BIO-EFFICACY OF SELECTED BEAN GENOTYPES AND SOME PLANT POWDERS AS PROTECTANT AGAINST THE SEED BEETLE, *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: CHRYSOMELIDAE) IN STORAGE

A dissertation submitted to the Department of Crop and Soil Sciences in partial
fulfillment of the requirements of the award of the Master of Science Degree in
Crop Science (Crop Protection)

By

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DECLARATION

This research was carried out at the Entomology laboratory, Crop and Soil Science Department in the University of Agriculture and Natural Resources (BUAN), Gaborone under the supervision of Dr B. Tiroesele and Dr G. Malambane. I Oabile Lecage Tlale declare that

1. The content of this dissertation, excluding where noted, comes from my own original research.;
2. This dissertation has never been submitted in any other University for any grading or examination;
3. This dissertation does not include any data or other information that belong to any person, unless they were accredited as having been acquired from others;
4. This dissertation does not comprise writings from other, unless it is acknowledged as being obtained from other researchers. Where other written sources have been quoted:

   a) Their words have been re-written, but the general information attributed to them has been referenced; and

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DEDICATION

I dedicate this paper to my late father Ekitlanye Carter Tlale who passed away before I could complete my undergraduate studies. I also dedicate my work to my mother Patricia Tlale and my siblings Onthatile Julie Tlale, Koketso Tlale and Kesego Tlale who have always supported and encouraged me during every step of my academic career.

ABSTRACT

Common bean *(Phaseolus vulgaris* L.*)* is one of the utmost significant grain legumes in developing countries, however its production in Botswana has been failing to retain pace with annual growth rates due to post harvest challenges in storage. Seeds for common bean are most likely attacked by beetles commonly known as seed beetles (*Callosobruchus maculatus Fabricius*), which cause substantial damage to the stored common beans. Therefore, the main objectives of this study were to; (1) screen new *P. vulgaris* bean genotypes for resistance against *Callosobrushus maculatus;* (2) to evaluate the efficacy of botanical plant powders peppermint (*Mentha piperita L.*), garlic (*Allium sativum L*), fever tea (*Lippia javanica*) and marigold (*Tagetes minuta*) in the control of *Callosobrushus maculatus* in stored common bean (*Phaseolus vulgaris)*; (3) to determine the effect of botanical plant powders on bean genotype germination. A total of 3 new bean genotypes CAL96, DAB520 and X-genotype and blackeye cowpea as control were used. The seeds resistance experiment was arranged in a Complete Randomized Design (CRD) replicated four times. The seeds were evaluated on the basis of number of eggs laid, number of adult emergences, number of adult mortalities, seed weight loss and Dobie Susceptible Index (DSI). The observed results showed that CAL96, DAB520, X-genotype and black cowpeas landrace (control) supported *C. maculatus* oviposition but only control supported adult emergence while the other seeds recorded 0% of adult emergence. CAL96, DAB520 and X-variety also had an average seed weight loss ranging from of 1%, to 2%, and based on the DSI which measures resistance and susceptibility. CAL96, DAB520 and X-variety recorded an index of 0% which suggests they are resistant while control recorded an index of 8.3% making it susceptible. Biochemical traits, ash, moisture, crude fat, protein, carbohydrates, crude fiber, tannins, sodium, potassium, calcium and magnesium contents were investigated to determine their effect on susceptibility index. Only sodium, potassium, ash and magnesium proved to be responsible for the susceptibility and or resistance of the genotype to *C. maculatus*. On the second experiment, different plant powder extracts were screened for efficacy in controlling *C. maculatus* on stored bean seeds. The experiment was arranged in a split plot design, with plant powder treatments (garlic, peppermint, fever tea and marigold) as the main plot and genotype (CAL96, DAB520, X-variety and black cowpeas landrace (control) as the subplot. This was laid out in a Complete Randomized Design and it was replicated three times. Four grams of all the plant powder extracts were observed to be significantly effective against *C. maculatus* in the bean genotypes with respect to oviposition percentage, adult emergence, and adult mortality. The extracts of marigold, fever tea and garlic decreased seed beetle oviposition on all the seeds, while peppermint extract increased seed beetle oviposition in all the seeds. Emergence of the F1 from the eggs laid on the seeds was only observed in the blackeye seeds. Peppermint powder treatment recorded the lowest emergence percentage with an average of 1.05% emergence, while fever tea had the second lowest adult emergency and garlic had the third and marigold the fourth with an average of 2.9%, 3.48% and 11.75% adult emergence respectively. Adult mortality percentage among the treated and untreated control were observed in the present study, where the peppermint and marigold powder treatments were the only treatments to have recorded a significant adult mortality with an average of 1% and 6.67% respectively. In seed weight loss experiment, marigold powder and the untreated control were the least effective as they recorded 0.5% and 1.33% reduction in seed weight due to *C. maculatus* infestation. The results from the DSI indicate that all the powder treatments had an index value of 0%, which means all genotypes were resistant against *C. maculatus*. The results from the present study indicates that garlic, peppermint and fever tea and marigold powders have anti-ovipositional properties, insecticidal effects, repellent activity, and permanent protective properties on CAL96, DAB520, X-variety, and blackeye cowpeas against damage from *C. maculatus*. Effects of plant powder fever tea, peppermint, garlic and marigold on germination on seeds of CAL96, DAB520, X-variety and blackeye cowpea were evaluated. The experiment to evaluate the efficacy on germination was also arranged in a split plot design, laid out in a Complete Randomized Design with 3 replicates. Seeds treated with garlic, fever tea and marigold treatments were observed to have recorded the highest average germination percentages which were 100, 99.17 and 97.5 percent respectively, peppermint recorded the second lowest average germination percentage which was 85.83 percent. The untreated control recorded the lowest average emergency percentage of 77.5. Growth activity on all the seeds by plant powders were reported. This study demonstrated the efficacy and the high control potential of plant powders against *C. maculatus* in storage while they are effective in promoting seeds germination in all the seeds. According to Dobie’s Susceptibility Index, garlic and peppermint performed very well.

Contents

LIST OF SYMBOLS AND ABBREVIATIONS

BUAN	Botswana University of Agriculture and Natural Resources
DAFF	Department of Agriculture, Forestry and Fisheries
DSI	Dobie Susceptibility Index
FAO	Food and Agricultural Organization
IPM	Integrated Pest Management
USA	United States of America

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CHAPTER ONE

1.1 INTRODUCTION

Common bean *(Phaseolus vulgaris* L.*)* is one of the most important grain legumes belonging to the family Fabaceae that has been documented to have originated from Central and South America. According to Wortmann (2006), since beans are a traditional important, particularly in nations like Mexico, Brazil, and Central America, it is currently one of the most extensively grown main food crops in many tropical and subtropical regions of America, Europe, Asia, and Africa, with Latin America being the top consumer and producer. Since *Phaseolus vulgaris* thrives in warm settings with typical temperatures ranging between 18°C and 24°C, it is widely recognized to flourish in tropical, subtropical, and dry tropical zones. According to Ferris and Kaganzi (2008), based on population density, bean output is steadily increasing globally, with poor and emerging nations using the most beans since they are a cheaper source of protein than meat.. Studies have shown that in most African developing countries, beans are largely grown for subsistence. They are consumed as mature grain and immature seeds as well as green pods and leaves taken as vegetables. Huge (proportion) of bean crops is for home consumption in smaller gardens and backyards and they are frequently intercropped with maize by smallholder farmers as secondary crops (Maredia, 2015). Studies by Maiti & Sigh (2007) established beans to be high and rich in starch, amino acids lysine, methionine, dietary fiber and to be an excellent source of potassium, selenium, thiamine, vitamin B6, and folic, as such making them complementary to cereals. Beans are genetically very diverse, adapted to local conditions and dietary preferences making them one of the best means of mitigating food nutrition problems experienced in most developing countries like Botswana. If kept in a cold, dry environment, beans may also be kept for three to four years, however as they desiccate and harden, their nutritional value and flavour deteriorate and cooking times increase (Ferris & Kaganzi, 2008)

Though beans are of nutritional and economic importance, its production trend has been failing to retain pace with the annual growth rate in most of the developing countries due to several abiotic, biotic and socioeconomic constraints (Kambewa, 1997; Forthcoming & Xavery, 2006). Diseases, pests, poor soil fertility, drought, price instability, an unsuitable market, a lack of capital, taxes, a low price for the good, and a lack of extension services are the majority of these restrictions (B. Beebe, Lachmann, Markese, & Bahrick, 2012; Hillocks, Madata, Chirwa, Minja, & Msolla, 2006).

Among the biotic constraints, attack by pest during storage is one of the most important, major and common constraint across Southern African countries like Botswana. The bean seeds are subject to attack by beetle commonly known as Seed beetles. The postharvest damage instigated by seed bean beetle during storage is diverse and varies from crop to crop depending upon the species of beetle and their biotype. In Botswana *Callosobruchus maculatus* species is the common primary seed beetle pest of beans encountered in storage. Unless beans are protected from these pests in storage, seed beetle damage can be very detrimental, rendering beans unfit for human consumption and for sowing. Most of the farmers plant their own home stored bean seeds and, in most cases, farmers cannot entirely protect the beans in storage from seed beetle attack which as a result reduces the storage life, quantity and quality of the stored beans.

There are several pest managements practices that have been used to control infestation of seed beetles in stored beans and they include techniques like physical control, biological control, mechanical control, chemical control, use of resistant host seeds and also the use of plants extracts. Of these techniques, the most common and popularly used technique is the chemical control which is viewed to be effective, quick and secure. Though it is the most commonly used technique it has some major downsides which include; undesirable effect on products and environment; constant danger of intoxication on humans and animals; presence of residues in different parts of the plants; and development of pesticide resistant pests (Jorg & Moltmann, 2000). These negative outcomes have necessitated research on the use of more eco-friendly control methods such as the use of host resistant plants and use of plant extract as control methods (Sarwar, 2013). Plant extracts with insecticide properties have been found to be environmentally friendly, cheap and safe to use as compared to synthetic pesticides as such their use needs to be exploited (Lale, 1992). The plants extracts have been reported to have negative effect on some of the insect biological parameters (developmental rate, oviposition, fecundity and egg viability and mortality rate). Studies on seed beetle management, which combine plant extracts and host resistance in beans within an integrated pest management are rather limited. For reducing the attack by seed beetle on stored beans, the primary objective of this study was to investigate the host resistance control method by assessing level of resistance of new common bean genotypes and the efficacy of botanical plants on *Callosobruchus maculatus*. The study further aimed to determine the influence of biochemical attributes mainly alpha amylase inhibitors activity in the resistant host plants.

1.2 Justification

The Department of Agricultural Research under the Ministry of Agriculture and Food Security in Botswana, which is mandated with the release of bean genotypes is currently carrying out studies on bean genotypes in preparation for their adoption, multiplication and release to the farmers. The release and adoption of these bean genotypes has to be preceded by information of factors that can hinder/affect the bean produce while in storage thus the reason for conducting this study. *Phaseolus vulgaris L.* is one of the utmost significant farm produces in most African countries including Botswana. However, its production is very low in Botswana. In addition to being readily available in supermarkets, beans are given to children in clinics and also are consumed in schools.

Though the demand and usage are sharply increasing, the produce in storage still has challenges when compared to storage of common crops like sorghum and maize. In order to compete with these commonly grown crops, common bean production and protection in storage needs to be ramped up to get better seed quality and quantity to keep up with the increase in demand. One of the major constraints for seed quality and quantity in storage in Botswana is the seed beetles, *Callosobruchus maculatus*. This insect causes economic damage to stored seeds, and this subsequently demotivate the farmers in production of the crops. To retain good quality and quantity of produce in storage, good storage practices are very important. Synthetic insecticides have been used as a mode of managing seed beetles in storage. Though they are good, their over-use lead to development of resistance and have been found to have high potential health hazard both to consumers. These chemicals are not environmentally friendly, and they also negatively impact untargeted organisms. For small scale farmers synthetic insecticides are expensive, need skill and knowledge to apply, and are not easy to find. Therefore, there is a need to search for cost-effective and ecofriendly bio-based pesticides that are safer, easy to access, low cost, and easy to use. There is not much information available on using plant products as an alternative to chemical pesticides. In several regions of the world, naturally occurring plant products have been utilised for a long time to protect agricultural goods against pests, and some writers have noted the insecticidal effects of plant products against a variety of pests. Alternatives to synthetic pesticides that are sustainable, ecologically friendly, and safe include using plant products. Studies by Keneni (2011) have shown that use of botanical plants as control is the best way of overcoming insect pests of common bean in an environment-friendly manner. There is a diversity of botanical plants with a variety of environmentally friendly active properties of which some have been used traditionally as natural pesticides in other countries like Kenya as per Keneni (2011). These plant extracts represent a great pool of bio-pesticides, which is largely untapped and not fully explored for utilization. There is paucity of information on the use of bio-pesticides against seed beetle on beans. In addition, no research has been done on common bean interaction with seed beetles in storage hence the need for this study. The outcome of this study might also contribute in policy formulation in relation to pest management without using synthetic chemicals.

1.3 Statement Problem

Seed beetle is one of the major pests that attack legumes in storage. The attack of these beetles can be very detrimental resulting in poor quality and quantity of produce that is unfit for human consumption and for sowing. The produce will also be of low market value and if seed beetles are not controlled, they can lead to 60 to 100 % storage losses (Pereira, 1983). There is no available information on storage pest of new bean genotypes that are in the process of introduction and adoption to farmers by Department of Agricultural Research (DAR).

1.4 Objectives

1.4.1 Main Objective

1. To evaluate the resistance level of new bean genotypes and the efficacy of grounded plant powders against *Callosobruchus maculatus* infestation on stored common bean (*Phaseolus vulgaris).*

1.4.2 Specific Objectives

2. To screen new bean genotypes for resistance against *C. maculatus*.
3. To determine the biochemical compounds underlying bean genotype resistance to *C. maculatus.*
4. To evaluate the efficacy of botanical plant powders (mint, garlic and marigold) in the control of *C. maculatus* in stored *P. vulgaris*
5. To determine the effect of botanical plant powders on bean genotype germination

1.4.3 Hypotheses

1. H_0 = New bean genotypes are not resistant to *Callosobruchus maculatus* infestation in storage.

H_a = New bean genotypes are resistant to *Callosobruchus maculatus* infestation in storage.

2. H_0 = Biochemical contents do not have any effect on resistance of genotypes against *Callosobruchus maculatus*

H_a = Biochemical contents have effect on resistance of resistance of genotypes against *Callosobruchus maculatus.*

3. H_o = Botanical plant powders are not effective as bio-pesticides against *Callosobruchus maculatus* on bean genotypes in storage.

   H_a = Botanical plant powders are effective as bio-pesticides against *Callosobruchus maculatus* on bean genotypes in storage.

4. H_o = Plant powders do not have any effect on germination of bean genotypes.

   H_a = Plant powders have effect on germination of bean genotypes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bean Origin and Geographical Distribution

Phaseolus vulgaris L. commonly called common bean is a diverse new world leguminous crop which falls under the Fabaceae family, that encompasses extensive variety of species which display different forms. It is known that they came from a vast arc that stretched from what is now northern Mexico (Chihuahua), through Central America, and the Andes Mountains, to northwest Argentina (Broughton, 2003). Through studies based on archaeological, ethnobotanical, morphological, biochemical, genetic and isoenzyme evidence it was also established that Mexico is the center of origin, diversification and domestication of the common bean (Asfaw, 2009). Presently, the common bean is distributed in Europe, Asia and Africa, where it presents resemblances to Andean and Mesoamerican gene pools or forms hybrids between both gene pools. The distribution pathways of beans into and across Africa and other continents were very complex and occurred through several introduction events from the New World combined with direct exchange between African, European and Mediterranean countries (Angioi, 2010).

The climatic condition in which common beans are established to have originated in sub-tropical to temperate with definite wet and dry seasons, and they have been reported to perform well in regions documented to experience moderate rainfall (Beebe, Rao, Devi, & Polania, 2014). DAFF (2011) observed that the crop performs well in the tropics at an altitude of about 1,250 m with rainfall ranging from 400 to 650 mm, and produce well in deep loamy sandy soils with a pH ranging between 5.8 to 6.5. The crop takes between 85 to 115 days to mature depending on cultivar and season (DAFF, 2011). Temperatures which exceed 30°C during flowering, might cause flower abortion (Beebe, Rao, Devi, & Polania, 2014).

2.2 Bean Growth Habitat and Taxonomy

Common bean falls under the family Fabaceae and genus Phaseolus encompassing extensive variety of species which include; shrubs, herbs and also some trees with climbing development properties (OECD Working Group, 2016). Though the Phaseolus genus includes a wide range of documented species with roughly 80 wild and also cultivated , and *P. vulgaris* has been reported to be the most widely cultivated species (OECD Working Group, 2016). Due to significant plant breeding, *P. vulgaris* cultivars have an extensive variety of agronomic and phenotypic traits, including variations in colour and size of seeds and also growth habit. (Singh & Schwartz, 2010). Determinate growth, which refers to decreased branching and twinning, fewer shorter internodes and most importantly, an enhanced allocation of biomass to reproductive development, is one of the features that is most frequently chosen (Kwak, 2012). A bean's fruit is a single-carpelled pod that comes in different sizes and forms and contains one to several seeds. Many species have a split pod that releases the seeds along either one or both of its borders, which are typically referred to as the placental and core sutures (Wortmann, 2006). This supports studies by Garcia (1997) who also had established that bean seeds and pods significantly smaller, and the pods feature an explosive dehiscence slit near the pedicel. Green bean harvesting is made simpler since they develop quicker, have been found to adapt better to shorter growing seasons, and yield pods over a quicker and more regular time. Determinate cultivars have demonstrated greater adaptability to mechanical cultivation and harvesting. (Kwak, 2012). Common beans normally grow lateral roots within 15 cm of the soil's surface and have a taproot-based root structure. The juvenile parts of the stems are always covered in small curled hairs, and the cultivar determines the concentration and length of the hairs on the stems (Freytag & Debouck, 2002). Beans' leaves are trifoliolate, rotting on the stalks, and have leaflets that are 8–15 cm × 5–10 cm and have little stipules. Wortmann (2006) also reported that although leaflet shapes vary between cultivars, they all tend to have wide bases and tipped tips, as well as more main stem branches with fewer nodes. Based on the cultivar, flowers are produced on terminal or axillary racemes and the colour is are either white, pink, or violet. The bisexual flowers are keeled, and keel ends in a coil with one to two twists.

2.3 Bean Nutritional Value

With an economic worth greater than all other legume crops put together, the common bean has over time become one of the most popularly utilized legumes in the world. It is also regarded as the most significant legume produced for direct human consumption. (Broughton, 2003; Porch, 2013). Mojica & de Mejía, (2015) reported common bean to be an excellent source of important nutrients such as iron, magnesium, copper, phosphorus, calcium, zinc, potassium, vitamins and have established that beans are low-priced sources of nutrients for people of lower socio-economic status in most developing countries. It has been established that amino acids lysine and tryptophan, mineral irons, copper, and zinc, as well as advantageous phytochemicals, antioxidants, and flavonoids are all abundant in beans (FAO, 1999). Studies by Munoz (2009) show that common bean is an outstanding protein source with a low carbohydrate level, and as assessed by cooking time there is high variability in protein content and grain hardness among improved varieties and landraces. Worldwide, common beans are used as a pulse in their mature state, dried seeds, and as a vegetable in their immature pods and seeds. The common bean is mostly grown and eaten as a pulse in tropical Africa. Although common beans are known for their nutritional qualities, they are also crucial for giving meals high in carbohydrates, like those made with maize or banana, variety and flavour. In some locations, common bean leaves and immature seed pods are occasionally consumed as vegetables, while the plant-derived straw are utilized as fodder. (Broughton, 2003). Majority of the time common beans are prepared before being consumed by cooking them in water, however certain beans are also consumed after roasting them and others are first ground into flour before being cooked (FAO, 1999).

2.4 Major Constraints of Beans in Storage

Each area has different common bean production constraints, as well as general challenges such farmers' marginal storage holdings in Asia, Latin America, and Africa. The storage of *P. vulgaris* has been documented to be subject to several constraints which includes rotting due to exposure to moisture, theft and also pest damage, with pests being the main constraints (Loko, Akpo, Orobiyi, Toffa, & Dansi, 2018). Studies by Soundararajan, (2012) indicated that storage pests such as *Callosobruchus maculatus* are the principal constraint of common bean in storage. The beetle produces qualitative damage, which is typically dependent on individual assessment and regionally recognized criteria of quality. Contaminants like uric acid and other nitrogenous wastes, adult *C. maculatus* inside the seed, exit holes, eggs adhered to the seed, different types of insect chitin, changes in color, coastal larval coat and texture, and alterations in taste can all render anything unsuitable for human consumption. The effects of seed beetles on stored beans results in economic losses as most of the product are usually deemed useless when damaged as they would have lost their quality. These seed beetles are reported to cause damage or losses of between 20 and 50% and sometimes the loss may even go up to 100% if the stored beans are not treated (Baldin & Souza, 2013; Lanka, 2019).

2.5 Seed Beetle Biology and Ecology

Callosobruchus maculatus which is also known as seed beetle belongs to the order Coleoptera and the family Chrysomelidae which has 74 genera with more than 406 species worldwide(Gavrilovic & Curcic, 2015). About 20 species are documented as devastating storage pests of different legume grain crops (Soundararajan, 2012). *Callosobruchus maculatus* has now become a major problem in the storage of legumes which includes bean, as such considered a major pest of storage sites. In the last 30 years, the species has also been recorded to have new host plants, such as *Pisum, Lens* and *Vigna* (Jarry and Bonet 1982).

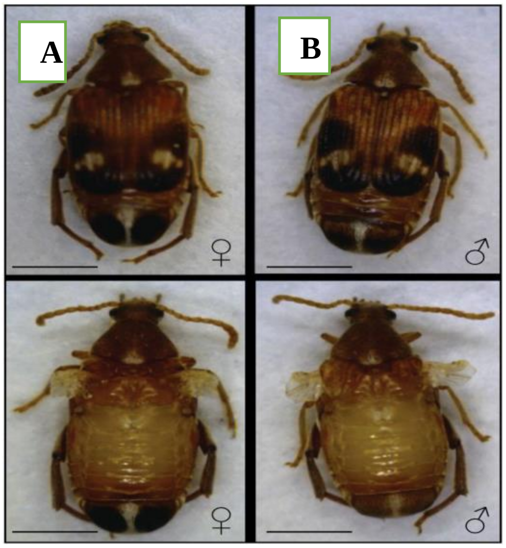

Figure 1: Adult *Callosobrushus maculatus* adults a) Female *C. maculatus* , b) Male *C. maculatus* Author: (Numajiri, 2020).

C. maculatus goes through a complete metamorphosis, which passes through four life stages which are adult, eggs, larva and pupa. The average mass of *C. maculatus* adults ranges from 4-6mg with the average body length ranging from 4-6mm. The male and female adults can be easily distinguished from each other through their physical appearance (morphology) (Akhiwu, 2020). The adult females are a bit different from the male as each side of the posterior dorsal abdomen of females have dark stripes while males don’t have the stripes (fig. 1). Females are commonly known to have significant markings on the elytra that are composed of two broad lateral black patches halfway along the elytra and smaller patches at the anterior and posterior ends, leaving a lighter brown cross-shaped region covering the rest while males are considerably less obviously marked (Mbata, 1997). The ventral side of each hind femur of *C. maculatus* has a pair of separate ridges (inner and outer), with a tooth on the apex of each ridge. The inner tooth has a triangle form and is often equal in length to or slightly longer than the outer tooth. (Mbata, 1997). Ramaswamy and Monroe (1997) described *C. maculatus* adults to have a unique chordotonal structure in the fore coxae. Mbata (1997) also reported that both sexes had slightly serrate antennas. *C. maculatus* can live its whole life cycle without water or any other food supply besides the dried beans on which the eggs are placed, and it can do so effectively. The females *C. maculatus* lays its eggs and attaches it to the surface of the bean. Eight to ten days after oviposition, the eggs hatch, and the larva (maggot) burrows into the bean.

The larva then spends the whole of its larval stage within the seed feeding on its cotyledon. Each larva can consume up to about 25% of the seed within from which they develop (Lanka, 2019). The larvae burrows into the seed through the seed coat and starts to feed on the embryo and endosperm as it molts into 4 more instars, while the egg shell remains on the surface of the seed (Arjanbhai, 2015; Mkenda and Ndakidemi 2014; Sarwar, 2012). The larva that tunnels into the seed leaves a round hole which is usually 1-2 mm and this is the hole that the adult *C. maculatus* emerge through after pupation. *C. maculatus* larva cannot move from one host to another as such it completes its development in one host as determined by female *C. maculatus* (Cope & Fox, 2003). Pupation and adult beetle emergence take place 25–35 days after an egg was placed, at temperatures between 25–30 °C. Adults reach maturity 24–36 hours after emergence, at which point they no longer require food and devote their brief lives (10–14 days) to mating and egg-laying. (Beck & Blumer, 2006; Shah, Pakhtunkhwa, Usman, Sohail, & Shah, 2016). Under these circumstances, adults may have a lifespan of 12 to 14 days, during which mating and oviposition take place. Developmental periods for *C. maculatus* depend upon the environmental conditions, thus having time and progeny production of minimum 18°C and 30 % RH, while complete development and maximum progeny production occurred at 31 °C and 70 % RH. The *C. maculatus* eggs develop rapidly at 70% R.H and 30°C environments, with the insects spending up to about 22-33 days inside the beans. Bhuiya & Peyara (1978) reported that the life-cycle of seed beetle was completed in 30-32, 22-23 and 44-46 days during early summer, mid-summer and winter seasons respectively.

2.6 Bean and Seed Beetle Interaction

For more than 350 million years insects and plants have been living together in the same ecosystem, they have both evolved strategies to avoid each other's defense systems as a way of survival. Plants have developed a complex defensive mechanism that can detect signals from damaged cells, much like animals can, and initiates the plant defense response against herbivores as a result of the evolutionary arms race involving insects and plants (Hare, 2011). The interrelationship between plants and insects have been recognized to be important for their survival ecologically. Insects always have sought out healthy host plants since they can offer them appropriate nutrition, might be good for mating and egg laying, and also give food for the progeny. Insects need the adequate nutrition from plants because, like any other species, any imbalance in how well they digest and use plant proteins has a significant impact on their physiology. The interactions between *C. maculatus* and legumes are highly specific, as one insect species feeds on a very few seed species (Somta, Ammaranan, Ooi, & Srinives, 2007).

The primary hosts for seed beetle *C. maculatus* are common bean and cowpea (*Vigna unguinculata* L. Walspers.) while other legumes like soybean and mung bean are secondary hosts (Milanovic, 1991. The females *C. maculatus* usually deposit and distribute their eggs uniformly (1 egg/seed) whenever the host bean seeds are overabundant (Messina, 1989; Horng, 1994). However, when bean seeds are limited the female *C. maculatus* voluntarily super parasitize by laying eggs on previously parasitized seeds. The first instar larva bores into a seed where the beetle spends its larval stages, as it develops into pupal stages, completely feeds a single seed, and digging a chamber. The final instar larvae excavate a chamber just below the seed coat and the presence of the larva may be detected by a small chance.

The loss due to seed beetle pest increases with how long the beans are kept, as such the length of storage is directly proportional to losses of common bean. Bean losses caused by seeds beetles are divided into two categories: qualitative, which refers to grains tainted with faeces or insect bodies, and quantitative, which is based on the quantity of seeds or sections of seeds devoured by insects. Because seed beetle's larval phase respiration increases temperature and relative humidity, which in turn creates favourable circumstances for the microorganism which decomposes the beans, qualitative losses are typically exacerbated by regular attacks from bacteria and fungus. The damage on beans is normally displayed in the circular holes when seed beetles emerge. The main cause of seed beetle infestation is normally known to be due to unclean storage conditions, and also storing beans with contaminated remains from previous harvests (Van Schoonhoven & Cardona, 1986). The term "nutritional loss" refers to the quantitative loss of the grain's nutritional value as a result of declining protein, hydrocarbon, and vitamin content. (Sing, 1997). The reduction of protein content on grains are normally due to seed beetles feeding on the cotyledons. The additional contamination of beans with uric acid from seed beetles is what causes detrimental deviations in their nutrient content, which result in a rise in fatty acids and a decrease in numerous vitamins, particularly thiamin and important amino acids (Salunkhe, 1985). Attack of *C. maculatus* on common bean causes damage by decreasing the mass and volume, the physiological quality of the bean, and the germination capacity. Unlike most of the other insect pest, the *C. maculatus* reproductive cycle is continuous as such being the one which causes more damages than other seed beetles.

2.7 Management of Seed Beetles in storage

Seed beetles inflict economic damage on stored beans as such due to inescapable losses, farmers are obliged to sell their grain a soon as they are done harvesting. Due to the extreme damages caused by seed beetle it is therefore necessary to find significantly justifiable ways of managing infestations by these beetles, thus farmers have tried several options to control and minimize the pest’s damage in storage (Giga, 2001). The most common management used by farmers has always been the synthetic pesticide control which has hazardous effects associated with it. Other effective control and or management /methods with minimal or no pesticides use that farmers can utilize to reduce the losses in storage by the seed beetle without damaging the environment include biological methods, cultural methods and host plant resistance (Scott & Maideni, 1998).

2.7.1 Cultural Control

Cultural control involves manipulating the host environment into being less suitable, favorable and appealing to insect pest existence, development and diversifying within the host of host environment. The cultural practices include removal of egg shells and dead larvae, removal of infested grains before storage of new grains, fumigation, disinfestation, white-washing and using repellent paints to paint the walls, ceilings and floors of empty stores with products such as coal tar. These practices significantly turn the host environment into an adverse environment for storage insect pest (Mishra 2017). These practices have proven to be the cheapest and reliable techniques especially for smallholder farmers, as they require standard post-harvest practices without the need for extensive labour. Sanitation plays an important role in this control method to reduce insect population. Controlling insect pests using the cultural methods is considered inexpensive, but for them to be effective they need to be cautiously timed and also need a great period of time planning. Storage and cultural control time as reported by Metcalf (1993), slows the rate of insect pest colonization, survival, reproduction and also delays development and phenotypic expression of the insect pest. In practical cultural control methods may lower the population number of insects pest below the economic threshold and even allow the ecological defenses to also take effect (Hill, 1989). Watson (1976) also revealed different insect pest may have different responses to cultural control methods as some cultural method that are effective against one insect pest may be unsuccessful with another pest insect of relative species.

2.7.2 Physical control

Physical control methods mostly are strategies that basically rely on the treatment of the seeds and insects using physical agents such as heat, moisture content, temperature and pressure (Mishra 2017). For each insect to be fully active and fully develop and reproduce it requires an optimal environment condition comprising temperature, humidity and photoperiod (Evans, 1987), which usually varies depending upon the stages of growth and reproduction of the insect. Thus, lowering or raising temperatures and altering relative humility from the insect's optimal range can reduce the rate of the development of bean seed beetles (Benz, 1987). Mbata *et al* (2005) established that a combination of low pressure and high temperature is more effective in killing the eggs, larvae of cowpea weevil and related *C. maculatus* in cowpea. The use of solar heating techniques has also been recommended for controlling seed beetles in beans and other legume seeds (Moumouni, 2014).

2.7.3 Biological control

Biological control of *C. maculatus* involves utilization of other living organisms which are usually natural enemies to the seed beetles. They are known as biological control agents, and they play an important role in maintaining the seed beetle populations below damaging level, so that no economic loss occurs. The biological control agents include pathogens, predators and parasitoids and actively participate in the effective control of the *C. maculatus* (Altieri, 2005; Mahr, 2008).

The biological pest control strategies are of three types: importation, augmentation and conservation. Biological control is considered to be a viable and acceptable strategy, where beneficial biological agents as formulated products are applied to the seeds infested with storage pests (Brower, 1996).

Biological control strategy is regarded as valuable control method where pest insects emerge naturally or farmers can release into the stored seeds when needed to parasitize *C. maculatus* larvae. In terms of use of parasitoids biological control agents, there has been minimal investigations in the degree to which they can be effectively utilized as biological control against seed beetles. Although use of biological agents to control may appear to be effective in controlling insect pest, their practice may prove to be complex for most small-scale farmers with their limited knowledge on types of predators, supplementation of parasitoids, and ways of preserving pathogens in their culture (Kananji, 2007). Parasitoids biological control also depends on specific stages of larvae development and also timing of releasing them so as to be active, and this can be challenging to most small-scale farmers who lack information on seed beetle larval stages and how to detect them. These biological control using parasitoids limitations have made this method uncommon to use against insect storage pests.

2.7.4 Chemical control

The use of synthetic pesticides have for so many years been established as primary and significant component in controlling and managing insect pests (Acrey & Kananji, 2007). Research by Harberd, (2004) have also described the ability of synthetic pesticides, such as fumigants, dusts, and sprays, to prevent *C. maculatus* pests and how using them in increasing dosages causes the buildup of hazardous remains in the items they are applied to. For the past six decades different groups of insecticides have been used to control pests both at the storage and field conditions (Mishra, 2018). Carbamates, organophosphates, and synthetic pyrethroid are the chemicals that are typically available commercially and are used most frequently to reduce *C. maculatus* infestations in storage legumes. It has been established that fumigants and dust insecticides are frequently applied to stored seeds to combat *C. maculatus* infestation.

Research by Gwinner, (1990), reported that the active substance of the dust formulation insecticides, which are frequently offered as ready to use, ranges from 0.1% to 5%. These details frequently contain added substances, which increment the adhesive intensity of the dynamic fixings to the stored grain. By using a scoop, dust insecticide formulas can be administered to floors, flat surfaces, and the area around the base of large containers. In order to accomplish effective control of *C. maculatus* s, dusts ought to be blended altogether and conveyed universally throughout the produce. The greater part of synthetic pesticides being used for obligated crisis activity against *C. maculatus* s when their populace draws near or surpasses financial edge level include: lindane, pirimiphos-methyl, Malathion, chloropicrin, deltamethrin and permethrin just to name few (Gwinner, 1990).

Another way to deal with insecticidal control in stored grains is by chemical fumigation, which are low atomic weight synthetic compounds, profoundly poisonous and unpredictable and are subsequently self-scattering and nonpersistent. This method of fumigation is one of the techniques that is mostly broadly rehearsed everywhere throughout the world to control *C. maculatus*, particularly in enormous storages. When fumigants are used efficiently, their tiny gas particles penetrate large amounts of grain right into the sand particles, attacking and destroying pests at all stages of life (Beebe, 2013). At least 16 substances have been certified as fumigants due to concerns about human safety, with the main fumigants currently used commercially for stored items being methyl bromide, carbon disulfide, methyl iodide, phosphine, and aluminium phosphide (Faruki, 2004). Research by Mebeasilassie (2004) reported that Methyl bromide treatment on exposed larvae of *C. maculatus* lowered fertility, the quantity of adult offspring, and there was a tendency for treated offspring to remain in their developmental stage longer. In any event, its fundamental flaws are that the best fumigants are extremely poisonous and hazardous to humans and other non-target living things, so when the commodity is exposed again there is no longer any guarantee against re-infestation (Mwanauta, 2015)

Besides these issues related with manufactured pesticides particularly pesticide obstruction, wellbeing risks and ecological impacts humans have made an overall enthusiasm for the advancement of alternative approaches for example the use of accessible host plant resistant through the instruments of biotechnology and breeding for seed beetles control legume crops. What's more, incases of subsistence farmers pesticide and pesticide sprays are unaffordable as they are unreasonably expensive, and they are also not easily accessible in some remote areas where farmers are based. Controlling insect pests involves fumigating farm stores, but in most villages the farm structural designs make fumigation difficult or even impossible to achieve.

2.7.5 Host resistance control

Host plant resistance is characterized as the ability of crops to naturally inherit resistance to insect pest infestation hence enhancing crop quality and yield (Kumar, 1984). Host plant resistance control method is considered to be environmentally friendly which makes it significant to integrated pest management, as continues to afford gradual protection from the insect pest, and it can as well be used in conjunction with other pest managing systems (Kogan, 1998). The in-built ability of cropping plants to contain, hinder or triumph pest infestations is what's known as host plant resistance, and results in improved both yield and quality of crop harvested and stored yield (Kumar, 1984). With a farmer's outlook using the host resistant varieties demonstrates a more suitable and simplified way of *C. maculatus* control. Crops which are considered to resistance to insect pest obtain their characteristics from combination of cultural, biological and natural suppression properties. These pest resistance properties interrupt the relationship of the host plants and the insects (Van Emden, 1997). The three characteristics that plant breeders follow when developing resistant cultivars, and they are, tolerance, antibiosis and antixenosis approaches. Cultivars in storage once damaged, the damage cannot be reversed as such tolerance approach is not applicable in storage, making antibiosis and antixenosis the most suitable approaches for insect pest resistance in storge. Local *C. maculatus* control methods possibly will be additionally active when used in union with cultivars with sufficient intensities of resistance. In a study by Baldin and Souza (2013), 50 cowpea genotypes resistance against *C. maculatus* were evaluated. Each of the genotypes were infested with *C. maculatus* and 9 of these cowpea genotypes were reported to be resistant to attack by *C. maculatus*. Seven of the 50 cowpeas genotypes showed a non-preference type resistance (oviposition and development) exhibiting antixenosis properties and the other 2 exhibited antibiosis against the seed beetles. In another study by Acrey and Kananji 2007, to test seeds for host resistance against the *Acanthoscelides obtectus Say* and *Zabrotes subfasciatus Boheman* beetles in storage 58 improved varieties and 77 landraces of bean genotypes were used. From the results it was established that against *Z. subfasciatus* 12% of the bean genotypes were resistant to moderately resistant and 88% were susceptible to highly susceptible. Whilst against *A. obtectus* 12.5% were resistant while 87.5% were moderately to highly susceptible. Acrey & Kananji 2007, additionally reported that antixenosis was a significant factor in the bean seeds' resistance to seed beetles. Using insect-resistance crop cultivars is not only ecologically sound but is also sparingly and naturally preserving (Kogan, 1998). By not procuring insecticides to use on vulnerable cultivars, money is saved, harvest is also kept from loss due to the insect pests. More often than not, seeds of pest resistant cultivars cost if not the same just slightly more than those of vulnerable varieties.

2.7.5.1 Antixenosis

Antixenosis is described as the variety's ability to use its chemical or morphological characteristics to resist insects pest infestation, either by decreasing in the insect's oviposition or feeding (Acrey & Kananji, 2007). Characteristics influencing susceptibility are kernel color, texture, its size and chemicals components. Externally, grains with a soft, plane and tiny kernel are more ideal for oviposition compared to those with rather rough, uneven, prickly and wrinkled kernels (Shaheen, 2006). The seed beetles will not oviposit on seed helium because of its spongy like texture (Somta, 2007). Studies have proven that external factors like the kernel coat toughness and kernel coat coarseness grant resistance of grains to seed beetles (Giga, 2002). A kernel with tough seed coat could effectively stopped larvae from successfully piercing into the seed, whilst coarse seed coat also makes it problematic for seed beetles as it glues the *C. maculatus* eggs on the seed testa, therefore establishing that *C. maculatus* pests do not desire rough seeds for laying eggs (Messina & Renwick, 1985). Cardona (1989) also reported that the seeds' testa may usually act as an external barrier feature accountable for the resistance against pest in the cotyledons.

Studies by Edde and Amatobi (2003) reported that the most preferred cowpea seeds for oviposition were the ones with undamaged seed coats as compared to ones without the seed coat and therefore it was concluded that when breeding cowpeas for resistance against seed beetle seed coat may not be one of the important factors to consider. This suggests that, just as a kernel coat may be sufficiently impenetrable, resistance to post-harvest insect activity depends on the connected component variables of antixenosis and non-preference and hard to stop boring of the seed to a certain extent, even though some primary pests have adjusted to break into an entire undamaged seed. Characteristics of the cellular makeup of leguminous plants' kernel covers like cowpea cultivar partly stops entry of the initial instar larvae of *C. maculatus* (Shade *et al*., 1996).

2.7.5.2 Antibiosis

Dent (2000) defined antibiosis as the mechanism by which a plant that is populated by pest is resistant to the pest due to its hostile effects on the development, survival and reproduction of the insect. The active antibiosis mechanism found on host resistant plants usually causes slow degree of development, reductions in production rate, insect pest mortality or even increases chances of the insect being exposed to its natural enemies (War, 2012). Antibiosis mechanism includes primary and secondary metabolites, and these biochemicals which usually cause antagonistic effects on *C. maculatus* as such affecting their feeding and development.

Chemical components of legume seeds have been reported to be important in conferring resistance against *C. maculatus* (Kosini, Nukenine, Saidou, Noubissié, & Dolinassou, 2019). Belay, (2017), has also documented some biochemical compounds to have an effect on resistance of cowpea against storage pest while other seed coat biochemical compounds are not associated with cowpea resistance to *C. maculatus*. Lattanzio, (2005) has reported that legume seeds rely on more than one chemical for defense against *C. maculatus*. Kpoviessi., (2021) documented those metabolites found in seeds such as tannin content, carbohydrates content and protein content are strongly related to cowpea resistant to seed beetle. In a study by (Lattanzio., 2005) different seeds of wild Vigna species were screened for their tannins as defensive mechanism against *C. maculatus*, and the results showed that with most of the seeds tannins had a negative correlation to resistance of seeds to *C. maculatus*. (Lattanzio, 2005) also reported that according to his research tannins should always be considered in biochemical defense of plants against seeds. Tannins have also been documented to play a role in resistance of cowpea seeds against seed beetle (Kpoviessi et al., 2021). Different cowpea genotypes were checked for resistance, cowpeas with high tannin content proved to be resistant to *C. maculatus*. Tannins my play a role as defense chemicals as they have a bitter taste which may affect palatability of pest and reduce consumption of the seeds. Tannins with proteins form complexes which reduce digestibility of the seed to insect pests.(Swain, 1977). Grain moisture content has also been reported to be favorable for growth and development of storage insect pest *Sitophilus zeamais* (Paneru, Thapa, Sharma, Sherchan, & Gc, 2018). In another study by Aryal, and Bhandari, (2019), to investigate moisture content on weevil *S. zeamais* susceptibility on maize seed, the results proved effect of moisture on weevil susceptibility to be highly significant. High level of moisture has been documented to result in rapid increase in the development and growth of insect pests in grain (Shepard, 1947). Shepard, (1947) reported that grain borer can develop successfully in grain that have high level of moisture. Osipitan, (2007) reported that moisture, ash and fat not to have any basis of resistance in maize against *Prostephanus truncatus.* Findings from Demissie, (2015), study indicated that crude fat, carbohydrates, protein and crude fibre had effect on resistance and susceptibility of different maize grains against S. cerealella. Kpoviessi., (2021) in a study to determine the biochemical compounds underlying 6 different cowpea genotypes resistance to *C. maculatus*, they reported positive correlation between DIS and protein content in cowpea seeds. the cowpea seeds with high protein content was recorded to be susceptible while those with low were recorded to be resistant. In another study cowpea seeds with high proteins were recorded to be resistant to *C. maculatus* as the protein proved to have a toxic effect on the *C. maculatus* (Souza, 2011). High content of proteins in seeds which are susceptible mean that the insect pest prefers seeds with much protein as it supports the development and growth of larvae as they are not toxic and could not harmfully affect *C. maculatus* larvae. High Carbohydrates content in cowpeas have also been documented to have effect on the resistant against *C. maculatus* (Kpoviessi, 2021). In another study to investigate biochemicals compounds effect on cowpea resistance against, carbohydrates were reported to do confer resistance of cowpea against *C. maculatus* by reducing the development of the pest (Belay, 2017). High level of carbohydrates increase hardness of the seed coat which in turn makes it hard for the larvae to penetrate into the seed. Nwosu, (2016), in his study reported crude fiber to be the base of maize seed resistance to *S. zeamais* in storage (Bergvinson et al. 2004; Nwosu 2016). Fiber has been documented as growth inhibiting substances against *S. zeamais* (Nwosu, Adedire, & Ogunwolu, 2015). High fiber content results in negative effect on the survival and development of insect pest. Ash is one of the biochemical components which proved to have an effect on resistance of seed against insect storage pest. in a study by Demissie, (2015) to investigate biochemical basis of resistance twenty maize varieties to the insect pest *Sitotroga cerealella*, seeds which had high ash were reported to be susceptible to *S. cerealella* while those which had low ash were resistant to the insect pest. Minerals such as Magnesium, calcium and sodium were also recorded to have an effect on the susceptibility of variety of maize against *S. zeamais*, as they indicated that an increase in these chemical constituents resulted in increase in susceptibility (Nwosu, 2016). The study also indicated that grains rich chemical constituents' protein and minerals except for potassium are susceptible to infestation and damage by *S. zeamais*. As such this paper will also evaluate some of the biochemical, ash, moisture, crude fat, protein, carbohydrates, crude fiber, tannins, sodium, potassium, calcium and magnesium factors that might play a part in the resistance/susceptibility of *P. vulgaris*. These biochemical factors have been documented by different studies to be of different content in common bean and cowpea seeds depending on the type of varieties seeds.

2.7.6 Control of Seed Beetle using Plant extracts

Botanical extract control involves the utilization of plant material with insecticidal properties to manage or control insect pests. For several decades use of botanical plants for grain legume preservation has been viewed to be a possible substitute to synthetic insecticides (Regnault-Roger, 2012). Plant extracts as pesticides have been recognized as safe than synthetic pesticides which studies have reported to cause increase in risk of ozone depletion, carcinogenic, neurotoxic, teratogenic and mutagenic effects in non-targets and cross- and multi-resistance in insects (Regnault-Roger, 2012). While certain plant families may only produce a few numbers of secondary metabolites with anti-insect capabilities, others may produce a large number of molecules with various structural kinds. Many different plants have been documented to have insecticidal effects on different insects in storage and also in the field as shown in Table 1. The plant as a whole can be used to control insect pests or different parts of the plant can be extracted and used like leaves, seeds or even bark depending on the plant species being used. Due to its appeal with organic producers and ecologically concerned growers, the use of botanical plants as pesticides has significantly expanded. Small scale farmers benefit more from botanical plants as control for seed beetles as they are locally available, inexpensive and biodegradable. A lot of emphasis has been directed to the need for comprehensive study on botanical plants as pesticides in order to fulfil requirements for IPM and pollution prevention (Mulungu, 2007). The most popular techniques for using botanical plants as insecticides in storage involve mixing powders, oils, and more filtered pesticides as well as using essential oils and naturally soluble plant components as fumigants and anti-agents (Harberd, 2004; Shaaya & Kostyukovysky, 2006).

Studies from different researchers have reported on how bioactive compounds of botanical plants have various effects on different insect pest including seed beetles and they are; reducing the life span of adults, repellants to the insect, anti-feedant action, oviposition deterrent, insect growth regulatory activity, inhibits juvenile hormone synthesis and intermediates are formed giving rise to larval-pupal, nymphal adults, and pupal-adult intermediates, and there is usually also a fractional interference with the respiration of the insects which as a results leads to suffocation (Pereira, 2006; Regnault-Roger, 2012). Studies by Don Pedro (1989) reported that the physical properties and toxic components of botanical plants causes change in the surface tension and oxygen tension within eggs of insect pests as such leading to egg mortality.

Table 1. Plants with insecticidal effects

Plant name (common & Scientific)	**Family**	**Target insects**	**Reference**
Neem, *Azadirachta indica*	Meliaceae	Maize weevil, Seed beetle	(Magano, Nchu, & Eloff 2011;Lanka 2019)
Sesame, *Ceratotheca sesamoides*	Pedaliaceae	Pulse beetle	(Laizu 2009; Said & Pashte 2015)
Goatweed, *Ageratum conyzoides*	Asteraceae	Wheat weevil	(Moreira, Picanço, Cláudio, & Barbosa, 2007)
Sunflower, *Helianthus annuus*	Asteraceae	Pulse beetle	(Said & Pashte, 2015)
Moringa, *Moringa oleifera*	Moringaceae	Maize weevil	(Zedan, 2018)
Garlic, *Allium sativum*	Amaryllidaceae	Pulse beetle, potato tuber moth, house fly.	(Islam, 2013; Tlankka, Mbega, & Ndakidemi 2020)

Mustard, *Brassica juncea L.*	Brassicaceae	Seed beetle, pulse beetle	(Laizu 2009; Prasanthi, Kumar, & Chakravarty 2017)
Black pepper, *Piper nigrum*	Piperaceae	Pulse Beetle, bean weevil	(Islam, 2013; Swella & Mushobozy 2007)
Marigold, *Tagetes minuta*	Asteraceae	western plant bug, silverleaf whitefly	(Id, Yool, & Spurgeon, 2020)
Tobacco, *Nicotiana tabacum*	Solanaceae	Seed beetles, pulse beetle	(Acrey & Kananji 2007; Lanka 2019)
Peppermint, *Mentha piperita L.*	Lamiaceae	Wheat weevil, black carpet beetle, rice weevil, red flour beetle	(Moreira, 2007)(Baker, Grant, & MAlakar-Kuenen, 2018)
Fever tea, *Lippia javanica*	Verbenaceae	Aphids, maize weevil	(Katsvanga & Chigwaza, 2004:
Eucalyptus, *Eucalyptus globulus*	Myrtaceae	Seed beetle	(Lanka, 2019)

The studies also demonstrated that developing embryos and first instar larvae are usually lethally affected as there is a reduction on the rate of gas exchange due to barrier effects. By far different botanical plants with insecticidal activities have been tested against several storage pest (Table 1). In some studies several edible and non-edible oils were tested for their biological activity against stored-seed insect pests, and the results showed that these treatment methods were successful in causing 80 to 100% adult mortality, reducing progeny emergence, and providing a high percentage of protection without having any negative effects on the viability of the seed. (Rajapakse & Emden, 1997), Khattak, 2001). Studies by Lanka (2019) where he tested insecticidal efficacy of botanicals neem (*Azadirachta indica)*, Chinese chaste tree (*Vitex negundo*), blue gum tree(*Eucalyptus globulus*) and sugar apple (*Annona squamosa lag*) against the insect pest, *Callosobruchus maculatus* (seed beetle) on stored grains; *Vigna unguiculata var. Waruni* (red cowpea), *Vigna unguiculata var. Dhawala* (cowpea with black eye), *Vigna radiate* (green gram) and *Cicer arietinum* (chickpea). In this study *A. indica* and *A. sativum* to be the significantly best along with 80 % of mortality in adult weevils and zero damaged seeds in all treated grains whereas *V. negundo* and *E. globulus* had average mortality effect (40-50 %) at the beginning of the study. Powder and the seed oil of *Khaya senegalensis* were also evaluated as possible treatment against *C. maculatus* on stored cowpea, and the results indicated that within 24 hours the mortality rate of *C. maculatus* was high (60-100%) (Bamaiyi,2007). Botanical plant extracts of mustard, sesame, ashanti pepper, neem, turmeric, and coconut also proved to be effective in controlling 60-100% of seed beetle in storage (Elatta & Ibrahim, 2002; Shaheen, 2006). Flavonoids could be valuable in pest-management strategy as they can play a significant role in the protection of stored seeds against insect pests (Acheuk & Doumandji-Mitiche, 2013). Simmonds, (2003) reported that by regulating the growth, reproduction, and behaviour of insect pests, flavonoids and isoflavonoids both defend the plant against pests. Diwan & Saxena (2010) also found that flavinoid glycosides isolated from purple tephrosia (*Tephrosia purpuria)* showed insecticidal property on *C. maculatus*. Isoflavonoids and proanthocyanidins are other classes of flavonoids responsible for plant protection against insects.

Besides protecting plants from different pest and diseases, several studies have reported botanical plant extracts to influence germination of different crops. In a study by Fritz,(2007) Germination and growth of lettuce was significantly inhibited by goatweed (*Hypericum polyanthemum*) plant extract. Ismail & Chong, (2002) also reported that extracts of Mikania decreased germination percentage, fresh weight and radical length of tomato. Besides the inhibitory effects, there are a lot of reports indicating positive effects of plant extracts on germination. Plant extracts of *Eugenia jambolana*, *Nerium oleander* and *Citrullus colocynthis* found to improve the growth and yield of lupine plants (Abdel-Monaim, 2011). Soaking of Bean seeds in leaf extracts of *Moringa oleifera* resulted in increased growth and yield in bean plants (Rady, 2013). Ashamo (2019) gave a list of plant products that have been found to be effective in controlling stored products insect pests in cowpea, maize, paddy rice, groundnuts amongst others. Plants such as Azadirachta indica, Piper guineense, Capsicum species, Citrus sinensis and Alstonia boonei have been found to have insecticidal properties against *C. maculatus*.

The botanical plants that will be used in this study to evaluate their efficacy in control of seed beetles on common bean are garlic, peppermint, fever tea and marigold. All these botanical plants are known to poses insecticidal properties and have been used traditionally as pesticides in storage in some countries.

2.7.6 .1 Garlic (*Allium sativum* L.)

Allium sativum, L. which is a botanical plant commonly known as garlic is a species that falls under the family Alliaceae. Garlic is recognized as multicultural plant that has a recorded history for having different uses which are therapeutic, culinary and also used for religious purposes (Chopra, 1986). Dawit, (2005) also reported that garlic is a widely cultivated plant which has been used throughout history by Egyptians and Romans for religious and traditional medicine purposes but commonly used as condiment in cooking.

Garlic is barely a perennial monocotyledonous plant which has long pointed and broad linear flat leaves, with a multiple bulb composed of several fractional bulbs usually known as cloves enclosed in a common membrane (Minnesota, 1999). Garlic has narrow level upright leaves attached to the base about 1cm wide getting thinner towards the tip, and grows and develops to a height of about 60cm. the flower stem is well known to have round head of greenish-whit or pale pink blossoms (Thompson & Kelly, 1957). Usually, the small garlic bulbs can be found in the flowers as such can be propagated vegetatively by planting cloves or bulbs (Purseglove, 1972).

Garlic has allicin as one of its different active components, having main active ingredients which include propylene, thioacrrolin, 2-propene sulphenic acid and 2-propenthion. These main ingredients give garlic its features of odor and or insecticidal properties (Gurusubramanian & Krishna 1996). The garlic cloves contain the enzyme alliinase which is released when the garlic is crushed, and leading to a process in which allicin, pyruvic acid and ammonia are produced. The enzyme alliinase converts the alliin which is an odorless compound into allicin which has a more powerful smell (Williamson, 2003). The pungent odor from allicin enables garlic to have antifeedant and repellency effect against some storage insect pests (Amhara Regional State Agriculture Bureau, 2001). Mechanism by which garlic control insect pest include deterrence, antifeedant, phagostimulants, oviposition modifier effect and disruption of major metabolic pathways leading to rapid death. Garlic may also accelerate or retard insect development or even delay its life cycle (Bell, 1990). Studies by Don-Pedro (1989) reported that garlic oil also has the properties of blocking respiration of insect's eggs leading to death. Willis (1958) in his research also indicated that a pined allicin of garlic was the main active principle which affected the activity of important insect metabolic enzymes. On other studies by Glob, (1999), garlic extracts were also discovered to prevent *C. chinensis* from causing damage to legumes, as 2% mixed with legume grains caused a reduction in percentage of damage caused by larvae. Garlic oil also displays antifungal, antibacterial, amebicidal and insecticidal qualities (Owens, 2002), and the constituents of the essential oil accountable for these characteristics are allicine and sulphide (Buss & Park-Brown, 2002).

2.7.6 .2 Peppermint (*Mentha piperita* L.*)*

Mentha piperita L. commonly known as peppermint belongs to the family Lamiaceae and popularly known to be widely distributed in Europe, Asia, Africa, Australia, and North America (Lawrence, 2006). Peppermint grows well in cool temperate regions and needs long days with warm to hot conditions and cool nights to guarantee the right balance of essential oil compounds, produced during the growing phase. The optimum temperature for peppermint growth and flowering ranges between 21 and 26 °C (Ringuelet, 2003). Peppermint is recognized as one of the many herbs that are known for their medicinal and aromatherapeutic properties since ancient times (Khan & Abourashed, 2010).

The flowering tops, live plant, dried leaves, and essential oil of peppermint are frequently used as culinary seasonings and as a flavoring agent and antitumor in medicine. (Merck, 2015). Being the fifth most produced essential oil in the world, peppermint is now one of the most researched sources of essential oils. (Franz & Novak, 2009). A multitude of variables, such as plant parts, age, temperature, environment, day duration, irrigation, and extraction process, affect the composition of essential oils and their output. (Lawrence, 2007; Franz & Novak, 2009; Khan & Abourashed, 2010). Peppermint is not only known for medical and food uses as peppermint oil is also a major source of menthone and menthol which are used in many personal care products such as toothpaste, fragrances, mouthwashes, soaps, hand lotions and even in tobacco products (Hayes, 2007). Peppermint also has compounds like menthyl acetate and menthofuran which are also present in significant amounts. Other constituents of peppermint include limonene piperitone, viridiflorol, bisabolene, isomenthone, isomenthol, neomenthol, ledol, pulegone, caryophyllene, and bicycloelemene, among others (Khan & Abourashed, 2010).

Peppermint has also been known to have insecticidal properties against ants, wasps, cockroaches, hornets and mosquitos (Worwood, 1993). The components found in peppermint essential oils are reported to have properties that are antifungal, antibacterial, and anti-cancerous, making it more worth exploring further (Lee, 2001; Bakkali, 2008; Tyagi & Malik, 2010). The prospects of peppermint to be used in food for seasoning makes it more effective and safer to use as insect repellent. Though they are viewed to be used for seasoning in food, few reports mutagenic activity and toxicity at high concentration, which makes it important to have the knowledge of dose and procedure of application (Franzios, 1997; Gardiner, 2000).

Post-harvest pests like *Sitophilus oryzae* (rice weevil), *Attagenus fasciatus* (black carpet beetle), *Tribolium castaneum* (red flour beetle), *Rhyzopertha dominica* (false powderpost beetle), *Oryzaephilus surinamensis* (sawtoothed grain beetle), and *Lasioderma serricorne* (cigarette beetle) have also been reported to be effectively managed by the use of peppermint oil (Gardiner, 2000). Misra & Kumar (1983), reported that red flour beetle populations was effectively reduced by peppermint oil in the form of fumigant, against the first, second, third, and fifth instar larvae. in a study carried out by Li & Tian (2020) to evaluate the effect of peppermint essential oil on the pest Chinese pear psylla, and they revealed that the essential oils have a 78% repellency rate against the adults of pear psylla. In another study by Lanka (2019) efficacy of peppermint essential 10% oil as control against *C. maculatus* in storage was tested, and reported that there was 80% mortality on adult *C. maculatus*.

2.7.6.3 Marigold *(Tagetes minuta)*

Tagetes minuta L. commonly known as tree marigold, Mexican sunflower, shrub sunflower or Japanese sunflower is an annual aromatic herb that belongs to the family Asteraceae and the genus Tagetes which is one of the most abundant plant taxonomical groupings (Sadia, 2013). The plant is commonly known to be a tropic and sub-tropic plant which is native to the temperate grasslands and montane regions of southern South America (Soule, 1993). Marigold is identified as a strong scented annual herb which consist of erect and most of the times highly branched stems (Shahzad, 2012). Throughout history marigold has been recognized as a medicinal plant that has always been used widely in folk medicine to treat various illnesses. Botanical surveys and research by Rungeler

, (1998) further described marigold to have medical properties and that marigold extracts exhibited antibacterial, antiproliferation, anti-inflammatory and antidiarrhetic properties. Rungeler (1998) study likewise demonstrated the marigold to be effective in the treatment of wounds. The leaf is reported to contain components like sequiterpene lactones taginin C which are active against inflammatory activity (Tona, 1998). Wells (1993) reported marigold plant to have insecticidal properties due to the insecticidal components in its flowers, roots and the leaves.

Alpha terthienyl is one major light sensitive compound found in marigold roots, which normally has the ability to overwhelm the population of nematodes and also able to improve the growth of other plants like tobacco and tomatoes (Miller & Ahrens 1969; Ijani & Mmbaga, 1988). Singh & Singh, (2002) reported marigold to have a strong larvicidal effect as such has been used traditionally for repelling mosquitos. The essential oils from the fresh and dried plants of Tagetes minuta were reported to be highly effective against the larvae of mosquito (*Anopheles stephensi)* (Hadjiakhoondi, 2008). Marigold essential oil have been screened for potential toxicity against stored insect pests which included, *Tribolium castaneum* and *Sitophilus oryzae,* and they were found to effectively control them (Alok, 2005). Hadjiakhoondi (2008) reported that marigold essential oils from fresh and dried plant materials had high efficacy against larvae of *Anopheles stephens.* More studies demonstrated that the marigold extracts have repellent and antifeedant activity against diamond backmoth*, Plutella xylostella L.* (Reddy, 2015)

2.7.6.4 Fever Tea (*Lippia javanica*)

The plant species *Lippia javanica*, sometimes known as fever tea, is a member of the Verbenaceae family, which has about 32 genera and 840 species (Marx., 2010). It is an aromatic perennial shrub woody that can grow up to 2m. Fever tea has been documented to naturally occur in southern, eastern and central Africa, and also found in the tropical Indian subcontinent (Mkenda. 2015; Narzary & Basumatary 2015). This species is reported to be native to sub- Sahara Africa countries which are Angola, Botswana, Central African Republic, Democratic Republic of Congo, Ethiopia, Kenya, Malawi, Mozambique, South Africa, Swazi- land, Tanzania, Uganda, Zambia, Zanzibar, and Zimbabwe (Maroyi, 2017a). It is usually found grasslands on hillsides and banks of streams, roadsides, forest edges, stream banks and bushveld (Anjarwalla Parveen, Jamnadass, Ram, Belmain, Koech 2015). Involatile oils found in fever tea include caryophyllene, carvone, ipsenone, ipsdienone, limonene, linalool, myrcene, oci-menone, p-cymene, piperitenone, sabinene, and tagetenone. (Maroyi, 2017b)

Based on its alleged therapeutic and physiological benefits, it has been discovered to have a variety of traditional applications in tropical Africa as an indigenous tisane, hydrating beverage, or food ingredient. It has also been documented to be regularly used as a traditional medicine to treat minor ailments and microbial infections such as coughs, colds, fungal infections, respiratory diseases and skin infections. (Anjarwalla Parveen, Jamnadass, Ram, Belmain, Koech 2015). It is used as a caffeine free tea in countries like Botswana and in countries like Zimbabwe and Malawi it is documented to be used mainly as a nerve tonic (Manenzhe, Potgieter, & Van Ree, 2004). Fever tea essential oils have been found to have good insecticidal effects as such has been reported to be used in pre and post-harvest management in crops and also ecto parasite control in livestock (Manenzhe, 2004; Parveen, 2015). In a study by Magano, Nchu, & Eloff (2011) effects of fever tea extracts using the in vitro tick climbing repellency bioassay on adults of Hyalomma marginatum rufipes Koch ticks were evaluated, and the study found that they caused a repellency index of 100%. In another study fever tea powdered leaf extracts indicated to have insecticidal propertieswith potentialto control damage from coleoptera pests by 21 to 33% (Chikukura, et al, 2011). Katsvanga & Chigwaza (2004), From their study also reported fever tea to be effective in control aphid’s species *Brevicoryne brassicae* as they found out that that 1:1 powdered aqueous leaf extract of fever tea reduced aphids by 53.2%. In another study the pesticidal effects of fever tea leaf powder against rape aphids (*Brevicoryne brassicae*) and tomato red spider mites (*Tetranychus evansi*) was evaluated, and fever tea reduced the red spider and aphids by 63% and 12.5% respectively (Belmain, Stevenson, & Mhazo, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the experimental site

This experiment was carried out in the Entomology laboratory, Department of Crop and Soil Sciences at Botswana University of Agriculture and Natural Resources (BUAN). The University is located 3km away from capital city Gaborone, which located in the Southeast district of Botswana.

3.2 *Callosobruchus maculatus* culturing

The rearing of *Callosobruchus maculatus* was carried out as described by Kestenholz *et al.* (2007) in the Entomology laboratory under natural conditions where the temperature ranged between 25ºCand 32ºc, with humidity ranging between 30% and 50% during summer. The *C. maculatus* beetles that were used in the experiment were collected from the laboratory stock culture on Tswana cowpeas. The method that was used for mass production of *C. maculatus* was adopted from Ousman *et al*. (2007), where 1kg of black-eyed cowpeas (which has been documented to susceptible to *C. maculatus)* was collected from the Horticulture Laboratory and was used as a medium for culturing the *C. maculatus*. The black-eyed cowpeas were then disinfected by keeping them in the refrigerator at temperature of 0 ºc for seven days.

The 1kg of the black-eyed cowpeas was then placed in 1litre plastic jar (Appendix 1) and hundred adults of mixed female and male (50:50 proportion) of *C. maculatus* collected from the stock culture was placed on the beans and the glass jar was covered with a muslin cloth for the seed beetles not to escape and also for aeration. The adult *C. maculatus* was left for a week in the laboratory conditions to mate and lay eggs, and once the oviposition was observed the adult *C. maculatus* were removed from the glass jar by serving them with a 2 mm sieve. The beans with the oviposited ova were then left on the glass jar until the emergence of new adult beetles. A day after emerging the adults was sexed according to shape of abdomen and markings on elytra (Van der Meer, 1979). Females have a more oval-shaped abdomen with contrasting “eye” marks on the elytra and two dark stripes on the tip of the abdomen, whilst males have a rounder abdomen and more consistently patterned elytra, with a consistently pale abdominal tip. The emerged adults were then used for the experiments.

3.3 Screening Genotypes for Resistance Experiment

3.3.1 The experiment material

The experiment was arranged in a completely randomized design (CRD) replicated four times. The experimental units were seeds of genotypes CAL96, DAB520, X-variety and Black eye as positive control (Table 1). The CAL 96 and DAB 520 genotypes that were screened for host resistance were collected at the Department of Agricultural Research, Sebele, the X-variety genotype was bought from Botswana Agriculture Marketing Board (BAMB), while the black eye was purchased from one of the local markets. Prior to the start of the experiment all the seeds were disinfected by being placed in a refrigerator at 4 ºc for 7 days. Then 50g of seeds of each genotype was each placed in transparent feeding jars, and the feeding jars were clearly labelled to indicate the date the experiment began, the names for varieties of seeds and also the replication numbers.

3.3.2 Infestation of the bean seeds

All the genotypes were then artificially infested with ten pairs of *C. maculatus* adults of 1 to 2 days old which were collected from the seed beetle culturing jar. A muslin cloth was utilized to cover the top of the feeding jar so that the feeding jar remain ventilated while the *C. maculatus* unable to escape.

The *C. maculatus* were then allowed to oviposit on the beans for ten days before they were removed and discarded. The number of eggs laid were then counted and recorded and they were kept for adult emergence. Other parameters that were recorded to evaluate the susceptibility of the bean genotypes to *C. maculatus* are; number of hatched eggs; total number of adult emergencies; number of dead beetles; initial weight of bean sample; and final bean weight. The data collected was then used to determine seed beetle adult emergency percentage; seed beetle mortality percentage; bean weight loss percentage; and Dobbie Susceptible Index (DSI).

For the negative control, 50 g of each common bean varieties were placed in a jar and no infestation was done. Each variety was replicated four times and was kept in the same conditions as in other treatments above. This experiment was repeated twice to confirm the results.

Table 2. Local bean varieties and landraces

TREATMENT	GENOTYPE
1	CAL 96
2	DAB 520
3	X-genotype
4 (Control)	Blackeye cowpeas

3.3.3 Proximate and mineral analysis of bean genotypes

This was laboratory experiment which was conducted in Animal Nutrition laboratory at Botswana University of Agriculture and Natural Resources. Eleven biochemicals were extracted from each of the four genotypes namely, CAL96, DAB520, X-genotype and Blackeye. Each genotype extraction was replicated three times. The methodology for extraction of each biochemical components is as follows:

3.3.3.1 Procedure for extraction and analysis of percentage moisture and ash

100g of each of the genotypes was ground into powder using piston and mortar in three replicates. Method used was adopted from AOAC, (1995), where 1g of each genotype powder was accurately weighed in a crucible of known weight and dried in a hot air oven for 24 hours at 105ºC. The crucible with sample was then cooled in desiccators and then weighed and the dry matter (DM) was recorded. The samples in the crucible were then dried in hot air oven again at 105ºC for 24hours, and the crucible with sample was then cooled in desiccators and then weighed. The weight obtained was used to calculate the percentage moisture. Dry matter and moisture were then calculated using the following formula (AOAC, 1995);

$$\textbf{\textit{Dry matter (\%)}} = \frac{Wf - Wi}{Ws} \times 100$$

Where; W_f = weight of Crucible + Dried Sample

W_i = weight of Crucible

W_s = weight of Fresh sample

$$\textbf{\textit{Moisture (\%)}} = \frac{(\mathrm{Ws} + \mathrm{Wi}) - \mathrm{Wf})}{\mathrm{Ws} + \mathrm{Wi}} \times 100$$

Where; W_f = weight of Crucible + Dried Sample

W_i = weight of Crucible

W_s = weight of Fresh sample

A similar method was followed for ash, where 1g powder sample of all the genotype were each placed into a crucible of known weight and the samples were then completely burned (to ashes) in a muffle furnace at 550-600 ºC for 4 hours. Then sample was then weighed and the weight was used to calculate ash. The following formula was used to calculate percentage ash:

$$\textbf{\textit{Ash (\%)}} = \frac{\mathrm{Wb} - \mathrm{Wi})}{\mathrm{Wf}} \times 100$$

Where; W_f = weight of dried sample

W_i = weight of Crucible

W_b = weight of crucible + ash

3.3.3.2 Procedure for extraction and analysis of crude fat

Filter bags were weighed then 2g of each ground sample was added into the labeled filter bag, replicated three times. The filter bags were then heat sealed then placed in an oven at 100ºC for 2 hours. The samples were then allowed to cool for an hour and then weighed. The samples were then placed into the fat extractor at 90ºC for 40minutes. And after extraction process was completed, the samples were cooled then placed in the oven at 100ºC for 30 minutes. The samples were cooled then weight. The % crude fat content of the sample was calculated as (AOAC, 1995):

$$\textbf{\textit{Crude fat (\%)}} = \frac{100\,(W2-W3\,)}{W1}$$

Where: W_1 = original weight of sample

W_2 = weight of pre-dried sample with the filter bag and pan

W_3 = weight of dried sample and filter bag after extraction

3.3.3.3 Procedure for extraction and analysis of total protein

The method used to determine crude protein was the Kjeldahl, (2016) Technique, where; 1.25 g of the dried ground sample of each genotype was placed onto lens tissue on an analytical balance replicated three times. The lens tissue was then wrapped up and placed in digestion tubes, with an extra 2 check samples (empty lens tissue). Then 20 ml of 72% sulfuric acid (H_2SO_4) mixed with selenium was added to each digestion tube and they were placed on a test-tube rack which was then put on a digestion block in a fume cupboard. The digestion block was switched on and temperatures were set as per (Kjeldahl, 2016) and then left for four hours. Thereafter, 4ml hydrogen peroxide (H_2O_2) was then added and the digestion block was switched back on with temperature set then was left for 2hours. The contents on the digestion tubes were then transferred into numbered 250 ml volumetric flasks. An aliquot of 25 ml from each solution was then distilled with sodium hydroxide over 4% boric acid and then titrating against 0.01N sulfuric acid using a Kjeldahl titration unit. The amount of nitrogen in the solution was calculated based on the amount of acid required to neutralise the nitrogen. From the nitrogen content, the crude protein content (dry matter basis) was then calculated. Nitrogen was calculated using the following formular (Kjeldahl, 2016);

$$\textbf{\textit{Nitrogen (\%)}} = \frac{[\text{ml acid x acid N} - (\text{ml base titrated x base N}) \text{ x } 0.014 \text{ x } 100]}{\text{Sample Weight (g)}}$$

Protein was then calculated using the following formular;

$$\textbf{\textit{Crude Protein (\%)}} = \text{Nitrogen (\%)} \times 6.25$$

3.3.3.4 Determination of total carbohydrates

Moisture, ash, protein was used to calculate carbohydrates. Carbohydrates were calculated using the following formula (Kjeldahl, 2016):

$$\textbf{\textit{Carbohydrate (\%)}} = 100 - (\text{Moisture} + \text{Ash} + \text{Fat} + \text{Protein})$$

3.3.3.5 Procedure for extraction and analysis of crude fiber

The method used for crude fiber was adopted from (Vereenigde, 2000), where; F57 filter bag were weighed and the weight was recorded and then 1.0 g of air-dried ground samples were placed into the filter bags each replicated three times. The bags were then sealed and then introduced to 500ml bottle filled with acetone, and the container was shaken for 10minutes. This process was repeated again with fresh acetone and then air dried. The bags were then placed in ANKOM fiber analyzer and 2000 ml of ambient temperature acid (0.255 N H_2SO_4) was added, and heat and agitation were turned on with timer set for 45 minutes. After 45 minutes agitation and heat were turned off and the acid drained, then approximately 2000 ml of hot (100ºC) rinse water was added and Agitation turned for 5mins. The water was drained and 2000 ml of ambient temperature base (0.313 N NaOH) solution was added to the ANKOM fiber analyzer vessel and Agitation and Heat was turned on with the timer set for 45 minutes. After 45minutes agitation and heat was turned off and the acid drained, then approximately 2000 ml of hot (100ºC) rinse water was added and agitation turned for 5mins. The bags were then soaked in acetone for 5 minutes then air dried. The bags were then completely oven dried at 100ºC, then weighed. After weighing the bags were then placed in crucibles then burned in a furnace for 2hour at 550ºC, then they were weighed after cooling. The following formula was used to calculate crude fiber (Vereenigde, 2000):

$$\textbf{\textit{Crude fiber (\%)}} = \frac{(W4 - (W1 \text{ x } C2)) \text{ x } 100}{W2 \times DM}$$

Where: W_1 = weight of bag

W_2 = sample weight

W_3 = weight after extraction process

W_4 = Weight of organic matter (OM) (Loss of weight on ignition of bag and fibre residue)

C_2 = Ash corrected blank bag (Loss of weight on ignition of bag/original blank bag)

3.3.3.6 Procedure for extraction and analysis of content of tannins

Finely ground weighed 200 mg genotype samples were placed in a container with 50% aqueous methanol in conical centrifuge tubes. The samples were then placed in a shaking water bath for 2hours at 30ºC. Then the samples were centrifuged for 20mins at 4ºC at 3000rpm. 1ml of supernatant from each sample were then placed in 100×12mm tubes, and then 6.0 ml of the butanol - HCl reagent and 0.2 ml of the ferric reagent were added to each test tube. The tubes were covered and placed in a heating block at 100ºC for 1hour. The samples were then allowed to cool and absorbance was measured using a visible spectrophotometer. The formula used to calculate tannins is as follows (AOAC, 1995):

$$\textbf{\textit{Tannins (CEmg/g)}} = \frac{\text{A550nm} \times 78.26 \times \text{Dilution factor}}{\text{\% Dry Matter)}}$$

3.3.3.7 Analyzing mineral content (Sodium, Potassium, Calcium and Magnesium)

Method used to obtain digest content to use for analyzing sodium, potassium, calcium and magnesium was adopted from (Kjeldahl, 2016), as per the crude protein analyzing digestion process. A Corning Flame Photometer 410 was used to analyze the digest liquid content (AOAC, 1995).

For Potassium (K) the standards used were 1000 ppm K obtained by dissolving 1.907g dried potassium chloride (KCL) in 1000 ml 2.4N H_2S0_4. In order to obtain 200ppm K, 100ml of the 1000 ppm K standard was dilute to 500ml with 2.4N H2S04. Series dilution of 0, 2, 4, 6, 10 and 15 ml of the 200 ppm K was then diluted into 100ml volumetric flasks. All samples and standards 1/10 of their original concentration were diluted using distilled water and emission on a flame photometer was measured and recorded.

For Sodium (Na) the standards used were 1000 ppm Na, and they were obtained by dissolving 2.542g dried (1400C) NaCl in 1000ml 2.4N H_2S0_4. To obtain 20 ppm Na, 2ml of the 1000 ppm Na standard was diluted to 100ml and 2.4 N H2S04 was used to make to mark. Series dilution of 0, 2, 4, 6, 10, and 15ml of the 20ppm Na was diluted into 100ml volumetric flasks and made to mark using 2.4 N H_2S0_4. Emission on a flame photometer was then measured. The same processes were carried out in analyzing calcium and magnesium, with only the difference of dissolving of Calcium chloride for calcium and magnesium chloride for magnesium in 1000ml 2.4N H_2S0_4.

3.4 Screening of efficacy of botanical powders experiment

3.4.1 Preparation of botanical powders

The botanical plant extracts that were used in this experiment as treatments were from marigold (stem and leaf powder), garlic (bulb powder) peppermint (leaf powder), Fever Tea (stem and leaf powder) (Table 4). The botanical plants which included garlic and peppermint were bought from the local markets, while marigold was collected from a local garden and fever tea was collected from one of the local farms. Preparation of extracts was done by thoroughly rinsing the plant parts in distilled water three times to remove dirt, and then the plant parts were dried at room temperature in the Entomology laboratory for 7 days. The plant materials were then oven dried at $60 \pm 1^{\circ}C$ for 48 hours and each were preserved in their own clear airtight bottle which was then placed in a refrigerator ($4 \pm 2^{\circ}C$).

3.4.2 Experimental set up

The seeds of different bean genotypes were used to test the efficacy of the botanical plants in controlling *C. maculatus*. This was a split plot design laid out in a Complete Randomized Design with three replicates (Table 4), with plant powders (marigold, fever tea, garlic and peppermint) as the main plot and genotype (CAL96, DAB520, X-variety and black cowpeas landrace (control)) as the subplot. In the feeding jars, the botanical powders were used to treat each of the seeds. The method for infestation was identical to that described in 4.3.2 above.

About 50 g of seeds for each of the genotypes was placed into rearing jars. Four (4) grams of each botanical plant powder was then introduced into rearing jars of the seeds. To evenly cover each seed's surface with the protective powder, the plant powders of each treatment material were introduced to the seeds in the rearing jars. The seeds were then combined and shook for three minutes. Then just like in the test for host resistance experiment, all the feeding jars were then artificially infested with ten pairs of *C. maculatus* adults of 1 to 2 days old which were collected from seed beetle culturing jar. And all the feeding jars were enclosed with a muslin cloth so that *C. maculatus* would not escape and were left in the Entomology laboratory to mate. For the control treatment, no botanical plant powder was added and was replicated three times also.

Table 3. Botanical plants used as treatments

Treatments	Common Name	Scientific name	plant parts used
1	Garlic	*Allium sativum L*	Bulb
2	Peppermint	*Mentha piperita L.*	Leaf
3	Marigold	*Tagetes minuta*	Leaf and stem
4	Fever tea	*Lippia javanica*	Leaf and stem
5	**Untreated genotype (control)**		

Table 4. A split plot design laid out in a Complete Randomized Design with three replicates

Treatment	Factor A	Factor B	Replications
1	CAL 96	Control	3
2		Mint	3
3		Garlic	3
4		Marigold	3
5		Fever Tea	3
6	DAB 520	Control	3
7		Mint	3
8		Garlic	3
9		Marigold	3
10		Fever Tea	3
11	X- variety	Control	3
12		Mint	3
13		Garlic	3
14		Marigold	3
15		Fever Tea	3
16	Black eye	Control	3
17		Mint	3
18		Garlic	3
19		Marigold	3
20		Fever Tea	3

The *C. maculatus* were then allowed to oviposit on the beans for ten days before they were removed and discarded, and there after the data collected was similar to the one done in experiment for testing seeds for host resistant to *C. maculatus* i.e.; number of eggs laid; number of hatched eggs; total number of adult emergencies; number of dead beetles; initial weight of bean sample; and final bean weight. The experiment was terminated after 50days where final measurements were taken. The data collected was also used to determine the seed beetle adulty emergence percentage; seed beetle mortality percentage; bean weight loss percentage; and Dobie Susceptible Index (DSI). Both the collection of data and the calculations used in this experiment was the same as those used in the screening genotypes for resistance experiment.

3.5 Data Collection

3.5.1 Fecundity (Oviposition) and number of hatched eggs

The number of eggs deposited was recorded immediately after adult *C. maculatus* have been removed from the bean samples and a magnifying glass and microscope were used to count the eggs sticking on the surface of the beans. In addition, once the adult *C. maculatus* have stopped emerging the number of hatched eggs and unhatched were counted under the magnifying glass and microscope and these were recorded.

3.5.2 Adult emergency and mortality

Every day, all the adults that have emerged from the seeds were counted. Both dead and living emerged adults were recorded. After recording the beetles, they were then discarded. This continued every day for two months. The beetles observed and recorded on the bean samples were further differentiated into two categories, that is, those found dead and those found alive. Percent Mortality of *C. Maculatus* was calculated as follows;

$$\text{Percentage mortality (\%)} = \frac{NDB}{TNEB} \times 100$$

Where;
NDB = Number of dead beetles

TNEB = Total number of emerged beetles

Assumption: beetles which failed to exit the beans were regarded as dead

3.5.3 Bean weight

The weight of seeds of each genotype in the rearing jar was recorded at the beginning of the experiment, before they were infested with *C. maculatus* and was recorded as the initial bean weight. Fourteen days after emergence of beetles the adult beetles were discarded, the same seeds of each genotype in the rearing jar were weighed and this was recorded as the final bean weight

The economic loss indicator or weight loss will be calculated as follows;

$$\text{Bean weight loss (\%)} = \frac{(IGW - FGW)}{IGW\ of\ sample} \times 100$$

where;

FGW = final grain weight

IGW = initial grain weight for the sample.

3.5.4 Dobie susceptibility index (DSI)

Bean susceptibility to *C. maculatus* was calculated by using the Dobie susceptibility Index (Dobie, 1974), this makes use of the overall number of adult seed beetle that emerged and the median development period for each bean sample/feeding jar. The number of days from the first day of oviposition to the first day of adult emergence was used to determine the median development period. Bean samples with high DSI are documented to be susceptible while those with low DIS are resistant to *C. maculatus.*

The formula for calculating DSI is as follow (Dobie, 1974; Siwale *et al*., 2009);

$$\text{DSI} = \frac{Loge\,Y}{t} \times 100$$

Where;

Y = total number of adult seed beetles emerged

t = median development period

The scale that was used to determine the DSI (Dobie, 1974; Siwale *et al*., 2009) is:

0–4.0 = resistant, 4.1–6.0 = moderately resistant, 6.1–8.0 = moderately susceptible, 8.1–10.0 = susceptible and ≥ 10.1 = highly susceptible

3.6 Effect of botanical powders on germination of beans

3.6.1 Experiment setup

Germination test was carried out on beans which had been treated with the 4 plants (pepper mint, fever tea, marigold and garlic) powder and left in storage for a month. The Germination test was carried out as per the standard germination test for seed viability. The germination of *P. vulgaris* that has been mixed with the plant extract was assessed. Upon termination of experiment 2 (4.4) the seeds were then taken for germination test. Ten seeds of each bean variety were randomly collected then they were soaked in water for 24hours, and then placed in Petri dishes over a moist germination paper in a split plot design arranged in a Complete Randomized Design with three replicates. The petri dishes were then kept in a dark place in the Entomology laboratory under ambient conditions, and number of emerged beans was taken at the end of seven days. The germination percentages were then calculated according to Ogendo *et al.* (2003) as follows;

$$\text{Germination percentage (\%)} = \frac{NSG}{TNST} \times 100$$

Where;

NSG = number of seeds germinated

TNST = total number of seeds tested in the petri dish

3.7 Data Analysis

The Data collected on various parameters from the experiments were entered into Microsoft excel and the statistical analysis was performed using the SAS software package. The data was subjected to analysis of variance (ANOVA) procedure and the means were separated using the Least Significant Difference (LSD). The level of significance that was used for the F test was P = 0.05, and the critical difference values were calculated for treatment comparisons. The arcsine square root was used to convert the % values of adult emergency, adult mortality, seed weight loss, DSI, and germination in order to stabilize the variance [arcsine √(percent x/100] (Bromiley & Thacker, 2002) and then they were subjected to analysis. Pearson correlation was performed on the relationship between biochemical components and pest susceptibility index.

CHAPTER FOUR

4.1 RESULTS

4.1.1 Screening Genotypes for Resistance Experiment

Table 5 shows the evaluation of genotype resistance against *Callosobruchus maculatus*. The genotypes were compared based on the number of beetle eggs laid, percentage beetle adult emergence and percentage adult mortality. It was established that there was significant difference ($F_{3,24} = 7.97$, $P = 0.0007$) in the number of eggs laid among the genotypes tested. Interestingly, the bean genotypes were not significantly different in the number of eggs laid, however, they were all significantly different from the black eye cowpea genotype (Table 5).

Table 5: Genotype Comparison for Resistance Against *Callosobruchus maculatus*

Genotype	Number Of Eggs Laid	Beetle Adult Emergence (%)	Adult Mortality (%)
CAL96	$52.88^{b} \pm 1.31$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
Black eye	$150.88^{a} \pm 0.85$	$47.90^{a} \pm 0.72$	$8.41^{a} \pm 0.28$
DAB520	$66.50^{b} \pm 3.14$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
X-variety	$75.75^{b} \pm 2.47$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
F	7.97	8	7.98
P	0.0007	0.0007	0.0007
LSD	45.43	24.72	4.34

Means within the same column followed by the same small letter do not differ significantly, ($P<0.05$), LSD Test. NB: mean results for all genotypes which were not infested with any C. maculatus are not included in the table as they were all observed to be zero (0).

The fecundity as a measure of number of eggs laid by females, ranged from 52.88 to 75.75eggs. Though there was no significant difference observed on the bean genotypes, it is worth noting that the minimum number of eggs were laid on CAL96 (52.88 ± 1.31), followed by DAB520 (66.50 ± 3.14) and then X-variety (75.75± 2.47) while the maximum number of eggs laid was recorded on

Black eye (150.88 ± 0.85). Similarly, when the genotypes were tested for beetle adult emergence, it was discovered that they were also significantly different in the percentage beetle adult emergence. The bean genotypes, CAL96, DAB520 and X-genotype, were observed not to be significantly different ($F_{3,24} = 8$, $P = 0.0007$) from each other in percentage beetle adult emergence but these bean genotypes were all found to significantly differ from the black eye cowpea genotype. In all the bean genotypes, there was no adult beetle emergence recorded. Comparatively, the black eye cowpea genotype had the highest percentage of adult beetle emergence (47.90 ± 0.72). Comparison of percentage adult mortality was also done among the four genotypes. The findings of the study revealed that the percentage adult mortality among the genotypes was significantly different ($F_{3,24} = 6.98$, $P = 0.0007$). However, there was no significant difference observed in percentage adult mortality among the bean genotypes, CAL96, DAB520 and X-genotype, but these bean genotypes were all found to significantly differ from the black eye cowpea genotype. All the bean genotypes did not show any adult mortality while the black eye genotype had the highest percentage adult mortality (8.41 ± 0.28).

Table 6 presents the analysis of variance for the DSI and seed weight loss as measure of genotype resistance. It was established that there was significant difference in DSI ($F_{(3,24)} = 8, P = 0.0007$) among the genotypes in the study. There was no significant difference observed for DSI in bean genotypes CAL96, DAB520 and X-variety, however these were all significantly different from black eye genotype. The individual means for DSI ranged from 0 to 4.15, and it was noted that minimum DSI was recorded in CAL96, DAB520 and X-variety (0 ± 0) and maximum was recorded in black eye (4.15 ± 0.07). It was also established that there was significant difference in seed weight loss ($F_{(3,24)} = 6.42, P = 0.0024$). No significant difference was indicated between the genotypes CAL96, DAB520 and X-variety, however they were established to be all significantly different to black eye genotype. The individual means for seed weight loss varied from 1 to 9. The bean genotype with minimum weight loss was observed to be DAB520 (5.74 ± 1.00), followed by CAL96 (7.04 ± 1.08) and X-variety (9.10 ± 1.08). Black eye was observed to have the highest seed weight loss (17.46 ± 1.15).

Table 6 Means of parameters (DSI and Seed weight loss) for Genotypes resistance to *C. maculatus*

Genotype	Seed Weight Loss (%)	DSI (%)
CAL96	7.04^{b} ± 1.08	0.00^{b} ± 0.00
Black eye	17.46^{a} ± 1.15	4.15^{a} ± 0.07
DAB520	5.74^{b} ± 1.00	0.00^{b} ± 0.00
X-variety	9.10^{b} ± 1.08	0.00^{b} ± 0.00
F	8	6.42
P	0.0007	0.0024
LSD	4.29	3.11

Means within the same column followed by the same small letter do not differ significantly, (P<0.05), LSD Test. *NB: mean results for all genotypes which were not infested with any C. maculatus are not included in the table as they were all observed to be zero (0).*

4.1.2 Correlation between susceptibility parameters and biochemical properties

Table 7 depicts the correlation of bean susceptibility parameters to biochemicals characteristics which are percentage moisture, percentage ash, percentage crude fat, percentage protein, percentage carbohydrates, percentage crude fiber, tannins, sodium, potassium, magnesium and calcium. Table 7 and 8 presents biochemical contents of all the genotype of this study that were used to investigate their relationship with pest susceptible index. The correlation coefficient revealed significant and highly positive correlation between the DSI and the sodium content (r=0.97, P≤ 0.0289), and magnesium (r= 0.98, P≤ 0.0165). However a significant and highly negative correlation was observed between DSI and ash (r= -0.97, P≤ 0.0334), and potassium (r= -0.74, P≤ 0.0289). Non-significant and highly positive correlation was observed between DSI and percentage protein (r= 0.76, P≤ 0.2365). Non-significant and low correlation was observed between DSI and percentage moisture (r= 0.41, P≤ 0.5888). DSI indicated a non-significant high negative correlation with percentage crude fat (r= -0.97, P≤ 0.3249), percentage crude fiber (r= -0.83, P≤ 0.165) and tannins (r= -0.76, P≤ 0.2363).

Table 7 Estimates of seed genotype biochemicals content

Genotype	Moisture (%)	Ash (%)	Crude Fat (%)	Protein (%)	Carbohydrates (%)	Crude Fibre (%)	Tannins (CEmg/g)
CAL 96	0.80 ± 0.12	3.35 ± 0.13	2.17 ± 0.44	15.60 ± 0.85	78.07 ± 0.40	4.52 ± 0.18	1.90 ± 0.04
Black eye	1.53 ± 0.37	2.98 ± 0.08	0.67 ± 0.33	19.60 ± 1.10	75.22 ± 1.85	1.84 ± 0.13	1.33 ± 0.13
DAB 520	0.47 ± 0.07	3.28 ± 0.07	1.00 ± 0.00	18.20 ± 0.21	77.05 ± 0.14	3.52 ± 0.30	1.84 ± 0.08
X genotype	1.80 ± 0.31	3.25 ± 0.02	2.67 ± 1.20	17.33 ± 1.10	74.95 ± 1.13	3.05 ± 0.19	1.50 ± 0.08

Table 8 Estimates of seed genotype mineral biochemical content

Genotype	Potassium (mg/g)	Sodium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)
CAL 96	1.24 ± 0.03	0.10 ± 0.01	1.41 ± 0.03	1.53 ± 0.04
Black eye	1.10 ± 0.01	0.13 ± 0.01	0.84 ± 0.03	1.81 ± 0.05
DAB 520	1.24 ± 0.00	0.10 ± 0.00	0.82 ± 0.05	1.51 ± 0.01
X genotype	1.14 ± 0.01	0.10 ± 0.00	0.60 ± 0.02	1.46 ± 0.01

The correlation coefficient as reflected in table 9 also revealed that there was a significant and strong correlation between number of eggs laid and sodium (r= 1, P≤ 0.0046), and a significant and negative strong correlation between number of eggs laid and ash (r=-1, P≤ 0.0016). Non-significant and positive strong correlation was observed between number of eggs laid and the moisture (r= 0.53, P≤ 0.4736), protein (r= 0.85, P≤ 0.1529) and magnesium (r= 0.92, P≤ 0.0751). A non-significant and negative correlation was also recorded between crude fat (r= -0.63, P≤

0.3699), carbohydrates (r= -0.66, P≤ 0.3428), crude fiber (r= -0.93, P≤ 0.0672), tannins (r= -0.87, P≤ 0.1325), potassium (r= -0.84, P≤0.1611) and calcium (r= -0.36, P≤ 0.6450). A significant and positive correlation was found between beetle adult emergence and sodium (r= 0.97, P≤ 0.0289) and magnesium (r= 0.98, P≤ 0.0165). Significant and negative correlation was observed between beetle adult emergence and ash (r= -0.97, P≤ 0.0334). Correlation coefficient also revealed a non-significant and positive correlation between beetle adult emergence and moisture (r=0.41 , P≤ 0.5888) , beetle adult emergence and protein (r=0.76 , P≤0.2365), while a non-significant and negative correlation was revealed between beetle adult emergence and fat (r=-0.68 , P≤0.3250), beetle adult emergence and carbohydrates (r=-0.49 , P≤0.5089), beetle adult emergence and crude fiber (r=-0.83 , P≤0.1650), beetle adult emergence and tannins (r=-0.76 , P≤0.2363), beetle adult emergence and potassium (r=-0.74 , P≤0.2586), beetle adult emergence and calcium (r=-0.15 , P≤0.8506). There was a significant and strong positive correlation between adult mortality and sodium (r=0.97, P≤0.0289), adult mortality and magnesium (r=0.98, P≤0.0165), while there was significant and negative correlation between adult mortality and ash (r=-0.97, P≤0.0334). Non-significant and positive correlation between adult mortality and moisture (r=0.56 , P≤0.4424), adult mortality protein (r=0.76 , P≤0.2365), while there was non-significant and negative correlation between adult mortality and crude fat (r=-0.68 , P≤0.2365), adult mortality and carbohydrates (r=-0.49 , P≤0.5089), adult mortality and crude fiber (r=-0.83 , P≤0.1650), adult mortality and tannins (r=-0.76 , P≤0.2363), adult mortality and potassium (r= -0.74 , P≤0.2568) adult mortality and calcium (r= -0.15 , P≤0.8506). A significant and strong positive correlation was observed between seed weight loss and sodium (r= 0.96, P≤ 0.0351), while a significant and strong negative correlation was observed between seed weight loss and ash (r= -0.97, P≤ 0.0284). Non-significant and positive correlation was revealed between seed weight loss and moisture (r=0.56 P≤ 0.4424), seed weight loss and protein (r=0.74, P≤ 0.2628), seed weight loss and magnesium (r=0.94, P≤ 0.0559), while a non-significant and negative correlation was observed between seed weight loss and crude fat (r=-0.55, P≤ 0.4476), carbohydrates (r=-0.60, P≤ 0.4004), crude fiber (r=-0.87, P≤ 0.1319), tannins (r=-0.85, P≤ 0.1508), potassium (r= -0.84, P≤ 0.1604), calcium (r= -0.22, P≤ 0.7797).

Table 9 Pearson correlations for biochemical and susceptibility parameters of the bean genotypes

	Resistance Parameters				
Biochemical Parameters	DSI	NEL	BAE	AM	SWL
Moisture (%)	0.41	0.53	0.41	0.41	0.56
Ash (%)	-0.97	-1.00	-0.97	-0.97	-0.97
Crude Fat (%)	-0.68	-0.63	-0.68	-0.68	-0.55
Protein (%)	0.76	0.85	0.76	0.76	0.74
Carbohydrates (%)	-0.49	-0.66	-0.49	-0.49	-0.60
Crude Fiber (%)	-0.83	-0.93	-0.83	-0.83	-0.87
Tannins (CEmg/g)	-0.76	-0.87	-0.76	-0.76	-0.85
Potassium (mg/g)	-0.74	-0.84	-0.74	-0.74	-0.84
Sodium (mg/g)	0.97	1.00	0.97	0.97	0.96
Calcium (mg/g)	-0.15	-0.36	-0.15	-0.15	-0.22
Magnesium (mg/g)	0.98	0.92	0.98	0.98	0.94

DSI= Percentage of Dobie Susceptible Index; NEL= Number of Eggs Laid; BAE= Percentage of Seed Beetle Adult Emergence; AM= Percentage of Adult Mortality; SWL= Percentage of Seed Weight Loss

4.1.3 Efficacy of botanical powders experiment

A two-way analysis of variance was done to examine the effect of genotype and seed treatment on total number of *C. maculatus* eggs laid, beetle adult emergence and adult mortality, and the results are presented in Table 10.

The study results revealed significant difference in number of eggs laid for genotypes at ($F_{(3,40)}$=95.99, P < 0.0001).The average number of eggs laid for genotypes ranged between 27.60 ± 0.62 to 47.27 ± 1.75. Blackeye showed the highest average number of eggs laid (47.27 ± 1.75). However the lowest average number of eggs were laid on genotype CAL96 (27.60 ± 0.62), followed by DAB520 (31.87 ± 1.43), the X-genotype (33.13 ± 1.61. The mean range for the number of eggs laid for genotypes was 27.60 to 47.27. Mean number eggs laid for genotype CAL96 was the lowest with mean of 27.60, followed by DAB520 with 31.87, then X-genotype with mean of 33.13, and Black eye with the highest mean of 47.27.

Table 10 *C. maculatus* Eggs Number, Adult Emergence and Adult Mortality Percentage in bean genotype treated with different plant extracts

		Number Of Eggs Laid	Beetle Adult Emergence (%)	Adult Mortality (%)
Genotypes	CAL96	27.60^{c} ± 0.62	0.00^{b} ± 0.00	0.00^{b} ± 0.00
	Black eye	47.27^{a} ± 1.75	11.03^{a} ± 1.67	4.12^{a} ± 1.53
	DAB520	31.87^{b} ± 1.43	0.00^{b} ± 0.00	0.00^{b} ± 0.00
	X-genotype	33.13^{b} ± 1.61	0.00^{b} ± 0.00	0.00^{b} ± 0.00
	F	95.99	120.21	8.62
	P	<.0001	< 0.0001	0.0002
	LSD	2.49	1.44	2.01
Treatments	Garlic	7.83^{d} ± 0.63	0.87^{bc} ± 0.34	0.00^{b} ± 0.00
	Mint	92.67^{a} ± 1.96	0.26^{c} ± 0.003	0.25^{ab} ± 0.001
	Fever tea	10.83^{cd} ± 0.66	0.73^{bc} ± 0.56	0.00^{b} ± 0.00
	Marigold	12.83^{c} ± 0.82	2.96^{b} ± 0.65	1.67^{ab} ± 1.29
	Control	50.67^{b} ± 1.97	8.97^{a} ± 0.30	3.23^{a} ± 0.42
	F	1419.00	41.54	3.25
	P	< 0.0001	< 0.0001	0.0213
	LSD	2.784	1.61	2.24

Means within the same column followed by the same small letter do not differ significantly, (P<0.05)

The results of the study in table 10 also showed that the treatments were significantly different in the average number of eggs laid (($F_{(4,40)}$=1419, P < 0.0001). The average number of eggs laid for the treatments ranged between 7.83 ± 0.63 to 92.67 ± 1.96.. Samples treated with Garlic were observed to have significant highest mean number of eggs, followed by fever tea with mean of 10.83 ± 0.66, marigold with a mean of 12.83 ± 0.82, control with mean of 50.67 ± 1.97, and then mint having significantly the highest mean with 92.67 ± 1.96. These findings showed that fever tea and marigold treatments were no significantly different in eggs laid (Table 10). Similarly, garlic and fever tea did not differ in eggs laid, as they displayed lowest number of eggs laid.

The findings of this study also showed that the percentage adult beetle emergence was significantly different among the genotypes($F_{(3,40)}$=120.21, P < 0.0001). There was no significant difference in mean number of adult emergences for bean genotypes CAL96, DAB520 and X-genotype. Each of these genotypes had no adult emergence observed (0.00 ± 0.00). However, the average number of adult emergences for blackeye genotype was significantly different from that of the three bean genotypes (CAL96, DAB520 and X-genotype). The average number of adult emergences for the four genotypes ranged between 0.00 ± 0.00 to 11.03 ± 1.67. Similarly, the treatments were found to be significantly different in percentage adult emergence($F_{(4,40)}$=41.54, P < 0.0001).. The mean number of adult emergences for treatments garlic, fever tea and marigold were not significantly different. However, these three treatments were found to be significantly different to the control treatment. Garlic and Fever tea did not significantly differ from mint in percentage adult emergence. The average adult emergence percentage ranged from 0.26 ± 0.003 (mint) to 8.97 ± 0. 30 (control), The second lowest mean number of adult emergences percentage was for fever tea treatment with mean of 0.73 ± 0.56, followed by that of garlic treatment with 0.87 ± 0.34, and then marigold treatment with mean of 2.96 ± 0.65.

Table 10 also depicts comparisons of genotypes by percentage adult mortality. There was significant difference in percentage adult mortality among the genotypes ($F_{(3,40)}$=8.62, P = 0.0002). The study findings revealed that there was no significant difference in mean percentage of adult mortality for genotypes CAL96, DAB520 and X-genotype. Whilst the mean percentage adult mortality for control was significantly different from that of bean genotypes CAL96, DAB520 and X-genotype. The percentage of adult mortalities ranged between 0.00 ± 0.00 (CAL96, DAB520 and X-genotype) to 4.12 ± 1.53 (blackeye). The study also investigated the comparisons of treatment on percentage adult mortality. The percentage adult mortalities for treatments mint, marigold and control were not significantly different from each other. In addition, garlic, mint, fever tea and marigold were not significantly different in percentage adult mortality (Table 10). However, control was significantly different in percentage adult mortality to garlic and fever tea.

The percentage adult mortalities ranged from 0 ± 0.00 (garlic and fever tea) to 3.23 ± 0.42 (control. The second lowest mean number of adult mortalities was for mint treatment with mean percentage of 0.25 ± 0.001, and then followed by that of marigold treatment with 1.67 ± 1.29.

Table 11 DSI Values (%) and Percentage Seed Weight Loss of different genotypes treated with different plant powder.

		Seed Weight Loss (%)	DSI (%)
Genotypes	CAL96	0.13^{b} ± 0.12	0.00^{b} ± 0.00
	Black eye	1.07^{a} ± 0.12	1.45^{a} ± 0.15
	DAB520	0.00^{b} ± 0.00	0.00^{b} ± 0.00
	X-genotype	0.40^{b} ± 0.2	0.00^{b} ± 0.00
	F	10.13	163.53
	P	< 0.0001	< 0.0001
	LSD	0.426	0.162
Treatments	Garlic	0.00^{b} ± 0.00	0.00^{c} ± 0.00
	Peppermint	0.00^{b} ± 0.00	0.00^{c} ± 0.00
	Fever tea	0.00^{b} ± 0.00	0.09^{c} ± 0.07
	Marigold	0.67^{ab} ± 0.13	0.46^{b} ± 0.08
	Control	1.33^{a} ± 0.40	1.26^{a} ± 0.01
	F	12.80	71.46
	P	< 0.0001	< 0.0001
	LSD	0.476	0.182

Means within the same column followed by the same small letter do not differ significantly, (P<0.05)

Table 11 illustrates the DSI values (%) and percentage seed weight loss of different genotypes treated with different plant powders. There was a significant difference in the percentage seed weight loss among the genotype compared in the study ($F_{(3,40)}$=10.13, P < 0.0001). The genotype CAL96, DAB520 and X-genotype were observed not to be significantly different in percentage seed weight loss. However, these three genotypes were observed to be significantly different in seed weight loss percentage from control (blackeye cowpea). The lowest recorded percentage weight loss was 0.00 ± 0.00 (CAL96, DAB520 and X-genotype) and higher percentage seed weight loss was 1.07 ± 0.15 (blackeye cowpea). The effect of treatment of percentage seed weight loss was also investigated (Table 11). It has been revealed from this study that treatments significantly differed in percentage seed weight loss ($F_{(4,40)}$=12.80, P < 0.0001). Treatment with garlic, peppermint and fever tea did not cause any percentage seed weight loss (0.00 ± 0.00These three treatments did not differ significantly from one another. In addition, they were significantly different in causing seed weight loss to marigold (0.67 ± 0.13). However, garlic, peppermint and fever tea had significantly no effect on seed weight loss compared to control (1.33 ± 0.40).

The percentage DSI values from different genotypes and treatments were investigate (Table 11). This study depicts that the percentage DSI values for different genotypes were significantly different ($F_{(3,40)}$=163.53, P < 0.0001). . The DSI values for genotypes CAL96, DAB520 and X-genotypes were not significantly different from each other. However, they were significantly different from that of the control (Blackeye). The value of DSI percentage for genotypes ranged from lowest at 0.00 ± 0.00 (CAL96, DAB520 and X-genotype) to highest at 1.45 ± 0.15 (Blackeye). The study has also shown that percentage DSI values for different treatments were also significantly different ($F_{(4,40)}$=71.46, P < 0.0001). Garlic, peppermint and fever tea treatment were not significantly different in DSI value. However, there were different to both marigold and control (Table 11). Marigold treatment values of DSI were significantly different from that of the control treatment. The DSI values for treatments ranged from 0.00 ± 0.00 (garlic and peppermint) to 1.26 ± 0.01 (control). Garlic and peppermint treatments had the lowest DSI values, followed by fever tea (0.9 ± 0.07), then marigold (0.46 ± 0.08), and control seeds with the highest DSI mean (1.26 ± 0.01).

4.1.4 Germination Test Experiment

Table 12 illustrates the germination percentage of different genotypes treated with different plant extracts. The experimental findings revealed that there was significant difference among the seed germination in terms of germination percentage ($F_{(3,40)}$=95.99, $P < 0.0001$). Blackeye and X-genotype had significantly highest germination percentage compared to other genotypes, and they were not significantly different from each other. However, X-genotypes was significantly different in terms of percentage germination, from both CAL96 and DAB520. Germination percentage of CAL96 and Blackeye were not significantly different from each other, but they were significantly different from that of DAB520. More so, DAB520 and X-genotype significantly differed in germination percentage. The individual means of germination for genotypes ranged from 81.33 ± 0.29 (DAB520) to 98.00 ± 0.17 (X-genotype). Mean of germination for genotype CAL96 was the second lowest with 92.00 ± 0.23 followed by that of blackeye cowpeas with 96.67 ± 0.06. the study also showed that there was significant difference in germination percentage among the treatments ($F_{(4,40)}$=37.32, $P < 0.0001$). There was no significant difference was observed among treatments garlic (100.00 ± 0.00), fever tea (99.17 ± 0.06) and marigold (97.50 ± 0.19). In terms of germination percentage these treatments had the highest germination percentage. The three treatments showed high germination compared to mint (77.50 ± 0.32) and control (85.82 ± 0.29). However, mint and control had significantly different germination percentages (Table 12).

Table 12 Germination percentage of genotype treated with different plant extracts.

		Germination (%)
Genotypes	CAL96	$92.00^{b} \pm 0.23$
	Black eye	$96.67^{ab} \pm 0.06$
	DAB520	$81.33^{c} \pm 0.29$
	X-genotype	$98.00^{a} \pm 0.17$
	F	95.99
	P	< 0.0001
	LSD	0.415
Treatments	Garlic	$100.00^{a} \pm 0.00$
	Mint	$77.50^{c} \pm 0.32$
	Fever tea	$99.17^{a} \pm 0.06$
	Marigold	$97.50^{a} \pm 0.19$
	Control	$85.83^{b} \pm 0.29$
	F	37.32
	P	< 0.0001
	LSD	0.464

Means within the same column followed by the same small letter do not differ significantly, ($P<0.05$)

4.2 DISCUSSION

4.2.1 Resistance of bean genotypes to *C. maculatus*

The study has revealed that the beans genotypes tested were reasonably resistant to *C. maculatus* infestation. However, there were differences in the number of eggs laid on seeds by seed beetles, seed beetle adults' emergence, adult mortality, seed weight loss and also DSI values among the bean genotypes and also between these bean genotypes and the blackeye cowpeas genotype (control) implying variations in response to *C. maculatus* resistance. The significant differences with respect to oviposition among the genotypes are indicative of the existence of variability in the evaluated genotypes and the insects' preference of some genotypes for oviposition. The seeds used in the experiment, that is CAL96, DAB520, X-genotype and black eye cowpea, supported *C. maculatus* oviposition at varying levels. However, it is evident from the findings of this study that *C. maculatus* had high preference for egg oviposition on the black eye cowpea genotype than all the three bean genotypes. The control (blackeye cowpea genotype) was more vulnerable to *C. maculatus* oviposition compared to the bean genotypes seeds. These results are closely similar to that of Swella and Mushobozy (2009) who reported blackeye cowpeas to be more susceptible to *C. maculatus* infestation and oviposition as compared to common beans. Variations in oviposition on varieties of common bean and cowpea by seed beetle have been reported before (Girsil, 2017). The rate of oviposition on control was more than on bean genotypes and this may be associated with the preferential mode of *C. maculatus* species for egg oviposition. Tengey et al. (2022) reported that several authors have shown significant variations among different legume genotypes which are artificially infested with *C. maculatus* and this reinforces the point that different genotypes respond differently to *C. maculatus* infestation. Interestingly, the three bean genotypes (CAL96, DAB520 and X-genotype) showed significant variations in terms of *C. maculatus* egg oviposition preference. Low egg load was obtained on bean genotype CAL96, followed by genotype DAB520 and lastly the X genotype, which varied in seed coat color. This was probably due to the presence of oviposition deterrent biochemical factors (Cope & Fox, 2003; Sharma & Thakur, 2014), which are the main factors of antixenosis. The findings are closely similar to the study by Sisay et al. (2021), in which different genotypes showed differences in the number eggs oviposited by seed beetle. Although the surface area, seed coat color and seed coat texture were not determined in this study, they may be the main contributors to the variability in the number of eggs deposited on the seeds as Adebayo et al. (2016) suggested that the variability in the oviposition rate on the different hosts was associated with the surface area of the seeds. Further work on the mechanisms involved for egg oviposition is needed. Sisay et al. (2021), also conveyed that seed beetle females may use various chemical and physical signals like multiple sensory modalities, egg-marking pheromone, and larval feeding vibrations from the seed to select suitable number to lay on seeds which would be allowing only the emergence of larvae with good fitness. All the three bean genotypes (CAL96, DAB520 and X-genotype) recorded had no beetle emergence. This according to Togola et al. (2017) is a property of antibiosis, where compounds, such as 7S vicilins, α-amylase inhibitor, E-64 cysteine protease inhibitors, retard the development of the insects, and delayed emergence. These genotypes probably contained these compounds.

It has been observed from this study that an average of 48% of beetle adults emerged out of eggs laid on the control (blackeye cowpea) while the bean genotypes CAL96, DAB520 and X-genotype, did not record beetle adult emergence. This could suggest possibility of host resistance mechanisms of antibiosis on adult emergence that might be existent in the bean genotypes CAL96, DAB520 and X-genotype. Similar report was made in a study by Nhamucho et al. (2017), in which there was high progeny emergence in susceptible grain genotypes compared to the other resistant genotypes. They suggested antibiosis as the mechanism at play. Girsil (2017), it was also said that the number of eggs placed is not a useful measure of seed beetle resistance since a high number of beetle eggs oviposited on grain may not always suggest that there would be a proportionally high number of adult emergences. In another study by Ponnusamy et al. (2014), correlation coefficients were worked out among different biological parameters of *C. maculatus* which included egg laying, developmental period and adult emergence. It was observed that the egg laying did not show significant correlation with any other parameters. The study further described that the reduction in percent adult emergence is an indication of the presence of unfavorable chemical constituents inside the cotyledons of the beans. In our study the emerged seed beetles in the control (Blackeye cowpea genotype) were found moving around the seeds within the bottles, while other ones were lifeless and not moving. The eggs that did not hatch were still attached to the seed coat in all the treatments. The seeds of the control, which recorded higher adult emergence were observed to be damaged as they had holes, while those from the other seeds CAL96, DAB520 and X-genotype were not damaged. Sarwar (2012), reported that larva which hatched from eggs laid on susceptible grain tunnels into the seed leaving a round hole which was usually 1-2 mm. This was the hole that the adult *C. maculatus* emerged through after pupation. Though the control had significantly high adult emergence, an average of 16.8% of mortality for adults was observed. Some of the dead adults were found outside the seeds while others were found within the seed holes. There was also an unanticipated mortality of early-exiting larvae as observed in control seeds. Accordingly, this behavior may have been brought on by a high level of competition brought on by a numerous population of *C. maculatus* s in the seeds, as described by (Messina, Lish, Gompert, & Campbell, 2019). In another study Cheng et al. (2013), established that when the seeds were stored in an environment with high levels of carbon dioxide (CO_2) and hypoxia, 20–50% of adult C. maculatus larvae formed premature escape holes and dropped out of the seeds. In this study, however, 50g of seeds were placed in 300ml bottles that were covered with muslin thus allowing more space and enough air circulation to occur.

The highest seed damage and seed weight loss percentage that is a significant economic indicator, was observed on the control seed. The control seeds had an average seed weight loss of 17% and this indicated that the control seeds are great as host for *C. maculatus* development. The seed weight loss percentage observed from the bean genotype CAL96, DAB520 and X-genotype were not significantly high. The bean genotypes CAL96 and DAB520 had an average seed weight loss 7%and 5%, respectively. Similarly, X-genotype was recorded to be 9% weight loss. This low weight loss by the bean genotypes could suggest that there is a mechanism within the bean genotype CAL96, DAB520 and X-genotype offering resistance against damage from the seed beetle as compared to the control (Blackeye cowpea genotype), which could be said to be susceptible. The resistance of these three bean genotypes could be due to physical factors such as grain hardness or antibiosis as a result of biochemical compounds which are toxic to the insects as suggested by Mwololo et al. (2012). Sharma and Thakur (2014) also documented that the resistance of different legumes to *C. maculatus* damage and weight loss might have been due to antinutritional factors like proteins in high amount as compared to cowpeas which support the development and damage of *C. maculatus* resulting in quantitative losses. As there was no visibility of egg development into larvae in bean genotypes CAL96, DAB520 and X-genotype, the small percentage weight loss might have been a result of death of larvae after penetration into the cotyledon, as reported by (Sharma & Thakur, 2014). In related development, Ashamo (2019) showed the basis of resistance in some stored commodities attacked by stored products insects including C. maculatus. The implication of this is that if all these bean genotype seeds were left unprotected, they were not going to result in economic quantity losses as compared to the black eye cowpeas, which resulted in economic weight losses. These findings revealed that the seed damage and weight loss are clearly related to the number of adults that emerged.

Susceptibility level was categorized based on the Dobie's susceptibility index value of each genotype as proposed by Dobie (1974). Dobie's susceptibility index is a criterion used to separate varieties into different resistance groups (indicator of resistance). From this study only the control (blackeye cowpeas) was found to be susceptible to seed beetle attack in storage based on Dobie's index of susceptibility, whilst the bean genotypes CAL96, DAB520 and X-genotype were observed to be resistant to seed beetle attack. The seeds were grouped into either susceptible or resistance based on the index of susceptibility as a measure of resistance. The index of susceptibility gives a credible assessment of resistance levels since it is proportionately connected to the rate of increase and the logarithm of the quantity of insects pest that emerge during a specific period of time referred to as the development time (Dobie, 1974). The genotypes with lowest index values are graded highly resistant and those with highest values are graded susceptible. The index of susceptibility of the seeds in this study ranged from 0- 8.3, with black eye recording an index value of 8.3 whilst the bean genotype CAL96, DAB520 and X-genotype recorded and index value of 0. The 0-index value in this experiment indicated that there was no adult beetle emergence over the test period. This means the bean genotypes CAL96, DAB520 and X-genotype fall within the resistant range, as compared to the control which fall within the susceptible range, as per the DSI scale by (Dobie, 1974). From the study it was observed that the developmental period of the resistant seeds was shorter ranging between 25 and 28 days, while that of the resistant seeds was 0. The resistant seed's ability to break the development of the seed beetle may be due to antibiosis mechanisms within the seeds. According to Smith and Clement (2012) a halt or an increased span of time between the egg and adult phases, as well as by the reduction in adult emergence is a characteristic of antibiosis type of resistance. This study indicates that there is a relationship between DSI and beetle adult emergence.

4.2.2 Relationship of biochemical contents and susceptibility parameters

The results obtained from this study confirms that some biochemical contents have an effect on the resistance and susceptibility of common been seeds against storage pest *C. maculatus*. Among the biochemical attributes which were tested for correlation with susceptibility parameters of seeds only percentage ash, potassium, sodium and magnesium were indicated to be associated with susceptibility to *C. maculatus*. These results corroborates the reports of Kosini, at al. (2019) that indicated some biochemical attributes to be important in conferring resistance against *C. maculatus.* Lattanzio et al., (2005), also documented that more than one biochemical attribute are responsible for conferring resistance against seed beetles in seeds. The results obtained from this study indicate sodium to have a positive correlation with all the susceptibility parameters (DSI; seed weight loss; beetle adult emergence; number of eggs laid; and adult mortality) in seeds which suggests that an increase in sodium content in seeds would result in increased susceptibility to seed beetle. From this study the results obtained suggest that seeds with high sodium are prone to infestation and damage by *C. maculatus.* The results also indicate magnesium to have a positive correlation with some of the susceptibility parameters which are DSI, beetle adult emergence and adult mortality. These results suggest that seeds rich in magnesium content will have high DSI, high adult emergence percentage and high adult mortality. This further suggest that seeds high in magnesium content reduces resistance of seeds to *C. maculatus,* as seeds. These results indicates that *C. maculatus* performs best on sodium and magnesium rich bean genotype seeds. These results are supported by Nwosu (2016) which reported that sodium and magnesium had an effect on susceptibility on different maize varieties against maize weevil, as an increase in these biochemical components resulted in an increase in seed susceptibility to the storage pest *S. zeamais*. On the contrary ash from this study had significant negative correlation with all the susceptibility parameters whilst potassium had a negative correlation with only the susceptibility index. These results indicates that an increase in ash contents results in a decrease in number of eggs laid, DSI, seed weight loss and beetle adult emergence. The results also prove that an increase potassium results in decrease in the susceptibility index of bean genotypes against *C. maculatus*. These results suggest that ash and potassium have an effect on the resistance of seeds against *C. maculatus*. In a similar study where association of biochemical contents with resistance of seeds against storage pests were studied, Demissie, (2015) also reported ash to have an effect on resistance of different maize varieties against *S. cerealella*. Unfortunately results on their study were different on the effectiveness of ash on susceptibility, as they reported that an increase in ash resulted in an increase in susceptibility. In another study Nwosu, (2016) reported pottasium to have an inverse correlation with susceptibility index of maize seeds to *S. zeamais* as it indicated that an increasee to potasium resulted in an increase in resistance against storage pest. They further documented that grains rich in minerals except for potasium are susceptable to infestation and damage by weevils. In this study the positve correlations between susceptibility parameters and percentage moisture, protein were not significant. The negative correlation observed between susceptibility parameters and percentage crude fat, percentage crude fiber, and tannins were observed to be non significant. This non-significant correlations means these biochemical factors are not likely to be the base of resistance fo the seeds against *C. maculatus*. The results coroborates studies by Belay et al. (2017) which suggested that though some compounds have an effect on resistance of seeds against *C. maculatus* some are not associated with seed resistance to *C. maculatus*. This study also doesn't agree with observations from other studies in which crude fat, crude fiber, protein, moisture, and tannins were recorded to be strongly related to grain's resistance to *C. maculatus* (Aryal, Pudasaini, and Bhandari 2019; Belay et al. 2017; Kpoviessi et al. 2021; Lattanzio et al. 2005;Nwosu 2016; Souza et al. 2011).

4.2.3 Efficacy of Plant Extracts in controlling *C. maculatus*

Botanical plant extracts have been documented to have effects on growth, development, and oviposition of insect pests, and also have an antifeedant and arrestant effects on insect's pest. Because they offer no risk to the environment or to human and animal health, botanical insecticides have always been hailed as appealing alternatives to synthetic chemical pesticides for pest management (Said & Pashte, 2015). This study was designed to screen bean genotypes and seed treatment for their effect on total number of *C. maculatus* eggs laid, beetle adult emergence and adult mortality. The current study's findings showed that the tested plant powders were considerably effective against *C. maculatus* in the bean genotypes with regard to adult mortality, adult emergence, and oviposition percentage. The results of the present study implied that when plant powder were added to any of the genotypes there was either a decrease or increase in seed beetle oviposition. A number of plant powders from different plants have been documented to have characteristics that are insecticidal to a variety of crop insects pest (Moreira et al., 2007). Some plant extracts have insecticidal qualities such as repellent action, antifeedant, anti-ovipositional function, and lasting protection against *C. maculatus* (Saxena & Sayyed, 2018). A total of 5 treatments (marigold, peppermint, fever tea, garlic and a control (no plant powder was used) were used in this experiment. The garlic, fever tea and marigold were significantly superior in controlling the oviposition, while peppermint was significantly inferior to the control. Among the five treatments, garlic powder had significant effect against egg laying capacity of *C. maculatus* as it had the lowest rate of oviposition. Fever tea and marigold powders were observed to be not significantly different to each other and provided the second and third lowest rate of oviposition respectively, whilst the mint powder provided the highest rate of oviposition. The efficacy of the plant powders on the oviposition rate differed depending on the source of their active ingredients. The results obtained in this present study indicate garlic powder had a significant anti-oviposition effect against seed beetles. Garlic was effective in controlling *C. maculatus* as it partially or completely prevented egg-laying. These results might be due to *C. maculatus* sensitivity to garlic which delayed males from locating and mating with the females. These results indicate that garlic powder negatively affect the reproduction process of *C. maculatus*. This is supported by what was reported by Ishag et al. (2018) who suggested that reduction in egg laying is induced by garlic extracts by harming reproductive system of the test insects or behavioral effects by obscuring the recognition of host. The results from the present study might also be associated with the insecticidal effect of garlic powder, as some of the *C. maculatus* adult which infested garlic treatment began to die 3 days after infestation. These results agree with study by Ho et al. (1996), who documented garlic to be toxic to *Tribolium castaneum* adults making it effective in reducing adult reproduction. Fever tea and marigold also demonstrated to have negative effects on the oviposition rate as they recorded significantly low oviposition as compared to the control treatment. This suggest that fever tea and marigold have anti-ovipositional properties against *C. maculatus. C maculatus* infested in both of the treatments started to oviposit 2 to 8 days after infestation. Some of the *C. maculatus* adults were observed to be non-active 4 days after infestation which suggests that both treatments had an insecticidal/toxic effect on the adult *C. maculatus*. The low rate of oviposition by fever tea might also be due to volatile chemistry which negatively affect reproduction of *C. maculatus*. Fever tea has been reported to have contact toxicity, repellent effect and as well as oviposition deterrent properties towards some storage pest (Sands 1977; Peixoto et al. 2015) . Fever tea extract have also been documented to contain an essential oil, osdienen, which has been known to have a repellent and toxic effect on insect pests (Katsvanga & Chigwaza, 2004). Suthisut et al. (2011) also reported fever tea to have a compound camphor which is known to have biological activity against other insect species including storage pests. Marigold treatment was observed to also have an anti-oviposition effect on *C. maculatus*. Some of the adults infested into the experiment became inactive few days after infestation, which was supported by (Cosmas et al., 2012) who reported marigold to inhibit weevil movement. The results suggest that marigold might have reproduction sterility effect on the *C. maculatus* adults, which suggests that marigold consist of chemical compounds that interferes with the reproduction process of *C. maculatus* as reported by Weaver et al. (1994). Peppermint was recorded to have significantly the highest ovipositional rate when compared to control and the other plant treatments. The peppermint treatment had a significantly high egg laid per seed ratio (3 to 6 eggs per seed). Adult seed beetles were observed to have started to oviposition 2 days after infestation. The infested adult *C. maculatus* laid a high number of eggs in short period and they all died within 3 to 7 days after infestation. This present experiment indicates that peppermint enhances ovipositional rate of *C. maculatus*. The high number of oviposition might be due to *C. maculatu*s response to mortality risk caused by chemical compounds from peppermint as reported by (Javoiš & Tammaru, 2004), who reported that insect *Scotopteryx chenopodiata* L had an increase in oviposition rate due to mortality risk. Another study on mortality risk due to seed chemical composition by Loolaie (2017) agrees with this study, where peppermint was reported to produce the highest level of toxicity to the adults and larvae of the black carpet beetle (*Attagenus fasciatus*) and cigarette beetle (*Lasioderma serricorne*). Reda et al. (2010) reported the monoterpene compounds of peppermint to be highly toxic when they penetrate insect cuticle or the respiratory system.

Irrespective of the treatments, only blackeye cowpea genotype showed emergence of the F1 from the eggs laid on the seeds. The seeds of other genotypes, that is CAL96, DAB520 and X-genotype, did not have any recordings of adult emergence. The results from this present study on ‘Genotype resistance’ indicated that the seeds of bean genotypes, CAL96, DAB520 and X-genotype, might be possessing ovicidal effects on *C. maculatus*. These results also indicate that the plant powder treatments had an effect of the seeds’ ovicidal effects on *C. maculatus*. A reduction in F1 progeny emergence was observed in treated seeds when compared to untreated seeds and this might be due to the increased ovicidal and larvicidal effects due to properties of the plant powders. Getahun et al. (2020) documented that the low or absence of F1 progeny of insect pest weevil on grain indicated the efficacy of plant extracts as control against maize weevil. Even though peppermint recorded the highest oviposition rate than all the treated and untreated seeds, it recorded significantly, the lowest emergence percentage as compared to the other treatments. Peppermint treatment recorded an average of 1.05% emergence, which suggests that it does not significantly support emergence. This result indicates that peppermint has high ovicidal and or larvicidal effects. The findings of the present study agrees with what was reported in a study by Loolaie (2017), in which peppermint was significantly effective in killing the larvae of black carpet beetle and cigarette beetle. Peppermint was also found to be highly effective against pulse beetle in terms of ovicidal effect (Kumari, Mukherjee, Kumar, & Kumar, 2014). The results obtained on peppermint treatment from this study might be due to chemical compound menthone which is found in peppermint. Li and Tian (2020) reported menthone to be consistently a major constituent of peppermint that had a strong contact toxicity on Pear Psylla nymph and larvae. Fever tea had the second lowest adult emergence of *C. maculatus*, while garlic had the third and marigold the fourth. Fever tea, garlic and marigold treatments recorded an average of 2.9%, 3.48% and 11.75% adult emergence, respectively. These treatments were observed to deter the emergency of *C. maculatus* adult, which indicates that they might be having ovicidal and or larvicidal effects on *C. maculatus*. The low percentage of emergence of the F1 progeny from these treatments on blackeye seeds maybe associated with high mortality of larvae and mortality of eggs due to chemical compounds found in these plant powders. These findings are in agreement to those of Ho et al. (1997) who reported garlic to cause mortality on larvae and eggs of *T. castaneum*, with eggs being more susceptible than larvae. In other studies, Garlic was also observed to reduce hatching and proved to have ovicidal effects on some insect eggs (Boivin 1997; Ishag, Mohammed, and Hammad 2018; Ahmed et al. 2019) while Gurusubramanian and Krishna (1996) reported that garlic extracts caused the egg shells of storage pests to harden making it hard to hatch. The observations in the present study also agree with Elango et al. (2009) who reported that marigold showed 100% ovicidal activity against *Anopheles subpictus*. The larval mortality from marigold might be due to antifeedant effect from marigold. Cosmas et al. (2012) reported that marigold had an antifeedant activity on the maize weevils. Results from fever tree treatment might also be due to larval of *C. maculatus* being unable to feed because of antifeedant and repellent properties of fever tea. These observation are in line with study by Manenzhe et al. (2004), who reported that fever tea has repellent, toxic, and antifeedant effects on insect pest *B. brassicae* and *T. evansi*. They further indicated that the insecticidal properties were from the essential chemical compounds found in fever tea.

Adult mortality percentage among the plant treatment and untreated control were observed in the present study. The data on the F1 progeny mortality was collected every two days after oviposition. The F1 progeny which were found dead, either outside the seeds or inside the seeds were recorded and used to calculate the adult mortality percentage. The results showed that the mean cumulative percentage mortality inflicted by some of the plant powders was effective. The peppermint and marigold were the only plant treatment to have recorded a significant adult mortality. Peppermint treatment recorded F1 progeny mortality with an average of 1% whilst Marigold recorded F1 progeny mortality with an average of 6.67%. Marigold and peppermint were statistically at par with each other in the present study on effect of F1 mortality. The mortality in the F1 progeny by these botanicals might be due to toxicity of their chemical compounds on *C. maculatus*. Other studies also showed mortality of *B. tabaci* on plants after treatment with marigold (Id et al. 2020; Weaver et al. 1994). Id et al. (2020) also recorded a significant mortality on both *L. hesperus* and *B. tabaci* adults after marigold treatment. Marigold has also been documented to be very effective in killing *Sitophilus zeamais* weevils (Cosmas et al., 2012). Weaver et al. (1994) reported marigold plant to have insecticidal properties due to the insecticidal components in its flowers, roots and the leaves. Similarly, peppermint has been document to be toxic to *Tribolium castaneum, Rhyzopertha dominica* and *Drosophila suzukii* (Renkema, Wright, Buitenhuis, & Hallett, 2016; Ukeh & Umoetok, 2011). Peppermint was also reported to be capable of blocking the spiracles of insects, thus impairing respiration leading to the death of insects (Getahun Debelo & Wondimu, 2020). Reda et al. (2010) also reported that peppermint cause suffocation and inhibition of various biosynthesis processes of the insects. Garlic and Fever tea treatment did not record any mortality of F1 progeny. The F1 progeny found in these treatments were not dead, however, they were observed to be less active. This result might be due to the less exposure time of the F1 progeny to the treatment. The insecticidal effects of the two treatments might have required a longer exposure time to take effect as recorded by Getahun Debelo and Wondimu (2020) in a similar work where they observed that the efficacy of the botanical powders on maize weevil increased with exposure time. In the study by Kifle Gereziher et al (2016), mortality rate of maize weevil treated with some botanical plants (neem and citric peel) increased with exposure time. The *C. maculatus* results from the present study suggest that garlic and fever tea treatment have of killing *C. maculatus* at a relatively slow rate.

Upon removal of the F1 progeny from the seeds, the weight of the seeds was recorded and was used to calculate seed weight loss percentage. Among all the treatments used on the seeds, the most effective were garlic, mint and fever tea treatment, which all recorded a zero percent reduction in seed weight loss caused by *C. maculatus*. This implies that all the seeds treated with garlic, peppermint and fever tea in the present study did not record any weight loss which suggest that if these seeds (CAL96, DAB520, X-genotype and blackeye cowpeas) were treated with the mentioned treatments and left unprotected, they wouldn't result in economic quantity losses as compared to marigold and untreated seeds. This is a good sign of effectiveness of plant products in protecting stored seeds against *C. maculatus*. Weight loss indicates the quantitative loss caused by insects feeding/damaging the seeds. Marigold and the untreated control were the least effective as they recorded 0.5% and 1.33% reduction in seed weight loss due to *C. maculatus* infestation, respectively. Despite the fact that these treatments recorded weighted losses, their percent weight loss was very low. Marigold treatment recorded seed weight loss only in blackeye cowpeas, but recorded a zero percent seed weight loss in bean genotypes CAL96, DAB520 and X-genotype. Due to failure to recording a significant reduction of seed weight loss in blackeye cowpeas, the zero percent weight reduction of seeds of genotype CAL96, DAB520 and X-genotype does not necessarily mean it was the effect of marigold as these genotypes didn't record any weight loss by *C. maculatus* in the untreated control. The results from the present study indicate that garlic, peppermint and fever tea treatment have a repellent function, an insecticidal property, , anti-ovipositional feature and lasting protectant property on CAL96, DAB520, X-genotype and blackeye cowpeas against damage from *C. maculatus*. These results agree with different studies which indicated garlic, peppermint and fever tea to have a persistent protective property against some insect pests (Peixoto et al. 2015; Mohammed and Hammad 2018; Ahmed et al. 2019; Lanka 2019;). The Dobie's susceptibility index was used to measure the resistance or susceptibility of the genotypes treated with plant powders. As reported by Dobie (1974), due to its proportional relationship between growth rate and the logarithm of the number of insects that emerge during a predetermined period of time, known as development time, this index provides an accurate estimation of resistance and susceptibility levels. As stated in our present study genotypes and treatments with lowest index values are graded highly resistant and those with highest values are graded susceptible. From the present study garlic and peppermint treatments recorded the lowest index value (0) and fever tea recorded the second lowest index (0.09). Garlic, peppermint and fever tea were observed to be resistant to *C. maculatus* infestation and were statistically in par with each other. Marigold treatment and untreated controls were also observed to be resistant as they recorded third lowest (0.46) and highest (1.26) index respectively, and they were also observed to be in par with each other. From all the seeds treated with the plant powders and untreated control, only blackeye cowpea seeds recorded an index higher than 0.0. The 0-index value in this experiment indicate that there was no adult beetle emergence over the test period. The blackeye cowpea seeds treated with marigold had a higher median development period and a smaller number of seed beetle emergence as compare to untreated blackeye seeds. This might explain the reasons for having smaller index number of seeds treated with marigold. This study strongly suggests that there is a relationship between DSI and seed weight loss percent.

4.2.4 Germination response of bean genotypes to different plant extracts

A germination test was performed on seeds treated with plant powder to investigate the effects of the treatments on their germination. The findings of this study revealed a significant effect of the plant powders tested on germination of all the seeds (CAL96, DAB520, X-genotype and blackeye). After 7 days the average germination percentage in different treatments and untreated seeds ranged from 77.5 to 100 percent. Garlic, fever tea and marigold treatments were observed to be statistically at par with each other and these were seen to have recorded the highest average germination percentages at 100, 99.17 and 97.5 percent, respectively. Peppermint recorded the second lowest average germination percentage at 85.83 percent, whilst the untreated control recorded the lowest average emergency percentage of 77.5. Observations from the present study have indicated that garlic can provide satisfactory protection and maintain quality needed for germination to host seeds against *C. maculatus* as shown by the 100 percent seed percent emergence in all the experimental units (CAL96, DAB520, X-genotype and blackeye cowpeas). The results of the experiment might be due to allelopathic properties of garlic which cause the seeds to emerge faster than when they are not treated with anything. Garlic treated seeds were observed to have started germination on the 4th day after germination test commenced. The observation of this study agrees with Perelló et al. (2013), who reported that garlic was observed to provide a growth promoting activity to wheat seed germination due to the effect of bioactive compounds found in garlic namely allicin. The present study has also shown that fever tea provided the second most satisfactory emergence with average percentage emergence of 99.17%. The germination percentage obtained in fever tea for each seed were 100 percent in both CAL96 and Blackeye, and then 96.7% in both DAB520 and X-genotype. Germination was observed to have started within the first five days of the germination experiment. These results indicate that fever tea improves the germination of seeds (CAL96, DAB520, X- genotype and black eye) when compared to untreated control, which might be due to chemical components found in fever tea. This is supported by studies in which fever tea was reported to have plant growth promoting properties (Maroyi 2017). Mashela et al. (2010) also reported fever tea to have growth promoting properties on tomato. Marigold had the third highest average germination percentage of seeds (97.5%). From the present study, the average germination percentage recorded from the seeds was 100% for both blackeye and X- genotype, 96.7% for CAL96 and 93.3% for DAB520. Germination from seeds treated with marigold were observed to have started within five days of the experiment. The result from this experiment indicates that marigold promotes seed germination when compared to untreated control. The results agree with those of Baličević et al. (2014) who reported marigold to have germination promoting and growth properties in weed. Alpha terthienyl, which is one major light sensitive compound found in marigold, was shown to be capable of enhancing the development of other plants, such as tomatoes and tobacco (Ijani & Mmbaga, 1988). Peppermint recorded the lowest average percentage germination of all the plant powders with an average of 77.50%. The average germination percentage of seeds treated with peppermint were 96.7 for X-genotype, 86.7 for CAL96, 83.3 for blackeye cowpeas and 43.3 for DAB520. The seeds treated with peppermint were observed to have started germinating within five days of the experiment. Peppermint was observed to reduce the percentage germination in seeds as it recorded the lowest average emergence percent compared to the untreated control, which had an average percent of 85.83%. The chemical compounds found in peppermint might be responsible for the reduction in germination percentage as suggested by Mozdzeń et al. (2019), where they reported the allelopathins of mint to have a germination inhibiting properties on tomato.

4.3 CONCLUSION

From the results of our study, it can be concluded that *C. maculatus* has a selective host characteristic. The current study has revealed that *C. maculatus* could not complete its life cycle in all of the bean seed genotypes tested. The bean genotypes were equally exposed to *C. maculatus* of same age and number, with same ratio of male to female adults with the environment being conducive to the insect development. The seeds of bean genotypes, namely CAL96, DAB520 and X-Genotype, were found to be resistant to damage by *C. maculatus,* while the blackeye cowpea (control) seeds were susceptible to *C. maculatus* damage. The susceptibility of the control treatment (blackeye cowpea genotype) is able to validate the fitness of the *C. maculatus* used in this experiment. Therefore, on the ovipositional basis, black eye cowpea genotype is the most preferred and suitable host for *C. maculatus* as compared to CAL96, DAB520 and X-Genotype. In terms of their susceptibility the four seeds can be ranked as follows blackeye cowpeas > x-Genotype >DAB520 >CAL96. From the current study it can be concluded that seeds for genotype CAL96, DAB520 and X-Genotype are safe to be stored without any treatment as they have shown to be resistant to *C. maculatus* pest damage in storage. This study further provides evidence that some of the biochemical contents found in the genotypes, particularly potassium, sodium, ash and magnesium are responsible for resistance against C. maculatus. These biochemicals were strongly associated with the susceptibility index (DSI). However, the other biochemicals proved not to be effect in resistance against *C. maculatus*

The plant powders garlic, peppermint, fever tea and marigold tested against *C. maculatus* on different seeds revealed to have high insecticidal activities towards the *C. maculatus* on different seeds. The reduced *C. maculatus* emergence suggest that antibiosis might be the mechanism as reported in this study. The plant powders garlic, fever tea and marigold proved to be the best treatments in reducing oviposition from *C. maculatus* in all the seeds and peppermint had a high ovicidal activity in all the seeds. The plant extracts also had a significant effect on the germination of seeds used in the current study. Marigold, garlic and fever tea have all the qualities to be considered as potential use in management of *C. maculatus* on the new genotype seeds as they have good insecticidal activity and also have a positive effect on germination of the seeds. Peppermint though it has good insecticidal properties against *C. maculatus,* it significantly reduces germination percentage of seeds as such it would not be recommended to use in management of *C. maculatus* on seeds.

5.0 APPENDICES

Appendix 1. *C. maculatus* culture in the Entomology Lab

Appendix 2. Cushing marigold (A) plant and mint plant (B) into powder with piston and mortar

Appendix 3. F1 adults removed from DAB520 seeds 14 days after infestation.

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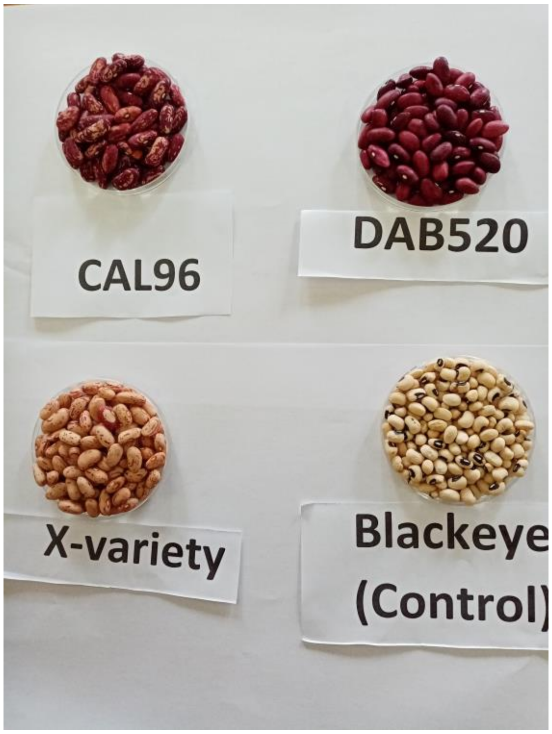
Appendix 4. Seeds germination test inside the seed germinator -test of CAL96, DAB520, X-genotype and blackeye seeds treated with plant powder.

Appendix 5. Germination test after 7days- germinated seeds of blackeye cowpea treated with garlic

Appendix 6. Variety of Seeds being screened for resistance against *C. maculatus*



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