher oil contents. The high seed yield of gomenzer in combination with its high oil content makes gomenzer a highly desirable oilseed for the Ethiopian highlands. Additional advantages of gomenzer over other oilseed crops for production of oil for local consumption are the ease of oil extraction using screw press and high oil recovery rates during oil extraction for local consumption. Gomenzer seed oil contains approximately 40% erucic acid, and the seed has high glucosinolate content. Oils from rapeseed contained 95.8% nonpolar and 4.2% polar lipids. Oil of *Brassica* species contained palmitic, oleic, linoleic, linolenic, eicosenoic and erucic acids (Getinet, 1996).

The oilseed crop *Brassica carinata* is grown in the highlands of the Ethiopia plateau and its cultivation is believed to date back in the 4th to 5th Millennia BC. Ethiopian mustard (*Brassica carinata*, A. Braun) is an amphidiploids with the BB genome derived from *Brassica nigra* and CC genome from *Brassica oleracea*. It is mainly self-pollinating *Brassica* oilseed crop constitute the third most important source of vegetable oil in the world , as well as in Ethiopia after noug (*Guizotia abyssinica*, Cass) and linseed (*Linum asitatisimum* L.) both in terms of area and production(Eyasu, 2007).

Ethiopian mustard (*Brassica carinata* A. Braun) has long been known to be one of the oldest crops in the plateaus of east Africa. The crop has good agronomic traits like high yielding, better resistance to disease, insect pests and seed shattering than any one of the oilseed crops adapted to comparable areas, with the additional agronomic advantage of its better tolerance to semiarid conditions. However, it possesses high erucic acid in the oil and high glucosinolate content in the meal. Recently, researchers in Canada, Spain and India showed interest to this crop due to its tolerance to biotic and abiotic stress under semiarid conditions (Abraha *et al.*, 2008).

2.5.8. Gime, *Chenopodium ambrosoids* L. Leaf

Chenopodium ambrosoids is an herb native to Central America, South America, and southern Mexico. It is an annual or short-lived perennial plant, growing to 1.2 m tall, irregularly branched, with oblong-lanceolata leaves up to 12 cm long. The flowers are small and green, produced in a branched panicle at the apex of the stem. In Nigeria *C. ambrosoids* is only used in the preparation of traditional herbal remedies against intestinal worms, although recent observations showed that villagers have fewer mosquito bites when the leaves are hung on the door posts than when it is absent (Odugbemi, 2006). It has, therefore, become necessary to investigate the bioactivity of the shrub against various insect groups and non-target species. Analysis of *C. ambrosioides* constituents showed that the test plant species contains sabinene (1.50%), β -pinene (0.29%), α -terpinene (55.55%), p-cymene (16.71%), limonene (1.09%), (E)- β -ocimene (0.27%), γ -terpinene (0.97%), 1,4-epoxy-p-menth-2-ene (17.72%), 1,2,3,4-diepoxy-p-menthane (0.14%) and phytol (0.38%) (Abiodun *et al.*, 2010).

Among the plants used as insect control agents, *C. ambrosioides* L. (Chenopodaceae) is a common choice. Except for a few reports such as Denloye *et al.* (2009), there is a scarcity of studies on the insecticidal properties of *C. ambrosioides*. Also, the few studies of the plant against storage insects are concentrated on laboratory evaluations of its toxicity to adults of test species. The powder, aqueous extract, ethanolic extract and essential oil of *C. ambrosioides* were tested for toxicity against the adults of three storage insects causing huge losses to grains in Africa namely: *Sitophilus zeamais* M. (Coleoptera: Curculionidae), *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and

Tribolium castaneum J. (Coleoptera: Tenebrionidae). Further tests were carried out to show the effect of *C. ambrosioides* products on oviposition, egg hatching and survival of *C. maculatus* larvae. The ability of the powder to protect cowpea and maize grains from insect infestation in typical traditional storage systems was also evaluated. The powder formulation was more toxic to *S. zeamais* than either *C. maculatus* or *T. castaneum* with 48 h LC₅₀ values of 0.46 g/kg, 1.60 g/kg and 2.14 g/kg, respectively. Ethanol extract was more toxic to *C. maculatus* with a 48 h LC₅₀ value of 0.023 g/l, than other test insect species. The essential oil treatment demonstrated higher fumigant toxicity against *C. maculatus* than *S. zeamais* with 24 h LC₅₀ values of 1.33 µL/l and 1.90 µL/l respectively. The oil vapour showed activity against *C. maculatus* egg, but had not causes appreciable larval mortality. The weight loss of grains admixed with *C. ambrosioides* powder was lower than the controls after 150 days of field storage (Abiodun *et al.*, 2010).

2.5.9. Cotton, *Gossypium hirsutum* L. Seed oil

Cottonseed oil, a by-product of cottonseed, is a valuable source of edible oil. The whole cottonseed contains 15-20% oil and about 30- 38% of kernel, depending on the quality of seed and the species. Of the four primary products produced by cottonseed processing plants, oil is the most valuable. On the average it accounts for about 40-50 percent of the total value of all four products. Cottonseed oil is used almost entirely as a food for man. In recent years, industry-wide yields of products per ton of seed have averaged about 320 pounds of oil, 910 pounds of meal, 540 pounds of hulls, and 167 pounds of linters, with manufacturing loss of 63 pounds per ton. These average yields vary from area to area, year to year and mill to mill, depending upon the character of the seed, the type of process used, and market conditions. Crude cottonseed oil has a better condition stability due to the presence of segment named gossypol and must be refined to remove gossypol, a naturally occurring toxin that protects the cotton plant from insect damage and therefore, unrefined cottonseed oil is sometimes used as a pesticide (Savanam *et al.*, 2011). Pyrethrum extracts stabilized with cotton and neem seed oils showed a marked increase in bio-efficacy against maize weevils. Cotton seed oil however, had the highest stabilizing effect compared to the pyrethrum extract (Wanyika *et al.*, 2009; Savanam and Bhaskara, 2011).

2.5.10. Malathion dust (5%) formulation

Malathion is an organophosphorus insecticide used in public health, residential, and agricultural settings as early as 1950. Over 100 food crops can be treated with Malathion and about half of total applications in the United States (U.S.) are on alfalfa, cotton, rice, sorghum, and wheat. Malathion is formulated as an emulsifiable concentrate (EC), a dust (D), a wettable powder (WP), a pressurized liquid (PrL) and as ready-to-use liquids used for ultra-low-volume (ULV) application. Examples of common product names include Agrisect, Atrapa, Bonide, Prentox, Clean Crop Malathion, Acme Malathion, Black Leaf Malathion spray, Eliminator, Fyfanon, and Gowan Malathion dust. Malathion has a broad range of use with target pests in the orders dipterans, lepidoptera, hemiptera, coleoptera, and other orders. Malathion is a slightly toxic compound in EPA toxicity class III and labels for Malathion products must carry the signal word “CAUTION” (Kay Lynn, 2006). This is a safe insecticide which can be admixed to or sprayed on shelled (threshed) or unshelled (unthreshed) grains. On stored produce only premium grade Malathion must be used. ($LD_{50} = 1400$ mg). The general recommendation is to mix 100150 9 2% with 100 kg produce. The limitations of Malathion dust includes the product must be dry, (moisture content not higher than 13.5%), short shelf life (not more than 6 months) (WHO, 2011).

2. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), South Western Ethiopia from September to December 2011. Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) is located 354 km south west of Addis Ababa at an approximate geographical coordinates of latitude 06°36' N and longitude of 37°12' E at an altitude of 1710 meters above sea level. The mean maximum and minimum temperatures are 26.8⁰C and 11.4⁰C, respectively and the mean maximum and minimum relative humidity are 91.4% and 39.92%, respectively.

3.2. Maize Grain Used for the Experiment

In all experiments, clean and well sieved maize grain of the variety 'BH-660' was used which was obtained from Ethiopian Seed Enterprise, Wollega branch and frozen at -6⁰C for seven days to kill any live insects. It is the most commonly grown maize hybrid developed by the National Maize Research Program based at Bako, Western Ethiopia and now days is considered as one of the susceptible maize varieties to insect pests (Abraham, 2004). Then it was adequately dried in ultra violet light for six hours and not previously treated with pesticides. The grains were graded manually and almost only larger grains were used in the study. The grains were cleaned of broken kernels and debris removed by hand and by using a 4.76-mm round holed sieve.

3.3. Collection and Preparation of Botanicals and Cooking Oils

Fresh and matured leaves, kernels and barks of *Azadirachta indica*, leaves of *Tagetes erecta*, *Chenopodium ambrosioides* and *Cymbopogon citrates* as well as seeds of *Maesa lanceolata*, stems of *Allium sativum*, roots of *Echinops kebericho*, purified cooking oils of *Brassica carinata*, and

unrefined cooking oils of *Gossypium hirsutum* were gathered and brought immediately to the laboratory from different localities (Table 1). *Tagetes erecta* and *Cymbopogon citratus* were harvested from the campus of Jimma University, while *A. indica* (leaf, bark and kernel) was collected from Melka Werer research centre of Afar region, and *C. ambrosoids*, *M. lanceolata*, and *E. kebericho* were gathered from the natural habitat of eastern Wollega zone while *A. sativum* is purchased from market. The purified cooking oils of *Brassica carinata* and unrefined cooking oils of *Gossypium hirsutum* were brought from Hararghe Fadis and Addis Mojo oil industry of eastern showa zone, respectively. The leaves and plant materials were air and shade dried for 14 days in the entomology laboratory of Jimma University College of Agriculture and Veterinary Medicine. The dried leaves, kernels and roots were pulverized using a micro pulverizer machine and were sieved through a 0.25 mm pore size mesh sieve to obtain uniform particle size which is similar with the procedures followed by (Araya, 2007; Parugrug and Roxas, 2008).

Table 1. Description of botanicals and cooking oils used in the experiments

Descriptions	Dose used	Local name	Common name	Parts used
Control	-	-	-	-
Malathion dust (5%)	0.125g	-	-	dust formulation
<i>Azadrachta indica</i> leaf	0.5g	Mimi Zaf / Nimia	Neem	Leaf powder
<i>Azadrachta indica</i> bark	0.5g	Mimi Zaf / Nimia	Neem	Bark powder
<i>Azadrachta indica</i> seed	0.5g	Mimi Zaf / Neem	Neem	Kernel powder
<i>Cymbopogon citratus</i>	0.5g	Lomi sar	Lemon grass	Leaf powder
<i>Tagetes erecta</i>	0.5g	Yeferenji Adey	Mery gold	Leaf powder
<i>Allium sativum</i>	0.5g	Nech Shinkurt	Garlic	Leaf powder
<i>Maesa lanceolata</i>	0.5g	Kelewa/Abayi	-	seed powder
<i>Chenopodium ambrosoids</i>	0.5g	Gime/Ajaye	-	Leaf powder
<i>Gossypium hirsutum</i>	0.5ml	Tit/ Jirbi	Cotton	seed oil
<i>Brassica carinata</i>	0.5ml	Gomenzer	Ethiopian mustard	seed oil
<i>Echinops kebericho</i>	0.5g	Kebericho	-	Root powder

The resulting powders (Figure 1) were kept separately in glass containers with screw cap and stored at room temperature in dark prior to use. The amounts of powder mixed with the maize grain were

calculated on weight by weight bases that is weight of powder/weight of grain (w/w) (0.5g of botanicals to 250 g of maize grains).



A)



B)



C)



D)



E)



F)



G)



H)



I)

Figure 1: The processed botanical powders used against *S. zeamais* and *S. cerealella* in the experiment

For the cooking oils, 0.5ml mixed with 2ml acetone (95%) to 250g of maize grains. The synthetic chemical Malathion super dust (5%) was used as standard check chemical in this study.

3.4. Rearing of the Experimental Insects

The initial generations of *Sitotroga cereallela* and *Sitophilus zeamais* were obtained from maize store culture of Jimma town stalk culture with maize grains and allowed to then further reared in an incubator at 27 °C and 50-70 r. h. in JUCAVM Parasitological laboratory in experimental jars. The collections of about two thousand male and female adult maize weevils were secured from the storage which is free from pesticides and introduced in to the rearing jars. After introduction of the insects in to maize grains, they were kept in two separate rearing jars with 500g on top of each jar there is perforated hole for ventilation purposes.

The Angoumois grain moth, *Sitotroga cereallela* were reared on 500g maize grains in two larger jars approximately 2 litre volume capacity and kept in an incubator for 35 days. Then after, the jar was screwed with plastic jar cover screw which was pinned with an electric pinning machine. The hole was sealed with nylon sheath cloth to prevent the escaping of insects and entry of mites and other insects. It also allows the exchange of gases in and out of the container. They were left undisturbed for 35 days. Finally, the early emerged moths were transferred to the experimental jars using smaller test tubes, insect net and locally prepared aspirator like material prepared from Kenyan bic pen tube.

3.5. Experimental Designs and Treatments

The experimental design was completely randomized design and percentage data were transformed using *Square-root transformation* ($\sqrt{x} + 0.5$). Thirteen treatments were used for the study, namely 1) Control (no treatment applied), 2) Malathion super dust, 3) *Azadrachta indica* leaf, 4) *Azadrachta indica* kernel, 5) *Azadrachta indica* cork, 6) *Tagetus erecta*, 7) *Cymbopogon citratus*,

8) *Alium sativum*, 9) *Maesa lanceolata*, 10) *Chenopodium ambrosioids*, 11) *Gossypium hirsutum* seed oil, 12) *Brassica carinata* seed oil, and 13) *Echinops kebericho*.

3.6. Bio-Assay Procedures

Each prepared botanicals was weighed (0.5g) and introduced in to 250g of maize grains in each jars. Jars were arranged 5-10 cm apart on a flat table and left undisturbed at 20-25°C for oviposition. The 20 early emerged adult insects of almost the same age were added to each jar (Parugrug and Roxas, 2008). Oils (0.5ml) were separately dissolved with 2ml acetone before mixing with the maize seed and allowed to evaporate for 2hrs. The jar contents were shaken thoroughly for about five minutes to ensure uniform distribution of the solution over grain surface. One set of the jars recieved no treatment and used as a negative control. Malathion super dust at the rate of 0.5% (0.125g/250g) to maize grain was applied as a standard check. After releasing of the adult insects (six per jar), the toxicity effect of each botanicals were inspected at one, two, three, four, five, 10, 15, and 20 days intervals. After 20 days of adult introduction, all the live and dead insects were removed from each jar to monitor F₁ progeny emergency until 40 days. On 45th days, samples of grains were taken from each experimental jar to check for number of seeds perforated (number of holed grains), the weights loss and percent germination.

3.6.1. Adult Mortality Test

Adult mortality of the botanicals was assessed throught adult mortality percentage (using formula below) on an interval basis (one, two, three, four, five, 10, 15 and 20 days) after introduction of the two insects by removing all the insects and counting dead and alive ones (Parugrug and Roxas, 2008).

$$\text{Percent Mortality} = \frac{\text{Number of dead insets}}{\text{Total number of insects}} \times 100$$

3.6.2. Progeny Emergence Test

Twenty days after the introduction of both insects to each jar, all dead and live insects were removed from each container and the seeds were returned to their respective containers to further assess F₁ progeny emergency at one, two, three, four, five, 10, 15 and 20 days interval. Inspection of the progenies was made on each assessment day by displaying the seeds on paper and sieving the contents of the jars (Govindan and Jeyarajan, 2009; Waktole and Amsalu, 2012).

3.7.3. Damaged seeds (seeds with holes)

Damage seeds were assessed on 45th day after adult introduction by randomly taking 10 seeds from the total seeds in each jar and counting wholesome and bored or seed with insect emergent holes. The damaged seeds were expressed in number out of ten seeds.

3.7.5. Grain weight loss

Percentage weight loss was assessed, for both insects, by measuring the initial and final weight of the grain as described by Ileke *et al.* (2011).

$$\text{Percentage Weight Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

3.6.6. Germination percentage (viability index) test

Germination test was carried out on 111 seed samples by randomly taking 18.50 % (46.25g/250g) out of the total grain seeds from each jar. The seeds were placed in Petri dishes containing moistened filter paper (Whatman No.1) and arranged in an incubator at 30⁰C in JUCAVM School of Veterinary parasitological laboratory. The number of emerged seedlings from each Petri dish was counted and recorded from 7-10 days. The percent germination was computed according to Ogendo *et al.* (2004) using the following formula:

$$\text{Germination Percentage} = \frac{\text{Number of Seed germinated}}{\text{Total Grain Sampled}} \times 100$$

3.7. Bio-assay for the Best Treatments (Cooking Oils)

Four concentrations (0.2ml, 0.3ml, 0.4ml and 0.5ml) of the two cooking oils were tested for their effectiveness on both insects along with two controls (no treatment and Malathion dust). The treatments were arranged in CRD replicated thrice. Data on adult mortality, F₁ progeny emergency and grain infestation (bored seeds, weight loss and germination percentage) were collected at different intervals (one, two, three, four, five, 10, 15 and 20 days) to determine the best concentration of the cooking oils in a similar way as expressed under section 3.7. Treatments were applied to the jars by Micro-pipettes.

3.8. Data Analysis

All data were transformed using square root transformation to homogenize the variance (Gomez and Gomez, 1984) before analysis. Data were analysed using one-way Analysis Of Variance (ANOVA) using SAS version 9.2 Software packages. USEPA Probit analysis version 1.5 was used for analyzing percent mortality and median lethal time. Mean separations were conducted using Turkey's studentized (HSD) test at 5% level of significance when treatments were found significant. All variables recorded were analyzed according to one-way ANOVA statistical model, i.e.,

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where; Y_{ij} = is the response, μ = is the general mean effect, T_i = is the i^{th} treatment effect and E_{ij} = is the experimental error.

4. RESULTS AND DISCUSSIONS

Results and discussion of the experiments conducted to assess the effectiveness of seven locally available botanical insecticides and two cooking oils against two storage insect pests, maize weevil and augonoumis grain moth, of maize grain is presented under different subheadings hereunder.

4.1. Experiment on *Sitophilus zeamais*

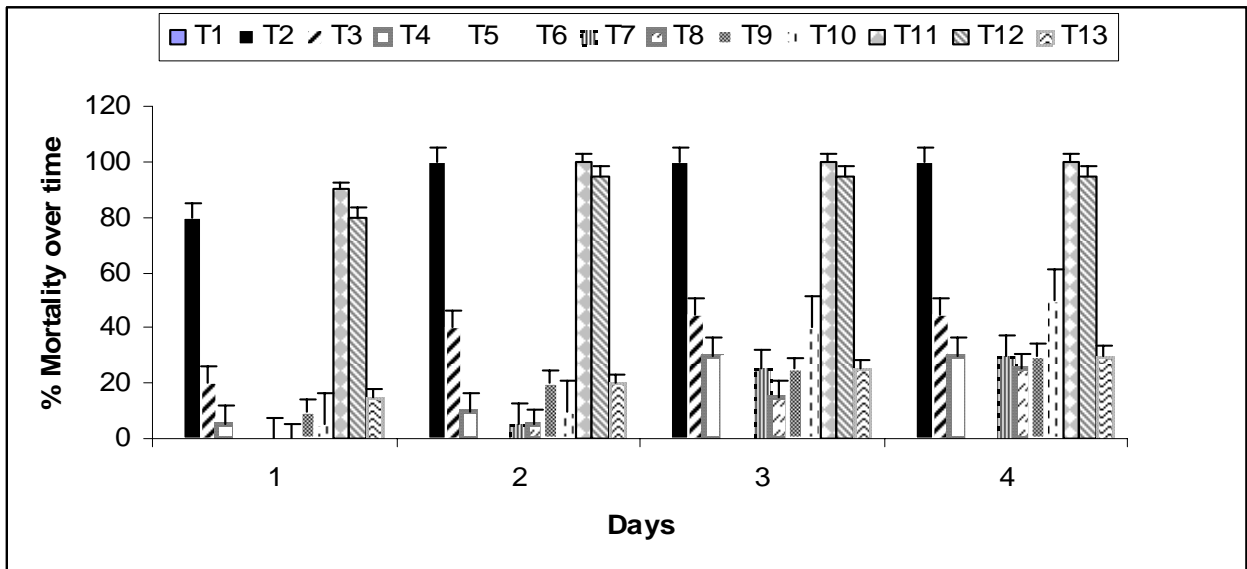
4.1.1. Cummulative toxicity of botanicals and cooking oils

The cumulative toxic action, at different interval, of the test materials against *Sitophilus zeamais* from 1-20 days were significant ($P < 0.05$) (Fig. 4 and Annex 1-8). On day one exposure, all the test materials except *T. erecta* and *A. sativum* showed comparable mortality among each other, however, *T. erecta* and *A. sativum* also resulted with a significant mortality compared to the untreated control. Maximum adult weevils mortality percentage was registered from *Gossypium hirsutum* (90%) followed by *Brassica carinata* oils (80%) and the standard check (Malathion super dust) (80%). *A. indica* leaf powder and *E. kebericho* root powders resulted in 20% and 15% adult weevil's mortality. Similarly, on the second days of weevil's exposures to the botanicals, there was a highly significant difference among the treatments with respect to toxicity of the botanicals. Maximum percent mortality (100%) was recorded from cotton seed oil and Malathion followed by Ethiopian mustard oil (95% mortality) and there was no death of weevils from the untreated check. From the third day onward, percent mortality from cotton seed oil, Malathion and Ethiopian mustard remains constant registering 100%, 100% and 95 % respectively. Other botanicals toxicity effect was better than untreated control showing an increasing trend from the second day to the last day. On the fourth days of introduction of weevils, *C. ambrosoid* killed 50% followed by *A. indica* leaf powder (45%), *A. indica* kernel powder (40%), *M. lanceolata* (30%), *A. indica* bark powder (30%), *C. citratus* (25%), *T. erecta* (30%) and *A. sativum* (25%). Finally, on the 20th days after introduction, *C. ambrosoid*, *T. erecta*, *A. indica* leaf and bark registered higher adult mortality (70%) followed by *A. indica* kernel powder (65%), *C. citratus*, *M. lanceolata* and *E. kebericho* (55%) and *A. sativum*

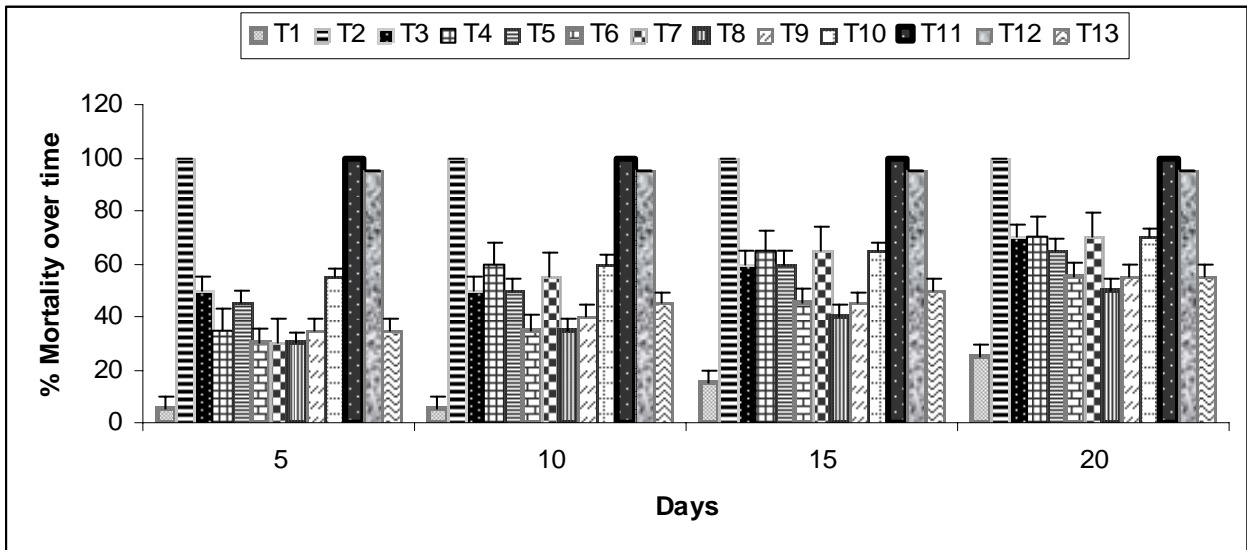
(50%) (Table2). Greater adult weevils mortality due to the application of the botanicals was observed as the exposure time of the pest to the treatment increased. As exposure time precedes, there was a progressive increase in the toxicity of the botanicals to the test insect registering appreciable control of *S. zeamais*. The two cooking oils had potent biocidal effects on *S. zeamais* with the same efficacy with Malathion super dust inducing 100% cumulative toxicity to the test insect over 20 days exposure time. The present investigation is in agreement with the report by Mekuria (1995) who stated that *C. ambrosoids* leaf and inflorescent parts applied at the rate of 4% w/w induced 100% mortality against maize weevil. Araya and Emana (2009) reported that *C. ambrosoids* leaf powder at higher rate (15g/150g) application to haricot bean weevil's resulted in 100% mortality of the weevils. Hence, the current findings are in agreement with the previous works, suggesting that the efficacy of botanical powders (*C. ambrosoids*) could serve as alternative maize weevil controlling material especially when applied at an higher rate (more than 0.5 g). The insecticidal activities of the botanical powders are broad and variable and dependent on different factors like the presence of bioactive chemicals which need to be identified, isolated and manufactured in the factory for pest management. The plant powders may act as fumigant, repellent, stomach poison and physical barrier (Mulungu *et al.*, 2007; Law-Ogbomo and Enobakhare, 2007).

Similarly, Odeyemi (1993) found that *C. citrates* essential oil applied at 0.7 ml /50g of maize increased the mortality of maize weevil compared to the control. Solvent extracts of different lemon grass parts were reported to have toxicant, repellent and fumigant activities against storage pests. Araya (2007) found that fresh *C. citratus* essential oil exhibited high (85-100% mortality) acaricidal activity. Study conducted by Paranagama *et al.* (2003) pointed out that *C. citratus* oil treatment reduced the germination capacity of rice paddy as compared to the control.

The two cooking oils, *Gossypium hirsutum* and *Brassica carinata* seed oils, exhibited higher toxicity to the adult weevils throughout the storage period similar to the standard check. The toxicity of these cooking oils may be due to their active components responsible for the insecticidal properties against the insect pests including weevils.



a)



b)

Figure 2. Percent adult mortality (Cumulative) of *Sitophilus zeamais* by botanicals and cooking oils at different time intervals (days)

Key for the legends:

[T₁-Control, T₂-Malathion, T₃-*Azadrachta indica* leaf, T₄-*Azadrachta indica* bark, T₅- *Azadrachta indica* Kernel, T₆-*Cymbopogon citratus* leaf, T₇-*Tagetes erecta*, T₈-*Alium sativum* bulb, T₉-*Maesa lanceolata* seed, T₁₀-*Chenopodium ambrosoids* leaf, T₁₁-Cotton seed oil, T₁₂-*Brassica* seed oil and T₁₃-*Echnops kebericho* root]

The treatments were found significant ($P < 0.05$) with respect to the median lethal time (LT_{50}) (Table 2). Among the treatments the time required to kill 50% of the test insect was less than one day for cotton and Ethiopian mustard oil statistically on par with Malathion super dust. Thus the most potent botanicals against maize weevils in storage are the two cooking oils. On the other hand, *C. citratus* required longer time (18.30 days) to kill 50% *S. zeamais* which was statistically on par with *A. sativum* (18.0 days), *M. lanceolata* (16.2 days) and *E. kebericho* root (14.5 days) indicating their less effectiveness against *S. zeamais*. *A. indica*, *T. erecta* and *C. ambrosoid* with LT_{50} values ranging from 6.0 to 9.2 days are considered as moderately effective botanicals.

4.1.2. *Sitophilus zeamais* progeny emergence

There were no progenies emerged from the first day to fourth days of introduced weevils removal from the experimental jars. The botanicals and cooking oils were significant ($P < 0.05$) in terms of maize weevil progeny emergency on 25th, 30th, 35th and 40th days of weevils introduction to the experimental jars (Table 3). Maximum mean numbers of progenies were emerged on 25th days from the jars that received no treatment, *A. sativum*, *M. lanceolata* (2 adults). But, there were no progeny emerged from the grains treated with Malathion super dust, *A. indica* kernel powder, *G. hirsutum* oil, *B. carinata* oil and *E. kebericho* root powder. Generally, similar trends were observed for adult weevil's emergency on the 30, 35 and 40 days. On the 40th day of adult weevils introduction (20 days from adult removal), maximum and significantly different number of progenies, 14 adults, were emerged from the jars that received no treatment. On the other hand, there was no progeny emergency from the jars that received Malathion, cotton and Ethiopian mustard oils. This indicates the efficacy of the two cooking oils against *S. zeamais* in preventing laying of eggs on maize seed. The total number of *S. zeamais* adult emerged from untreated control increased with time of exposure compared to the other treatments. All the botanical powder treatments induced significant reduction in F_1 adult emergence of *S. zeamais* compared to the untreated check although the plant materials vary among themselves. Accordingly, *C. ambrosoid*, *T. erecta*, *A. indica* leaf and bark were superior in reducing the production of F_1 progeny among the plant powders. However, *C. citratus*, *M. lanceolata* and *E. kebericho* leaf powder were less effective compared to other plant powder treatments, which was not significantly different from the

untreated control. The reduction in F₁ progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested leaf and seed powders of the botanicals (Araya and Eman, 2009). But, still higher dosages and longer exposure periods are needed to achieve appreciable control as has been reported by several authors. Tapondjou *et al.*, (2002) noted that, all concentrations of dry ground leaves of *C. ambrosoids* resulted in complete (100%) inhibition of oviposition and subsequent progeny production by *C. chinensis*, *C. maculatus* and *A. obtectus* and may kill the larvae hatching from eggs laid on grains, preventing feeding and damage. Likewise, it was reported that *Chenopodium* leaf powder mixed with maize and sorghum grains at the rates of 2 and 4% w/w caused complete reduction in F₁ progeny production by maize weevil (Mekuria, 1995; Asmare, 2002). The act of weakening of adults by botanical powders may make them lay fewer eggs than the normal leading to less hatchability to larvae and adults.

Large numbers of weevils from the untreated check and most of the botanical plants were emerged on the 40th day of adult introduction. This indicates that the total development period (TDP) of the maize weevil, *S. zeamais* is 40 days on an average under Jimma condition. This means that the test plants did not affect the growth and development of maize weevil inside the grain. This finding coincides with the work of Parugrug and Roxas (2008) who reported 39 days. Based on the result of the study, powdered form of the test plants might not be effective in inhibiting growth. This might be because of the fact that the active compound of the plants with insecticidal characteristics could not penetrate well inside the grains, thus, did not affect the development of the weevil inside the seeds.

Maize weevil is an internal feeder and the different life stages developed successfully inside the grain. The growth and development of the weevils from the untreated corn grains and those insects from grains treated with powdered test plants had almost similar number of days of development. Therefore, it could be assumed that the test plants did not affect the insect development, except, the two oils and Malathion. These three treatments completely inhibited growth of the weevils leading to no emergency of weevils.

Table 2. Percent adult mortality (cumulative) and Median Lethal Time (LT₅₀) of botanicals and cooking oils against *Sitophilus zeamais*

Treatment descriptions	Mortality (%)	Median Lethal time(LT ₅₀) in days	Confidence Interval		Slope[±SE]
			Lower	Upper	
Control	25	40.8 ^a	23.7	382.8	2.33±0.77
Malathion Dust	100	<1.0 ^c	-*	-	-
<i>A.indica</i> leaf	70	6.0 ^b	3.2	13.4	0.82±0.25
<i>A.indica</i> bark	70	8.4 ^b	6.2	12.4	1.67±0.29
<i>A.indica</i> kernel	65	8.8 ^b	6.0	15.5	1.3±0.27
<i>C.citratus</i> leaf	55	18.3 ^{ab}	10.3	77.5	1.01±0.27
<i>T. erecta</i> leaf	70	9.2 ^b	7.02	12.9	1.95±0.31
<i>A.sativum</i> stem	50	18.0 ^{ab}	11.65	42.9	1.42±0.31
<i>M. lanceolata</i> seed	55	16.2 ^{ab}	9.2	70.0	0.95±0.26
<i>C. ambrosoids</i> leaf	70	6.5 ^b	4.7	9.6	1.49±0.27
<i>G. hirsutum</i> oil	100	<1.0 ^c	-	-	-
<i>B. carinata</i> seed oil	95	<1.0 ^c	-	-	-
<i>E.kebericho</i> root	55	14.5 ^{ab}	8.3	58.1	0.93±0.26

*No confidence interval for the oils and Malathion, because of the very low LT₅₀ obtained which are beyond the computing capacity of the soft ware (USEPA probit analysis program)

4.1.3. Maize grain damage by *Sitophilus zeamais*

Grain damage by *S.zeamais* was assessed in terms of counting perforated holes, percent weight loss and viability loss caused by adult weevils and larvae feeding inside the seeds. The treatments were significantly different (P<0.05) with respect to the number of perforated seeds, percent weight loss and grain viability (Table 4). Mean numbers of perforated seeds were maximum (2.1 out of 10 seeds) and significantly different from untreated check which was on par with jars that received *A. indica* kernels and *C. citrates* (1.8 holed grains out of 10 grains) leaf powder.

Regarding percentage grain weight loss, the highest weight loss was recorded from untreated grains (1.6%) followed by grains treated with *A. indica* bark, *A. sativum* stem and *M. lanceolata* seed powder each with 1.2% weight loss. No grain weight loss was recorded from the two cooking oils on par with the standard check (Mmalathion). Some treatment effects were significantly different to others after 45 days of grain storage and the untreated grain suffered highly significantly greater grain damage as well as weight loss than grains treated with Malathion 5%

dust and all other treatments, except *C. citratus* leaf powder. The untreated grains had consistently higher percentage weight losses than the treated grains. However, there were no significant differences in weight losses among oils and the standard check insecticide, Malathion throughout the storage period.

Table 3. *Sitophilus zeamais* progeny emergence from maize grains treated with botanicals and cooking oils at different intervals (days)

Treatments	Time interval after exposure (days)			
	25	30	35	40
Control	2 (1.53) ^{b*}	6 (2.03) ^{a*}	10 (3.1) ^{a*}	14 (3.3) ^{a*}
Malathion Dust	0 (.93) ^c	0 (0.7) ^c	0 (0.7) ^e	0 (0.7) ^d
<i>A.indica</i> leaf	1 (1.03) ^c	3 (1.47) ^{ab}	5 (1.5) ^{bc}	6 (1.47) ^b
<i>A.indica</i> bark	1 (1.2) ^{abc}	2 (1.4) ^b	3 (1.27) ^{bcd}	4 (1.3) ^{bc}
<i>A.indica</i> kernel	0 (0.87) ^c	0 (0.7) ^c	1 (0.87) ^{de}	2 (0.87) ^{cd}
<i>C.citratus</i> leaf	1 (1.03) ^c	2 (1.4) ^b	4 (1.6) ^{bc}	5 (1.2) ^{bcd}
<i>T.eracta</i> leaf	1 (1.13) ^{abc}	2 (1.2) ^{bc}	3 (1.2) ^{cd}	4 (1.27) ^{bc}
<i>A.sativum</i> stem	2 (1.67) ^a	4 (1.47) ^{ab}	6 (1.73) ^{ab}	7 (0.87) ^{cd}
<i>M.lanceolata</i> seed	2 (1.53) ^{ab}	3 (1.2) ^{bc}	5 (1.6) ^{bc}	6 (1.3) ^{bc}
<i>C.ambrosoid</i> leaf	1 (1.27) ^{abc}	2 (1.27) ^{bc}	3 (1.03) ^{de}	4 (1.03) ^{bcd}
<i>G. hirsutum</i> seed oil	0 (0.7) ^c	0 (0.7) ^c	0 (0.7) ^e	0 (0.7) ^d
<i>B.carinata</i> seed oil	0 (0.7) ^c	0 (0.7) ^c	0 (0.7) ^e	0 (0.7) ^d
<i>E.kebericho</i> root	0 (0.87) ^c	1 (1.3) ^b	2 (1.03) ^{de}	3 (1.03) ^{bcd}
P value	0.0001	0.0001	0.0001	0.0001
HSD	0.612	0.576	0.554	0.54
CV (%)	18.5	15.64	16.13	16.1

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different ($P<0.05$)

The germination percentage of the grains ranged from 86.5 to 95.5% in the untreated and Malathion treated jars, in that order. The effect of the botanicals in powder form on the viability/germination rate of the treated grains indicated that none of the plant powders mixed with the grains adversely affected the germination of the maize grains compared to the untreated control. Most of the treated seeds were germinated. But, statistically significantly higher germination percentage of seeds were recorded from grains treated with Malathion (95.5%) which was statistically on par and followed by *G. hirsutum* seed oil (94.6%), *B. carinata* seed oil (94.6%) and *C.ambrosoid* leaf powder (91.0%).

Table 4. Grain hole number, weight loss and germination percentage of maize grains infested with *Sitophilus zeamais*

Treatments	Hole Number/ 10 seeds	Weight Loss (%)	Germination (%)
Control	2.1(1.6) ^{a*}	4.6 (2.1) ^{a*}	86.5 (9.8) ^{c*}
Malathion Dust	0.0 (0.7) ^f	0 (0.7) ^f	95.5 (10.3) ^a
<i>A.indica</i> leaf	1.1(1.3) ^{cd}	0.8 (1.7) ^{bc}	90.1 (10) ^{bc}
<i>A.indica</i> bark	1.3 (1.3) ^{cd}	1.2 (1.8) ^{abc}	90.1 (10) ^{bc}
<i>A.indica</i> kernel	1.6 (1.4) ^{ab}	0.8 (1.47) ^{cde}	90.1 (10) ^{bc}
<i>C.citratus</i> leaf	1.8 (1.5) ^a	0.8 (1.5) ^{abc}	90.1 (10) ^{bc}
<i>T.eracta</i> leaf	1.1(1.3) ^{cd}	0.4 (1.3) ^{de}	90.1 (10) ^{bc}
<i>A.sativum</i> stem	1.1(1.3) ^{cd}	1.2 (1.8) ^{abc}	88.3 (9.9) ^c
<i>M.lanceolata</i> seed	1.1(1.3) ^{cd}	1.2 (1.9) ^{ab}	90.1 (10) ^{bc}
<i>C.ambrosoid</i> leaf	0.3 (0.9) ^e	0.4(1.2) ^e	91 (10.1) ^a
<i>G. hirsutum</i> seed oil	0.0 (0.7) ^f	0 (0.7) ^f	94.6 (10.3) ^a
<i>B.carinata</i> seed oil	0.0 (0.7) ^f	0 (0.7) ^f	94.6 (10.3) ^a
<i>E.kebericho</i> root	0.9 (1.2) ^d	0.8 (1.6) ^{bcd}	90.1 (10) ^{bc}
P value	0.0001	0.0001	0.0001
HSD	0.748	0.36	0.14
CV (%)	6.64	12.57	0.48

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different ($P<0.05$)

The germination test demonstrated that the plant materials tested against *S. zeamais* did not show any visible adverse effects on germination capacity of the grains. Some of the treatments were infected by moulds of fungal pathogens grown on the soft paper kept on the underside of the petridishes, which resulted in a reduced germination percentage. Asmare (2002) showed that powders of *D. stramonium*, *J. curcas*, *P. dodecondra* and *A. indica* used in the control of *S. zeamais* did not show any significant effect on the germination capacity of sorghum. Araya and Emanu (2009) explained that some of the treatments applied against *Z. subfasciatus* were infected by moulds which resulted in a reduced germination percentage.

The result of simple linear correlation studies among the variables revealed that there existed an association between percent toxicity, mean progeny emergency, number of perforated holes on the grains, percent weight loss and germination percentage of maize grains infested with the same number of *S. zeamais* (Table 5). Percent adult mortality was inversely and significantly correlated with mean progeny emergency ($r=-0.47^*$), number grains perforated ($r = -0.71^{**}$), percentage

weight loss ($r=-0.79^{**}$) but positive and significantly correlated with germination percentage ($r=0.74^{**}$). With an increasing trend of weevil's adult mortality; there is a decreasing trend of progeny emergency, number of perforated holes on the grains, percent weight loss. On the other hand, an increasing adult mortality is associated with an increasing germination percentage.

Table 5. Pearson correlation coefficients among different variables of maize grains infested by *Sitophilus zeamais*

	Mortality (%)	Progeny Emerged	Hole Number	Weight Loss (%)	Germination (%)
Mortality (%)	1	-0.47*	-0.71*	-0.79*	0.74*
Progeny Emerged		1	0.66*	0.71*	-0.69*
Hole Number			1	0.90*	-0.84*
Weight Loss (%)				1	-0.91*
Germination (%)					1

*Significant at 5% and 1% level of probability respectively

4.2. Experiment on *Sitotroga cereallela*

4.2.1. Cumulative toxicity of botanicals and cooking oils

The cumulative toxic effects, at different time interval, of all the plant powders and cooking oils against *S. cereallela* in stored maize grains are presented in Table 6 and fig. 5. The cumulative toxicity of the treatments were significantly different ($P<0.05$) on each day (Annex 9-16). After the first 24 hours of exposure (on day one), few plant materials showed comparable mortality among each other but gave a significantly higher mortality over the untreated control (Fig. 5). Maximum mortality, 94.4%, after 24 hours of exposure was recorded from the standard check, Malathion super dust. Among the other treatments, the two cooking oils, *G. hirsutmn* and *B. carinata* seed oils, registered 77.8% mortality to the grain moth after 24 hours of exposure. On the 5th days of exposure, *A. indica* kernel, *C. citrates* and *C. ambrosoids* gave 33.3% cumulative toxicity (mortality). The trends in cumulative toxicity from the first to the 20th days of exposure of grain moth to the botanicals powder showed slow increasing trend attaining maximum mortality on the last day of exposure as shown by survival curve. The cumulative toxicity (on 20th days of exposure) of Malathion super dust (94.4%) and the two cooking oils (77.8%) were found significant on the

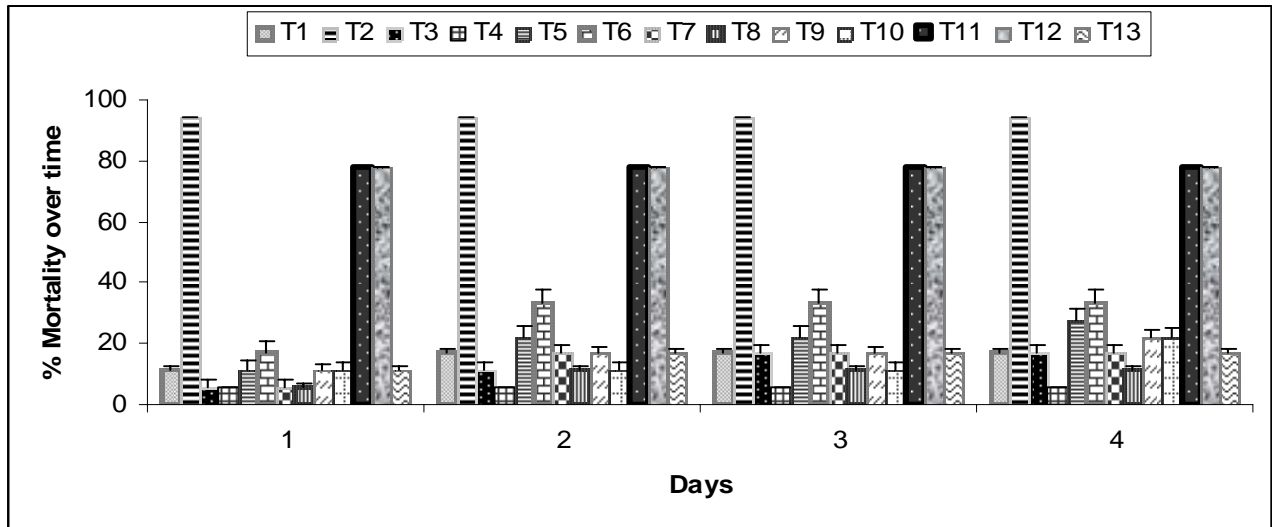
basis of ANOVA result (Annex 16) but said to be similar based on the probit analysis result (Table 6).

The treatments were found significant ($P < 0.05$) with respect to the median lethal time (LT_{50}) (Table 6). Among the treatments less than one day LT_{50} was taken to kill 50% of *S. cerealella* for cotton and Ethiopian mustard oil statistically on par with Malathion super dust. Therefore, these two cooking oils are the most potent botanicals against *S. cerealella* in storage. On the contrary, maximum time (338.1 days) was required for *E. kebericho* root powder to kill 50% of *S. cerealella* which was assumed to be the same, based on the probit analysis, with control (338.1 days), *M. lanceolata* (219.8 days) and *C. citratus* (171 days). *A. indica*, *T. erecta*, *A. sativum* and *C. ambrosoid* with LT_{50} values ranging from 14.7 to 49 days are considered as intermediate ones.

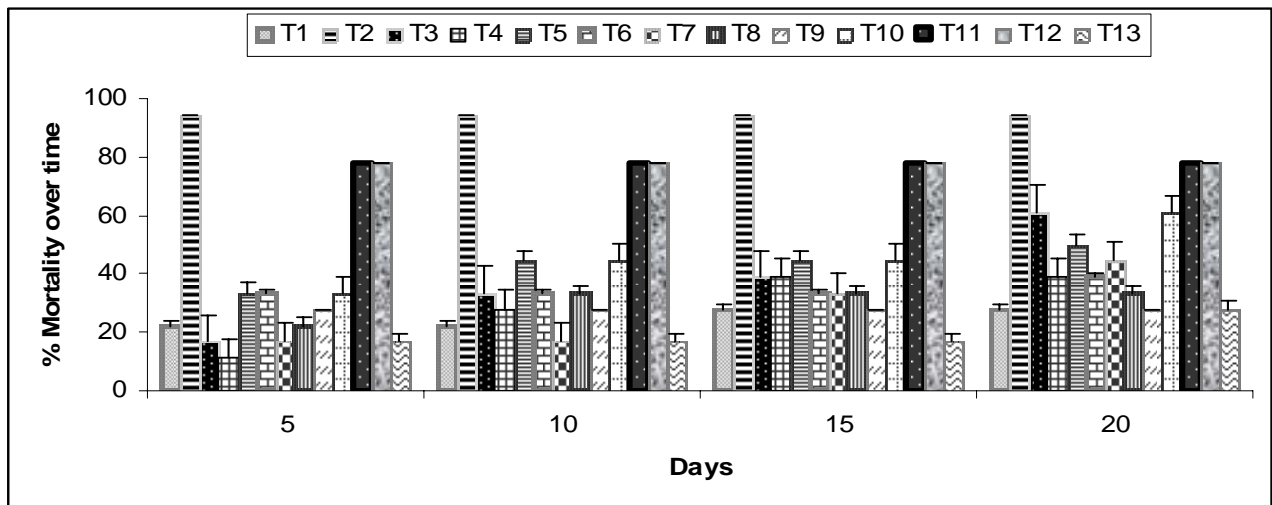
The result observed on the toxicity of *S. cerealella* is in agreement with the study of Javed *et al.* (2010), that extracts of *Acorus calamus*, sweet flag, *Azadirachta indica* and *Curcuma longa* (turmeric) prepared in petroleum ether, acetone and ethanol and evaluated as growth inhibitor against *Sitotroga cerealella*. Petroleum ether extract of sweet flag at application rates of 1000, 500 and 250 $\mu\text{g/g}$ and its acetone extract at 1000 and 500 $\mu\text{g/g}$ completely inhibited emergence of adults. Zaidi *et al.* (2003) compared extracts of 'neem', turmeric and sweet flag as insect repellents against *Sitotroga cerealella*, under laboratory conditions and found that the acetone-extract of neem was the most effective botanical insecticide.

The effect of *G. hirsutum* and *B. carinata* oils were comparatively more visible on earlier stages (larvae) of metamorphosis. Such an effect is reported by Girma *et al.*, (2008) on *S. zeamais* using different cooking oils at Bako research center, Ethiopia. This phenomenon may be due to the presence of gossypol in *G. hirsutum* and erucic acid about (40%) in Ethiopian mustard, *B. carinata* oil. In addition to the existence of erucic acid in *B. carinata*, it contains high glucosinolates which are the active components found in the oil. This efficacy probably indicates that the oils contain higher content of the active components responsible for the insecticidal properties including the above mentioned compounds.

Bamaiyi (2007), reported that application of oils occlude seed funnels leading to the death of the developing insect by asphyxia. A significant protection for maize grains against attack by *S. cereallela* was provided by cooking oils of *G. hirsutmn* and *B.carinata*. This suggests their protection potential even for other storage insect pests.



a)



b)

Figure 3. Percent adult mortality (Cumulative) of *Sitotroga cereallela* by botanicals and cooking oils at different time intervals (days)

Key for the legends:

[T₁-Control, T₂-Malathione as standard check, T₃-Azadrachta indica leaf, T₄-Azadrachta indica bark, T₅- Azadrachta indica Kernel, T₆-Cymbopogon citratus leaf, T₇-Tagetes erecta, T₈-Alium sativum bulb, T₉-Maesa lanceolata seed, T₁₀-Chenopodium ambrosoids leaf, T₁₁-Cotton seed oil, T₁₂-Brassica seed oil and T₁₃-Echnops kebericho root]

Table 6. Percent adult mortality (Cumulative) and Median Lethal Time (LT₅₀) of *Sitotroga cereallela* by botanicals and cooking oils

Treatment descriptions	Mortality (%)	Median Lethal time(LT ₅₀) in days	Confidence Interval		Slope[±SE]
			Lower	Upper	
Control	27.8	338.1 ^a	.*	.*	1.17±0.29
Malathion Dust	94.4	<1.0 ^c	-	-	-
<i>A.indica</i> leaf	61.1	18.8 ^b	11.6	54.2	1.34±0.32
<i>A.indica</i> bark	39	30.4 ^b	16.73	147.9	1.39±0.36
<i>A.indica</i> kernel	50	18.2 ^b	9.5	140.1	0.89±0.28
<i>C.citratus</i> leaf	39	171 ^a	-	-	0.31±0.26
<i>T. erecta</i> leaf	44.4	49 ^b	18.4	5623	0.87±0.31
<i>A.sativum</i> stem	33.3	42.1 ^b	17.8	1216.6	0.97±0.31
<i>M. lanceolata</i> seed	27.8	219.8 ^a	-	-	0.47±0.29
<i>C. ambrosoids</i> leaf	61.1	14.7 ^b	9.3	37.1	1.27±0.30
<i>G. hirsutmn</i> oil	77.8	<1.0 ^c	-	-	-
<i>B. carinata</i> seed oil	77.8	<1.0 ^c	-	-	-
<i>E.kebericho</i> root	27.8	338.1 ^a	-	-	0.30±0.30

.*No confidence interval for the oils and Malathion, because of the very low LT₅₀ obtained which are beyond the computing capacity of the soft ware (USEPA probit analysis program)

4.2.2. *Sitotroga cereallela* progeny emergence

Mean number of *S. cereallela* adult progeny emergency from grains treated with various botanical extracts and cooking oils at constant application rate is presented in Table 7. The different plants powders and cooking oils significantly (P<0.05) reduced *S. cereallela* progeny emergency. Up to the 4th days after adult removal (from day 21-24), no progeny was emerged from all jars including the untreated control. On 25th days of adult introduction (fifth days of introduced adults removal), no maize grain moth was emerged from the jars that received Malathion dust and the two cooking oils. This was true throughout the experimental period with no adult grain moth emergency from these three treatments indicating the effectiveness of the two cooking oils against *S. cereallela* similar to Malathion dust. In all the other treatments, there was an increasing trend of adult emergency from the 25th to 40th days of adult introduction indicating less effectiveness of these botanicals.

Petroleum ether extract of neem was next to sweet flag in reducing adult emergence of *S. cereallela*. Studies with several insects indicated that IGR effects are induced by azadirachtin applications. It is known that neem interferes with many life processes. Feng-Lian (2011), also reported on garlic essential oil, diallyl disulfide and diallyl trisulfide and had significant fumigant activity with 50% lethal concentration values at 1.33, 0.99, and 1.02 $\mu\text{L/L}$ air space, respectively. The aromatic chemicals found in the oils completely inhibit growth of insects.

All the botanical powder treatments induced significant reduction in F1 adult emergence of *S. cereallela* compared to the untreated check although the plant materials vary among themselves. Accordingly, powder treatments of *C. ambrosoid*, *A. indica* leaf, bark and kernel were superior in reducing the production of F1 progeny. However, *C. citratus*, *A. sativum*, *M. lanceolata* and *E. kebericho* root powder were less effective compared to other plant powder treatments, which is not significantly different from the control treatment. The powders of *C. ambrosoid* leaf, *A. indica* leaf, bark and kernel indicated the adequate toxicity to the larvae of the moth. In contrarily, the highest number of moth was emerged from untreated control inflicting maximum grain weight loss (5%) followed by *E. kebericho* and *T. erecta* (Table 8). The number of grain moth progeny emergence was significantly reduced from the grain treated with *G. hirsutmn* and *B. carinata* oils. Practically, plant oils coating can be effective in reducing progeny production by storage insect pests. Hence, the current findings regarding the use of cooking oils in reducing the emergence of F1 progeny of *S. cereallela* was in agreement on par with the earlier findings. Javed *et al.*, (2010) studied the possible cause for reduction of F1 progeny production of *S. cereallela* in treated grains with cooking oils were likely that immature stages of the insects were killed physically by oil coating and impairing respiration through blockage of spiracles thereby resulting in inhibiting immature stages survival or reduced longevity of adult females.

The number of grain moth progeny emergence was significantly reduced from the grain treated with *G. hirsutmn* and *B. carinata* oils. Practically, plant oils coating can be effective in reducing progeny production by storage insect pests. Hence, the current findings regarding the use of cooking oils in reducing the emergence of F1 progeny of *S. cereallela* was in agreement on par with the earlier findings. Javed *et al.*, (2010) studied the possible cause for reduction of F1 progeny

production of *S. cereallela* in treated grains with cooking oils were likely that immature stages of the insects were killed physically by oil coating and impairing respiration through blockage of spiracles thereby resulting in inhibiting immature stages survival or reduced longevity of adult females.

Table 7. Progeny emergence of *Sitotroga cereallela* from maize grains treated with botanicals and cooking oils at different time interval (days)

Treatments	Time interval after exposure (days)			
	25	30	35	40
Control	2 (1.58) ^{a*}	5 (2.35) ^{a*}	8 (2.92) ^{a*}	11 (3.39) ^{b*}
Malathion Dust	0 (0.71) ^c	0 (0.71) ^d	0 (0.71) ^d	0 (0.71) ^e
<i>A.indica</i> leaf	0 (1.22) ^c	0 (0.71) ^d	1 (0.87) ^{cd}	2 (1.58) ^c
<i>A.indica</i> bark	1 (1.2) ^{ab}	2 (1.58) ^d	4 (0.87) ^{cd}	7 (2.74) ^c
<i>A.indica</i> kernel	1 (0.87) ^{bc}	2 (1.58) ^d	4 (0.87) ^{cd}	7 (2.74) ^c
<i>C.citratus</i> leaf	1(1.2) ^{ab}	3 (1.87) ^{bc}	8 (2.92) ^a	15 (3.94) ^a
<i>T.eracta</i> leaf	1 (1.2) ^{ab}	3 (1.87) ^{cd}	6 (2.55) ^{bc}	14 (3.81) ^{ab}
<i>A.sativum</i> bulb	1 (0.87) ^{bc}	4 (2.12) ^{ab}	9 (3.08) ^{ab}	15 (3.94) ^a
<i>M.lanceolata</i> seed	1 (1.2) ^{ab}	4 (2.12) ^{ab}	9 (3.08) ^{ab}	17 (4.18) ^a
<i>C.ambrosoid</i> leaf	1 (0.87) ^{bc}	2 (1.58) ^d	3 (1.87) ^d	4 (2.12) ^d
<i>G. hirsutum</i> seed oil	0 (0.7) ^c	0 (0.71) ^d	0 (0.71) ^d	0 (0.71) ^e
<i>B.carinata</i> seed oil	0 (0.7) ^c	0 (0.71) ^d	0 (0.71) ^d	0 (0.71) ^e
<i>E.kebericho</i> root	1 (0.87) ^{bc}	3 (1.87) ^{cd}	6 (2.55) ^{bc}	11(3.39) ^b
P value	0.0001	0.0001	0.0001	0.0001
HSD	0.49	0.55	0.41	0.53
CV (%)	16.63	17.85	12.18	14.6

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different (P<0.05)

4.2.3. Maize grain damage by *Sitotroga cereallela*

Grain damage by *S. cereallela* was assessed in terms of counting perforated holes, percent weight loss and percent germination reduction caused by adult moth and larvae feeding inside the seeds on 45th days of adult moth introduction. The treatments were significantly different (P<0.05) with respect to the number of perforated seeds, percent weight loss and grain viability (Table 8). Mean numbers of perforated seeds were maximum (0.5 out of 10 seeds) and significantly different from untreated check which was on par with jars that received *A. indica* leaf and bark (0.3 holed seeds

out of 10 seeds) powder. However, there were no holes observed from grain treated with the Malathion and the two cooking oils indicating their efficacy against *S. cereallela*.

Maximum weight loss was recorded from untreated grains (5.0 %) followed by grains treated with *M. lanceolata* seed powder with 2.0% weight loss. No weight loss was recorded from the two cooking oils on par with the standard check (Mmalathion). Some treatment effects were significantly different from others after 45 days of grain storage and the untreated grain suffered highly significantly greater grain damage as well as weight loss than grains treated with Malathion 5% dust. The untreated grains had consistently higher percentage weight losses than the treated grains. However, there were no significant differences in weight losses among oils and the standard check insecticide, Malathion throughout the storage period affirming the effectiveness of the two cooking oils against *S. cereallela* similar to the standard chemical.

Table 8. Grain hole numbers, percent weight loss and percent germination of maize grains infested by *Sitotroga cereallela*

Treatments	Hole number /10 seeds	Percent Weight Loss	Percent Germination
Control	0.5 (1.0) ^{a*}	5 (2.3) ^{a*}	86.5 (9.8) ^{f*}
Malathion Dust	0.0 (0.7) ^d	0 (0.7) ^d	99.1 (10.5) ^a
<i>A.indica</i> leaf	0.3(0.9) ^{ab}	1 (1.2) ^c	93.7 (10.2) ^{cde}
<i>A.indica</i> bark	0. 3(0.9) ^{ab}	1 (1.2) ^c	93.7 (10.2) ^{cde}
<i>A.indica</i> kernel	0.2 (0.8) ^{bc}	1 (1.2) ^c	91.9 (10.1) ^{de}
<i>C.citratius</i> leaf	0.2 (0.8) ^{bc}	1 (1.2) ^c	90.1 (10.0) ^e
<i>T. erecta</i> leaf	0.2(0.8) ^{bc}	1 (1.2) ^c	91.9 (10.1) ^{de}
<i>A.sativum</i> bulb	0.2 (0.8) ^{bc}	1 (1.2) ^c	90.9 (10.1) ^{de}
<i>M.lanceolata</i> seed	0.2 (0.8) ^{bc}	2 (1.61) ^b	90.1 (10.0) ^e
<i>C.ambrosoid</i> leaf	0.1 (0.8) ^{cd}	1 (1.2) ^c	94.6 (10.3) ^{bc}
<i>G. hirsutmn</i> seed oil	0.0 (0.7) ^d	0 (0.7) ^d	97.3 (10.4) ^{ab}
<i>B.carinata</i> seed oil	0.0 (0.7) ^d	0 (0.7) ^d	97.3 (10.4) ^{ab}
<i>E.kebericho</i> root	0.2 (0.8) ^{bc}	1 (1.2) ^c	91.9 (10.1) ^{de}
P value	0.0001	0.0001	0.0001
HSD	0.63	0.297	2.28
CV (%)	14.55	8.7	0.143

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different (P<0.05)

The effect of plant powders on the viability (germination rate) of treated grains discloses significant difference among treatments and compared with control 86.5%. But, the highest germination rate was observed from the two cooking oils, *G. hirsutum* and *B. carinata* with 97.3% comparatively with the standard check chemical with 99.1%. The mean percent germination of other botanicals ranges from 86.5% - 99.1% (Table 8).

The result of simple linear correlation studies among the variables revealed that there exists an association between percent toxicity, mean progeny emergency, number of perforated holes on the grains, percent weight loss and germination percentage of maize grains infested with the same number of *S. cerealella* (Table 9). Percent adult mortality was inversely and significantly correlated with mean progeny emergency ($r=-0.40^*$), number of grains perforated ($r = -0.73^{**}$), percentage weight loss ($r=-0.52^{**}$) but positively and significantly correlated with germination percentage ($r=0.56^{**}$). With an increasing trend of moth's adult mortality; there is a decreasing trend of progeny emergency, number of perforated holes on the grains, percent weight loss. On the other hand, an increasing adult mortality is associated with an increasing germination percentage.

Table 9. Pearson correlation coefficients for maize grains infested by *Sitotroga cerealella*

	Mortality (%)	Progeny Emerged	Hole Number	Weight Loss (%)	Germination (%)
Mortality (%)	1	-0.40*	-0.73*	-0.52*	0.56*
Progeny Emerged		1	0.48*	0.68*	-0.75*
Hole Number			1	0.56*	-0.61*
Weight Loss (%)				1	-0.87*
Germination (%)					1

*=Significant at 5% and 1% level of probability

4.3. Toxicity of (Cumulative) *G. hirsutum* and *B. carinata* Seed Oils against *Sitophilus zeamais*

Results regarding mortality effects of chemical fractions of *G. hirsutum* and *B. carinata* seed oils on *S. zeamais* are presented in Table 10. *G. hirsutum* and *B. carinata* being the most promising growth inhibitors & were divided in to four promising fractions (0.5ml, 0.4ml, 0.3ml and 0.2ml) each dissolved in 2ml acetone (95%) for complete dissolution. Out of these, fraction-1 and fraction-2 completely inhibited the growth of *S. zeamais* within 72 hours of exposures, i.e. 100%

mortality by *G. hirsutum* and 90% mortality by *B. carinata* seed oil (Table 10). Fortunately, the present study showed that no botanicals cause any noticeable adverse effect on the germination capacity of the maize grain. This indicates that most of the treatments do not interfere with viability of seeds and can be applied for the protection of stored grain for food and seed purposes. The two cooking oils namely, *G. hirsutum* and *B. carinata* seed oils at the higher rates (0.5ml and 0.4ml/250g) were seen to be the best out of the concentrations tested.

Like those tested botanical powders, the activity of cooking oils against adult *S. zeamais* and *S. cerealella* appeared to be directly related to level of application and exposure periods. In general, these results indicate that effective control of *S. zeamais* and *S. cerealella* using cooking oils is possible although there appeared variation of efficacy due to concentration and longer exposure period. Recently, in elsewhere the use of different plant extracts and plant derived oils for the control of stored-product insect pests has been reported. The present investigation is in agreement with the result obtained by Girma *et al* (2008) that he evaluated the other cooking oils mixing with maize grains against *S. zeamais*.

Table 10. Percent adult mortality (Cumulative) and Median Lethal Time (LT₅₀) of different concentrations of *Gossypium hirsutum* and *Brassica carinata* against *Sitophilus zeamais*

Treatment	Treatment Levels	Mortality (%)	Median Lethal time(LT ₅₀) in days	Confidence Interval		Slope
				Lower	Upper	
<i>G. hirsutum</i> oil	0.2 ml	30	25.8 ^c	13.9	94.9	1.43±0.29
	0.3 ml	45	8.4 ^b	-*	-*	0.78±0.54
	0.4 ml	85	0.5 ^a	-	-	1.18±0.69
	0.5 ml	100	0.6 ^a	0.0	0.92	3.33±1.56
<i>B. carinata</i> oil	0.2 ml	35	50.9 ^c	13.9	94.9	1.43±0.23
	0.3 ml	25	15.5 ^b	-	-	0.78±0.80
	0.4 ml	80	1.3 ^{ad}	0.18	2.2	1.18±0.40
	0.5 ml	90	0.6 ^{ad}	0.01	0.23	3.33±0.44
Malathion	0.125g	90	0.14 ^b	-	-	1.01±1.03
Control	-	5	-	-	-	-

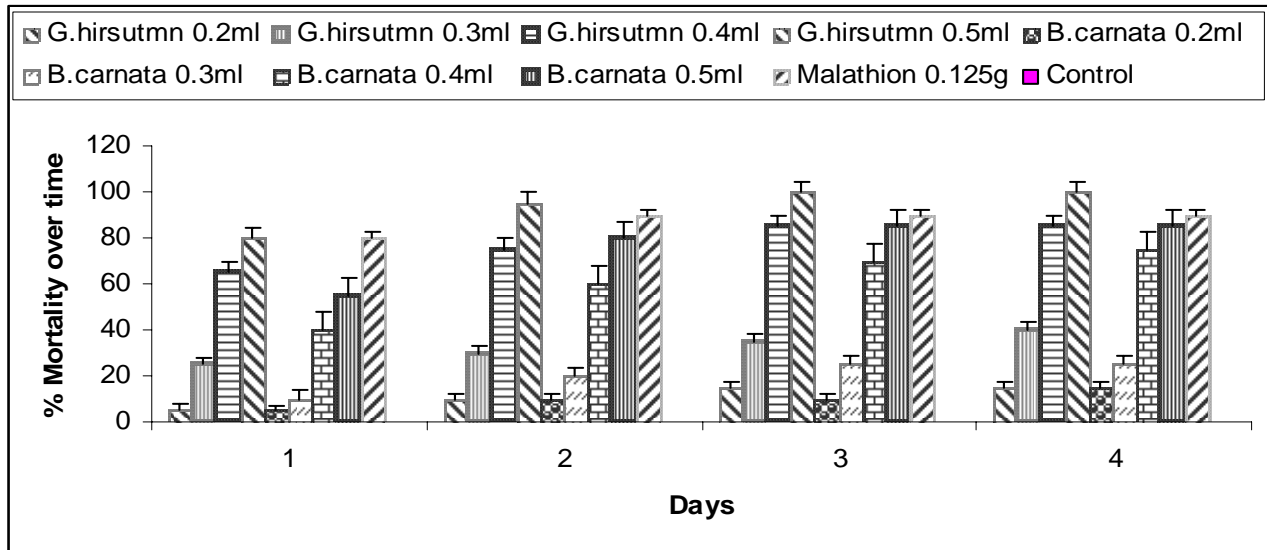
*The confidence interval for the cooking oils and malathion could not provide, because of the very low LT₅₀ obtained which are beyond the computing capacity of the soft ware (USEPA probit analysis program)

By the last 20 days, 0.6 (LT₅₀) *G. hirsutmn* at concentration of 0.5ml and 0.5 (LT₅₀) for *G. hirsutmn* at concentration of 0.4ml was recorded. Similarly, 0.6 (LT₅₀) by *B. carinata* seed oil at 0.5ml concentration and 1.3 (LT₅₀) at 0.4ml concentration of *B. carinata* seed oil was among the results recorded. On the other hand, the oils were seen to be the promising chemical compared to standard check chemical malathion dust which was with (90%) at toxicity at 0.14 (LT₅₀) (Table 10).

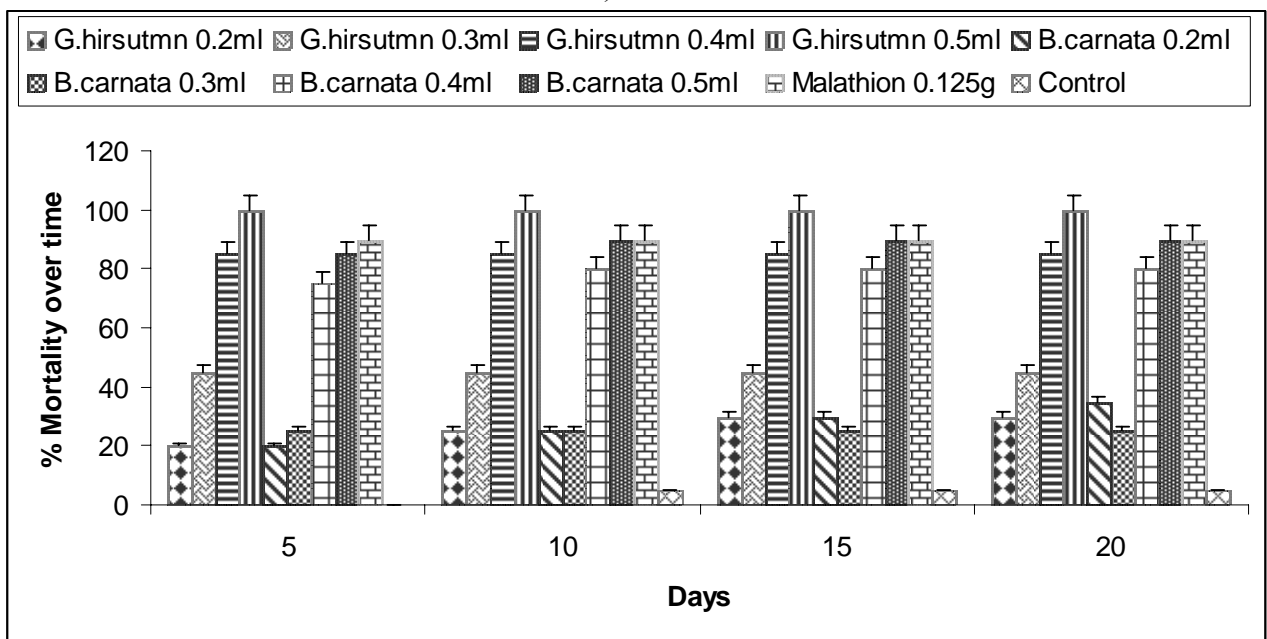
Among the treatments, *G. hirsutmn* and *B. carinata* were highly significant in reducing maize grain damage at all concentrations. It appears therefore that the pure oils screened have pesticidal properties which account for much higher levels of their effectiveness in reducing the feeding damage of *S. zeamais*. Additionally, most of botanical powders admixed to the maize been seed provided significant reduction of grain weight losses compared to the untreated check, suggesting that the presence of chemical factors that can interfere with the feeding habit of the *S. zeamais*.

4.4. *Sitophilus zeamais* Progeny Emergence after Grains Treated with *G. hirsutmn* and *B. carinata* seed oils

Mean number of *S. zeamais* adult progeny emergency from grains treated with different concentrations of cooking oils is presented in Table 11. The different rates of cooking oils significantly ($P < 0.05$) reduced *S. zeamais* progeny emergency. Up to the 15th day after adult removal (from day 21-35), no progeny was emerged from all jars including the untreated control. On 40th day of adult introduction (20th day of introduced adults removal), no maize grain weevil emerged from the jars that received Malathion dust and the two cooking oils. This was true throughout the experimental period with no adult grain weevil emergency from these three treatments indicating the effectiveness of the two cooking oils against *S. zeamais* similar to Malathion dust. In all the other treatments, there was an increasing trend of adult emergency at 40th days of adult introduction indicating very effectiveness of these cooking oils. The treatments with minimum application rate (0.2ml) had comparably higher number of adult emerged at the final 40th days of adult introduction. Similar result was reported by Javed *et al.*, (2010) that the three botanical extracts significantly reduce the F1 progeny emergence in wheat grain.



a)



b)

Figure 4. Percent adult mortality (Cumulative) of *Sitophilus zeamais* by different concentrations of *G. hirsutum* and *B. carinata* seed oils at different time intervals (days)

The reduction in F_1 progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested cooking oils. But, still higher dosages and longer exposure periods are needed to achieve appreciable control as as been reported by several authors. Girma *et al.*, (2008) noted that, the different application rates of the cooking oils other than

G. hirsutmn and *B.carinata* seed oils resulted in complete (100%) inhibition of oviposition and subsequent progeny production of *S.zeamais* in maize grains.

Table 11: *Sitophilus zeamais* progeny emergence from maize grains treated with different concentrations of *G. hirsutmn* and *B.carinata* seed oils at different time intervals (days)

Treatments	Levels	40th day after adult introduction
<i>G. hirsutmn</i> oil	0.2 ml	3 (1.9) ^{b*}
	0.3 ml	0 (0.7) ^d
	0.4 ml	0 (0.7) ^d
	0.5 ml	0 (0.7) ^d
<i>B. carinata</i> oil	0.2 ml	1 (1.2) ^c
	0.3 ml	0 (0.7) ^d
	0.4 ml	0 (0.7) ^d
	0.5 ml	0 (0.7) ^d
Malathion	0.125g	0 (0.7) ^d
Control	-	8 (2.9) ^a
P value		0.0001
HSD		0.37
CV(%)		15.3

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different ($P<0.05$)

4.5. Maize Grain Damage by *Sitophilus zeamais* after Treated with *G. hirsutmn* and *B. carinata* Seed Oils

Grain damage by *S. zeamais* was assessed in terms of counting perforated holes, percent weight loss and percent germination reduction caused by adult weevil and larvae feeding inside the seeds on 45th days of adult weevil introduction. The treatments were significantly different ($P<0.05$) with respect to the number of perforated seeds, percent weight loss and grain viability (Table 12). Mean numbers of perforated seeds were maximum (0.5 out of 10 seeds) from the least concentrations of *G.hirsutmn* and untreated check which was significantly different from jars that received higher rates (no holed seeds out of 10 seeds) were seen on par with Malathion dust formulation.

Maximum weight loss was recorded from untreated grains (0.8 %) followed by grains treated with least concentrations 0.4% weight loss. No weight loss was recorded from the higher rates of the two cooking oils on par with the standard check (Mmalathion). Some treatment effects were significantly different from others after 45 days of grain storage and the untreated grain suffered

highly significantly greater grain damage as well as weight loss. Similar trends were followed for the percent germination assessed.

Table 12. Hole number counted, percent weight loss and percent germination as infested by *Sitophilus zeamais*

Treatments	Levels	Hole Number/ 10 seeds	Weight Loss (%)	Germination (%)
<i>G. hirsutum</i> oil	0.2 ml	0.5(1.0) ^{a*}	0.4(1.2) ^{b*}	86.5(9.8) ^{fg*}
	0.3 ml	0.2(0.8) ^d	0(0.7) ^c	89.2 (10) ^{de}
	0.4 ml	0.1(0.8) ^d	0(0.7) ^c	92.8 (10.2) ^{bc}
	0.5 ml	0.0(0.7) ^e	0(0.7) ^c	95.5 (10.3) ^a
<i>B. carinata</i> oil	0.2 ml	0.3(0.9) ^b	0.4(1.1) ^{bc}	88.3 (9.9) ^{ef}
	0.3 ml	0.2(0.8) ^c	0.4(0.9) ^{bc}	89.2 (9.97) ^{de}
	0.4 ml	0.1(0.8) ^c	0(0.8) ^c	91.9 (10.1) ^{cd}
	0.5 ml	0.0(0.7) ^e	0(0.7) ^c	93.7 (10.2) ^{ab}
Malathion	0.125g	0.0(0.7) ^c	0(0.7) ^c	95.5 (10.3) ^a
Control	-	0.54(1.0) ^a	0.8(1.6) ^a	84.8 (9.7) ^g
P value		0.0001	0.0001	0.0001
HSD		0.28	0.37	0.11
CV(%)		6.75	13.9	0.4

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different (P<0.05)

4.6. Toxicity of (Cumulative) *G. hirsutum* and *B. carinata* Seed Oils against *Sitotroga cerealella*

Results regarding mortality effects of cooking oils (*G. hirsutum* and *B. carinata* seed oils) against *S. cerealella* are presented in Table 13. Results revealed that, *G. hirsutum* was seen the most promising growth inhibitors which cause maximum mortality at 0.5ml of 100% and minimum toxicity at 0.2ml with 66.7%. The other cooking oil *B. carinata* recorded 100% mortality at 0.5ml concentration and minimum mortality (50%) at 0.2ml at 20th days of exposures. But, highly significance differences were observed comparatively with that of untreated control that records (11.1 %). For the 20 days exposures, the median lethal time (LT₅₀) was <0.5 for the highest(100%) mortality revealed from fraction-1 in both cases that completely inhibited the growth of *S. cerealella* which is similar with that of Malathion super dust formulations (100%) and still highly significant difference was observed compared with that of control treatment (Table 13).

Table 13. Adult mortality (Cumulative) and Median Lethal Time (LT₅₀) of *Sitotroga cereallela* by different concentrations of *Gossypium hirsutum* and *Brassica carinata* seed oils

Treatment	Treatment Levels	Mortality (%)	Median Lethal time(LT ₅₀) in days	Confidence Interval		Slope
				Lower	Upper	
<i>G. hirsutum</i> oil	0.2 ml	66.7	1.8 ^c	0.3	3.0	1.60±0.68
	0.3 ml	77.8	0.5 ^b	-*	-*	1.57±0.93
	0.4 ml	83.3	0.6 ^b	-	-	1.57±0.96
	0.5 ml	100	<0.5 ^a	-	-	-
<i>B. carinata</i> oil	0.2 ml	50	2.6 ^b	-	-	1.67±0.91
	0.3 ml	61.1	1.6 ^b	-	-	0.50±0.46
	0.4 ml	94.4	0.6 ^{cd}	0.0	1.0	2.40±0.20
	0.5 ml	100	<0.5 ^a	-	-	-
Malathion	0.125g	100	<0.5 ^a	-	-	-
Control	-	11.1	68 ^d	-	-	1.17±0.66

*The confidence interval for the oils and malathion could not provide, because of the very low LT₅₀ obtained which are beyond the computing capacity of the soft ware (USEPA probit analysis program)

4.7. Progeny Emergence of *Sitotroga cereallela* after Grains Treated *G. hirsutum* and *B. carinata* Seed Oils

Mean number of *S. cereallela* adult progeny emerged from maize grains treated with *G. hirsutum* and *B. carinata* seed oils at different application rates were presented in Table 14. The result discloses that no adult was emerged from the first through 10-days from the days of adult removal i.e. the different rates of cooking oils significantly (P<0.05) reduced *S. cereallela* progeny emergency. But, starting from the next 15th days from adult removal, few progenies were started to be seen and significant difference in progeny number between all concentrations were recorded. Treatments with highest ratios resulted with no progeny emerged which was with significantly different number of progeny from the untreated control treatment.

The reduction in F₁ progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested cooking oils. But, still higher dosages and longer exposure periods are needed to achieve appreciable control as as been reported by several authors. Girma *et al.*, (2008) noted that, the different application rates of the cooking oils other than

G. hirsutum and *B. carinata* seed oils resulted in complete (100%) inhibition of oviposition and subsequent progeny production of *S. zeamais* in maize grains.

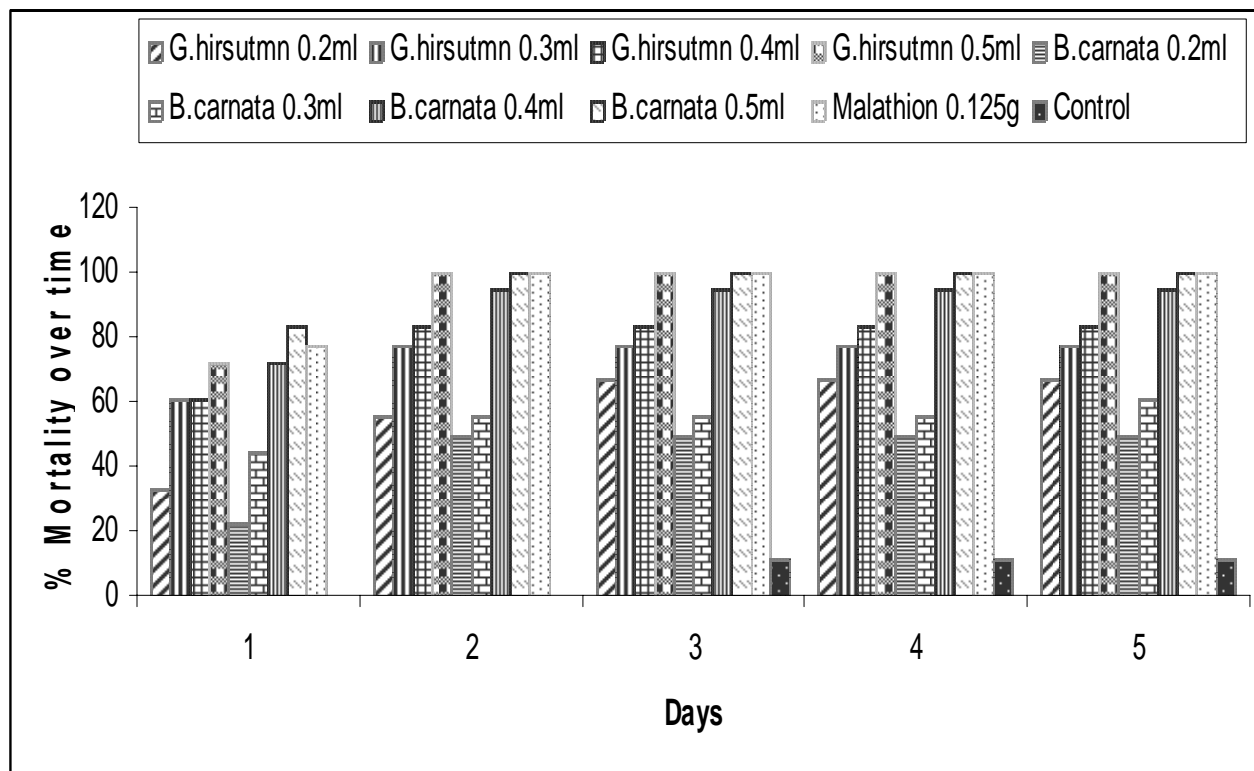


Figure 5. Percent adult mortality (Cumulative) of *Sitotroga cerealella* by different concentrations of *G. hirsutum* and *B. carinata* seed oils at different time intervals (days)

The number of grain moth progeny emergence was significantly reduced from the grain treated with *G. hirsutum* and *B. carinata* oils. Practically, plant oils coating can be effective in reducing progeny production by storage insect pests which is similar with Javed *et al.*, (2010), that he reported the possible cause for reduction of F1 progeny production of *S. cerealella* in treated grains with cooking oils. This was likely that immature stages of the insects were killed physically by oil coating and impairing respiration through blockage of spiracles thereby resulting in inhibiting immature stages survival or reduced longevity of adult females.

Table 14. *Sitotroga cereallela* progeny emergence from maize grains treated with different concentrations of cooking oils at different time intervals (days)

Treatments	Levels	Progeny emerged after 40 th days
<i>G. hirsutmn</i> oil	0.2 ml	2 (1.6) ^{b*}
	0.3 ml	1 (1.2) ^{bc}
	0.4 ml	0 (0.7) ^c
	0.5 ml	0 (0.7) ^c
<i>B. carinata</i> oil	0.2 ml	2 (1.6) ^b
	0.3 ml	0 (0.7) ^c
	0.4 ml	0 (0.7) ^c
	0.5 ml	0 (0.7) ^c
Malathion	0.125g	0 (0.7) ^c
Control	-	4 (2.1) ^a
P value		0.0047
HSD		0.37
CV(%)		13.5

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different (P<0.05)

4.8. Maize Grain Damage by *Sitotroga cereallela* after Treated with *G. hirsutmn* and *B. carinata* Seed Oils

Grain damage by *S. cereallela* was assessed in terms of counting perforated holes, percent weight loss and percent germination reduction caused by adult weevil and larvae feeding inside the seeds on 45th days of adult weevil introduction. The treatments were significantly different (P<0.05) with respect to the number of perforated seeds, percent weight loss and grain viability (Table 15). Mean numbers of perforated seeds were maximum (0.2 out of 10 seeds) from untreated check which was significantly different from jars that received higher rates (no holed seeds out of 10 seeds) were seen on par with Malathion dust formulation.

Maximum weight loss was recorded from untreated grains (0.63 %) and the higher concentrations (0.5ml) which scored 0.5% weight loss was from grains treated with least concentrations. No weight loss was recorded from the higher rates of the two cooking oils on par with the standard check (Mmalathion). Similar trends were followed for the percent germination assessed. That means, higher germination percentages were recorded from the two promising cooking oils with higher ratios which are (95.5%) *G. hirsutmn* and (93.7%) *B. carinata* at 0.5ml, compared to

(88.3%) *G. hirsutmn* and (88.3%) *B. carinata* at 0.2ml level and comparatively to standard check chemical Malathion (95.5%). But, significantly different results were revealed compared to the untreated control with (85.6%) (Table 15). Similar investigation was reported by Eler and Cetin, 2009; Mulungu (2007) and Maribet and Aura (2008).

Table 15. Hole number counted, percent weight loss and percent germination of maize grains treated with different concentrations of *G. hirsutmn* and *Brassica carinata* Seed Oils by *Sitotroga cereallela*

Treatments	Levels	Hole Number /10 seeds	Weight (%)	Loss	Germination (%)
<i>G. hirsutmn</i> oil	0.2 ml	0.1(0.8) ^{ab*}	0.51(1.27) ^{ab*}		88.3(9.9) ^{de*}
	0.3 ml	0.1(0.8) ^{bc}	0.41(1.03) ^{bc}		90.1(10) ^{cd}
	0.4 ml	0.0(0.8) ^c	0.31(0.77) ^c		93.7(10.2) ^a
	0.5 ml	0.0(0.8) ^c	0.28(0.7) ^c		95.5(10.3) ^a
<i>B. carinata</i> oil	0.2 ml	0.1(0.8) ^{ab}	0.52(1.3) ^{ab}		88.3(9.9) ^{de}
	0.3 ml	0.1(0.87) ^{bc}	0.41(1.03) ^{ab}		89.2(9.97) ^{cd}
	0.4 ml	0.0(0.7) ^c	0.37(0.93) ^{bc}		91.9(10.1) ^{ab}
	0.5 ml	0.0(0.7) ^c	0.31(0.9) ^c		93.7(10.2) ^{ab}
Malathion	0.125g	0.0(0.7) ^c	0.31(0.9) ^c		95.5(10.3) ^a
Control	-	0.2(0.8) ^a	0.63(1.1) ^a		85.6(9.77) ^e
P value		0.0001	0.0001		0.0001
HSD		0.41	0.495		0.14
CV (%)		14.79	16.7		0.5

*The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different (P<0.05)

The cooking oils at all dosage rates nevertheless offered better protection than the control. The reduced damage recorded by the bio-pesticide (cooking oils) is an indication of their efficacy against grain moth infestation, hence, damage and seem to follow the trend of potency of the trial insecticides on insect mortality (Knock down). The effective protection offered by the two cooking oils seems to be consistent with the findings of Girma *et al* (2008) who found other cooking oils as a promising locally available grain protectant against *S. Zeamais* and caused over 50% mortality of the weevils up to the six months of storage. Shaheen (2006) also reported the neem seed powder applied at the rate of 1% w/w caused 100% mortality against pulse beetle within four days.

Generally, results in the present study on neem seed powder against *S. zeamais* and *S. cereallela* were in agreement with the previous studies in reducing the number of adult insects in general;

eventhouth the efficacy is not as effective as the previous studies. This reduced in efficacy of the neem powder might be because of several factors including the harvesting time of the seed, other ecological factors (temperature, rainfall and soil type), the concentrations used, and variations in insect behavior and species susceptibility.

5. SUMMARY AND CONCLUSIONS

The current findings demonstrated that most of the botanical plant powders and cooking oils tested against maize weevil, *S. zeamais* and *S. cereallela* possess insecticidal properties that can be used in the control of both insect pests in maize grain storages. The cooking oils from *G. hirsutmn* and *B. carinata* seed oils exhibited the most promising potent botanicals in toxicity action against maize weevil, *S. zeamais* and Angoumois grain moth, *S. cereallela* in maize storages. The two cooking oils in their higher concentrations (0.5 ml and 0.4 ml) caused mortality ranging from 77.8% - 100% against *S. cereallela* and 94.5 – 100 % against *S. zeamais* within one to two days of exposures. In general, no or few progenies were emerged from the maize grains treated with higher concentrations of the oils. As a result, no significant weight losses were observed and no significant impact observed over the germination rate of the grains treated with oils.

Among the powders of botanicals tested, the *C. ambrosoids* was observed to be the most bio-potent botanical and the rests of botanicals revealed to be moderately toxic against the two insect pests over 20 days exposures. In addition, *A. indica* leaf and kernel powders, as well as *T. erecta* leaf powder also caused higher mortality among all the botanicals used at the first day to 48 hours exposure, even compared to *C. ambrosoids* leaf powder at this point. Although eggs were not actually seen, oviposition of the maize weevils and moths were not totally poisoned as indicated by the presence of adult emergence. The average total development period (TDP) of *S. zeamais* noted about 40 days and 42 days for the moths, *S. cereallela* that emerged from maize grains treated with botanicals and cooking oils which was almost similar with the TDP from untreated control. The availability of these botanicals in or around the farm of most maize growers is another additional value for which botanical powders are preferred to other control methods, particularly the use of synthetic insecticides.

6. RECOMMENDATIONS

Farmers can save their stored-grains, capital, health and environment by using the cooking oils from cotton, *G. hirsutum* seed and Ethiopian mustard, *B. carinata*. Solutions of the oils should be sprayed over the maize grains, 0.2ml-0.5ml / 250g, before storing. In addition, the use of the evaluated oils is also needed to further determine their potential as insecticide against maize weevil and Angoumois grain moth.

Brassica carinata and *G. hirsutum* seed oil is generally extracted by using harsh chemical solvents and heat which may alter the chemistry of the oil. Most nutritionists are still uncertain about the long-term implications of these changes. *G. hirsutum* is high in Vitamin E, which is an antioxidant which works against the free radicals that cause cell damage aging. In addition, cottonseed oil contains gossypol, a substance that has been shown to cause sterility in rats. For this reason, it has been used in parts of the world as a contraceptive and has been seen as a threat to men's fertility. Gossypol still has toxins that decrease spermatogenesis and sperm motility in men. So, the effect of gossypol from cotton seed oil on human body needs to be studied further.

The cause for a considerable protection of maize grains against the insect attack could be due to the presence of different chemicals which interfere with the feeding habit of the pest. Identification of the chemicals responsible should be an immediate research agenda. It should be appreciated at government level, to achieve meticulous practical benefits.

Contribution of cooking oils in the IPM programs must be promoted to make our food products according to the standards, which recognize the key elements ensuring the food safety up to the point of final consumption. Further research is required to explore some new indigenous organic sources of the insecticidal allelo-chemicals, which can, more efficiently, be utilized for the food-safety purposes and to overcome the dilemma of health hazards and environmental pollution. It is recommended therefore that a similar study should be conducted up on the two insects separately by using other parts of the test plants like roots, flowers or even the whole plant to further evaluate their efficacy against maize weevil and grain moth and other important stored product pests. Finally the cost benefit analysis of the best botanical products should be done for the future directions.

There are a number of botanical plants known for their insecticidal value, but there was no much progress in isolating toxic substance from the plants and do some scientific work to promote them to the level of synthesizing them in the industry, so that they can be marketed to generate extra revenue to the community and country. It is widely believed that the identification of the active compounds and their modes of action against insect pest would contribute a lot to their use in stored maize protections. In such practice (botanical screening and rating) need the involvement of chemists, biochemists and environmental scientists that may enhance the development of products which may even play a great role in the economic development of a given country.

Their transformed products may also become important supplements to imported synthetic pesticides to control stored pests. Botanicals also present many farmers with large number of options for controlling insect pests that attack their products as they are cheap and based on local materials. Because, some of the botanical plants tested are considered as noxious weed by farmers and anti-fungal growth on the fruits and vegetables in the post harvest management, there should be a scientific rationale for the incorporation of these botanical plants into the grain protection practice of resource-poor farmers. It is also essential that further work to isolate, improve their efficacy and reliability and appropriate technological systems need to get priority concern.

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APPENDICES

Annex 1. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 1 day's exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	2.93076923	0.24423077	4.94	0.0003
Error	26	1.28666667	0.04948718		
Corrected Total	38	65.42307692			
		CV		9.791632	
		Alpha		0.05	
		Error Degrees of Freedom		26	
		Error Mean Square		0.049487	
		Critical Value of Studentized Range		5.13931	

Annex 2. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 2 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	3.88923077	0.32410256	10.53	<.0001
Error	26	0.80000000	0.03076923		
Corrected Total	38	4.68923077			
		CV		12.32622	
		Alpha		0.05	
		Error Degrees of Freedom		26	
		Error Mean Square		0.030769	
		Critical Value of Studentized Range		5.13931	
		Minimum Significant Difference		0.5205	

Annex 3. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 3 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	15.00256410	1.25021368	53.00	<.0001
Error	26	0.61333333	0.02358974		
Corrected Total	38	15.61589744			
	CV			10.89089	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.02359	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.4557	

Annex 4. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 4 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	8.45025641	0.70418803	54.93	<.0001
Error	26	0.33333333	0.01282051		
Corrected Total	38	8.78358974			
	CV			10.10499	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.012821	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.336	

Annex 5. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 5 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	2.77589744	0.23132479	7.91	<.0001
Error	26	0.76000000	0.02923077		
Corrected Total	38	3.53589744			
CV				16.38288	
Alpha				0.05	
Error Degrees of Freedom				26	
Error Mean Square				0.029231	
Critical Value of Studentized Range				5.13931	
Minimum Significant Difference				0.5073	

Annex 6. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 10 days exposures

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	12	11.22307692	0.93525641	35.76	<.0001
Error	26	0.68000000	0.02615385		
Corrected Total	38	11.90307692			
CV				12.74169	
Alpha				0.05	
Error Degrees of Freedom				26	
Error Mean Square				0.026154	
Critical Value of Studentized Range				5.13931	
Minimum Significant Difference				0.4799	

Annex 7. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 15 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	4.34769231	0.36230769	10.39	<.0001
Error	26	0.90666667	0.03487179		
Corrected Total	38	5.25435897			
	CV			14.65363	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.034872	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.5541	

Annex 8. ANOVA table for showing percent mortality of *Sitophilus zeamais* at 20 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	4.13641026	0.34470085	10.50	<.0001
Error	26	0.85333333	0.03282051		
Corrected Total	38	4.98974359			
	CV			14.33146	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.032821	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.5375	

Annex 9. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 1 day exposure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	12.02307692	1.00192308	1302.50	<.0001
Error	26	0.02000000	0.00076923		
Corrected Total	38	12.04307692			
	CV			1.811835	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.000769	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.0823	

Annex 10. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 2 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	1.74974359	0.14581197	3.87	0.0019
Error	26	0.98000000	0.03769231		
Corrected Total	38	2.72974359			
	CV			17.09178	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.037692	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.5761	

Annex 11. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 3 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	2.26974359	0.18914530	13.66	<.0001
Error	26	0.36000000	0.01384615		
Corrected Total	38	2.62974359			
	CV			13.03727	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.013846	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.3491	

Annex 12. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 4 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	0.14102564	0.01175214	0.92	0.5445
Error	26	0.33333333	0.01282051		
Corrected Total	38	0.47435897			
	CV			15.60382	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.012821	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.336	

Annex 13. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 5 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	1.63025641	0.13585470	6.71	<.0001
Error	26	0.52666667	0.02025641		
Corrected Total	38	2.15692308			
	CV			16.82023	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.020256	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.4223	

Annex 14. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 10 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	0.92307692	0.07692308	3.00	0.0092
Error	26	0.66666667	0.02564103		
Corrected Total	38	1.58974359			
	CV			19.95207	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.025641	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.4751	

Annex 15. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 15 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	0.93589744	0.07799145	6.08	<.0001
Error	26	0.33333333	0.01282051		
Corrected Total	38	1.26923077			
	CV			14.57386	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.012821	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.336	

Annex 16. ANOVA table for showing percent mortality of *Sitotroga cereallela* at 20 days exposures

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	1.81230769	0.15102564	7.46	<.0001
Error	26	0.52666667	0.02025641		
Corrected Total	38	2.33897436			
	CV			16.32552	
	Alpha			0.05	
	Error Degrees of Freedom			26	
	Error Mean Square			0.020256	
	Critical Value of Studentized Range			5.13931	
	Minimum Significant Difference			0.4223	