COMPARATIVE EFFICACY OF NEEM (*Azadirachta indica* L.)AND PEPPERMINT (*Mentha piperita* L.) LEAF POWDERS AS A CONTROL OF ADULT MAIZE WEEVIL (*Sitophilus zeamais* Motsch) IN STORED SORGHUM GRAINS

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Comparative Efficacy of Neem (*Azadirachta indica* L.) and Peppermint (*Mentha piperita* L.) Leaf Powder as a Control of Adult Maize Weevil (*Sitophilus zeamais* Motsch.) in Stored Sorghum Grains

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By

Jemal Gebi

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APPROVAL SHEET

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As thesis Research advisors, we hereby certify that we have read and evaluated this Thesis, prepared, under our guidance by Jemal Gebi entitled: **Comparative Efficacy of Neem (*Azadirachta indica* L.) and Peppermint (*Mentha piperita*L.) Leaf Powders as a Control of Adult Maize Weevil (*Sitophilus zeamais* Motsch.) in stored Sorghum Grains.** We recommend that it be submitted as fulfilling the 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 and evaluated the Thesis prepared by Jemal Gebi and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirements for the degree of Master of Science in the field of Biological Sciences.

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External examiner	Signature	Date

DEDICATION

This piece of work is dedicated to my beloved family: my Father Gebi Wodesso, my mother Jelane Dalu, and to all my sisters and brothers.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this M.Sc Thesis is my own work. I have followed all ethical and technical principles of scholarship in conducting studies, data collection, data analysis, and compilation of this Thesis. Any scholar matter that is included in the Thesis has been given recognition through citation.

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TABLE OF CONTENTS

TABLE OF CONTENT (*CONTINUED*)

LIST OF TABLES

Table **Page**

` LIST OF FIGURES

Comparative Efficacy of Neem (*Azadirachta indica* L.) And Peppermint (*Mentha piperita* L.)Leaf Powders as Control of Adult Maize Weevil (*Sitophilus zeamais* Motsch.) in stored Sorghum Grains

ABSTRACT

Plant extracts in powder or essential oil form from different bioactive compound producing plants are known to be effective repellents and insecticides against different storage pests of grains. Post-harvest losses are one of the major causes of food insecurity in the developing world. , the present study was planned to investigate the effect of neem and peppermint leaf powders in control of maize weevil and the prolonged storage of the powders on their insecticidal activity. A 2 × 2 x 3 factorial experiment was laid in a Complete Randomized Design (CRD) with two replications. The results indicated that all the treatments had shown high repellence against maize weevil at all amounts used (0.5, 1, and 1.5 grams) for the whole duration storage. However, there was no significant difference among 0.5, 1, and 1.5 grams for all the treatments including neem, mint, and combination of neem and mint powders. Mean separation for maize weevil mortality rate during 48 days of treatment of sorghum grain with different amounts of neem, mint and combination of neem and mint leaf powder had shown that there was no mortality of maize weevils recorded for control (0gram). However, significant mortality was recorded for each treatment during 45 days of storage. The highest mean mortality rate was recorded for neem leaf powder than for mint leaf powder, and combinations of neem and mint leaf powders showing insecticidal property of neem leaves. Maize weevil progeny emergence rate during extended duration of sorghum grain storage as treated by neem, mint, and combinations of neem-mint leaf powders indicated that the highest progeny emergence rate was recorded for control group in all treatments. Only after 48 days was the progeny emergence recorded for experimental groups. It can be concluded from the present study that neem and mint leaf powders could be used as effective insect repellents. Even though the neem and mint leaf powder retards population growth of maize weevil, the powders cannot be recommended for extended storage duration.

Keywords: *Insecticidal activity, Mortality, Plant extracts, Repellence, Storage duration.*

1. INTRODUCTION

Sorghum, Sorghum *bicolor* (L.) Moench is an important cereal crop grown worldwide for food and feed purposes. It is mostly cultivated in semi-arid tropics where water is scarce and drought is frequent (Mailafiya, 2003; Beshir, 2011). The crop is environment-friendly as it is water efficient, requires little or no fertilizers. Sorghum ranks second among cereals and fifth among all crops in terms of production in Africa. Globally, sorghum, (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop after maize, rice, wheat and barley (FAOSTAT, 2013). It is a major food security crop in sub-Saharan Africa supporting some 300 million people. It is grown in drought-prone and marginal areas in semi-arid zones where other crops cannot grow reliably. In Ethiopia, sorghum is a major staple food crop, ranking second after maize in total production. It ranks third after wheat and maize in productivity per hectare, and after teff and maize in area cultivated. It is grown in almost all regions, covering a total land area of 1.8 million ha (CSA, 2015).

Among the key constraints to improving food security in Africa are losses resulting from poor post-harvest management of grains (Charles *et al*., 2016). The average grain losses due to storage pests is about 12% of the total grain produced but in some cases the losses could rise to 50% (Gabriel and Hundie, 2006). Deterioration of stored grains results from the interactions of several factors such as physical, chemical and biological variables existing the overall chains from production to consumption (Dubale *et al*., 2012). Deterioration of grain due to infestations of insects, mites, and fungi is the main post-harvest factor affecting the nutritional quality and marketability of stored grain. Grain storage pests are major concerns for farmers worldwide but especially in developing countries because large percentage of the crop may be lost to storage pests. Prior to any pest control interventions it is vital to assess the pest status and extent of losses that have occurred or likely to occur during storage (Togola *et al*., 2013).

Post-harvest losses are one of the major causes of food insecurity in the developing world. In Africa, at the farm level, producers store their grains for three purposes: for consumption until the next harvest, as seed for planting in the next season and for selling when prices become favorable. In many developing countries, including Ethiopia, grain storage practices involve traditional structures, which are largely ineffective in the prevention of deterioration of stored products (Abraham, 1995). The majority of farmers in Ethiopia (93.3%) use traditional storage containers that exposes stored grains to storage insect pest, mold and other los factors (Dubale *et al*., 2012).

Insect pests cause heavy losses to stored grains, especially in humid and warm areas of the world. Nature keeps a partial check on these insect pests by the action of predators, parasites, parasitoids and pathogens. Insect pest control in stored grains such as sorghum has relied heavily on synthetic insecticides due to their instant effectiveness and ease of application. However, extensive use of chemical/ industrial insecticides results in environmental pollution carry potential health hazards, pest resurgence, pest resistance and lethal effects on non-target organisms, increasing cost of application and erratic supply in developing countries due to foreign exchange constraints (Kolterman *et al*., 2000). Hill (1997) suggested that integrated pest management (IPM) approach in maintaining pest population below economic injury level (EIL) is necessary in order to reduce hazards associated with chemical application in stored cereals. This leads to a search for eco-friendly methods such as the use of botanicals in the control of insect pests.

A study conducted by Tadeos (2018) on traditional underground pit for storage of sorghum in Eastern Ethiopia has found significant infestation of stored sorghum grains with maize weevil (*Sitophilus zeamais*), angoumois grain moth (*Sitotroga cerealella*) and flour beetle (*Tribolium castenum*), flat grain beetles (*Cryptolestes ferugineus*)) and Saw-toothed grain beetle (*Oryzaephilus surinamensis*). Among the commonly recorded insects pest two of them, namely: maize weevil (*S. zeamais*), angoumois grain moth (*Sitotroga cerealella*) were highly abundant and damaging in major stored items. There were also maximum grain damage and reduced seed germination capacity in grain samples collected from these traditional underground pit storage systems. Therefore, efforts should be initiated on activities leading to improvement of traditional storage system and there by reduction in infestation, grain damage and losses.

The neem tree (*Azadirachta indica* L.) is a tropical evergreen plant with wide adaptability and known resistance to insect infestation (Oparaeke*et al.*, 2006). The tree is known to possess some compounds such as limonoids, which gives it a bitter taste while the principal bioactive content, Azadirachtin, is a repellent and anti-feedant to many insects (Ruchi*et al.*, 2014).Many plants can protect themselves against insects by producing their own chemical defenses that are toxic or repellent (Ileke and Oni, 2010). The consideration for the use of extracts of plants origin is that they are easily biodegradable, effective on some pests and considered safe in pest control operations as they minimize pesticide residues, ensure safety of the consumers of the treated grains and the environment. Further, the production of organic extracts of plant origin for pest control may be easier and less expensive than the synthesis of some complex chemical formulations (Dancewicz and Gabrys, 2008). Examples of these important bioactive compound producing plants are catnip, basil, artemisia, borage, dahlia, ginger, hyssop, chrysanthemum, lime, black pepper, clove, neem and garlic, and a host of others. These plants are known to contain organic compounds which possess bio-pesticidal properties in their bioactive components (Dancewicz and Gabrys, 2008).

Temperate countries suffer much less from stored grain pests as compared to tropical countries especially the areas with high humidity. More than half the cases of poisoning and about three-fourth of deaths documented took place in third world countries which together consume only 15 % of the total pesticide output in the world (Anonymous, 1992).Thus, losses due to pests should be reduced to minimum. In order to reduce serious losses experienced during storage, various techniques and control methods have been developed and more are still being developed. The destructive activities of insects and other storage pests have been adequately subdued by synthetic chemical control methods comprising fumigation of stored commodity with carbon disulphide, phosphine or dusting with Malathion, carbaryl, pirimiphosmethyl or permethrin (Ileke and Oni, 2010). However, there are problems associated with the use of these synthetic chemicals. The problems of many synthetic insecticides include high persistence, poor knowledge of application, increasing costs of application, pest resurgence, and genetic resistance by the insect and lethal effects on non-target organisms in addition to direct toxicity to users (Mondal and Khalequzzaman, 2010).

Though synthetic insecticides are being in use since 1950s and are of great help since inception, the adverse effects on human, non-target organisms and the environment has become of great concern. The concerns have made some countries to ban the use of some pesticides in a view to protect the ecosystem. It is evident that some plants possess insecticidal properties thus could be considered as natural suitable replacement for synthetic chemicals used in the control of pests. Plant extracts in powder or essential oil form from different bioactive compound producing plants are known to be effective repellents against different economic storage pests of grains, even for stored cereals (Owusu, 2001). There is strong need to establish acceptable daily intake (ADI), permissible limit (PL) and maximum residue limit (MRL) for neem products. The most active insecticidal ingredients are present mostly in the seeds, leaves and other parts of the neem tree (Sonalkar*et al*., 2014). Its various plant parts have been traditionally used to control in door insects, pests in stored grains, crops, human and livestock medicine. These properties have been attributed to hundreds of chemicals present in the tree. Neem is safe, available and renewable source of insecticide and fits in well in sustainable agriculture. Farmers in developing countries should be encouraged to use neem as substitute to synthetic insecticides. Hence, the present study was planned to investigate the effect of neem and peppermint leaf powders in control of maize weevil and prolonged storage of the powders on their insecticidal activity.

General Objective

To investigate the effect of neem, peppermint and neem and peppermint leaf powders in control of maize weevil.

Specific Objectives

- To determine the repellence effectiveness of neem, peppermint and neem-peppermint powders;
- To assess control effectiveness of neem, peppermint and neem-peppermint powders;
- To evaluate progeny emergency of storage pests from stored grains treated with neem, peppermint and neem-peppermint powders;
- To determine seed quality after treatment.

2. LITERATURE REVIEW

2.1. Losses of Food Grain Due to Pests

Global losses due to insect and non-insect pests such as birds, rodents etc. are estimated to be about 10% or even more, five percent of this is attributed to insect pests. These may appear small in term of quantity but if saved may solve our food problem (FAO, 1994). The losses in these commodities due to pests (10%) comes to 199745.1 thousand metric tons (FAO, 1994).In terms of value the estimated global losses due to various factors is more than $US 100 billion. Of this, 10% or 10 billion in term of value is attributed to various pests during storage. The losses due to insects (estimated to be 5%) then comes to $US 5 billion. These estimated losses are on global basis and not on the basis of country or the continent. The losses due to insect pests' in Asia and Africa, unlike cold climate countries, where mites are problem, must be much more as most developing countries in both the continents are very conducive for insect multiplication. Also sizeable amount of food grain (60-70 %) are still stored in traditional storage structures which are far from satisfactory (Radha, 2014).

2.2. Traditional Uses of Neem (*Azadirachta indica* L.) as Grain Protectant

Pest control or repelling organic extracts of plant origin offer protection with minimal impacts on the ecosystem and repel the insect pests from the treated materials by stimulating receptors (such as the olfactory receptor) of the insect. Repellent when effective causes the target pest to make an oriented movement away from the source of stimulus, and in cases where escape is not possible, over stimulation of the receptors leads to death of the pest. Use of neem dates back to pre-historic period as its use is mentioned in *Sanskrit* Language which is one of the oldest languages of the world. Keeping of neem leaves between folds of cloths leather goods and mixing them with grain destined for storage, generation of smoke to drive away mosquitoes are well known traditional practices. Of the different parts of the neem tree it is the neem leaves which have been used extensively in India and some other neighboring countries. Their use, however, differ indifferent regions**.** It is evident from the foregoing that neem is an effective alternative to contact synthetic insecticides and fumigants for management of stored grain pests. In certain cases it has even proved better than fumigants. Recently, there is a steady increase in the use of plant products as a cheaper, renewable and ecologically safer means of controlling insect pests of stored cereals and grains especially in the tropics where resource poor farmers are found (Ileke and Oni, 20011). Such plant materials include powders from parts of the neem tree (*Azadirachta indica* L.). Mixing dried neem leaves with grain in storage is a classic example of natural product use that has been practiced by farmers in many countries for many years. Neem is well known for its insecticidal properties and it is very effective against a wide range of insect pests (Radha, 2014). Since large variations occur in the bio-efficacy of neem products due to variations in neem trees, some degree of standardization and determination of shelf life of crude products become necessary for effective utilization.

Neem has been evaluated mostly as preventive measure and not as curative measure. Wheat seed infested with *Trogoderm granarium* when admixed with deoiled neem kernel powder completely checked its further multiplication (Singh, 1988). This shows that neem can be used as substitute to fumigants for disinfecting infested grains too. Traditionally neem leaf paste has been used for painting storage structures to prevent entry of insect pests. Farmers may be advised to use seed kernel paste which may provide effective preventive measure than leaf paste. A survey conducted in 8 Indian states and 2 Pakistan states showed that farmers used ‘handful’ to 5-10 kg. Of air dried neem leaves per 100 kg of grain (paddy/ rice, wheat, corn and sorghum) either alone or in combination with leaves of *Vitexnegundo* or *Pongamia pinnata* for protecting stored grain(Ahmed *et al*, 1988).Users found it effective in preventing entry of beetle and weevils if neem leaves are mixed with freshly harvested grains immediately on storage. The surveyed farmers were satisfied with protection obtained for their 3-6 months storage period. Also the treatment did not adversely affect the grain’s cooking quality as most of the residues of leaves get removed during winnowing (Singh and Srivastava, 2015).

With notable exception of *Oryzaephilus surnamensis* which feeds and breeds well on neem kernel powder all other insects infesting food grain, spices, tobacco, etc. are susceptible to neem products (Prakash and Rao, 1985). Neem leaves, seed kernel powder, deoiled neem kernel powder (cake) and seed oil are commonly used product for preservation of various agricultural commodities (rice, pulses, wheat, maize sorghum etc. Barring leaves all the other products are derived from seeds which are naturally shed by the tree. Leaves on the other hand are not always shed by the tree. The tree is deciduous in certain drier regions but ever green in other parts of the word. Plucking of large quantities from the tree is bound to effect health of the tree and consequently fruit yield.

Ripened fruits (yellow) which have dropped to the ground just have to be collected. Fruits are depulped and washed by rubbing the fruits with hand in a tub containing water (depulping become easy if fruits are allowed to ripen further for 24h. Keeping the beyond 24may affect seed quality). Seeds thus obtained are sun-dried for about 12 hrs and then for several days in shade to bring down the moisture content to less than 10%.. In case of rain, hot air blower may be used. Rapid drying at high temperature (70^{o}C) is not good. Drying of seeds is very important as wet seeds easily gomouldy.*Aspergillus* spp. destroys seed kernel and its most important active ingredient such as azadirachtin. One of the species *A. flavus*is most dangerous fungus on neem kernel as it produces highly toxic and possibly carcinogenic mycotoxin, aflatoxin. The U.S. aflatoxin limit is 20 parts/billion).Dried seeds are stored in low humidity area in airy containers such as jute bag, cloth bag, basket etc. but never in metal or plastic container. Seeds stored in this way may remain biologically active for one year (Singh and Srivastava, 2015).

After the oil has been expressed, the left over material (cake/deoiled powder) show high biological activity as it contains several biologically active compounds including azadirachtin. The cake obtained by manual pressing contains about 10% or more oil. The solvent extracted kernel powder on the other hand has almost no oil. In drier parts leaves are shed during spring. Large quantities of naturally fallen leaves are thus available in these areas which can be gathered and put to use. Fresh Green leaves which are plucked from the trees are dried under shade until they turn brittle and then pounded in a mortar to get fine powder for mixing (Singh and Srivastava, 2015).

2.3. Limitations of Use of Synthetic Insecticides in Developing Countries

The first and the foremost drawback of synthetic insecticides is its high toxicity to human beings and other forms of life specially, when it is used in close proximity as most storage structures are located in the premises. Unlike field conditions where residue of insecticides dissipates quickly it continue to persist on grain and other treated surface (Singh and Srivastava, 2015). Even if farmers are aware about the persistence of insecticides on the food grains they do not hesitate in selling if the price of the commodity goes up. It is practically impossible to fumigate grains under the condition they are stored. For fumigation air-tight container/room is necessary. In India phostoxin a known fumigant is now given under certificate because large number of people died because of leakage of phosphene gas from storage structures. Prohibitive cost of synthetic insecticides is yet another important factor (Oni and Ileke, 2008).

2.4. Peppermint (*Mentha piperita* L.) as Bio-pesticide

The use of herbs as a base for the preparation of natural products protecting the crops is one of the safest (both for human and other living organisms) ways to fight the pests. Their production does not require high costs and specialized equipment (Isman, 2000). The effectiveness of protective measures using extracts of plants is determined by both the concentration of the substance, type and quality of plant material and the applied solvent (both aqueous and alcohol plant extracts are popular). Substances contained in herbaceous plants can act as a disincentive for further feeding of the pest in crops, cause disorders in development cycle and fertility, and even the death of the pest (Wawrzyniak and Dębek-Jankowska 2010).Peppermint, or medical spearmint (*Mentha piperita*L.), is a plant species belonging to the mint family (*Lamiaceae* Lindl.), which is originally a blend of two other species – aquatic mint (*Mentha aquatic* L.) and spearmint (*Mentha spicata* L.), bred and introduced into cultivation in England (Cavelius, 2005). The spread of this plant in the crops in most regions of the world was determined by its ease of cultivation, as well as vegetative propagation by rhizomes division, which causes an excessive expansiveness of peppermint, sometimes treated as a weed in other crops (Burnie, 2005).

Peppermint is a valuable medicinal plant, since it is characterized by analgesic, sedative, antibacterial, choleretic and diastolic activity; it also affects nerve receptors, causing a cooling sensation. Therefore, it is used in the food industry as an additive in beverages or food products. Intensely fragrant mint essential oil contained primarily in the leaves of this plants, can also be useful in the production of natural repellents in organic farming (Ciesielska*et al*., 2011). The composition of peppermint oil mainly includes: menthol (monoterpene type) (above 50%), menthofuran, menthone (about 20%), menthol esters – menthol acetate and valerate (about 5%), phellandrene, pinene, cineole, menthofuran, piperitone, jasmone, tannins (6–12%), flavonoids (luteolin, apigenin, diosmetin) as well as mustard, phenolic acids and mineral salts (Iscan*et al.*, 2002). The studies indicate the effectiveness of peppermint extracts in certain aphid species combating, e.g. peach-potato aphid (*Myzuspersicae* Sulzer) (Ikeura*et al*., 2012). In turn, the essential oils of peppermint were tested for toxicity against mosquitoes, as reported by Ansari *et al.* (2000) and Kumar *et al*. (2012).

2.5. Economic importance of the maize weevil

It has been established by several workers that the maize weevil, *S. zeamais* causes severe quantitative and qualitative losses in stored maize grain in Africa (Nwosu*et al.*, 2015a; Nwosu*et al.*, 2015b). As a primary pest of stored maize, *S. zeamais*is capable of penetrating and infesting intact kernels of grain, in which immature stages develop (Lale and Ofuya, 2001) leaving the maize emptied of its nutritional and seed values, culminating in outright loss of visual appeal at the local and international markets. Conventional synthetic insecticide is the chief weapon in the fight against the weevil infestation in stored maize (Nwosu, 2016).

2.6. The use of synthetic insecticides

Different storage systems and arrays of pesticides (usually in the form of fumigants) have been used to preserve maize grain against pests since 1950s (Olakojo and Akinlosotu, 2004). Recently, concerns about the effects of these conventional synthetic chemicals on human health and ecosystem have been on the increase. It is fairly well-documented that the use of synthetic chemicals in the preservation of food and food products causes loss of life and has negative impact on human health (Nwosu, 2012). The widespread use of chemicals as insecticides for the management of storage pests is reportedly of global concern due to associated environmental hazards, development of resistance to the chemicals, presence of chemical residues in food, broad-spectrum actions on non-target organisms, and exorbitant cost of procurement of the chemicals. The increased public awareness and concern for environmental safety has compelled reorientation of research focus to the development of alternative management strategies such as the use of resistant maize varieties, plant-products, and bio pesticides against the storage pests (Abebe*et al.*, 2009).

Host-plant resistance is an indispensable component of integrated pest management (IPM) to reduce storage losses and poor quality grains. Indeed, resistant varieties when combined with the use of effective plant-derived insecticides seem to hold strong promise for weevil control in stored maize. Gradual withdrawal of the commonly used synthetic insecticides and focus of research on reduced or risk-free alternatives to conventional insecticides have been advocated (Arthur and Throne, 2003), but unfortunately, this has not yielded sustainable results. This is because most of the plant and animal materials tested for insecticidal activities lack quick action and residuals effects. In addition, identifying and synthesizing the active ingredients into commercially available insecticides have been at low ebb. Therefore, more effort is needed in finding sustainable alternative to synthetic insecticides. Since the use of chemicals in grain protection is inseparably associated with a number of demerits (Nwosu, 2016) and resistance or tolerance is absent in the local maize varieties, concerned bodies have been working and improving the genetic resistance of maize varieties to storage pests since 1956 (Iken and Amusa, 2004).Since then, new maize varieties and cultivars that confer some level of resistance to weevils and constitute eco-friendly option have been released and confirmed. Much information is present in online literature (Suleiman *et al.*, 2015; Nwosu*et al.*, 2016). This effective and environmentally friendly management alternative has not been sustainably explored, harnessed, and applied, as storage pests such as *S. Zeamais* still invade and cause varying degrees of damage to the so-called genetically improved maize varieties.

2.7. Maize Quality and Safety after application of the grain Protectants

Maize grain used for human consumption should be safe and of good quality (Befikadu, 2014). Therefore, the grain protectant used against pest infestation should not be toxic to human beings. Also, pest activities and environmental factors should not adversely affect the quality of the grain for food use. Due to safety issues, the use of synthetic insecticides is being discouraged to favor the use of natural materials in insect pest control (Olotuah, 2013). The safety of the natural materials as well should not be overlooked because they are of natural origin. Any insecticide that will ensure the safety of maize for food use after weevil control should be toxicologically investigated and approved. Otherwise, health hazards (such as food poisoning) associated with chemical insecticides could surprisingly occur after pest-control process with a natural material (Singh and Srivastava, 2015).

The quality properties of a grain are affected by the type and nature of grain protectant. Indeed, maize quality includes a range of properties definable in terms of physical, sanitary, and intrinsic quality characteristics (Befikadu, 2014). The physical quality attributes which are of measurable include moisture content, broken kernels, total damaged kernels, stress cracking, test weight, kernel size, and breakage susceptibility (Lale, 2002). The sanitary quality characteristics include fungi and mycotoxin count, insects and insect fragments, pest excreta, poisoned seeds, pesticide residue, odour, and dust (Suleiman *et al.*, 2013; Befikadu, 2014). Intrinsic quality characteristics include protein content, oil value, hardness, density, and viability (Suleiman *et al.*, 2013; Befikadu, 2014).

In successful control, the insecticide kills adult insects and eggs, suppresses oviposit ion, development, and emergence of new progenies, and this maintains the quality of maize grain by preventing direct consumption of kernels (Olotuah, 2013; Tiptoe, 2014). Accumulation of insect exuviae, excreta, and cadavers taints the grains, causes odour, and inadvertently affects grain quality adversely (Yallappa*et al.*, 2012; Olotuah, 2013). High levels of insect debris can result in maize grain that is unfit for food use (Yallappa*et al.*, 2012). However, insect cadavers can easily be sieved out before the grain is used. The use of synthetic chemicals in grain protection causes residue problem and health risks to maize consumers and their use is either not permitted or restricted (Yallappa*et al.*, 2012).

The use of natural materials such as medicinal plants and bone charcoal dusts of animals in the protection of maize grain against weevil infestation appears to favor grain quality and safety. The powders are usually applied in dry state and do not taint the grains or cause increase in grain moisture. It is known that plant and animal powders are not readily changed by chemical or biochemical reactions and can easily be sieved out before food use. Although the effect of botanicals on the viability of maize grains and other intrinsic quality characteristics has largely been untested, most undamaged grains protected with botanicals often germinate when planted (laboratory and field experience)(Singh and Srivastava, 2015).

2.8. Effect of applied neem powder on Seed Germination

Germination tests conducted with kernel powder -treated wheat (Jotwaniand Sircar, 1965) mungbean, cowpea chick pea, pea (Jotwani and Sircar, 1967) and paddy (Satori and Rao, 1977) did not show any impairment due to treatment. Neem oil at 0.5%, however, caused 27% reduction in germination of cowpea as against 56% in phostoxin and 2% in control (Zeherer, 1984). Rice seedlings raised from seeds treated with 2.5% neem seed kernel or 2% neem cake had higher root and shoot growth than untreated control (Kareem *et. al.*, 1989). Mixing of seed kernel at 3% did not affect cooking quality or germination of paddy (Akouedi, 1984). Khare*et al.*, (1992) found no adverse effect on the germination after 100 days of storage of neem oil-treated pigeon pea seeds. Low germination of green gram was reported by Babu*et.al*.(1989) after treatment with neem oil. Organoleptic tests carried out with pulses treated with kernel powder showed no taste or smell of neem (Jotwani and Sircar, 1967). Neem oil - treated cowpea, however, tasted slightly bitter which was considered as minor (Zeherer, 1984). Kernel powder did not in any way affect cooking quality of paddy (Savtri and Rao, 1977). Wheat seeds treated with neem oil (5ml/kg) did not affect germination of the seeds (Gupta *et al*., 1988).

3. MATERIALS AND METHODS

3.1. Study Area

The experiment was conducted in Biotechnology laboratory, School of Biological Sciences and Biotechnology at Haramaya University. The University is found at 525 km away from Addis

Ababa to the east. It is located at latitude of 9^{o}26'N, longitude of 42^{0}03'E and altitude of 1980 m.a.s.l. (FAO, 2009).

3.2. Experimental Materials and Laboratory Procedures Used for Preparations

Sorghum grain was bought from market in Haramaya town. The grain was sieved to remove dead seed, dirty and broken particles. Five kilogram of sorghum grains was randomly sampled and stored in a freezer at -4^{o}C for 2weeks to kill any prior source of the *S. zeamais* inoculum and eggs which might be already pre-existing in the grain according to the procedures followed by Parugrug and Roxas (2008). After 2weeks in the freezer, subsamples of 200g grain were placed in 375ml capacity bottles with perforated lids to prevent weevils from escaping and for aeration. Neem leaves were collected from neem tree in Dire Dawa city, while peppermint was obtained from streams of Doba district, West Hararghe zone. The plant parts were air-dried under shade at an ambient temperature to avoid photo degradation of active ingredient by ultra-violet rays as per the recommendation of Salako (2008). The air-dried materials were then ground into fine powder using grinding machine and sieved with a 10mm sieve. The fine plant powder was kept in air-tight containers until required.

3.3. Treatment and experimental Design

A 2× 2 x3 factorial experiment was laid in a Complete Randomized Design (CRD) with two replications for adult mortality test (Fig 1).

Where: 2 levels of treatment (i.e. 5gm & 10gm of the test material)
2 replications
3 test materials (i.e. neem powder, mint powder & neem-mint powder)

3.4. Preparation of Insect Culture

The parent stocks of *Sitophilus zeamais* were obtained from infested sorghum grains. The insects were cultured under ambient temperature of 28±2^{o}Cand 75±5% relative humidity. The food media for the insect culture was sorghum for *S.zeamais*. One kilogram (1kg) of sorghum food medium was weighed into three different glass jars. Forty adult insect pests were introduced into each culturing medium. The culturing spanned for 45 days and at the end about 120 adult insect pests were randomly selected for the study.

3.5. Data collection

3.5.1. Test for Weevil Mortality and Grain Loss

Two hundred gram of sorghum grains which was adjusted to 10% moisture content (MC) was treated with 5 and 10g of each of the test treatment in 12 cm high x 6.5 cm diameter glass jars as in Fig 1. In the first treatment only neem leaf powder was used while in the second experiment only peppermint powder was used, and in the third treatment admixture of both neem leaf and peppermint powders was used. The admixtures was shaken manually for 5 minutes and then tumbled for 15 minutes in a mechanical tumbler. The treated sorghum grains were left undisturbed for an hour. Thereafter, mixture of 20 adult maize weevils, were introduced per treatment. The glass jars were covered with filter paper and sealed with molten wax to keep the insects. Untreated sorghum grains were served as control. Each treatment was replicated two times. Weevil mortality rates were measured by physically counting dead weevils at 5, 10, 20 and 30 days after exposure to the treatment.

Figure1. Experimental set-up for adult mortality test

The mortality counts were done during the day when the weevils were highly active due to high temperatures and relative humidity. Percent adult mortality was determined by counting the number of dead insects divided by the total number of insects introduced multiplied by 100. Grain loss assessment was determined by using Thousand Grain Weight (TGW) method (Ahmed Ibrahim, 2014) as follow:

$$TGW= \frac{initial\ \mathrm{TGW} - final\mathrm{TGW}}{\mathrm{initial\ TGW}} \times 100$$

Mass of 1000 grains at the beginning of the storage period was compared with mass of 1000 grains at the 2 week intervals during the experiment (Ahmed Ibrahim, 2014).

3.5.2. Test for Repellence

The method employed by Garcia (1990) with some modifications was followed. Transparent plastic tubings, 13cm long x 1.3 cm diameter as test cylinders were used in the experiment (Fig 2). Each test cylinder was plugged at one end with fine mesh tulle containing 0.5g, 1 and 1.5grams of neem leaf powder, mint leaf powder and admixture of both neem leaf and mint powders, while the other end was plugged with clean cotton ball which served as control. About20weevils were introduced at the middle of each test cylinder through a hole at the middle portion of the cylinder. The hole was covered with nylon tulle mesh to keep the insects inside the cylinder. The cylinders were grouped accordingly to represent the treatments and replications. Each treatment consisted of three cylinders and replicated three times.

Figure 2.General view of experimental set-up for repellency test

The cylinders were left undisturbed and the number of weevils that moved towards the untreated halves of the cylinders was counted and rated every hour for the first five hours and at24, 48 and 96 hours thereafter. Repellency rating was calculated following the formula (Garcia 1990).

$$\text{Repellency rating} = \frac{n\ (1) + n\ (3) + n\ (5) + n\ (7)}{N}$$

Where: n= number of insects stayed 0, 1-2, 3-4 and 5-6 cm from the center of the cylinder towards the untreated cotton plug, respectively. 1, 3, 5 and 7 = rating scale on the reaction of the insects on different test materials. N= total number of insects introduced per cylinder. The degree of repellency of each test material will be based on the following scale (Table 1).

Table 1. Scale for the determination of the degree of repellency of the test materials

Rating	Distance (cm) from center of the cylinder towards the untreated plug	Description

1	0	Ineffective
3	1-2	Slightly repellant(SR)
5	3-4	Moderately repellant (MR)
7	5-6	Highly repellant(HR)

3.5.3. Progeny Emergence and grain safety test through germination test

The treated grains and the control were kept in containers in conducive culture conditions for 45days. At the end of the culture period, the number of emergent insect pest for each treated grain and the control was recorded. Germination test was conducted by allowing sorghum seeds to germinate on cotton fiber.

3.6. Data Analysis

Mean and percentage were used for data analysis. Percentages and mean mortality/migration rate of adult insect pest which occurred was calculated and the number of progeny that emerged from the treated grains after six (6) weeks was reported. Data were presented in tables and figures.

4. RESULT AND DISCUSSION

4.1. Repellence test

Mean values for repellence test of maize weevil against neem and mint leaf powder as indicated in Table 2 All the treatments have shown high repellence against maize weevil at all amounts of treatments (0.5, 1, and 1.5 grams) in the whole durations. However, there was no significance difference among 0.5, 1, and 1.5 grams for all the treatments including neem, mint, and combination of neem and mint powders. Related study by Parugrug and Roxas, (2008) has indicated powdered leaves of neem and lantana were noted to be highly repellant. *Sitophilus zeamais* is a key pest of stored maize causing serious economic damage. The predominantcontrol of this pest is the use of synthetic residual pesticides, which have adverse effects on consumers and environment. The use of phytochemicals for controlling storage pests constitutes an attractive alternative to synthetic products, since plant may be more biodegradable and safer.

Table 2 Mean values for repellence test against with different amounts of neem and mint leaf powder

After 5hours				After 48 hours		
Treatment	Neem	Mint	Neem+Mint	Neem	Mint	Neem+Mint
0.5gm	5.78±0.53 a	5.50±0.14 a	6.35±0.21a	6.65±0.21 a	6.45±0.07 a	6.55±0.35a
1gm	5.55±0.07 a	6.20±0.21 a	6.45±0.07a	6.35±0.21 a	6.75±0.21 a	6.75±0.07a
1.5gm	5.75±0.64 a	5.65±0.21 a	6.45±0.07a	6.75±0.21 a	6.60±0.14 a	6.75±0.21a
After 24hours				After 90hours		
Treatment	Neem	Mint	Neem+Mint	Neem	Mint	Neem+Mint
0.5gm	6.25±0.21 a	6.10±0.14 a	6.70±0.14a	6.15±0.07 a	6.45±0.07 a	6.70±0.28a
1gm	6.50±0.42 a	6.35±0.07 a	6.70±0.28a	6.55±0.35 a	6.75±0.21 a	6.60±0.14a
1.5gm	6.55±0.21 a	6.60±0.14 a	6.950.07a	6.60±0.28 a	6.60±0.14 a	6.60±0.28a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on DMRT (Duncan's Multiple Range Test).

4.2. Test for Weevil Mortality Rate

Mean separation for maize weevil mortality rate during 48 days of treatment of sorghum grain with different amounts of neem, mint and combination of neem and mint leaf powder as indicated in Table 3 It was observed that there was no mortality of maize weevils recorded for control (0gram). However, significant mortality was recorded for each treatment. The highest maize weevil mortality was recorded for 15grams than for 10 and 5grams in all treatments. It was also observed from Table 3that the highest mean mortality rate was recorded for neem leaf powder than for mint leaf powder, and combinations of neem and mint leaf powders showing insecticidal property of neem leaves. Related study was conducted by Binia s*et al* (2017) who found aqueous extracts of dry and fresh peppermint significantly limited the feeding of pea leaf weevil females, and the extracts from dried material were more effective. Extracts from peppermint caused mortality of black bean aphid larvae compared to wingless females to a greater extent, and the higher the concentration of the extract, the more beneficial effect was observed.

Table 3.Weevil mortality rate after 48 days as sorghum grains treated with different amounts of neem and mint leaf powder

Treatment	Neem (%)	Mint (%)	Neem + Mint (%)
0gm	0.00d	0.00b	0.00c
5gm	22.50±3.54c	7.50±3.54b	12.5±3.54b
10gm	32.50±3.54b	17.50±3.54a	25.0±7.1a
15gm	42.50±3.54a	22.50±3.54a	17.5±3.54ab

Means followed by same letter within a column were not significantly different at 0.05 probability level based on DMRT (Duncan's Multiple Range Test).

Maize weevil progeny emergence rate during extended duration of sorghum grain storage as treated by neem, mint, and combinations of neem-mint leaf powders as indicated in Table 4.It was observed that the highest progeny emergence rate was recorded for control group in all treatments. Only after 48 days that progeny emergence was recorded for experimental groups. The minimum progeny emergences were recorded for 15gm neem leaf powder followed by mint leaf powder while the highest progeny emergence was recorded for combinations of neem-mint leaf powders. Generally, it was observed among experimental groups that the highest progeny emergence rate was recorded for 5gram powder while the least progeny emergence was observed for 15gm powder indicating that increase in amount of treatment powders have inhibitory effect on weevil population. This finding was supported by Tilahun and Daniel (2016) works on the effect of neem leaf and seed powders against adult maize weevil (*Sitophilus zeamais* Motschulsky) mortality. Related study was also done by Suleiman*et al* (2018) who reported powders of botanicals from *Euphorbia balsamifera*, *Lawsonia inermis* L., *Mitracarpus hirtus* and *Senna obtusifolia* suppressed the development of *Sitophilus zeamais* Motsch.in stored sorghum grains.

Table 4. Maize weevil progeny emergence rate during extended storage time

Treatment	After 48days	After 90days		
		Neem	Mint	Neem+Mint
0gm	1.50±0.28a	4.98±0.32a	4.98±0.32a	4.98±0.32a
5gm	0.00	2.08±0.18b	3.30±0.42b	3.43±0.39b
10gm	0.00	1.50±0.14b	2.43±0.25bc	2.73±0.25bc
15gm	0.00	0.80±0.28c	1.83±0.32c	2.18±0.32c

Means followed by same letter within a column were not significantly different at 0.05 probability level based on DMRT (Duncan's Multiple Range Test).

4.3. Grain Weight Loss and germination Test

Grain loss and germination test due to damage by maize weevil (*Sitophilus zeamais*) on sorghum grains after 45 days of treatment with neem and mint leaf powders as indicated in Table 5.It was shown that there was significant difference between control and treated groups in both grain weight loss and percent germination. The highest grain loss and the least percent germination was recorded for control group (with zero treatment) indicating that neem and peppermint leaf powders can be used to reduce grain loss during postharvest storage. Among treatments the least percentage grain loss (14.50%) and the highest percentage germination

(94.0%) was recorded for combinations of neem and peppermint leaf powders indicating the combination of neem with peppermint leaf powder was more effective than separate treatments. This finding was in accordance with Danga*et al*. (2015) treatments completely hindered or significantly reduced progeny emergence, percentage grain damage, grain weight losses, but did not affect maize grain germination after 4 months of storage.

Table 5.Grain loss and germination test due to damage by maize weevil (*Sitophilus zeamais)*on sorghum grains after 45 days of treatment with neem and mint leaf powders

Treatment	Grain weight loss (%)			Germination test (%)		
	N	M	NM	N	M	NM
0gm	45.03±1.13a	45.03±1.13a	45.03±1.13a	69.50±3.53c	69.50±3.53c	69.50±3.53c
5gm	27.82±7.20b	33.22±5.73b	27.58±6.00b	81.00±4.24b	74.00±1.41bc	81.50±2.12b
10gm	27.52±8.22b	31.32±5.08b	18.95±2.66bc	82.50±3.54b	78.50±3.54b	88.0±5.66ab
15gm	22.50±2.12b	25.29±1.00b	14.50±0.71c	92.50±2.12a	88.00±1.41a	94.00±1.41a

N=Neem; M= Mint; NM= Neem plus Mint. Means followed by same letter within a column were not significantly different at 0.05 probability level based on DMRT (Duncan's Multiple Range Test).

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

All the treatments have shown high repellence against maize weevil at all amounts of treatments (0.5, 1, and 1.5 grams) in the whole durations. However, there was no significance difference among 0.5, 1, and 1.5 grams for all the treatments including neem, mint, and combination of neem and mint powders.

Mean separation for maize weevil mortality rate during 48 days of treatment of sorghum grain with different amounts of neem, mint and combination of neem and mint leaf powder was shown that there was no mortality of maize weevils recorded for control (0gram). However, significant mortality was recorded for each treatment during 45 days of storage. The highest mean mortality rate was recorded for neem leaf powder than for mint leaf powder, and combinations of neem and mint leaf powders showing insecticidal property of neem leaves.

Maize weevil progeny emergence rate during extended duration of sorghum grain storage as treated by neem, mint, and combinations of neem-mint leaf powders was indicated that the highest progeny emergence rate was recorded for control group in all treatments. Only after 48 days that progeny emergence was recorded for experimental groups. The minimum progeny emergences were recorded for 15gm neem leaf powder followed by mint leaf powder while the highest progeny emergence was recorded for combinations of neem-mint leaf powders.

5.2. Conclusion

The general outcome of this study suggested that neem and mint leaf powders could be used as effective insect repellents. The highest mean mortality rate was recorded for neem leaf powder than for mint leaf powder, and combinations of neem and mint leaf powders showing insecticidal property of neem leaves.

Therefore, it can be concluded that, the neem and mint leaf powder retards population growth of maize weevil, the powders cannot be recommended for extended storage duration.

5.3 Recommendations

Even though the neem and mint leaf powder retards population growth of maize weevil, the powders cannot be recommended for extended storage duration.

Based on high repellence rate, neem and mint leaf powders can be used as control for damage caused by maize weevil (*sitophilus zeamais*). Hence, the use of neem and peppermint leaf powders as an alternative control option in integrated storage pest management strategies by small holder farmers. Further studies are required to check the effectiveness of repellence activity of neem and peppermint leaf powders as repellence of foliar insects.

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