INFLUENCE OF COWPEA PLANT AND SEED CHARACTERISTICS AND PACKAGING MATERIAL IN STORAGE ON COWPEA WEEVIL (*Callosobruchus maculatus*) INFESTATION

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Influence of Cowpea Plant and Seed Characteristics and Packaging Material in Storage on Cowpea Weevil (*Callosobruchus maculatus*) Infestation

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This Thesis has been submitted for examination with our approval as University supervisors

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DEDICATION

This thesis is dedicated to my beloved husband James Ogutu, my children Bethel, Minchah and Euodia and to my parents Mr. and Mrs. Buleti and Mr. and Mrs. Aloo

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ABSTRACT

Cowpea (*Vigna unguiculata*) is a self-pollinated popular legume. It is only propagated through seed. Cowpea is one of the popular African Indigenous Vegetables that can be consumed as vegetable and grains stew because it is rich in protein. Quality seed is a challenge facing African indigenous vegetable production in Kenya. Cowpea weevil is the main pest of cowpea seed causing damage during storage causing loss up to 100 % when the seed is stored without any form of treatment and improper packaging. This research was carried out to identify ways of minimizing seed damage through characterization of cowpea so as to identify superior lines, and to determine appropriate packaging material that can store cowpea seed, with minimum damage. Experiments were set up in October 2016 to August 2017 where plant characteristics were evaluated in field experiments. Upon harvesting the seed, characteristics were determined at the plant physiology laboratory in Jomo Kenyatta University of Agriculture and Technology. Morphological characteristics were assessed based on the cowpea descriptors of the International Board for Plant Genetic Resources. Seed biochemical composition including protein, tannin and amino acid were analyzed using the Association of Official Analytical Chemists methods. Amino acid composition was evaluated using Liquid Chromatography-Mass Spectroscopy at International Centre of Insect Physiology and Ecology for selected set of lines. An experiment to determine extent of damage among the lines due to cowpea weevil damage was carried out by storing the seed in cellulose bags for a period of three months in the laboratory in JKUAT. To assess effect of packaging material, a repeated experiment was carried out. A single cowpea variety was used to evaluate the efficacy of packaging materials; cellulose paper bags, gourds, grain storage bags, polyethylene bags and glass bottles. Completely Randomized Design method was used with each storage material being replicated thrice. Data was obtained on the number of seeds with holes, mean number of holes per seed, seed weight loss, damage score, and seed germination. Analysis of Variance was used to determine variation among cowpea lines for plant and seed characteristics, biochemical composition, seed damage and the variation in types of packaging materials for seed storage. Principal Component Analysis was used to classify cowpea lines. Correlation analysis was used to determine the relationship between plant and seed characteristics and pest infestation and relationship among seed damage parameters. From the evaluated characteristics, cowpea seed were classified based on skin characteristics. Significant differences ($p \leq 0.05$) on cowpea seed damage based on in plant and seed characteristics were observed. Cowpea lines had significant variation ($p \leq 0.05$) in protein level, tannin content and amino acid concentration. Both plant and seed characteristics had an influence on cowpea weevil infestation. Protein level and total amino acids were positively correlated ($r = 0.698$ and $r = 0.897$ respectively) with cowpea weevil infestation while tannin content had a negative correlation ($r = -0.666$) with cowpea weevil infestation. There was significant variation ($p \leq 0.05$) among storage materials for the respective measured parameters for pest damage. The parameters used to evaluate damage were positively correlated except germination. Seed stored in glass bottles showed highest germination rate (92.7%); whereas, germination rates were less than 52% for the other seed packaging materials. Glass bottles were ranked the best packaging material (0.01% weight loss and no emergence holes), followed by polyethylene bags (7.5% weight loss and 5 emergence holes) and finally grain storage bags (7.5% weight loss and 5 emergence holes). Plant and seed characteristics among the cowpea varieties studied had an influence on the *Callosobruchus maculatus* infestation. Airtight packaging material effectively reduced weevil damage and preserved seed quality. Use of hermetic storage allows long term storage of cowpea and minimizes damage caused by the cowpea weevil. This information is important for farmers and breeders involved in cowpea improvement for weevil management, seed quality and sustainable production.

Keywords; seed storage, morphological characteristics, biochemical composition, hermetic seed storage, smallholder farmers

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cowpea (*Vigna unguiculata*) is a self-pollinated popular pulse belonging to the Leguminosae family. Cowpea is produced in Europe, Asia, America and Africa specifically parts of East Africa (Coulibaly *et al*., 2008). Cowpea is produced in Makueni, Kitui, Kwale, Machakos, Kilifi, Homabay, Busia, Siaya, Kisii, Taita Taveta, Kakamega, Vihiga, Mombasa, Meru and Nyamira counties of Kenya (HCDA, 2016). Grain Cowpea production in Kenya increased from 0.65 tons/ha to 0.76tons/ha (FAOSTAT 2018). Cowpea is mainly popular due to its diversity with regard to consumption. There are three types of cowpea that is vegetable cowpea, grain cowpea and dual purpose cowpea. The leaves, grains and a combination of both are utilized for consumption respectively. The foliage can also be used in manufacturing animal forage (Timko *et al*., 2007). Cowpea is a very nutritious crop; it's a source of dietary fiber necessary for digestion and a source of cheap protein. The leaves and grains of cowpea contain 34.2g and 24 grams per 100 grams of protein respectively (Grubben *et al*., 2014). Agronomic benefits include ability to fix up to 30kg/ha of nitrogen which results in increase in yield for the intercrops (Khan *et al*., 2007).

Cowpea is mainly propagated through seeds making seed an important aspect in its production. Cowpea production is faced by challenges of weeds, pests and diseases, occurrence of mixed types due to self-fertilization and 15% cross pollination (Thooyavathy *et al*., 2013). Among the major challenges facing production of this crop is poor seed and or unavailability of quality seeds (Biemond *et al*., 2012). Lack of quality seeds at planting time and low yielding varieties is a common problem to cowpea farmers. This can be due to use of "farmer saved seeds".

Challenges facing cowpea production in the tropics include pests and diseases, fungi, and storage mechanisms; seeds in storage are affected mainly by cowpea weevil (*Callosobruchus maculatus)* (Yakubu, 2012). This weevil is a field to storage pest that infests during the later stage of pod development. The adult weevil lays eggs on the seed testa which develops into a larva that penetrates through the testa which grows and matures and emerges hence causing holes within the seed (Swapan, 2016). Presence of holes is reported to cause reduced eating quality, low germinability, low vigor, and low economic values for farmers (Umeozor, 2005).

Biological control methods including cultural, timing of harvest, varietal selection and crop hygiene are suggested as methods used in cowpea weevil management in Kenya (Van, 1991). Studies on control of cowpea weevil during storage through use of synthetic pesticides have been done (Oluwafemi, 2012) but popularity in use has largely reduced due to potential toxic effects of pesticides on the human body (Muhammad, 2015). Plant products like chilies garlic and pepper have also been used but have been found to provide short term solution for seed storage (Tiroesele *et al*., 2015). Farmers also use their own storage methods including pots, gourds, and sacks (Francis and Waithaka, 2015). These methods are however not economical for large scale producers and they are less effective (17% of seed is lost) in storage of seed and protection against cowpea weevil (Ilesanmi and Gungula, 2013).

1.2 Statement of the problem

Production of African leafy vegetables (cowpea included) in Africa is about 12tons/ha per annum; with an average population of 540 million people there is need to produce more cowpea (Grubben *et al*., 2014). Apart from pests and diseases, climate change and post-harvest losses seed quality deterioration during storage is a major challenge facing production of African indigenous vegetable (Keatinge *et al*., 2015). Cowpea is one of the popular African Indigenous Vegetables. Pests are a leading cause of global grain/seed losses; in the world 25% to 33% of grain is lost during storage due to insect pests (Muhammad, 2015). Biemond *et al*., (2012) reported that shortage of seed for planting of cowpea is a common occurrence. Seed shortage occurs mainly because of poor storage which makes the seed vulnerable to pest infestation (Morad, 2013). Pest infestation in cowpea seed has the potential to interfere with the seed germination (65%), viability and ultimately poor seed performance in the field, reduced yield and reduced income for farmers (Bourtey *et al*., 2016). Since most farmers do not have appropriate packaging and storage materials they prefer selling their cowpea immediately after harvesting to avoid risk of storage losses hence low returns (Carlos, 2004). Packaging material used for storage can influence moisture content of seeds, pest infestation and general seed quality characteristics (Susmithar & Rai, 2017). Cowpea weevil infestation during storage can result to 50 % loss and 100% seed damage in less than 6 months (Oluwafemi, 2012). Farmers stand a risk of losing all or part of their stored produce which could eventually lead to malnutrition and hence Food insecurity for the nation (FAO, 2018).

1.3 Justification

Sub Saharan Africa accounts for 70% of cowpea production because cowpea can be consumed as vegetable, grain and even forage for animals. According to Gennari *et al*., (2013) 96% of cowpea is produced in Africa indicating that it has wide adaptability and is tolerant to heat. Cowpea contains different nutrients including crude fiber (0.97%), protein (20 %), low fat (2%) and carbohydrate (66.21%) for every 100g providing an alternative to animal proteins which are expensive (Oyeyinka *et al*., 2013). Cowpea is reported to have capacity of lowering cholesterol levels when consumed alongside high fatty acid foods (Frota *et al.*, 2008), thus it can be included in daily diets as management for lifestyle diseases which are on the rise. Horticultural Crop Directorate Authority reported in 2016 that there is an increase in consumption of African indigenous vegetables in Kenya. This is because of the sensitization and promotion of their nutritional value (CTA and FARA, 2011). It is further reported that cowpea is the leading crop in terms of value at 43.31% of the total value of indigenous vegetables produced worth 2,434,852,370 Kenyan Shilings (HCDA, 2016). This is an indication that cowpea is a prefered crop because of the many diverse ways of utilization. Malnutrition and Food insecurity is a global challenge. In 2018 FAO reported that 820 million people in the world did not have enough food to eat in 2018 compared to 811 million people in 2017. Similarly in East Africa, it has been reported that 30.8% of the population is undernourished. Cowpea being a crop rich in protein content and can be used as a substitute for animal protein is a crop of importance regarding reduction in obesity and curbing undernutrition among our populations (CTA and FARA 2011).

Cowpea has other beneficial attributes like nitrogen fixation when intercropped it has potential to reduce costs incurred for procurement of fertilizers. It can also be used to control Striga, a noxious weed causing enormous maize yield losses (Khan, 2007). Sustainable production can increase production of other staple food like maize and sorghum. Cowpea is only propagated through seed and therefore the growth and productivity is directly linked to the quality of seed used (Francis & Waithaka, 2015). Sustainable production is anchored on quality seed which is a challenge in a crop that is self-pollinating. Effects of poor seed quality could eventually lead to food and nutrition insecurity due to the rapidly increasing population

Among the popular legumes, cowpea is reported to be the most damaged by storage pest *Callosobruchus maculatus* (Sharma and Thakur, 2014). Cowpea weevil is the most economically important pest during storage of cowpea; it can cause up to 100% seed loss when seeds are not treated (Cruz *et al*., 2015; Carlos, 2004). Harvesting time and intercropping have been reported as cultural methods of pest control in Kenya (Olubayo and Port, 1997). The ability of seed to retain its production potential during and after storage depends on its individual characteristic and the storage mechanisms (Morad, 2013). Use of traditional storage techniques is a contributor to cowpea weevil proliferation and multiplication during cowpea storage (Yakubu, 2014). A large percentage of cowpea farmers (76%) plant their own saved seed (Njonjo *et al*., 2019) and they face a challenge from field pests and poor varieties (Owade *et al*., 2020). They also face problems with selection and storage because there is limited information on the characterization of their cowpea varieties (Hutchinson *et al*., 2017). Polythene bags are used in storage of cowpea beside pots, baskets, and gunny bags in Coastal (Taita) and Eastern (Makueni) regions of Kenya

(Njonjo *et al*., 2019). Polyethylene bags and gourds formed part of the material tested in storage for cowpea. Use of hermetic storage in storage of cereals has been reported in Kenya (Groote *et al*., 2013). Hermetic storage bags including Purdue Improved Cowpea Storage (PICS), Grain Pro and metallic silos are reported to be useful in cereal storage in Kenya (Kumar and Kalita, 2017). There is need therefore to establish if famers' accessions and other cowpea material available are resistant or susceptible to cowpea weevil infestation and the properties of the cowpea lines. It is also important to establish alternative methods which can prolong storage time and still maintain the quality of cowpea seed. This information is expected to inform farmers and breeders in selection criteria of cowpea.

1.4 Objectives

1.41 Overall objective

- To determine possible ways of minimizing cowpea seed damage through identification of plant and seed characteristics for identifying superior lines and to determine appropriate packaging material for seed storage with minimum damage by *C.maculatus*.

1.42 Specific objectives

- To determine variation among cowpea morphological characteristics and their influence on *Callosobruchus maculatus* infestation
- To evaluate the influence of biochemical composition of cowpea on *Callosobruchus maculatus* infestation
- To determine the effect of selected packaging material on storage of cowpea seed

1.5 Hypotheses

- There is no variation among cowpea morphological characteristics hence no influence on *Callosobruchus maculatus* infestation in cowpea
- Cowpea seed biochemical properties have no influence on the *Callosobruchus maculatus* infestation in cowpea
- Type of seed packaging material has no effect on *Callosobruchus maculatus* infestation in cowpea

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of utilization of cowpea

Cowpea (*Vigna unguiculata*) is classified into three categories based on consumption. The vegetable cowpea; leaves and young stems are consumed, grain cowpea; whole seed used for stew preparation and dual purpose cowpea; both the leaves and the grains are consumed (Wambugu & Muthamia, 2009). In Kenya cowpea is listed as one of the most popular African indigenous vegetables and it's widely grown in the country; this is because of its diverse potential for utilization (CTA & FARA 2011). It is the second most important legume after beans in Kenya (Wambugu and Muthamia 2009). It is a very nutritious crop and in addition a readily affordable source of protein; leaves contain 34.2 grams per 100 gram and the grains contain 24 g per 100 gram of serving (Grubben *et al*., 2014). Cowpea leaves are reported to be rich in flavonoids, carotenoids, antioxidants and carbohydrates (Kirigia *et al*., 2018).

2.1.1 Growing conditions

Cowpea is adapted to a wide range of agro-ecological conditions most importantly the dry savannas with low soil fertility and drought conditions (Gennari *et al*., 2013). Cowpea requires a pH range of 4 to alkaline conditions on heavy textured soils. The crop can survive in areas with annual rainfall of 650-2000mm and warm season of mean daily temperature of 25-35^{0}C (Ajeigbe *et al*., 2009). West Africa is the leading producer of cowpea, other areas include East Central Africa, India in Asia, South and Central America (Directorate Agricultural information Services, Department of Agriculture and Fisheries, 2011). Cowpea originated in West Africa but currently production is in all parts of Africa, some parts of Europe, India, China, South East Asia and tropical America (Ngalamu *et al*., 2014). In Kenya it is produced mainly in Coastal and Eastern parts of the country (Owade *et al*., 2020). Cowpea is also produced in Kakamega, Vihiga, and Kisii counties of Kenya (HCDA, 2016). Cowpea is a short day plant but day neutral cultivars also exist (Stefan *et al*., 2013).

2.2 Cowpea propagation and crop management

Seed of any seed-propagated plant is an essential precursor in plant growth and development thus its quality is important in determining the final quality of the harvested produce or the yield (CTA and FARA, 2011). Important crop management practices in cowpea seed production have been documented including planting, weeding rouging, pest and disease management, harvesting, threshing, processing, drying and storage (Thooyavathy *et al*., 2013). Olasoji *et al*., (2013) reported that physiological maturity of seed is a factor that determines seed growth, germination and seed harvesting stage. Cowpea is considered to be physiologically mature when more than 50% of the pods turn brown and the moisture content is about 12% (Ajeigbe *et al*., 2009)**.** Quality of seed determines its germination capacity, ability to establish in the field, vigor and eventually the overall productivity (Susmithar and Rai, 2017).

2.2.1 Seed Quality and Plant Defense

Differences in genetic makeup, morphological and biochemical characteristics in a collection of cowpea seeds have been reported to be associated with resistance or tolerance to pests (Sharma and Thakur, 2014). Seeds exploit different strategies and defense mechanisms against pathogens and insect pests (Titilope and Kannike, 2019). Seed coat is a very important part of seed which takes part in determining water absorption, development of the embryo and germination (Bourtey *et al*., 2016). The seed coat is known to synthesize products that are useful in plants for defense and general plant development (Moise *et al*., 2005)

2.2.2 Characterization of cowpea

Cowpea morphological attributes (IBPGRI, 1983) are used for characterization of cowpea. Of importance are seed and plant morphological characteristics. Among the cowpea seed characteristics; seed color, seed shape, eye color/pattern, seed coat thickness, texture and moisture content have been used to in evaluation and characterization of cowpea. The seed shape can either be kidney, ovoid, crowder, globose or rhomboid. The texture of the testa can be smooth, rough to wrinkled or wrinkled. The eye pattern which is presence of the shape of the pigment pattern which surrounds the hilum can be tan brown, red, blue to black mottled (IBPGRI, 1983).

Cowpea plant morphology is equally an important aspect of the crop with regard to cowpea weevil infestation. The attributes associated with plant morphology are evaluated at vegetative and reproductive stages. Growth habit include; acute erect where branches form acute angles with main stem), erect where branching angle is less acute than acute erect, semi-erect where branches are perpendicular to main stem, but not touching the ground and prostrate where plants lie flat on ground with branches spreading several meters. Growth pattern; determinate where apical bud of main stem is reproductive and matures at same time and indeterminate apical and lateral bud are reproductive and matures throughout season at different times. Twining tendency are either none, slight, intermediate and pronounced. Plant pigmentation can be none, very slight, moderate at the base and tips of petioles, intermediate, extensive or solid. Terminal leaf shape; globose, sub–globose, sub–hastate and hastate. Plant hairiness assessed on stems leaves and pods is reported as either glabrescent, short appressed hairs var. pubescence or Pubescent to hirsute. Days to flowering is determined by counting days from date of sowing to the stage when 50% of plants have flowered**.** Raceme position is recorded when peduncles have reached full length–either as mostly above canopy**,** in upper canopy or throughout canopy**.** Flowering pigment pattern of newly opened flowers is recorded as Not or pigmented (white), Wing pigmented; standard with light V-shaped pattern of pigment at top center, Pigmented margins on wing and standard, Wing pigmented; standard lightly pigmented, Wing with pigmented upper margin; standard is pigmented and completely pigmented. Flower color is recorded as white, violet, mauve–pink, or Other. Duration of flowering refers to days from first flowers to stage when 50% of plants have finished flowering, Number of pods per plant is determined by mean number of mature pods from 10 randomly selected plants. Pod width [cm] refers to mean width of the 10 pods measured for length. Pod wall thickness is perceived as thin, intermediate and thick. Pod color of mature pod can be pale tan or straw, dark tan, dark brown, black or dark purple. Days to first mature pods refers to days from sowing to stage when 50% of plants have mature pods**.** Immature pod pigmentation is pattern of pigment distribution on full grown immature pods which include None, Pigmented tip, Pigmented sutures, Pigmented valves, green sutures, Splashes of pigment, uniformly pigmented & other. Pod curvature of mature pods can be straight, slightly curved, curved or coiled. Pod length in cm refers to mean of the 10 longest mature pods from 10 randomly selected plants. Seed length [mm] refers to mean of 10 mature seeds excluding those from the extremities of pods, Seed width in [mm] refers to Mean width from hilum to keel of the 10 seeds measured for length, Seed thickness [mm] refers to mean thickness of the 10 seeds measured for length; measured perpendicular to length and width.

Vegetative qualities comprise of leaf color which is the intensity of green color; pale green, intermediate green and dark green. Leaf marking is presence/absence of V-mark on leaflets; absent or present. Terminal leaflet length is terminal leaflet whose shape was recorded earlier. Terminal leaflet width is the widest dimension of the terminal leaflet whose shape was recorded. Leaf texture can be coriaceous, intermediate or membranous. Number of main branches refers to the branches whose origin is in the leaf axils on the main stem; recorded in the 8th week after sowing. Number of nodes on main stem is recorded 3 – 4 weeks after sowing as a mean of 10 randomly selected plants (IBPGRI, 1983).

Cowpea preference by farmers is reported to differ based on growth habit, growth pattern, pigmentation, coloration of flowers and pods, earliness in maturity, pod yield, seed properties and overall productivity (Aysun and Erkut, 2013). Improvement of cowpea has been reported to result in higher number of branches, peduncles, pods per plant and seeds per pod than the local cowpea varieties (Kamai *et al*., 2014). Odireleng *et al*., (2016) reported peduncle length, seed width, seed thickness, pods per peduncle, and 100 seed weight as some of the parameters distinguishing one material from the other. It has been documented that male prefer early maturity, high grain yield, drought tolerance ease of harvesting and good leaf quality in cowpea while the females prefer high grain yield, drought tolerant, pest and disease resistance, ease of harvesting and the leaf quality respectively in Kilifi county (Ndiso *et al*., 2016).

Variation in days to 50% flowering (65 to 82) and 100 seed weight (7 to 15 grams) and positive correlation between numbers of pods per plant with yield of cowpea in East Africa has been reported (Menssena *et al*., 2017). Earliness in growth and development, growth habit, resistance to diseases drought tolerance, high and stable seed yield output, harvest index and good seed quality are traits of focus in breeding programs in the tropical areas (Abadassi, 2015). Among many preferred properties of cowpea, early flowering and maturity observed are reported to be an indication of drought resistance (Hutchinson *et al*., 2017).

The seed characteristics confer different adaptation attributes for protection against pests; seed coat may be too hard to allow penetration of insect larvae hence useful for defense against cowpea pests (Moise *et al*., 2005). In other cases, seed coat color can influence rate of pest infestation. Dense cowpea weevil egg oviposition and high percentage tolerance has been observed in white-coat seed and smooth coat as opposed to rough seed coat (Augustine *et al*., 2016). Cowpea weevil is reported to show preference to shiny seeds in chickpea varieties (Umadevi *et al*., 2018). Physically minute seed make it difficult for insect larvae to develop; nutrient content and composition may have an influence on growth and development of insect pests. Level of damage in sample of cowpea lines tested was reported not to be related to the physical characteristics of the seed but rather biochemical contents (Adebayo *et al*., 2016). Seed coat characters including color are reported to have positive correlation with cowpea weevil infestation in some cowpea varieties (Titilope and Kannike, 2019).

2.2.3 Biochemical composition of cowpea

Biochemical composition comprises of chemical substances that are derived from metabolic processes. In cowpea these biochemical substances are responsible for certain functions within a specific seed. Major characteristic biochemical compounds of cowpea include seed protein, tannins, carbohydrates, amino acids, alpha amylase inhibitor and trypsin inhibitor (Lattanzio *et al* 2005; Maina *et al*., 2015: Nikolova, 2016). The composition and level of the characteristic cowpea compounds differ in cowpea lines. Analysis of seed protein content of cowpea is reported to show variation in overall protein content and subsequently the globulins, albulins and prolamins (Tchiagam *et al.*, 2011). Nikolova, (2016) observed that crude protein, crude fiber and phosphorus content have an influence on resistance of peas to *Bruchus pisorum*; the bruchids caused limited damage on lines which had low protein and low phosphorus content. Oleiveira *et al*., (2018) reported a negative correlation between protein content and cowpea weevil infestation in four cowpea varieties tested with protein content less than 12%. The ability of a certain cowpea line to be resistant or susceptible is dependent on various individual plant and seed characteristics. Some lines' attributes interfere with oviposition, larval development or even adult emergence. In other cases, it is due to toxins present in the lines which kill larva before it grows to maturity (Lattanzio *et al*., 2005).

Legumes are rich sources of amino acids (Ade-Omowaye *et al*., 2015). Three major acids present in cowpea seeds include glutamine which is responsible for glutamic acid asparagine for aspartic acid and phenylalanine (Vasconcelos *et al*., 2010). Chemical and amino acid of cowpea flour were reported to be rich in leucine and iso leucine (Olaleke *et al*., 2006). Alpha amylase inhibitors have potential to alter the catalytic action of alpha amylase on starch and consequently slow down or stop the breakdown of starch to maltose (Kanahaiya and Kumar, 2010). Some amino acids are reported to cause 1-5% level of toxicity against bruchids including *C.maculatus* (Janzen *et al*., 1976)

2.2.3.1 Protein assay

Protein level is determined using semi Microkjeldahl method (AOAC 1995); using the 950.46 method 20.87-37.1.22. The principle is digestion of organic matter with sulfuric acid in the presence of a catalyst, rendering the reaction product alkaline then distillation and titration of the liberated ammonia. Calculation of the nitrogen content is done as a percentage then Multiplication of the result by the conventional factor 6.25 to obtain the protein content in % (Ubini *et al*., 2016).

2.2.3.2 Tannin assay

There are three methods of determining tannin content; vanillin hydrochloric acid assay, proanthocyanidin and protein precipitating phenolics. In vanillin-HCl assay, vanillin reagent reacts with certain types of flavonoids precursors to condensed tannin or any phenol that has unsubstituted resorcinol or phloroglucinol nucleus and forms a colored substituted product which is detected at 500nm (maximum uv absorption at 500nm wavelength of the electromagnetic spectrum. Results from this assay are expressed in Catechin equivalents. Catechin is a tannin precursor that does not precipitate protein (Price *et al*. 1978).

The proanthocyanidin method measures condensed tannin and nontannin monomeric flavonoids (Bullard *et al*., 1981). The standard used in this assay is quebracho tannin, commercially prepared tannin (Pilar River Plate Corp., Newark, New Jersey).

Determination of Protein precipitating phenolics in terms of tannic acid equivalents is described by Hagerman and Butler's (1978) procedure. The assay depends on a phenol measurement, and therefore depends not only on tannin content but also on tannin structure. Cowpea seed tannin content in this case was determined using vanillin hydrochloride method.

2.2.3.3Amino acid assay

The amino acid trade products or premix are dissolved in 0.1M HCl and diluted with sodium citrate buffer. Internal standard solutions are added and the amino acids are separated by an amino acid analyzer or by HPLC using a cation exchange resin and sodium citrate buffer eluent solutions. Standard solutions must contain only the amino acids being analyzed; the amino acids are measured colorimetrically following post-column reaction with ninhydrin, or by fluorescence detection after post-column reaction witho-phthaldialdehyde (OPA). Analysis by Liquid Gas chromatography-Mass spectroscopy process involves hydrolysis, evaporation of the hydrolysates to dryness and reconstitution in 1 ml 90:10 water: acetonitrile. Vortexing is done for 30 seconds, sonication (30 min), and centrifugation at 14,000 rpm. Serial dilutions of the authentic standards containing 18 amino acids (1-105 µg/µl) is analyzed by LC-MS to generate linear calibration curves (peak area vs. concentration) used for external quantification. The equations for the amino acids are identified and amounts in (mg/g) of sample recorded (AOAC, 1995).

2.3 Cowpea weevil

The cowpea weevil (*Callosobruchus maculatus*) is a field to storage pest (Baidoo *et al*., 2010). It has potential to infest and cause up to 100% losses of cowpea seed when stored under no treatment in a span of 3 to 5 months (Cruz *et al*., 2015). Cowpea weevil larvae is known to feed on the protein part which is the food reserve thus depleting nutrients thus causing poor germination, viability and vigor in the field (Olasoji *et al*., 2013). Cowpea weevil is reported as one of the pest causing poor quality seed in cowpea among farmers in Makueni and Taita Taveta (Njonjo, 2018). Bruchids infestation is reported as one of the challenges facing cowpea farmers in arid and semi-arid regions of Kenya because they use informal seed (Owade *et al*., 2020). The physical and chemical characteristics of cowpea line are reported to determine preference of cowpea weevil (Mogbo *et al*., 2014). Damage and losses caused by the cowpea weevil has forced farmers to adopt biological methods of control including use of garlic chilies and peppermint to reduce the cowpea weevil progeny in the stored seed (Tiroesele *et al*., 2015). To achieve cowpea weevil mortality, use of zeolite as a synthetic treatment in cowpea seed before storage is encouraged (Jianhua *et al*., 2017). There is a shift from use of harmful synthetic products to safe biological and plant products due to food safety (Muhammad, 2015). It has been reported that turmeric rhizomes, garlic bulb, sand paper leaves, bitter kola seeds, ginger rhizomes and essential oil reduce egg oviposition, percentage grain damage and increase mortality of cowpea weevil (Asawalam and Anaeto, 2014). Use of ash, paraffin, oil and smoke in cowpea weevil management has been reported in Kenya (Njonjo *et al*., 2019). The challenge with these products is that they only provide a short term solution to the control of this menace.

Cowpea weevils have ovipositional preference when subjected to different varieties of cowpea. Variation in resistance to *Callosobruchus maculatus* has been reported; preferred varieties are white in seed coat color and smooth coat surface (Augustine *et al*., 2016). Shiny and smooth chickpea were more infested (high susceptibility) by *C. maculatus* compared to the large sized wrinkled ones. Size and coat color are linked to cowpea weevil infestation even in other legumes (Kouser *et al*., 2017). Significant differences in cowpea weevil mean development period, adult emergence and weight loss being 32% have been reported (Edde and Amatobi, 2008).

2.4 Storage

At harvesting there is usually a lot of cowpea seed resulting in low prices hence low incomes for farmers. Seed is similarly stored for planting in subsequent seasons (Hutchinson *et al*., 2017). Seed storability is considered to be a management aspect of seed quality. The production potential of a seed is determined by how long the seed can be stored without losing its viability and other preferred characteristics (Morad, 2013). Stored seed is subject to physiological changes and damage from pest infestation that results in deterioration of the seed thus leading to reduced quality. The storage conditions of a seed should therefore be monitored since they have potential to induce seed deterioration (Carlos, 2004).

2.4.1 Storage material

Storage of seed is crucial for providing constant supply of seed and also for maintaining germplasm (Jyoti *et al*., 2017). Shade and ventilation are important considerations in storage of cowpea because they reduce heat that accelerates spoilage (Wambugu and Muthamia, 2009). Cowpea seed is prone to cowpea weevil (*Callosobruchus maculatus)* infestation which is the most economically important pest especially during storage. It can cause up to 80% seed loss in less than 6 months (Oluwafemi, 2012). Damaged seed is considered unfit for use as planting material since its viability and vigor are compromised. Packaging materials can have an effect on storability of the field crops like garden peas and cowpea (Hatice, 2017).

2.4.1.1 Use of plant products in C.maculatus management

Attempts have been made to test different treatment on seed for longevity and cowpea infestations. Use of traditional methods like ash alongside garlic in reducing cowpea progeny development has been on the rise (Njonjo *et al*., 2019). Plant products including, garlic, chilies and pepper mint are reported to significantly reduce grain weight loss, emergence holes and number of eggs laid by *C.maculatus* in cowpea (Tiroesele *et al*., 2015). Moringa and neem products have been found to minimize number of cowpea weevil eggs laid on stored cowpea seed though it is adopted minimally due to bitter taste and cumbersome method of extraction (Ilesanmi and Gungula, 2013). According to Carlos (2004) seed treatment prior to storage is important for the purpose of mitigating pest attack.

2.4.1.2 Management of C.maculatus using pesticides and polythene material

Behera *et al*., (2016) in a bid to test management of ground nut beetle in different storage materials noted that coal tar coated gunny bag and polythene-coal tar coated gunny bag recorded no infestation of groundnut beetle and impregnation of malathion kept seed for five months preventing adult penetration and development. This implies that packaging seed in polythene and gunny bag requires seed treatment. Though the commercial synthetic products (malathion and pirimiphos-methyl), significantly reduce pest infestation, they are expensive (Njonjo *et al*., 2019). Utilization of synthetic pesticides is limited by stringent measures on minimum residue levels in food products and emerging health and environmental concerns of pesticide use (Muhammad, 2015).

2.4.1.3 Controlled atmosphere storage

Controlled atmosphere has potential to be a solution to long-term storage of food grain which involves modified atmosphere of gaseous exchange, humidity and temperatures (Divya *et al*., 2016). Evaluation of hermetic conditions for storage of grains has been carried out for pigeon peas, maize, rice, and wheat (Muhammad, 2015). In Turkey, cellophane, polyethylene pierced cardboards and polyethylene bags have been evaluated for packaging of pulses prior to storage with the aim of increasing longevity and minimize damage due to pests (Hatice, 2017). The Purdue Improved Cowpea Storage bags (PICS) have been tried and well adopted in West Africa (Nigeria, Burkina Faso and Mali) and several other countries for cowpea storage (Divekar and Sharma, 2016). The PICS comprises 3 layered bags with the outer most made of woven propylene and the two inner layers made of 80-micron high-density polyethylene (Baributsa *et al*., 2009). Hermetic storage conditions (PICS and silos) have been applied in Kenya for maize storage against the maize weevil and large grain borer; PICS were reported to be effective for maize weevil (Groote *et al*., 2013). Seed handling techniques have an influence on seed quality. It is therefore important to obtain proper storage techniques to maintain seed quality at its optimum (Adetumbi, 2010). There is need to explore other methods of managing cowpea weevil which can minimize chemical use through identification of characteristics associated with resistance to cowpea weevil and appropriate packaging material for cowpea seed storage.

CHAPTER THREE

INFLUENCE OF PLANT MORPHOLOGICAL CHARACTERISTICS ON COWPEA WEEVIL SEED INFESTATION

Abstract

Cowpea; *Vigna unguiculata* is a nutritious legume consumed as vegetable, grains stew or fed to animals as forage. Seed is the main propagating material. Cowpea weevil is a field to storage pest causing damage and poor quality seed upon infestation. It can cause up to 100 % seed loss when stored without any form of treatment. This study was therefore carried out to determine the influence of plant and seed characteristics on *Callosobruchus maculatus* infestation of cowpea seed. Plant characteristics were evaluated in field experiments. After harvesting, seed characteristics were determined at the plant physiology laboratory in JKUAT. Plant and seed characteristics were determined according to cowpea descriptors. Descriptive statistics were used to describe the cowpea lines. Variation among cowpea lines was determined by Analysis of Variance using GenStat Version 17 and means separated by LSD at ($p \leq 0.05$) level of significance. Principal Component Analysis and multivariate analysis were evaluated in PAST Version 4.03 and were used to classify cowpea lines based on cowpea characteristics. Pearson correlation analysis was carried out in GenStat Version 17 to determine the relationship between plant and seed characteristics, and pest infestation. Based on seed coat characteristics, cowpea lines were classified as grey mottled, white, light red, red, black, cream, brown mottled and mixed types. ANOVA for plant and seed quantitative characteristics revealed that lines used in the trials were significantly different ($p \leq 0.05$). Plant and seed characteristics had an influence on *C. maculatus* infestation. Large and lightly colored seeds were more infested (>20%) compared to dark and small seeds (< 5%). Days to flower and days to maturity were positively correlated with weevil infestation. Plant and seed characteristics were shown to influence the cowpea weevil preference among the cowpea lines. Cowpea lines that were late maturing (>100days), light color (white, cream), large seed size (>14g) had higher cowpea weevil infestation. This information is important for farmers and breeders in selecting desirable lines and carrying out selection for cowpea improvement respectively.

3.1 Introduction

Cowpea is solely propagated through seeds making seed quality an important aspect in its production (Coulibaly *et al*., 2008). Among the major challenges facing production of this crop is poor seed quality, seed pest infestation and unavailability of quality seed (Njonjo *et al*., 2019). Lack of quality seed at planting time is a common problem to cowpea farmers in Kenya. This can be attributed to use of "farmer saved seeds" and damage caused by cowpea weevil (*Callosobruchus maculatus*) (Kumar and Kalita, 2017). Poor seed quality results in poor stand establishment, reduced productivity, hence leading to food insecurity and inability to meet the needs of rapidly increasing population (Njonjo *et al*., 2019).

Morphological characteristics including plant features and seed characteristics differ among the cowpea lines. The plant and seed characteristics are important in carrying out selection and breeding. Some of the preferred properties of cowpea among farmers include erect growth habit, resistance to diseases and early maturity (Abadassi, 2015). Variations in cowpea are well documented for growth pattern, flower color and seed coat characteristics (Aysun *et al*.,2013). Kamai *et al*., (2014) reported that branches peduncles and pods per plant are morphological characteristics targeted during cowpea crop improvement because they affect vegetable and grain yield. Studies on morphological diversity of cowpea show that variation exists among cowpea lines in days to 50% flowering, 100 seed weight and number of pods per plant (Menssena *et al*., 2017). Physical and chemical characteristics of cowpea have been reported to have an influence on growth and development of cowpea weevil (Mogbo *et al*., 2014).

In a bid to identify characteristics of resistant cowpea lines and management of cowpea weevil, an assessment was carried out to determine the influence of plant and seed characteristics on cowpea weevil infestation. The knowledge on relationship between cowpea seed characteristics and cowpea weevil infestation is expected to inform research and farmers in the identification and selection of appropriate lines that are resistant to *C. maculatus* infestation. Selected lines can be widely exploited by farmers in identifying materials for subsequent planting.

3.2 Materials and methods

3.2.1 Study site and source of material

The study was carried out in Jomo Kenyatta University of Agriculture and technology (JKUAT) from October 2016 to August 2017. In order to evaluate plant morphological characteristics, cowpea lines were planted at JKUAT farm in Juja. Juja is located 13 kilometers from Thika town and approximately 35 kilometers from Nairobi in Kiambu County at 1°11’ 0’’ S, 37° 7’ 0’’ E. The site is located in Agro-ecological Zone 4 (Foeken, 1994). The annual minimum and maximum temperature experienced is 10.4 and 22.7 degrees Celsius respectively. The mean annual rainfall is 856 mm; with potential evaporation of 5.0 mmd^{-1}. Individual cowpea line seed characteristics were determined at the plant physiology laboratory in JKUAT. Fifty cowpea seed varieties were used for the experiment. Thirty-four varieties were obtained from the National Gene Bank of Kenya, seven from farmers’ collection, eight lines from World Vegetable Centre through Hortinlea SP6 project, and one commercial line (Table 1). Cowpea lines were procured from regions that popularly produce cowpea that is Eastern Kenya (Machakos, Makueni, Kitui), Western Kenya (Vihiga), and Central Rift (Kabarnet, Marigat and Lambwe), World Vegetable Centre (Centre for seed improvement for vegetables in Arusha) and Kenkunde; a registered variety in Kenya.

3.2.1 Assessment of plant morphological and seed characteristics

Fifty lines were planted at JKUAT farm in a randomized complete block design with each line replicated three times. The plants were spaced at 60 cm by 30 cm between rows and within rows respectively. Two seeds were planted per hole, and subsequently gapping and thinning done to achieve one plant per hole at three-week stage as shown in Plate 1. Each plot measured 2.7 by 1.2 m each having 30 plants. The recommended agronomic practices were carried out except that no pesticides were applied during cultivation. Vegetative data was obtained in the 6th week after sowing and inflorescence data was collected when 50% of the plants in a plot began to flower (IBPGRI, 1983). Data was obtained for; plant growth pattern, plant growth habit, plant height, plant hairiness, plant pigmentation, leaf color, leaf shape, leaf texture, leaf length, leaf width, number of nodes, number of main branches, raceme position, flower color, flower pigment pattern, immature pod pigment, pod curvature, pod thickness, pod color, days to flowering, days to maturity and number of pods per plant as described by IBPGRI (1983). The seeds were harvested when pods were dry. They were cleaned and kept for further laboratory evaluation. Data on seed coat color, seed length, seed width**,** seed coat thickness, and 100-seed weight were determined as per IBPGRI (1983). Data on seed length, seed width, and seed coat thickness was obtained by getting the mean for 10 seeds of each line. 100-seed weight was obtained for each line by counting a total of 100 healthy seeds per line and measuring their weight.

Plate 1: Field layout of cowpea lines at 21 days (a), and 42 days (b) after planting

3.2.2 Pest infestation and damage of cowpea seeds

After harvesting cowpea seeds were cleaned and used for assessment of pest infestation. The insect damage was assessed on the 50 cowpea lines. The experiment was set up at JKUAT in the laboratory in a completely randomized design and replicated three times. The control line was Kenkunde which is a popularly grown registered variety in Kenya. The experiment was set up in ambient conditions of (20–28°C and 60%–90% relative humidity) (Tiroesele *et al*., 2015). Inoculum was based on inherent *C. maculatus* in the freshly harvested seed and infested seed available in the laboratory from untreated cowpea harvested in the previous season. The daily laboratory temperature ranged from 12-27^{0}C and relative humidity ranged from 65%-75%. The seeds were kept in cellulose paper bags for three months. The seed damage was evaluated by assessing damage caused by *Callosobruchus maculatus* by observing emergence holes. Number of seeds with emergence holes was obtained by counting the number of seeds with holes from a random subsample of 100 seeds. The damaged seeds per 100 seed sample were calculated as follows:

%damaged seed= (Number of seeds with holes/total number of seeds picked)*100 (Augustine *et al*., 2016).

3.3 Data analysis

Descriptive statistics was used to determine the distribution of cowpea lines used for the trial into various traits based on plant characteristics. Analysis of Variance (ANOVA) was determined in GenStat Version 17 to determine variation among cowpea lines and means separated using LSD at ($p \leq 0.05$) based for quantitative data. Principal component analysis (PCA) in PAST software version 4.03 was used to determine the parameters that significantly contributed to the observed variation. Multivariate cluster analysis was carried out in PAST software version 4.03 to classify cowpea lines based on seed characteristics. Pearson correlation analysis in GenStat Version 17 was done to determine relationship between plant and seed characteristics and pest infestation.

3.4 Results

Classification based on seed coat color of lines used in the experiment is presented in Table 1. The representative seed coats of the cowpea lines distributed into eight colors are presented in (Plate 2).

Table 1: Seed characteristics of the experimental material

Coat Characteristics	Grey Mottled	White	Light Red	Red	Black	Cream	Brown mottled	SP6 LINES
LINES	GBK003658	**GBK003656**	GBK0034722	**GBK003660**	**GBK003695**	**GBK003645**	GBK003 654	**9334**
	GBK003660	**GBK003659**	GBK003703	KAK 2	**GBK003697** GBK003699	GBK003650		ACC 20
	GBK003702	GBK003674	**GBK003814**	**KENKUNDE**	GBK003876 GBK026941	GBK003652	**GBK003 689**	**ACC 25**
	GBK003780	GBK003690 GBK003707	GBK026958	KOL 1	GBK026958	GBK003700	GBK003 723	ACC6
	GBK003820	GBK046540	GBK034732	**LAM 4**		GBK003702		**DAKAWA**
	GBK005173		KOL5	MAR 3		GBK003721	GBK003 724	EASEED
				MAR 5			**KAB1**	EX ISEKE
								GKKCP-2
Source	Gene Bank of Kenya							AVRDC and Kenyan Farmers

Classification of cowpea lines used in the experiment based on seed coat color and source of the lines

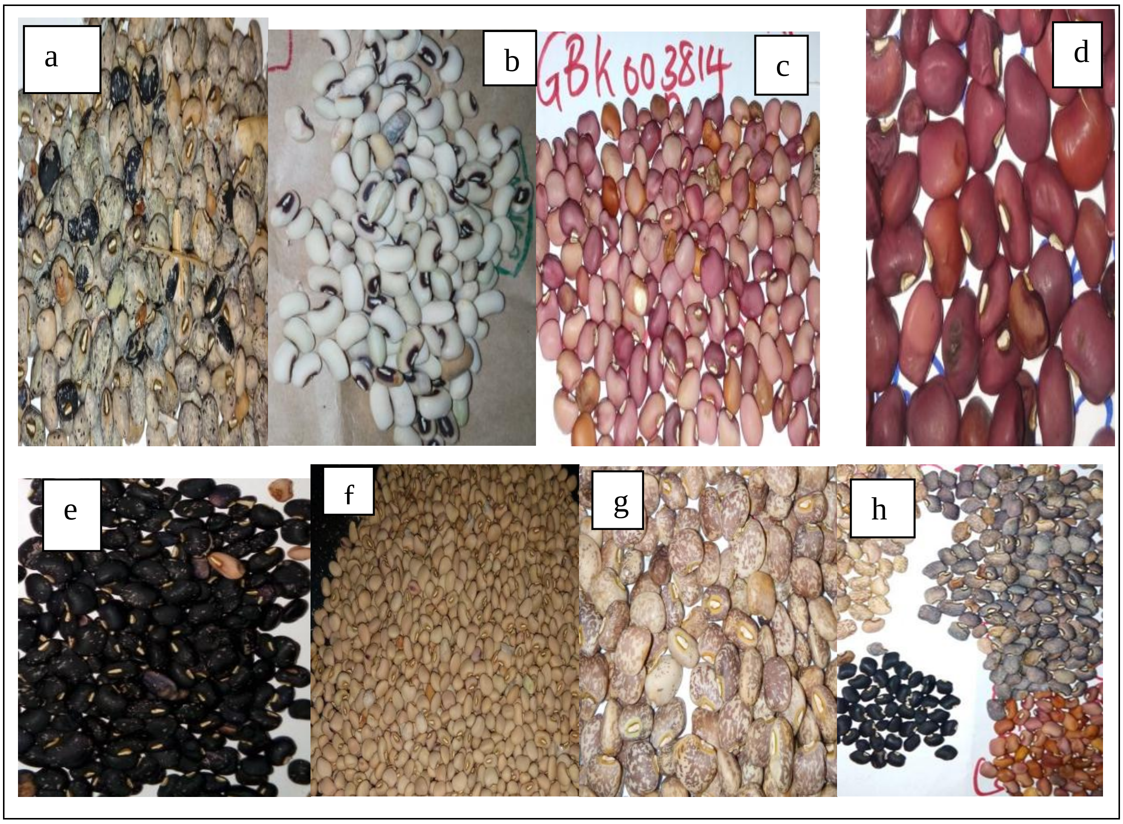

Plate 2: Representative characteristics of each of the classified groups of cowpea lines used in the trial.

Cowpea lines: a-grey mottled; b-white; c-light red; d- red, e-black, f-cream, g-brown mottled, h-mixed types(SP6)

The lines varied significantly ($p \leq 0.05$) for plant characteristics during the vegetative and inflorescence stages (Table 2). The leaf length ranged from 4 to 13.8 cm with the average leaf length being 8cm. cowpea lines comprising (16%) had broad leaves (above 10cm) and 84% were narrow leaved (Plate 3). The leaf mean width was 6.5 cm with a maximum of 7.7 cm and a minimum of 3 cm. Average plant height was 19.5 cm and the minimum was 16.0cm. Lines contributing to 22% did not attain the average height. The lines had a mean of 10 nodes each and the main branch mean was 6, the maximum number of main branches recorded was 11 and a minimum of 4. The frequent growth habit among the 50 lines was acute erect comprising 44%, followed by semi erect at 30% prostrate at 22% and the least was erect at 4%. Determinate growth pattern comprised of 90% of the lines while 10% displayed indeterminate growth pattern. Cowpea lines with no twining tendency were 52% while 48% had either slight or intermediate twining tendency. Extensive plant pigmentation was displayed by 20% of the lines, 14% were not pigmented and the rest had intermediate pigmentation. Globose type of leaflet shape was observed on 40% of the cowpea lines, 40% of the lines had the hastate and 20% had sub-globose leaflet shape. It was observed that the lines had seeds had seeds of different sizes (Table 2). The seed length ranged from 5.9-8.7 mm with a mean of 7.6mm, seed width ranged 4.8-7.1mm and a mean of 6.2mm and seed thickness ranged from 4.0-5.8mm and a mean of 4.8mm. 100-seed weight ranged from 8.6g (smallest seed) to 19.57g (largest seed) as shown in (Table 2).

Table 2: Distribution of cowpea lines among the respective traits of the evaluated characters at vegetative and inflorescence stage

Characteristic	Distribution (%)	Range	Mean
Leaf length (cm)	Broad leaf 16	4-13.8	8
	Narrow leaf 84		
Leaf width		3-7.7	6.5
Plant height (cm)	Above average 22		19.5
	Below average 78		
Nodes			10
Main branches		4-11	6
Growth habit	Acute erect 44		
	Semi-erect 30		
	Prostrate 22		
	Erect 4		
Growth pattern	Determinate 90		
	Indeterminate 10		
Twining tendency	No twining 52		
	Slight/intermediate 48		
Plant pigmentation	Extensive 20		
	None 14		
	Intermediate 66		
Leaflet shape	Globose 40		
	Hastate 40		
	Sub globose 20		
Plant hairiness	Glabrescent 100		
Leaf color	Dark green 32		
	Intermediate green 34		

Characteristic	Distribution (%)	Range	Mean
	Pale green 34		
Leaf texture	Intermediate 90		
	Membranous 10		
Raceme position	Above and throughout 76		
	Upper canopy 24		
Flower pigment pattern	Wing pigmented 78		
	Not pigmented 22		
Flower color	Violet 66		
	White 34		
Immature pod pigmentation	Uniform 10		
	Pigmented tip and splashes 90		
Pod curvature	Slightly curved 50		
	Curved 50		
Pod color	Dark tan 58		
	Pale tan 21		
	Green 21		
Pod thickness	Thick pods 56		
	Thin pods 44		
Seed length (mm)		5.9-8.7	7.6
Seed width (mm)		4.8-7.1	6.2
Seed thickness (mm)		4-5.8	4.6
100 seed weight (g)		8.6-19.57	13.5

Traits for the different characters when the plants were evaluated in the field and seeds in the laboratory

All cowpea lines had the same texture in terms of plant hairiness; glabrescent. Cowpea lines had dark green leaves (32%), 34% intermediate green and the rest were pale green 34 %. Intermediate leaf texture was observed in 90% of cowpea lines and 10% had membranous leaf texture. Cowpea lines were both narrow and broad (Plate 3).

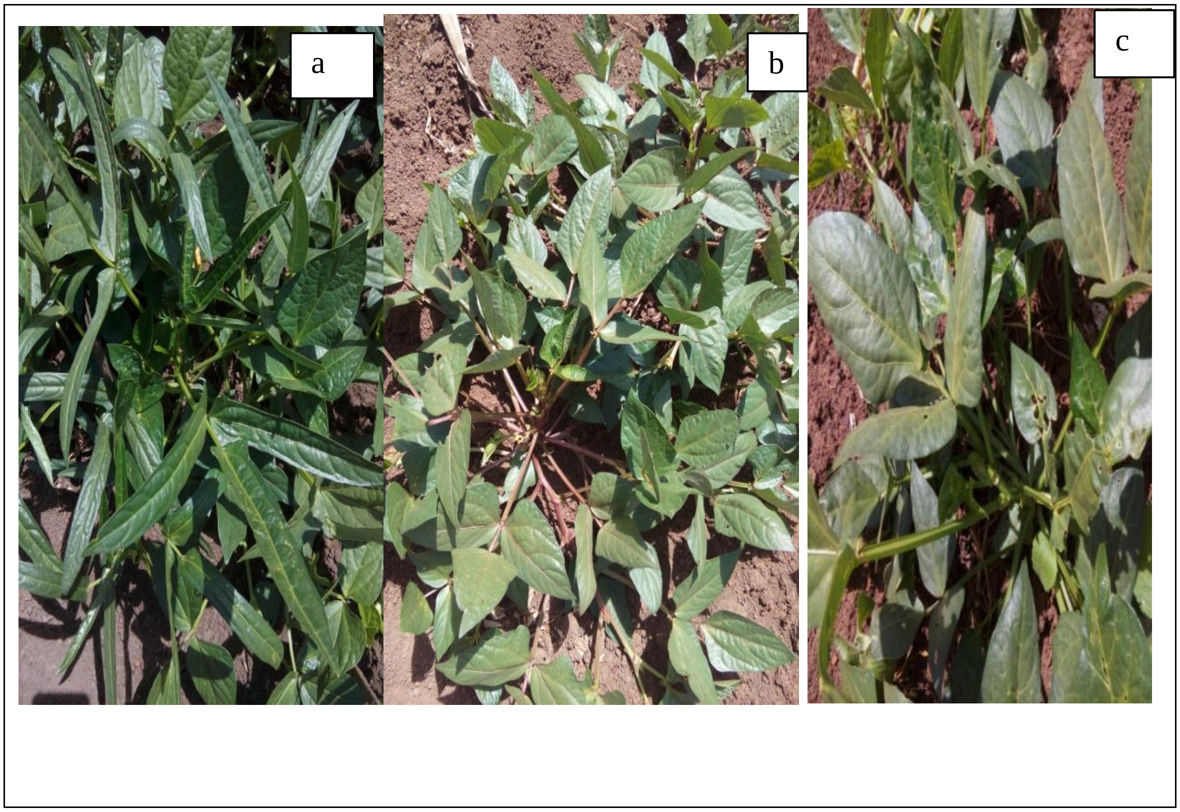

Plate 3: Variation in leaf characteristics among cowpea lines planted in the field

a- Narrow leaved and b-broad leaved pigmented, c-broad leaved non-pigmented.

Raceme position distribution among cowpea lines were; 76% mostly above canopy and throughout the leaf canopy and 24% was above the canopy (Plate 4).

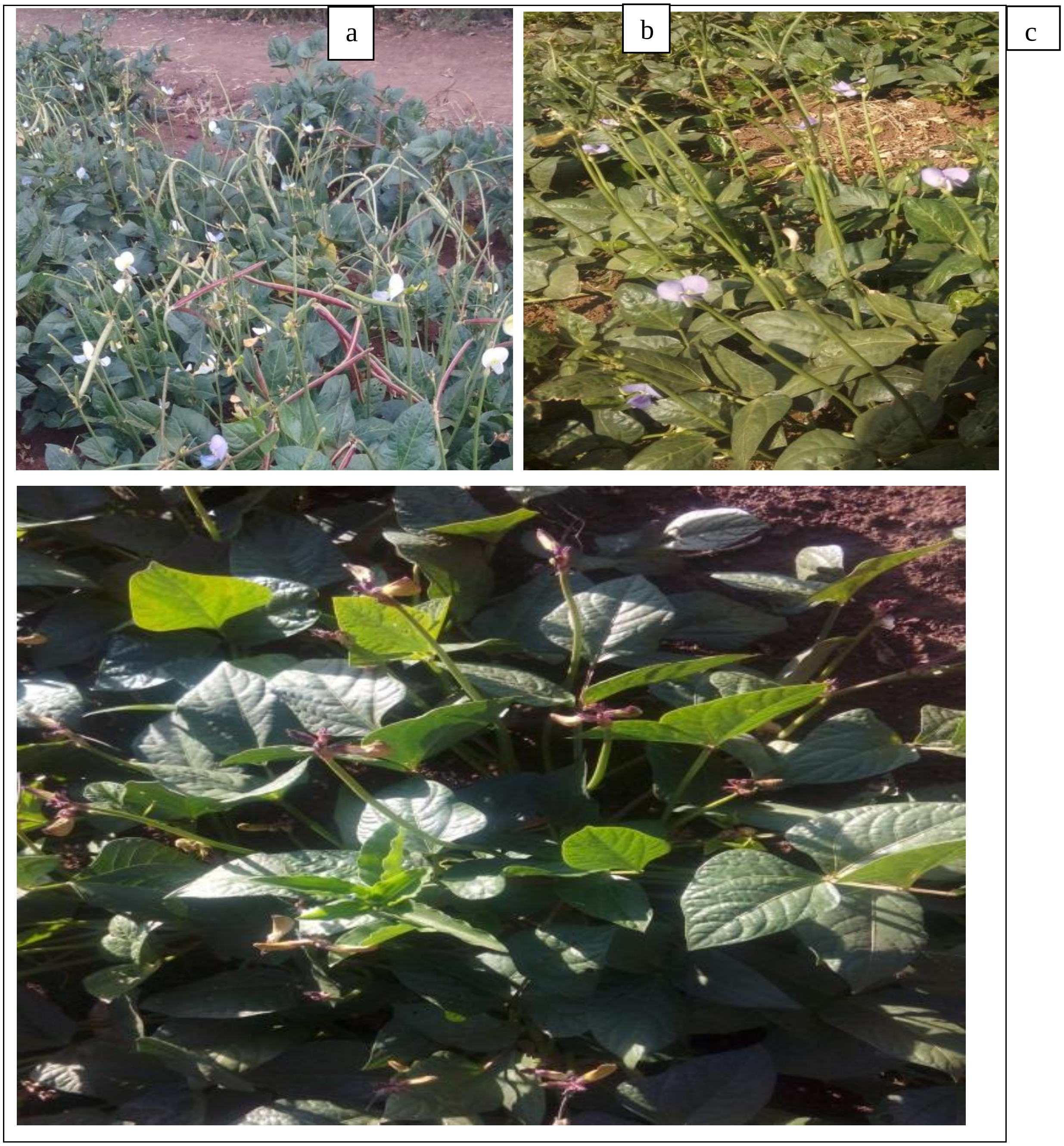

Plate 4: Variation in the position of the raceme in the cowpea lines planted in the field

a- mostly above canopy b-in upper canopy, c- throughout canopy

For flower pigment pattern, the wing pigmented were 78%, and not pigmented were 22%. Cowpea lines contributing to 66% had violet flowers and 34% had white flowers (Plate 5).

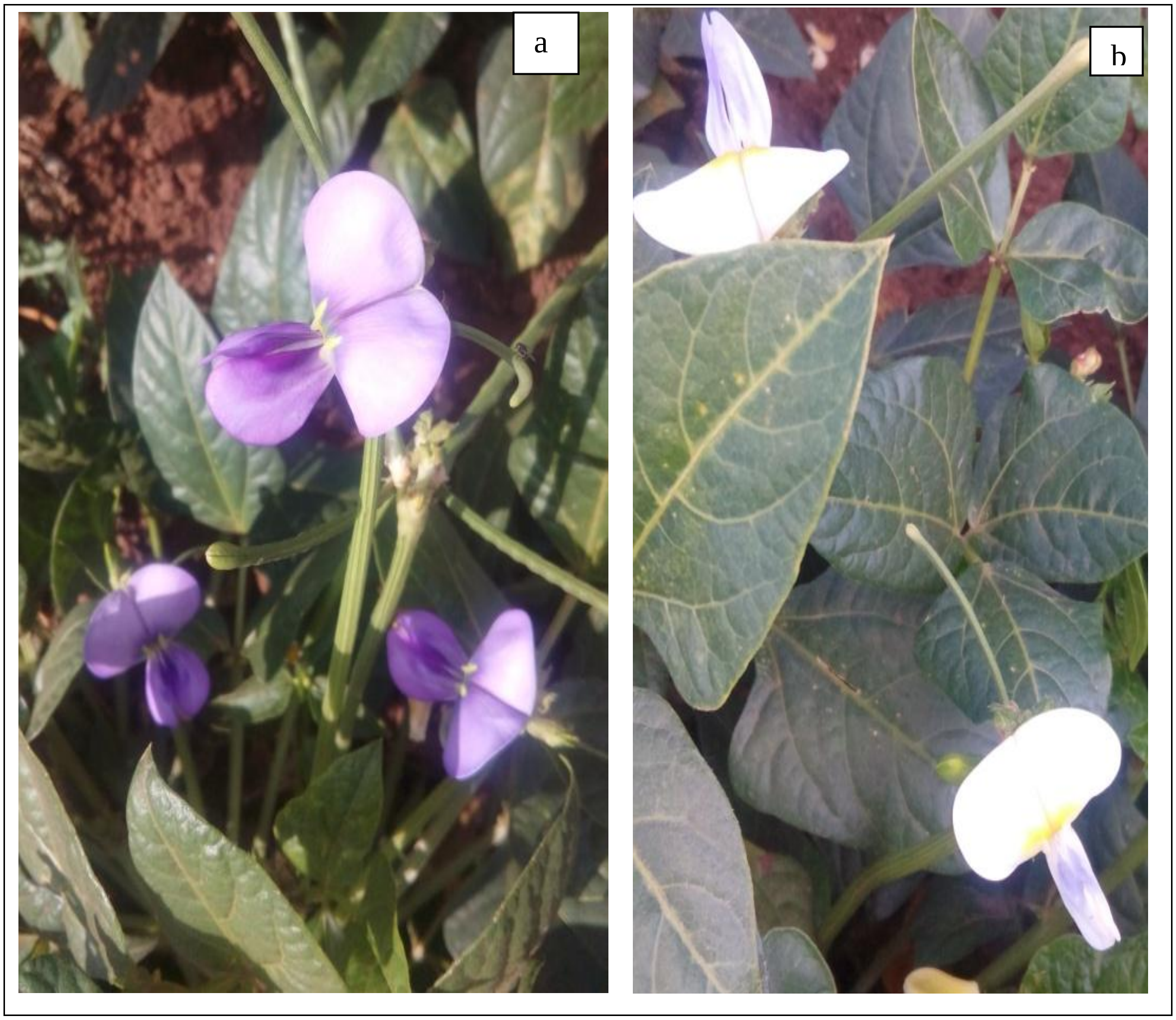

Plate 5: Variation in flower color for cowpea lines planted in the field

Flower color for the different cowpea lines assessed: a) violet b) white

Pattern of pigment distribution on full grown immature pods among the lines was 10% uniform pigmentation and 90% had pigmented pod tips and splashes of pigment (Plate 6). For pod curvature; 50% of the lines has slightly curved pods and 50% had curved pods.

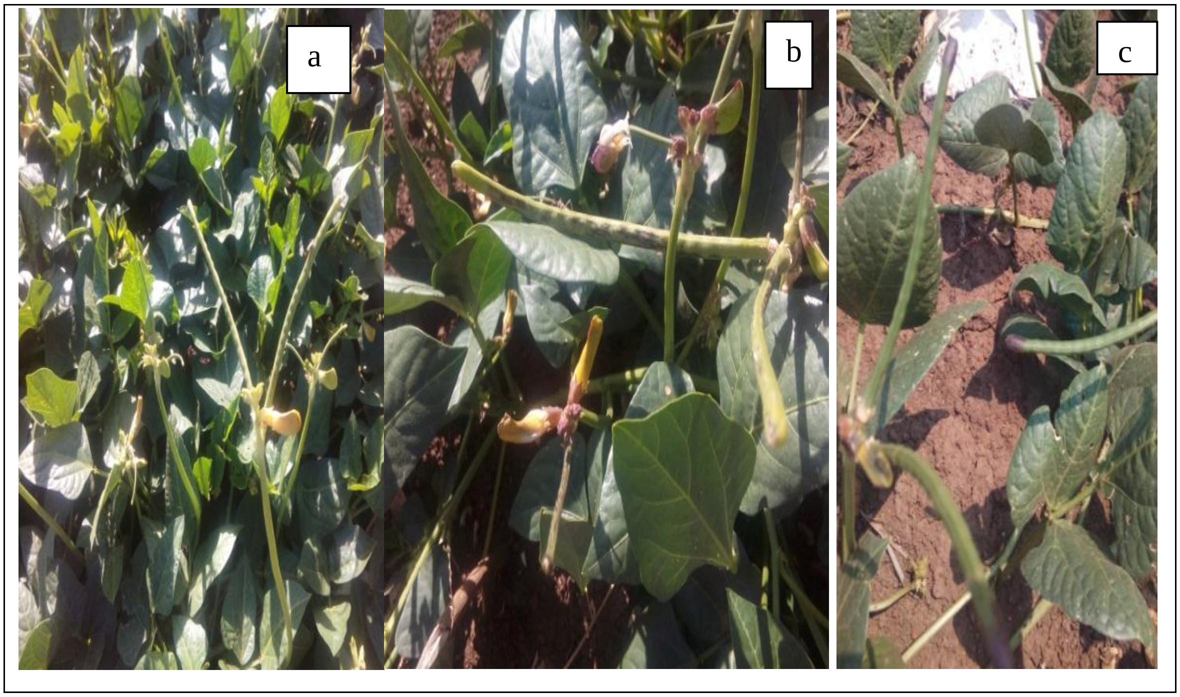

Plate 6: Variation in pattern of pigment distribution on fully grown immature pod

Pod pigmentation among cowpea lines a-none b- splashes of pigment, c- pigmented tip.

Dark tan color was perceived on 58% of cowpea lines, 21% were pale tan and 21% were green. For pod thickness, 56% of cowpea lines portrayed thick pods while 44% had thin pods (Plate 7).

Plate 7: Pod coloration at maturity of cowpea lines

Pod coloration among the cowpea lines as evaluated in the field a-dark tan b-pale tan/straw c- green.

Cowpea lines were significantly different ($p \leq 0.05$) in the respective characteristics at vegetative and reproductive stages (Table 3). The variation among the lines was significant ($p \leq 0.05$) for days to flowering, days to maturity, pod length, pods per plant and 100 seed weight. Cowpea lines maturity ranged from 72-113 days. The cowpea lines were also different in terms of pod sizes. Small seeded cowpea lines had low 100 seed weight while large seeded cowpea lines had high 100-seed weight ranging from (8.60-19.57g).

Table 3: Days to maturity, size of pods, number of pods and seed yield of cowpea lines

Plant Character/performance range	P value	Attributes/Cowpea line;	Attributes/Cowpea lines
Days to flowering	P≤0.001	**Early flowering**	**Late flowering**
63-75		Dakawa, GBK 3674, GBK3723, GKKCP-2, ACC20	GBK3660-1, GBK3700, GBK026958-2, 9334, GBK3656
Days to harvest	P≤0.011	**Early maturing**	**Late maturing**
72-113		ACC25, GBK3689, GBK3695, DAKAWA, MAR3	ACC6, GBK3652, GBK3707, GBK026941, 9334
Pod Length	P≤0.01	**Short pods**	**Long pods**
12.17-18.37 cm		GBK3659, GBK026941, GBK3697, GBK3645, EASEED	9334, MAR 5, GBK3689, GBK3660-2, DAKAWA
No. of Pods per plant	P≤0.047	**Least pod number**	**Highest pod number**
7-28		GBK3659, GBK3658, GBK3652,9334, GBK3700	MAR3, GBK3699, DAKAWA, 3656, KAB1
100 seed weight	P≤0.001	**Small seed size**	**Large seed size**
8.6 -19.57g		GBK3659, GBK3724, GBK3658, 9334, EASEED	KOL1, MAR3, LAM4, GBK3814, GBK3689, GBK5173

Variation in plant and seed parameters ($p \leq 0.05$) at inflorescence and maturity

Seed damage level was assessed on seed stored for three months in cellulose paper bags (Table 4). The cowpea lines significantly differed ($p \leq 0.05$) in damage caused by *C. maculatus* low at <4% while highest at 54%. Cowpea lines showed different levels of resistance and susceptibility based on inherent characteristics. The least damaged lines include; ACC 20, ACC 25, DAKAWA, KAB 1, KOL5, MAR 3, GBK 003674, GBK003697, GBK003699, GBK003702 and GBK 026941.

Most damaged lines were KAR 2, GBK034722, GBK003876, GBK003656, GBK003658, GBK003724, GKKCP-2; MAR 5 GBK003660-1 and KENKUNDE.

Table 4: Variation among cowpea lines based on damaged seed during storage per 100 seed sample.

Cowpea line	Seed damage (%)
9334	0^{a}
ACC 20	**2.6^{abcd}**
ACC 25	**2.57^{abcd}**
ACC 6	23.73^{jkl}
DAKAWA	**0.97^{abc}**
EASEED	6.1^{abcdef}
EX ISEKE	7.33^{bcdefg}
GBK003645	9.63^{defg}
GBK003650	6.33^{abcdef}
GBK003652	13.83^{ghi}
GBK003654	12.13^{fgh}
GBK003656	**33.93^{mn}**
GBK003658	**38.33^{no}**
GBK003659	18.8^{hij}
GBK003660-1	**51.03^{qr}**
GBK003660-2	4.1^{abcde}
GBK003674	2.7^{abcd}
GBK003689	0.5^{ab}
GBK003690	4.27^{abcde}
GBK003695	11.83^{fgh}
GBK003697	2.8^{abcd}
GBK003699	**0.8^{abc}**
GBK003700	13.93^{ghi}
GBK003702-1	1.53^{abc}
GBK003702-2	3.83^{abcde}
GBK003703	18.67^{hij}
GBK003707	7.2^{bcdefg}
GBK003721	7.9^{cdefg}
GBK003723	27.97^{klm}
GBK003724	41.77^{op}
GBK003780	3.13^{abcd}
GBK003814	2.97^{abcd}
GBK003820	10.47^{efg}
GBK003876	33.67^{mn}
GBK005173	6.23^{abcdef}
GBK026941	3.57^{abcde}
GBK026958-1	11.7^{fgh}
GBK026958-2	20.63^{ij}
GBK034722	30.47^{lm}
GBK034732	5.17^{abcdef}
GBK046540	21.23^{jk}
GKKCP-2	44.77^{opq}
KAB 1	3.8^{abcde}
KAR 2	28.13^{klm}
KENKUNDE	54.73^{r}
KOL 1	6.4^{abcdef}
KOL 5	2.53^{abcd}
LAM 4	9.23^{defg}
MAR 3	1.67^{abc}
MAR 5	48.2^{pqr}

Damage level for the 50 cowpea lines ($p \leq 0.05$). Letters represent significance. Numbers with the same letter are not significantly different.

The plant characteristics evaluated were reduced to two major principal components; the first-principal component accounting for 51.5% of the total variation was contributed by plant height, days to flower and days to maturity. Principal component two that accounted for 25.4% of the total variation was associated with plant pigmentation (Table 5).

Table 5: Plant morphological parameters associated with 76.9% of the total variation within the cowpea lines

Plant character	PC1 (51.5%)	PC2 (25.4%)
Leaf Shape	0.05238	0.01723
Leaf length	0.10571	0.36893
Leaf color	0.07087	0.03791
Leaf width	0.05802	0.20932
Leaf texture	0.02068	0.0404
Plant height	**0.97494**	0.06678
Plant pigment	0.01674	**0.70992**
Branches	0.05344	0.40174
growth habit	0.07734	0.05236
Nodes	0.13279	0.37964
Days to flower	**0.76428**	0.62313
Days to harvest	**0.64265**	0.75468
Pod length	0.00158	0.07913
Pod color	0.0359	0.03916
Pod pigment	0.01016	0.01047
Pod number	0.0384	0.18511

Principal component analysis for cowpea plant characteristics

Data on plant and seed characteristics subjected to cluster analysis distinguished the cowpea lines into two main groups indicating (33%) similarity among the lines (Figure 1). The cowpea lines were initially grouped into two main groups, one having 12 lines and the other having 38 lines. Group two was further subdivided into two other clusters (15%) (A and B) group A having 29 cowpea lines and group B having 9 cowpea lines.

The cowpea line in group 1 were observed to be large in size, light colored, those in group 2A were medium in size and red in color and mottled. The ones in group 2B were small sized and had low 100 seed weight; they were mostly dark colored (black) and were less damaged by the cowpea weevil (< 5%). While those in group 1 were more damaged by the cowpea weevil (above 25%)

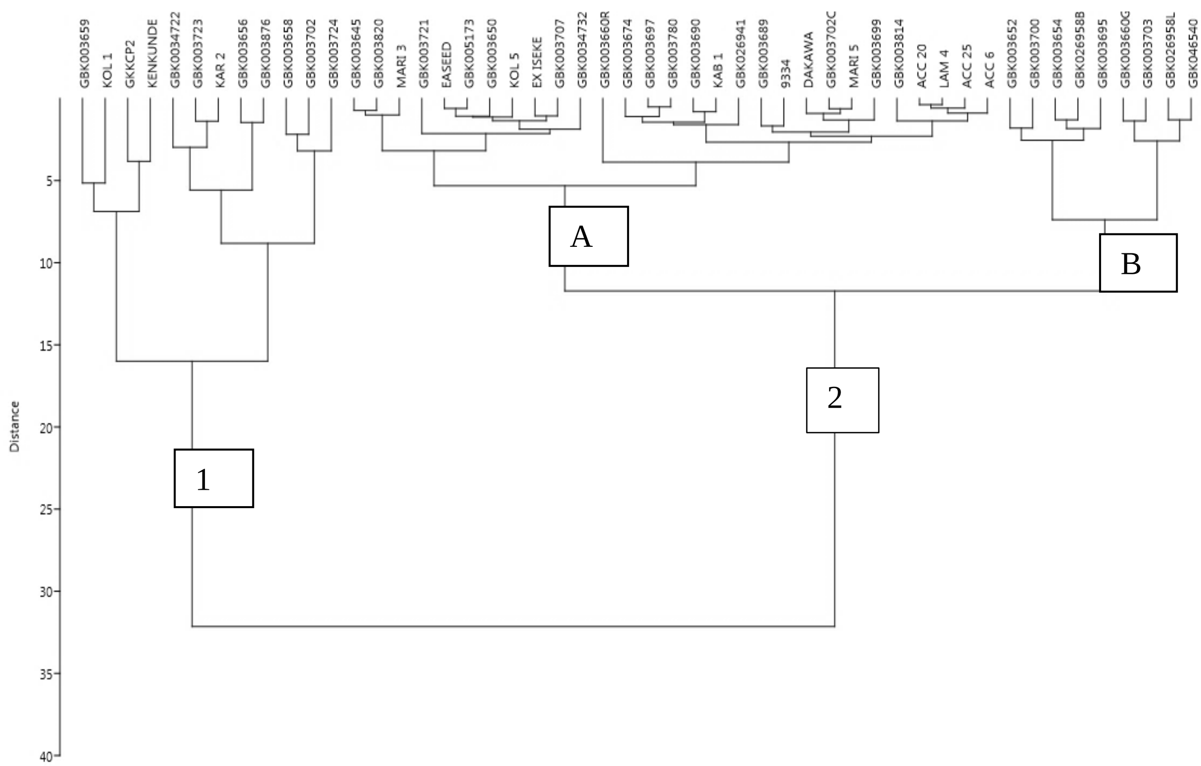

Figure 1: Classification of cowpea lines based on seed characteristics

There was significant positive correlation between plant characteristics and cowpea weevil infestation (Table 6). Days to flower (r=0.81, r^2 = 0.66), days to harvest (r=0.89, r^2 = 0.79) branches (r=0.8, r^2 = 0.64), and leaf length (r=0.76, r^2 = 0.58) were all positively correlated to cowpea weevil damage. The late maturing cowpea lines were more infested than the early maturing cowpea lines (above 25-54%).

Table 6: Pearson Correlation among cowpea lines characteristics at inflorescence and damage caused by cowpea weevil infestation

Parameter tested	**Value of coefficient of correlation(r)**				
%damage	-				
No of Days to flower	**0.81***	-			
No of Days to harvest	**0.89***	0.07	-		
No of Branches	**0.8***	0.41	0.11	-	
Leaf length	**0.76***	0.32	0.15	0.06	-
	% damage	**No of Days to flower**	**No of Days to harvest**	**No of branches**	**Leaf length**

Correlation between cowpea plant parameters and cowpea weevil infestation * indicate significant correlation

3.5 Discussion

Plant and seed characteristics differed significantly among the various cowpea lines. Leaf size and leaf texture are important for selection of vegetable cowpea (Hutchinson *et al.*, 2017) Indeterminate growth pattern is associated with continuous production and non-uniform maturity hence more yield while determinate growth pattern is associated with uniform maturity therefore allows for application of mechanization (Kumar *et al.*, 2015). Morphological characteristics including plant features and seed characteristics differ depending on the cowpea line. These plant and seed characteristics are important to farmers for the identification and characterization of preferred cultivars (Abadassi, 2015); early maturity is associated with drought tolerance. Development of new and improved varieties of cowpea is reported to focus on traits such as growth pattern, flower color and seed coat characteristics (Aysun and Erkut 2013). Kamai *et al*., (2014) established that branches, peduncles, and pods per plant are morphological characteristics that are considered for cowpea crop improvement because they determine the vegetative and grain yield. Contrary results have been reported by Gerrano *et al*., (2016); in their study it was noted that parameters contributing to variation in cowpea were; leaf area, leaf area index and the number of seeds per plant.

The findings in the current study are however similar to Manggoel and Uguru (2011) who established days to 50% flowering and maturity in cowpea as some of the traits contributing to the total variation. It has also been observed in rice lines that some of the traits contributing to variation are days to 50% flowering and days to maturity (Riaz *et al*., 2018). Shiny and smooth color in chickpea is an attractant attribute to cowpea weevil. Knowledge of cowpea seed traits is important to farmers because they select cowpea seed based on seed coat color, seed size, taste and relative resistance to diseases (Kumar *et al*., 2015).

Cowpea lines that take long to flower and eventually to mature are more predisposed to the weevil infestation while in the field prior to storage (Baidoo *et al*., 2010). Cowpea lines that had colored pigmented and thin pods were more infested eventually during storage. Thick pods create a physical barrier that hinders the weevil larvae from easy penetration. The observations are in agreement with what Sousa *et al*., (2015) established that thick pods in cowpea had a positive correlation with cowpea weevil infestation. Seed size influences weevil infestation; GBK046540, GBK003695 and GBK026958BL, GBK003699 in this experiment which were comparatively smaller and dark colored were less infested (<4%) than those that were large and light colored GKKCP2, KENKUNDE, GBK003656, GBK003658. These results are in agreement with Duraimurugan *et al*., (2014) who found that physical seed characteristics of green gram and black gram had the same influence on *Callosobruchus chinensis* oviposition and infestation. Similarly, in a study on the resistance of grains to pulse beetle in chick pea it was found that small seeds were less infested compared to those that had a high 100 seed weight (Chandel and Bhadauria, 2015). Chick pea varieties which is a legume classified as susceptible to cowpea weevil is reported to exhibit smooth seed coat and white coat color as observed in the current study (Kouser *et al*., 2017). Contrary results on correlation between hundred seed weight, seed color and seed sizes with cowpea weevil infestation have been reported (Fawki *et al*., 2012). Augustine *et al*., (2018) reported a positive correlation between seed size and oviposition preference of the pulse beetle in cowpea though not significant. In the current study cluster analysis grouped cowpea lines that were most damaged and their observed characteristics comprised of; late maturity, light color and high 100 seed weight. The relative resistance is based on the presence of components like tannins present in dark seeds that hinder oviposition. Kamble, *et al*., (2016) reported that pulse beetle preferred white to brown seeds as opposed to yellow seeds. The preference of white seeds to brown seeds is similar to the current study. Chickpea seed with white seed coat and smooth texture were more susceptible to cowpea weevil infestation compared to chickpea seeds that were brown with rough textured seed coat (Kouser *et al*., 2017).

3.6 Conclusions and recommendations

Plant and seed characteristics of cowpea lines differ and they have an influence on cowpea weevil infestation. The information on seed characteristics can be exploited in plant breeding programs and it is important for farmers in selection of seed. Farmers' lines ACC 20 and ACC 25 were superior to registered lines like Kenkunde and GKKCP-2 and lines from the gene bank since they experienced less damage (<4%) by the cowpea weevil. The following lines can be considered for production by farmers since they are relatively resistant to the cowpea weevil ACC 20, ACC 25, DAKAWA, KAB 1, KOL5, MAR 3, GBK 003674, GBK003697, GBK003699, GBK003702 and GBK 026941.

CHAPTER FOUR

INFLUENCE OF COWPEA SEED BIOCHEMICAL COMPOSITION ON COWPEA WEEVIL INFESTATION

Abstract

Seed biochemical components differ from one cowpea line to another. Cowpea is rich in both nutrient and anti-nutrient properties. These properties of cowpea can be exploited as a defense mechanism against pest infestation. Of importance are the proteins, tannins and amino acids besides alpha amylase inhibitor and trypsin inhibitor properties. An experiment was carried out on cowpea lines to determine variation among cowpea lines and the relationship between cowpea seed biochemical composition and cowpea weevil infestation. The experiment was carried out in JKUAT where the cowpea lines were planted out in the field prior to the laboratory analyses. The aim of the study was to determine biochemical contents among cowpea lines and the influence of these biochemical seed characteristics on *Callosobruchus maculatus* infestation. Seed biochemical composition (Protein, tannin and amino acid) were analyzed following the Association of Official Analytical Chemists protocol in 2017 to 2018. To assess damage, harvested seeds from different lines were stored in cellulose bags with inoculum available for a period of three months. Laboratory temperature ranged from 12-27^{0}C and relative humidity ranged from 65%-75%. Amino acid analysis of the 15 lines used in the trial was carried out at International Centre of Insect Physiology and Ecology. The cowpea lines were selected from each cluster. Variation among the lines was determined using Analysis of Variance from the GenStat Version 17. Pearson Correlation analysis was carried out to determine the relationship between seed characteristics and pest infestation. ANOVA revealed significant variation ($p \leq 0.05$) among the cowpea lines for protein level, amino acid and tannin content. Amino acids present in the 15 cowpea lines were; glycine, arginine, glutamic acid, proline, valine, aspartic acid, methionine, tyrosine, isoleucine, leucine, phenylalanine. Protein level and total amino acid were positively correlated with cowpea weevil infestation (r=0.698*and r=0.897**) respectively. Methionine had a negative correlation with cowpea weevil infestation(r=-0.603). Tannin content was negatively correlated with cowpea weevil infestation (r=-0.666*). Biochemical content has an influence on *Callosobruchus maculatus* infestation.

4.1 Introduction

Legumes are rich sources of proteins, carbohydrates, vitamins and essential amino acids (Olaleke *et al*., 2006). Amino acids, tannin, alpha amylase inhibitor, trypsin inhibitor, tannin, carbohydrate, seed protein are some of the components that are present in different cowpea lines (Nikolova, 2016). These biochemical components differ among cowpea genotypes. Differences in protein and tannin contents have been reported (Asante *et al*., 2004). Lattanzio *et al*., (2005) observed that tannin content and alpha amylase in cowpea lines differ. Differences in protein content have been reported in chick pea which is a legume; the protein is reported to have a relationship with cowpea weevil influence (Umadevi *et al*, 2018). Variation in tannin content of indigenous vegetables and grains in western Kenya has been reported (Alwala *et al*., 2014). Nikolova (2016) observed that crude protein; crude fiber and phosphorus contents have an influence on resistance of peas to *Bruchus pisorum*. Alkaloids, flavonoids and tannins contents have a relationship with cowpea weevil susceptibility and resistance (Adebayo *et al*., 2016). Amino acid content has been shown to differ among cowpea varieties with regard to valine, iso leucine and tryptophan (Carvalho *et al*., 2012). The three major acidic amino acids present in cowpea seeds include glutamine which is responsible for glutamic acid asparagine responsible for aspartic acid and phenylalanine (Vasconcelos, *et al*., 2010). In Nigeria for example, most people who cannot afford animal protein, are advised to explore option of plant protein like *Laganaria siceraria* and *Citrullus colocynthis* seeds; which are rich in glutamic acid, aspartic acid and arginine (Sabo *et al*., 2015). Cruz *et al*., (2015) established resistance of most stored cowpea lines due to larval interference on digestibility. This is because most pest infestation occurs during storage. Difference in biochemical content among other properties can be directly or indirectly linked to pest resistance. The current study was carried out to determine variation of protein, tannin and amino acid composition within different cowpea lines and their influence on cowpea weevil infestation and damage.

4.2 Materials and methods

Three biochemical components; proteins, tannins and amino acids were determined. Protein and tannin content were determined in 50 lines and amino acid content and composition was determined in 15 selected cowpea lines. These were evaluated on the freshly harvested and dried seeds that were obtained at the end of the growing season as described in chapter 3 section 3.2.1

4.2.1 Materials

Cowpea lines (Table 1) were used for evaluation of protein, tannin and amino acid content and their influence on cowpea weevil infestation. Cowpea lines in bold type font were used for amino acid analysis.

4.2.2 Methodology

Cowpea seed lines they were cleaned and used for assessment for pest infestation. Insect damage was assessed on the 50 cowpea lines. This is described in chapter 3 section 3.2.2.

Hundred grams of freshly harvested and dried seed for each cowpea line was ground into flour using a hand mill manufactured by Shimadzu (Shimadzu, Scientific Instrument Inc., Columbia, MD, AU Series Semi-Micro and Analytical Balances; Shimadzu, Kyoto, Japan) one cowpea line at a time. The ground samples were placed in separate bottles, labeled and stored for protein, tannin and amino acid analysis.

4.2.3 Determination of protein content

Crude protein level was determined using the semi Microkjeldahl method (AOAC 1995) following the 950.46 method 20.87-37.1.22. Freshly harvested dry cowpea ground sample (0.2 g) was placed in digestion flask and 0.5 g potassium sulphate, 0.5 g of copper sulphate and 15 ml of Sulphuric acid were added respectively to act as a combined catalyst allowing digestion to take place in a fume chamber until a blue color was obtained. The digest was allowed to cool to room temperature and then transferred to 100 ml volumetric flask and topped up to the 100ml mark with distilled water**.** A control comprising of blank digestion with the catalyst was prepared separately. A sample (10ml) of diluted digest was transferred into the distillation flask and washed with distilled water. Another sample (15 ml) of 40% NaOH was added to the contents and washed with distilled water. Distillation was done to 60 ml of distillate. Titration of the distillate was done using 0.02 N HCl until color changes from violet to green which signified the end point. This was carried out for each cowpea line.

The nitrogen level in the sample was calculated as follows;

$$\% \text{ Nitrogen} = \frac{(V1-V2)*N*F \div (V*100)}{S}$$

Where: V1 is the titre for sample in ml, V2 is titre for blank in ml; N= normality of standard HCL (0.02); f= molecular weight of HCl; V= volume of diluted digest taken for distillation (10 ml); S= weight of sample taken for distillation (0.2 g). The protein content was calculated as follows; % Protein= % Nitrogen * Protein factor (6.25)

4.2.4 Evaluation of tannin content

Tannin content was determined using vanillin hydrochloric method (Price *et al*., 1978). A percentage (8%) of HCl was dissolved in methanol, 4% vanillin in methanol and then the two were mixed making sure it is not colored, if red color appeared the mixture had to be discarded. Catechin-Stock standard solution was prepared by mixing Catechin and methanol in a ratio of 1:1. A working standard was prepared by diluting the stock solution 10 times from 10-100 ml (100mg/ml Cowpea ground sample (0.25g) was weighed and placed in 100.0ml of Erlenmeyer flasks. 10 ml of 4 % HCl –methanol was added, it was sealed with parafilm and the mixture was shaken for 20 minutes in a shaker. The resulting extract was centrifuged at 4500 rpm for 10 min. The supernatant aliquot was transferred into 25 ml volumetric flask. Second extraction of tannin was done by adding 5 ml of 1% HCl in methanol to the residue. It was shaken, and centrifuged. The second supernatant was added to the 1^{st} one in the 25ml volumetric flask. The supernatant was topped up to the 25ml mark using methanol. A set of Catechin standard solutions was prepared ranging from 0, 20, 40, 60, 80, 100 ppm using methanol as the solvent. Each respective standard (1 ml) and sample extract (1 ml) was pipetted into the test tube. A portion (5ml) of freshly prepared vanillin in HCl reagent was added into the test tubes with extract and standards. Sample blanks of 1ml were prepared and placed in different sets of test tubes and 5ml of 4%HCl in methanol was added to each. Absorbance of the standard solutions, sample extracts and blanks were read in UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) at 500nm, 20 minutes after adding vanillin- HCl reagent to the samples and standards. A standard curve from the reading of Catechin standard solutions was prepared. The absorbance for sample blanks were subtracted from samples absorbance and calculation of the concentration of tannin for the sample extract done. The percent Catechin equivalents was calculated as follows;

$$\text{Tannin content (\% CE)} = (CC * VM) / (VE * WT) * 100$$

Where; CE-Catechin equivalent; CC- Catechin concentration (mg/l); VM-volume made up (25 ml); VE-volume of extract (1ml); WT-sample weight (0.25g)

4.2.5 Determination of Amino acid content

The amino acid analysis was carried out at International Centre of Insect Physiology and Ecology (ICIPE). Amino acid content determination was carried out for 15 selected cowpea lines from different regions (Table 1) based on utilization and farmer preference The 15 samples were a representation of each of the coat color cluster (inclusive of farmers' varieties, improved lines from World Vegetable Centre, and a registered variety Kenkunde in Kenya). The previously ground cowpea samples were used for this analysis. Amino acid content was determined and quantified on liquid chromatography coupled to mass spectroscopy respectively (LC-MS) (Shimadzu, Scientific Instrument Inc., Columbia, MD, USA or LCMS 8040; Shimadzu, Kyoto, Japan).

A sample (100 mg) from each of the ground cowpea lines sample was weighed in triplicate. Each was transferred into a 5 ml reaction vial. 2ml of 6N HCl were added into each of the 5ml vials. Nitrogen gas was also introduced in and the contents closed completely. The samples were hydrolyzed for 24 hours at 110°C. The hydrolysates were evaporated to dryness under vacuum. The hydrolysates were reconstituted in 1 ml 90:10 water: acetonitrile. They were vortexed for 30 seconds, sonicated for 30 min, and then centrifuged at 14,000 rpm and the supernatant transferred to another vial. Serial dilutions of the authentic standards containing 18 amino acids (1-105 µg/µl) was also analyzed by LC-MS to generate linear calibration curves (peak area vs. concentration) used for external quantification. The equations for the amino acids were identified and amounts in (mg/g) of sample recorded. The samples were analyzed on LC-MS to determine the amino acids and the level of amino acids in each sample.

The flow rate was held constant at 0.5 ml min^{-1} and the injection volume was 3 µL. The chromatographic separation was achieved on an Agilent system 1100 series (MA, USA) using ZORBAX SB-C18, 4.6 × 250 mm, 3.5 µm column, operated at 40°C. Mobile phases used were made up of water (A) and 0.01% formic acid in acetonitrile (B). The following gradient was adapted: 0–8 min, 10% B; 8–14 min, 10-100% B; 14–19 min, 100% B; 19–21 min, 100-10% B; 21–25 min, 10% B. The LC was interfaced to a quadruple mass spectrometer. The mass spectrometer was operated on ESI-positive mode at a mass range of m/z 50-600 at 70eV cone voltage.

4.3 Data analysis

The data obtained for proteins, tannin, amino acids in mg/g and weevil damage were subjected to analysis of variance in GenStat software and means separated at $P \leq 0.05$ to find variation in protein and tannin for 50 cowpea lines and amino acids for the 15 cowpea lines and damage caused by the weevil for the 50 cowpea lines. Pearson's correlation was carried out in GenStat software to determine the relationship between biochemical seed characteristics and pest infestation.

4.4 Results

Analysis of variance revealed significant variation ($P \leq 0.05$) among cowpea lines based on protein level, tannin content, and damage levels caused by cowpea *C. maculatus* (Table 7). Protein level ranged from 2.8- 26.25 with an average of 13.5g/100grams of seed, tannin content ranged from of 0.032- 0.43mg/g with an average of 0.14mg/g tannin, damage level ranged from 0.5- 54%, and the average recorded was 14.7% of seed was damaged due to the cowpea weevil for the 50 cowpea lines. Cowpea lines that showed high levels of damaged (28% and above) include; KAR 2 (28.13%), GBK034722 (30.44%), GBK003876 (33.67%), GBK003656 (33.93%), GBK003658 (38.33%), GBK003724 (41.77%), GKKCP-2(44.77%), MAR 5 (48.2%) GBK003660-1 (51.03%) and KENKUNDE (54.73%). Those that had high protein content of 19% and above were; KAR 2(19.5%), MAR 5(19.24%), GBK003876 (19.02%), GBK003674 (20.07%), GBK003660 (21.05%), GBK003656 (22.75%), GBK003724 (22.93%), GBK003595 (26.25%). Cowpea lines with high tannin content include; GBK003697 (0.2087), GBK003699 (0.21), GBK026941 (0.24470), GBK003780 (0.257), GBK026958 (0.3397), GBK003695 (0.3873), ACC 20(0.432), KOL 1 (0.222) and KOL 5(0.214) (Table 7).

Table 7: Variation among cowpea lines based on damaged seed during storage, protein level and tannin concentration

Cowpea line	Seed damage (%)	Protein level/100g	Tannin level (mg/g)
9334	0^{a}	15.63^{pq}	0.0853^{jk}
ACC 20	2.6^{abcd}	11.02^{hij}	**0.4327^{L}**
ACC 25	2.57^{abcd}	12.22^{kl}	0.053^{c}
ACC 6	23.73^{jkl}	11.8^{jk}	0.1507^{x}
DAKAWA	0.97^{abc}	17.33^{tu}	0.0863^{k}
EASEED	**6.1^{abcdef}**	**4.73^{c}**	0.122^{r}
EX ISEKE	7.33^{bcdefg}	4.37^{bc}	0.088^{l}
GBK003645	9.63^{defg}	6.3^{d}	0.032^{A}
GBK003650	**6.33^{abcdef}**	**6.4^{d}**	0.1433^{v}
GBK003652	13.83^{ghi}	17.7^{u}	0.135^{t}
GBK003654	12.13^{fgh}	21^{x}	0.179^{B}
GBK003656	**33.93^{mn}**	**22.75^{y}**	0.1283^{s}
GBK003658	38.33^{no}	15.75^{pqr}	0.1647^{A}
GBK003659	18.8^{hij}	14.35^{o}	0.1123^{q}
GBK003660	**51.03^{qr}**	**21.05^{x}**	0.135^{t}
GBK003660	**4.1^{abcde}**	**2.8^{a}**	0.14^{u}
GBK003674	2.7^{abcd}	20.07^{w}	0.04^{b}
GBK003689	0.5^{ab}	12.95^{lm}	0.1277^{s}
GBK003690	**4.27^{abcde}**	**8.93^{f}**	0.0717^{h}
GBK003695	11.83^{fgh}	26.25^{z}	**0.3873^{K}**
GBK003697	2.8^{abcd}	15.23^{p}	**0.2087^{D}**
GBK003699	0.8^{abc}	17.5^{tu}	**0.21^{D}**
GBK003700	13.93^{ghi}	10.6^{gh}	0.112^{q}
GBK003702	1.53^{abc}	15.58^{pq}	0.134^{t}
GBK003702	3.83^{abcde}	3.75^{b}	0.1617^{z}
GBK003703	18.67^{hij}	8.5^{ef}	0.146^{w}
GBK003707	7.2^{bcdefg}	11.38^{hij}	0.0677^{e}
GBK003721	7.9^{cdefg}	14no	0.069^{f}
GBK003723	27.97^{klm}	8.04^{e}	0.1^{o}
GBK003724	**41.77^{op}**	**22.93^{y}**	0.094^{m}
GBK003780	3.13^{abcd}	13.2^{mn}	**0.257^{I}**
GBK003814	2.97^{abcd}	14no	0.085^{j}
GBK003820	10.47^{efg}	11.23^{hij}	0.065^{d}
GBK003876	33.67^{mn}	**19.08^{v}**	0.249^{H}
GBK005173	6.23^{abcdef}	11.55^{ijk}	0.098^{N}
GBK026941	3.57^{abcde}	16.1^{qrs}	**0.2447^{G}**
GBK026958	11.7^{fgh}	16.5^{rs}	**0.3397^{J}**
GBK026958	20.63^{ij}	15.75^{pqr}	0.145^{w}
GBK034722	**30.47^{lm}**	14.38^{o}	**0.0397^{b}**
GBK034732	5.17^{abcdef}	16.8st	0.0657^{d}
GBK046540	21.23^{jk}	10.05^{g}	0.08^{i}
GKKCP-2	44.77^{opq}	8.91^{f}	0.0703^{g}
KAB 1	**3.8^{abcde}**	**7^{d}**	0.1593^{y}
KAR 2	**28.13^{klm}**	**19.5^{vw}**	0.1813^{C}
KENKUNDE	54.73^{r}	10.85^{ghi}	0.2087^{D}
KOL 1	6.4^{abcdef}	15.63^{pq}	0.222^{F}
KOL 5	2.53^{abcd}	16.27^{qrs}	0.214^{E}
LAM 4	9.23^{defg}	13.83^{no}	0.103^{p}
MAR 3	**1.67^{abc}**	**3.68^{b}**	0.098^{n}
MAR 5	**48.2^{pqr}**	**19.24^{v}**	0.15^{x}

Damage, Protein, Tannin levels each bearing the same letters are not significantly different

Protein level was positively correlated (r= 0.698*) to cowpea weevil infestation (Figure 2).

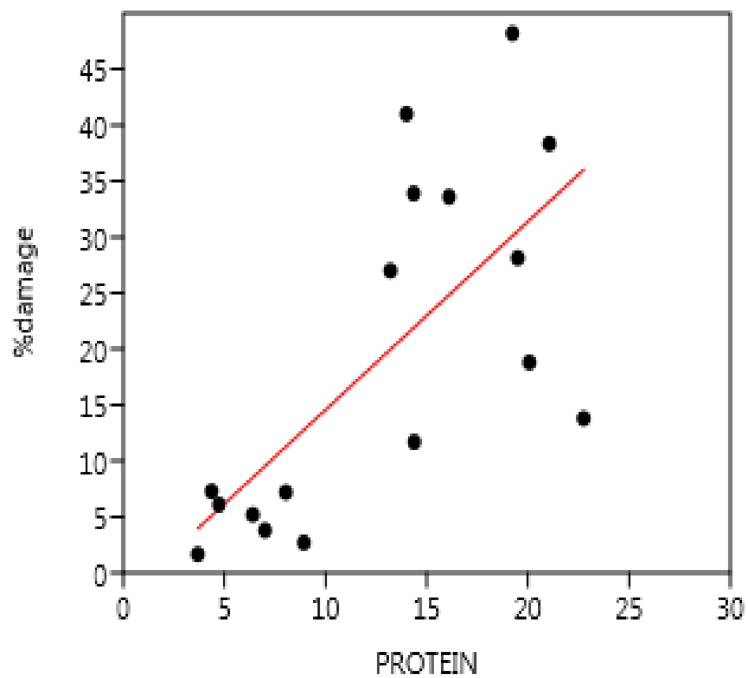

Figure 2: Pearson correlation between protein level in the cowpea and damage caused by the *Callosobruchus maculatus*

%damage- percentage damage caused by cowpea weevil, protein- protein level

Tannin content was negatively correlated (r= -0.666*) to cowpea weevil infestation (Figure 3)

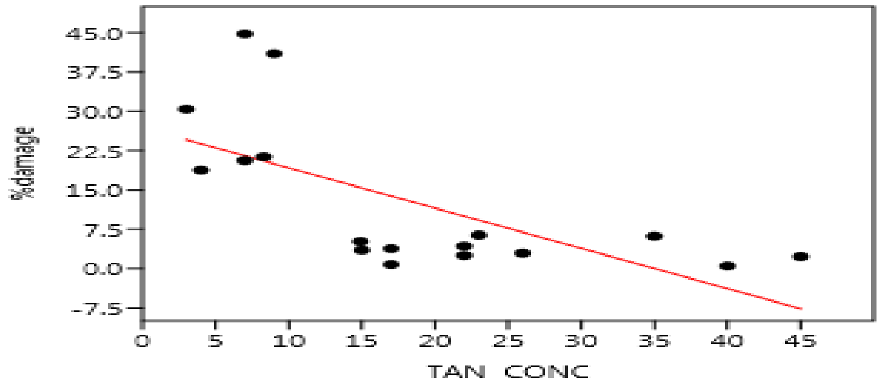

Figure 3: Pearson correlation between the tannin concentration and the damage caused by *Callosobruchus maculatus*

% damage- damage caused by cowpea weevil; TAN CONC- Tannin concentration

Eleven amino acids were detected in the cowpea seed including glycine, arginine, glutamic acid, proline, valine, aspartic acid, methionine, tyrosine, isoleucine, leucine, and phenylalanine. The amino acids that were found in greatest amounts across the cowpea lines were leucine, isoleucine and tyrosine respectively (Table 8). Peak curves showing amino acids quantification are presented in Appendix 1.

Table 8: Variation in detected amino acid in 15 cowpea lines and damage caused by cowpea weevil

	LINE	METHIONINE	ISOLEUCINE	LEUCINE	VALINE	PHENYL	TYROSINE	Glutamic acid	Aspartic acids	GLYCINE	ARGININE	PROLINE	Damaged/ 100g
1.	**9334**	6.633^{d}	11.5^{a}	15.54^{ab}	3.61^{bcde}	4.887^{f}	8.783^{f}	6.83^{bcde}	4.242^{cd}	2.38^{cde}	2.305^{cde}	4.28^{a}	0^{a}
2.	**ACC 25**	4.727^{abcd}	14.69^{ab}	13.4^{a}	4.413^{e}	4.745^{f}	6.1^{bcde}	7.633^{de}	2.097^{ab}	2.14^{bcde}	2.587^{de}	4.53^{a}	2.57^{abcd}
3.	**DAKAWA**	3.658^{abc}	11.65^{a}	20.3^{de}	3.85^{cde}	2.547^{bcd}	3.538^{ab}	7.07^{cde}	4.745^{cd}	2.41^{cde}	2.797^{e}	13.772^{b}	0.97^{abc}
4.	**GBK003645**	3.286^{ab}	13.82^{a}	21.52^{e}	4.307^{de}	4.543^{ef}	1.949^{a}	8.255^{e}	4.209^{cd}	2.53^{cde}	0.787^{ab}	2.476^{a}	9.63^{defg}
5.	**GBK003656**	1.777^{a}	12.71^{a}	15.19^{ab}	3.88^{cde}	4.953^{f}	4.962^{bc}	7.1^{cde}	3.886^{bcd}	0.217^{a}	0.547^{a}	3.892^{a}	33.93^{mn}
6.	**GBK003659**	2.697^{a}	12.97^{a}	13.49^{a}	3.027^{abcde}	4.493^{ef}	4.797^{bc}	6.247^{abcd}	1.754^{A}	0.883^{abc}	2.013^{bcde}	3.273^{a}	18.8^{hij}
7.	**GBK003660**	6.46^{cd}	13.34^{a}	14.56^{ab}	2.353^{abc}	3.565^{def}	9.028^{f}	5.573^{abc}	2.817^{abc}	1.123^{abcd}	1.048^{abc}	3.513^{a}	**4.1 ^{abcde}**
8.	**GBK003689**	4.165^{abcd}	14.74^{ab}	15.06^{ab}	4.493^{e}	3.687^{def}	5.967^{bcde}	7.713^{de}	3.757^{bcd}	1.163^{abcd}	0.778^{ab}	12.617^{b}	0.5^{ab}
9.	**GBK003695**	3.028^{ab}	17.5^{ab}	20.66^{de}	3.213^{abcde}	4.63^{ef}	6.076^{bcde}	6.433^{abcd}	3.625^{abcd}	2.943^{de}	1.4^{abcde}	19.312^{c}	11.83^{fgh}
10	**GBK003697**	6.45^{cd}	12.87^{a}	16.76^{bc}	1.773^{a}	3.228^{cdef}	8.861^{f}	4.993^{a}	4.46^{cd}	0.543^{ab}	0.468^{a}	3.667^{a}	2.8^{abcd}
11	**GBK003814**	5.78^{bcd}	14.74^{ab}	16.22^{bc}	2.633^{abcd}	0.56^{a}	8.273^{ef}	5.853^{abc}	4.283^{cd}	0.303^{a}	1.265^{abcd}	2.693^{a}	2.97^{abcd}
12	**GBK005173**	4.663^{abcd}	13.77^{a}	15.79^{ab}	2.593^{abc}	1.81^{abc}	4.703^{bc}	5.813^{abc}	4.493^{cd}	3.023^{e}	1.188^{abcd}	4.85^{a}	6.23^{abcdef}
13	**KAB 1**	4.26^{abcd}	13.32^{a}	15.84^{ab}	1.687^{a}	1.254^{ab}	7.489^{def}	4.907^{a}	3.67^{abcd}	1.673^{abcde}	0.99^{abc}	2.287^{a}	**3.8^{abcde}**

14	**KENKUNDE**	2.94^{ab}	13.28^{a}	18.57^{cd}	2.013^{ab}	2.98^{bcde}	4.354^{abc}	5.233^{ab}	3.55^{abcd}	1.557^{abcde}	1.65^{abcde}	4.407^{a}	54.73^{r}
15	**LAM 4**	2.91^{ab}	12.32^{a}	16.99^{bc}	3.143^{abcde}	2.633^{bcd}	6.542^{cdef}	6.363^{abcd}	4.965^{d}	1.743^{abcde}	1.753^{abcde}	3.433^{a}	9.23^{defg}

The 11 amino acids were detected in great amounts among the 15 cowpea line

Strong positive correlation(r=0.897***) was observed between total amino acid and cowpea weevil infestation (Figure 4)

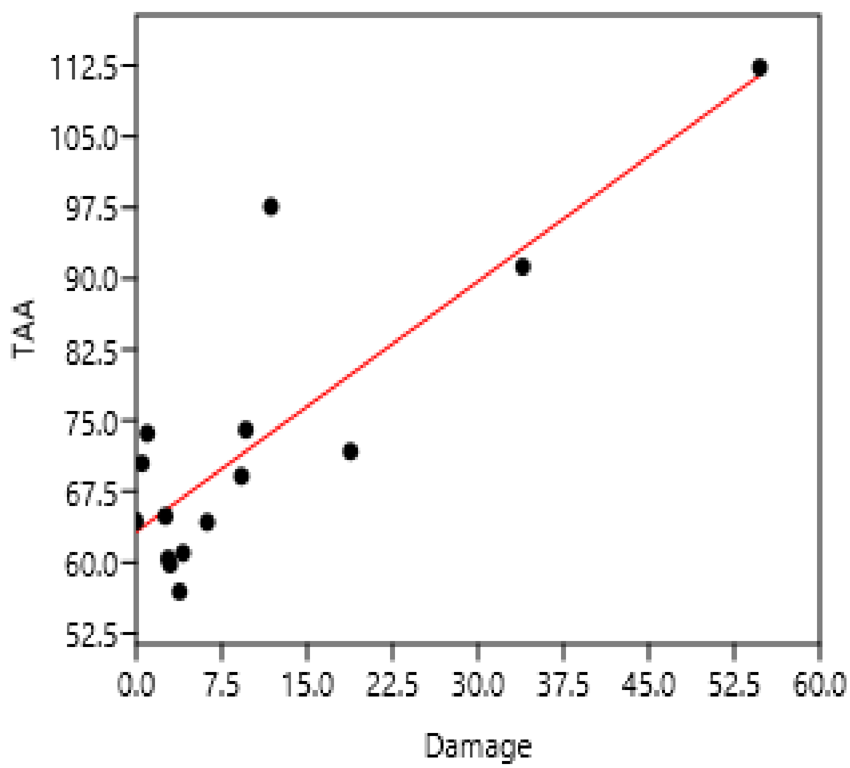

Figure 4: Pearson correlation between total amino acids in the cowpea lines and damage caused by *Callosobruchus maculatus*

TAA- Total amino acid, Damage- Damage caused by cowpea weevil expressed as a % of 100 seeds sampled

Only methionine had a negative correlation with damage caused by cowpea weevil (r= -0.603*) (Figure 5).

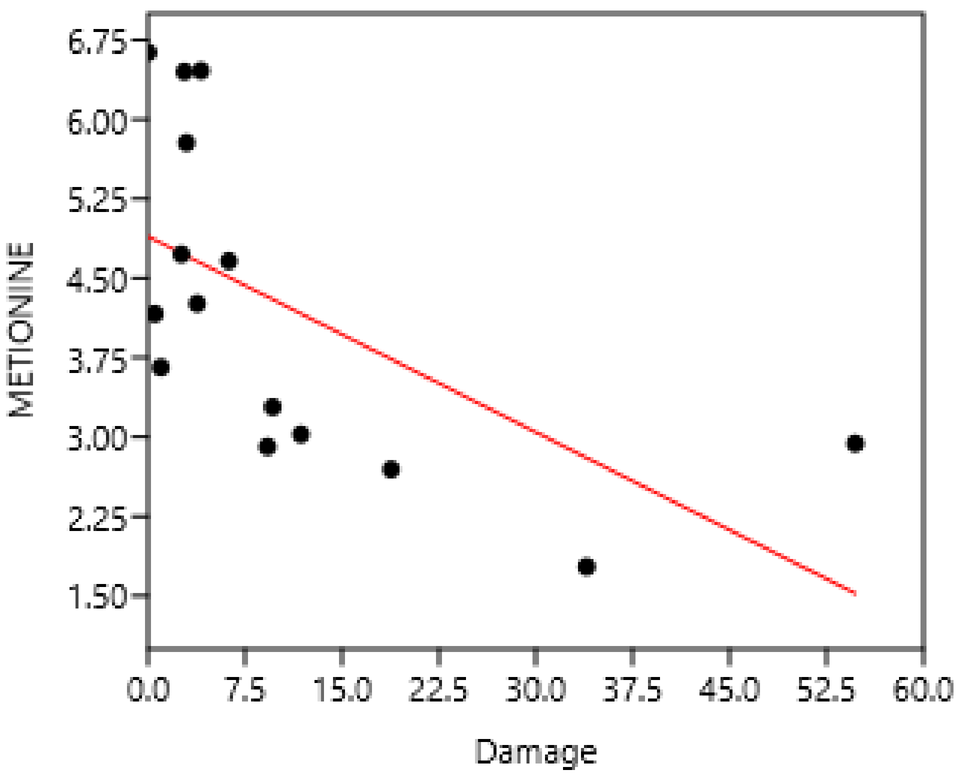

Figure 5: Pearson correlation between methionine content in the cowpea lines and damage caused by *Callosobruchus maculatus*

Ten amino acids were positively correlated to cowpea weevil but the strength of the relationship differed from one amino acid to another (Table 9)

Table 9: Pearson Correlation between cowpea line amino acids and damage caused by cowpea weevil infestation

	Methionine	**Iso leucine**	**Leucine**	**Valine**	**Phenyl**	**tyrosine**	**Glutamic acid**	**Aspartic acid**	**Glycine**	**Arginine**	**proline**	**Damage**
Methionine		0.698	0.296	0.287	0.405	0.001	0.273	0.784	0.826	0.96	0.426	0.017
Iso leucine			0.547	0.745	0.847	0.942	0.740	0.347	0.514	0.393	0.059	**0.883**
Leucine				0.813	0.891	0.063	0.571	0.0388	0.077	0.929	0.099	**0.647**
Valine					0.025	0.087	2.55E-11	0.801	0.389	0.308	0.231	0.493
Phenyl						0.467	0.025	0.119	0.672	0.697	0.501	**0.54**
Tyrosine							0.046	0.941	0.168	0.643	0.502	0.148
Glutamic acid								0.883	0.331	0.441	0.333	0.519
Aspartic acid									0.546	0.467	0.711	**0.555**
Glycine										0.121	0.133	0.448
Arginine											0.501	**0.714**
Proline												**0.639**
Damage												-

Amino acids were positively correlated with damage caused by cowpea weevil the strength of the correlation ($P \leq 0.05$) differed though from one amino acid to another. The values in the table are Coefficient of correlation

4.5 Discussion

Cowpea varieties differ in susceptibility to cowpea weevil based on seed loss, duration of emergence and median developmental period (Titilope and Kannike, 2019). Damage caused by *Callosobruchus maculatus* effect varied among cowpea varieties exposed to cowpea weevil infestation under treatment of African black pepper and cloves (Oyeniyi *et al*., 2015).

Ubini *et al*., (2016) observed variation in seed protein content of cowpea genotypes. Similar to the current study, protein level ranges (16-27%) in 32 cowpea accessions have also been reported (Asante, *et al*., 2004). Ovipositional preference by cowpea weevil has been observed in chickpea seed which is a legume; the weevil preferred chickpea with high protein level compared to those with low protein levels (Umadevi *et al*., 2018).

Tannin content variation (0.12 to 2.38) has also been reported Asante *et al*., (2004) brown and dark mottled seeds recorded the highest tannin levels. Their results are different from what was established in this study in terms of ranges because the lines used in this study were a representation of 7 cluster colors. Their results however agree that the dark colored seed had higher tannin levels compared to light colored seed. Abdelatief and El-asser, (2011) reported an average of 0.23% tannin content a result which is in range with tannin level 0.14%.

Amino acid content variation has been reported in cowpea (Gonçalves *et al*., 2016). Higher levels of amino acids; leucine, iso-leucine glutamic acid and tyrosine were similarly obtained among cowpea genotypes (Olaleke *et al*., 2006). Glutamine and asparagine were also found to be the highest amino acids present in the analysis of chemical composition of leguminous seeds (Grela *et al*., 2017). This was contrary to the current study where iso-leucine, leucine and tyrosine were amino acids present in highest amounts. Similar studies revealed that cowpea genotypes were rich in the essential amino acids and chemical composition (Harmankaya *et al*., 2015).

Significant positive correlation between protein level and damage caused by cowpea weevil infestation observed is in tandem with Umadevi *et al*., (2018); who reported that *Callosobruchus maculatus* preferred chickpeas with high levels of protein to those with low protein level. Contrary results of no relationship between protein content and cowpea weevil infestation have been reported (Oliveira *et al*., 2018), because the cowpea lines in this study had low protein content below 12%. Cowpea weevil infestation is directly associated with the level of protein in legumes; it has been established that protein and carbohydrate levels reduced in stored cowpea due to the cowpea weevil infestation (Sawalu *et al*., 2014). Protein level in cowpea is one of the intrinsic factors affecting infestation by the cowpea weevil (Arotolu *et al*., 2018). Legumes with high carbohydrates, proteins and anti-nutritional factors like soybean have been reported to be resistant to cowpea weevil compared to cowpea and chick pea which are high in protein and less in anti-nutritional factors (Sharma and Thakur, 2014) implying that cowpea is generally susceptible to weevil infestation compared to other legumes.

Cowpea lines with high tannin content experienced less *Callosobruchus maculatus* infestation, compared to lines with low tannin content. Dark seed coat color is associated with high tannin content (Oyeyinka *et al*., 2013). Seed tannin content is reported to cause negative impact on cowpea weevil by causing non palatability (Lattanzio *et al*., 2005). Phenolics are utilized by plants as a defense mechanism against insect pests. They may act as feeding deterrents or toxicants against insect herbivores (Schoonhoven *et al*., 2005). Tannins, for example are reported to act as feeding deterrents to many phytophagous insects (Barbehenn and Constabel, 2011).

Studies on amino acid composition and protein isolates in cowpea reveal that methionine which is hydrophobic group is a limiting amino acid in some cowpea seed (Elhardallou *et al*., 2015). Harmankaya, (2015) reported that apart from methionine, tryptophan and tyrosine are also limiting amino acids in cowpea seed. Cowpea line inherent characteristics including cystein protease content interfere with female oviposition, larval development and sometimes adult emergence (Cruz *et al*., 2015). Some beetles like cowpea weevil are able to detect nutrient concentrations within grains and thus infest. This is facilitated by the presence of neurosensory ducts in *Callosobruchus maculatus* (Chapman and Bernays, 1995). This explains why some lines were more infested than others. GBK 003656, Kenkunde and GBK003659 were more damaged by the cowpea weevil compared to Dakawa, ACC25, and GBK003689. In a study on relationship on amino acid and caterpillar in mango; a negative correlation was established between the mango weevil infestation and free amino acid levels; varieties that had least total free amino acid levels were more infested than those which had higher levels of total amino acids (Mainak, 2017). The results are contrary to what was established in the current study, the total amino acid content was positively correlated with damage caused by cowpea weevil infestation. Preference of cowpea weevil to high amino acid content has been reported in chickpea (Umadevi *et al*., 2018). Similar results have been reported that cysteine rich protein cowpea lines(high in methionine) were relatively resistant to cowpea weevil implying that individual amino acid have an influence on cowpea weevil infestation (Swapan, 2016).

4.6 Conclusions and recommendations

Significant variation among cowpea lines was observed for protein, tannin and amino acids content. Similarly, there was variation in damage of the cowpea lines.

Protein level and total amino acid had positive correlation with cowpea weevil infestation while tannin content had negative correlation with cowpea weevil infestation. Cowpea lines with high levels of protein and high total amino acid were more infested compared to their counterparts. Total amino acids were positively correlated with cowpea weevil infestation. Cowpea lines with high tannin concentration were less infested compared to their counterparts.

These cowpea lines can be used for breeding against resistance to the weevil. Cowpea lines that were relatively resistant included GBK 003689, GBK 003697, GBK003699, MAR 3, GBK003780, DAKAWA, ACC20 and GBK 3702.

CHAPTER FIVE

APPROPRIATE PACKAGING MATERIAL FOR COWPEA SEED STORAGE

Abstract

Quality seed is an important resource for any seed-propagated crop. Cowpea *(Vigna unguiculata)* seed is prone to cowpea weevil (*Callosobruchus maculatus*) infestation during storage resulting in 100% seed loss when stored without any treatment. Smallholder farmers in Kenya who save grains for food and seed for planting use traditional methods of storage; occasionally use pesticides treatment. Due to health concerns, pesticide use is discouraged. A study was conducted to determine effectiveness of packaging materials on reducing cowpea weevil damage during cowpea seed storage. A repeated experiment was carried using a single cowpea line each time. Packaging materials evaluated were; cellulose paper bags, gourds, grain storage bags, polyethylene bags and glass bottles. Completely Randomized Design was used and each treatment replicated thrice. Data were obtained on number of seeds with holes, mean number of holes per seed, seed weight loss, damage score, and seed germination. Quantitative data were subjected to Analysis of Variance in GenStat software version 17 and means separated using LSD ($P \leq 0.05$) to determine variation and correlation analysis to establish the relationships among parameters for seed damage. There was significant variation ($p \leq 0.05$) among storage materials for the respective seed damage parameters. Glass bottles, polyethylene bags, and grain storage bags showed significantly ($P \leq 0.05$) less seed damage compared to the gourd and cellulose bags in all the parameters tested except germination. The parameters used to evaluate damage were significantly and positively correlated except germination. Only seed stored in glass bottles showed high germination rates (92%); whereas, germination rates were generally less than 52% for the other seed packaging materials. Glass bottles were ranked the best packaging material (0.02% weight loss), followed by polyethylene bags (3.87% weight loss) and grain storage bags (5.7% weight loss). Hermetic seed packaging is the most effective in reducing cowpea weevil damage and preserving seed quality.

5.1 Introduction

Cowpea production is wholly dependent on seed as propagation material. The quality of cowpea seed is therefore an essential determinant of final quantity and quality of cowpea leaves and grain (CTA and FARA 2011). As a legume and a mostly self-fertilizing crop, farmers usually produce their own seed (Francis and Waithaka., 2015) and only occasionally purchasing commercial seed or procuring from development agencies during periods of crop failure. Few commercial companies therefore find it a sustainable commercial venture to produce process and sell cowpea seed thus most farmers depend on their own storage facilities (Francis and Waithaka., 2015) to store saved seed. Farmers use traditional forms of seed storage like hanging pods in the kitchen, use of gourds and pots and storage in sisal bags. Emerging technologies for seed storage are now available including Purdue Improved Cowpea Storage bags, metal drums, and polythene bags. The seed, if stored and not treated well, will be vulnerable to pest damage. Damaged seed is not fit for even germination (Olasoji *et al*., 2013).

Seed treatment is one of the alternative solutions to reducing cowpea seed loss in storage. Small scale farmers use the traditional methods of seed treatment; soot and ash, botanical components like garlic, neem and moringa prior to seed storage (Tiroesele *et al*., 2015). Application of bioactive agents, for example, *Xylopia aethiopica* and *Aframomum melegueta* has been reported to have ability to suppress oviposition and significantly reduce adult emergence in cowpea seed during storage (Adesina *et al*., 2015). Use of bitter leaf (*Vernonia amygdalina*) powder has also been exploited as a treatment for cowpea seed in storage against cowpea weevil (David, 2015). Bioactive jute fabrics have been applied in Egypt to alleviate cowpea seed losses associated with pest infestation though they have to be treated with chitosan and neem to increase effectiveness (Hashem *et al*., 2017). The adoption of these methods is however low because of the bitter taste, limitation to small-scale production and the uneconomical method of extraction (Ilesanmi and Gungula, 2013).

Commercial products significantly reduce pest infestation but their utilization is low due to stringent measures on minimum residue levels in food products and emerging health and environmental concerns of pesticide use (Muhammad, 2015). Food quality and safety is priority during food storage.

Susmithar and Rai (2017) reported that plastic bag is a better packaging material for storage of cowpea seed compared to cloth bag because it provides a relatively airtight condition. Polythene bags are also widely exploited in cowpea grain storage in Kenya (Njonjo *et al*., 2019). The only challenge is that plastics have to be accompanied by a combination of seed treatments including polymer coat, ferrous sulphate, *Trichoderma*, mancozeb and neem oil to increase efficiency (David, 2015).

Hermetic storage is a new technology of controlled atmosphere being used in storage of cereals and legumes. Evaluation of hermetic conditions for storage of grains has been carried out for pigeon peas, maize, rice, and wheat (Jyoti *et al*., 2017). Super bags have been tried and well adopted in Africa for cowpea storage (Divekar & Sharma, 2016). Purdue Improved Cowpea Storage (PICS) is an example of a super bag widely used in Nigeria, Burkina Faso and Mali for storage of legumes. PICS comprise 3 layered bags with the outer most made of woven propylene and the two inner layers made of 80-micron high-density polyethylene (Baributsa *et al*., 2009). Super bags have the potential to maintain quality of seed stored, germination capacity and infestation by weevils compared to polyethylene and jute bags, because the bags create a micro environment that ensures minimum qualitative and quantitative losses of stored products (Kumari *et al*., 2017). Hermetic storage conditions have been applied in Kenya for maize storage whereby PICS and metallic silos are used to protect maize against the maize weevil and large grain borer (Groote *et al*., 2013). Seed handling techniques have an influence on seed quality. It is therefore important to identify proper cowpea seed and grain storage techniques to maintain seed quality at its optimum (Adetumbi *et al*., 2010).

Cowpea is mostly self-fertilizing and Smallholder farmers usually save their own seed (Francis and Waithaka, 2015) or procure from their neighbors for the next planting. Storage of seed is important for availing seed at planting time and ensuring seed purity and sustainability of a variety; these greatly influences production, productivity and quality of the produce. It is therefore important to quantify seed damage and determine effective packaging material on reducing cowpea weevil damage during seed storage and thereafter determine alternative storage mechanisms in order to alleviate the challenge of cowpea seed loss and availability of seed to the smallholder cowpea growers.

5.2 Materials and methods

5.2.1 Experimental design, source of material and study site

Two laboratory experiments were carried out. A freshly harvested cowpea line was used each time for the two sets of experiments. One set of seed was produced at JKUAT and another procured from a farmer immediately after harvest without any application of pesticide. Packaging materials for storage of seed were polyethylene bags, cellulose paper bag (size 14), and glass bottles from Nairobi Plastics Limited, grain storage bags (AgroZ^{R}), and gourds from Maasai market (Plate 8).

The two sets of laboratory experiments were carried out at the Jomo Kenyatta University of Agriculture and Technology. Freshly harvested /procured seed of one line of cowpea was cleaned off plant debris and broken seed and used in each time. Seed was then measured (80g) using an electric balance (Shimadzu Scientific Instrument Inc., Columbia, MD, AU Series Semi-Micro and Analytical Balances; Shimadzu, Kyoto, Japan) and placed in each packaging material for storage. The experiment was laid out in a completely randomized design with five seed packaging material each replicated three times. Inoculum of *Callosobruchus maculatus* was based on inherent content in the seed. The laboratory daily temperature ranged from 12-27^{0}C during the time for the experiment. The seeds were stored for a period of 5 months. The seeds were assessed for presence of cowpea weevil eggs, average number of holes on seeds, damage score based on severity, cumulative weight loss and germination.

Plate 8: Packaging materials for seed storage

A glass bottles, B-cellulose paper bag; C- thin polyethylene bags; D-grain storage bags; E-gourds.

5.2.2 Data collection

Presence of eggs was evaluated by closely examining a handful of the stored seed using a hand lens and recorded as present or absent. The number of seeds with pest emergence holes was recorded as the number of seeds with holes from a random subsample of 100 seeds. The mean number of emergence holes per seed was also determined on a subsample of 10 seeds. The damage score was evaluated as the proportion of damaged seed to total number of seed in the 100 sample expressed on a scale of 1-5.

The weight loss was assessed after storing the seed for five months. The seeds were initially sieved to eliminate dust, chaff, completely broken seed and dead or alive insects. The seeds were then weighed to obtain the weight of seed at the end of the storage period. The cumulative weight loss was the difference between the initial weight and final seed weight.

Germination tests were carried out on stored seed end of the second trial according to Sikinyi (2010). A sample comprising 150 from each of the packaging storage material was soaked in distilled water for 24 hours to allow water imbibition. The seeds from each treatment were then divided into three samples of 50 each and placed on petridishes lined with Whatman's filter paper grade 42. The seed samples were incubated in a growth chamber at 25^0C for seven days (Plate 9).

Germination was assessed after every 24hours. The data collected were used to calculate speed of germination, mean germination time and overall germination rate.

Plate 9: Evaluation of germination in growth chamber for cowpea seed after storage for 5 months in different packaging materials.

Seed germination rate was calculated as the proportion of germinated seeds divided by the total number of seeds planted, expressed as a percentage. Speed of germination was calculated by the following formula

$$\text{Speed of Germination} = \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{d3} + \cdots$$ (Czabator, 1962).

Where, n = number of germinated seeds, d= number of days,

Mean Germination Time (MGT) was estimated according to Ellis and Roberts (1981) formula

$$\mathrm{MGT} = \frac{\mathrm{n1d1} + \mathrm{n2d2} + \mathrm{n3d3} + \cdots}{\text{Total number of days}}$$

Where n= number of germinated seed d = number of days

5.3 Data analysis

Number of seeds with emergence holes, number of emergence holes, weight loss data and germination were subjected to Analysis of Variance (ANOVA) using GenStat Version 17 and means separated using LSD at $p \leq 0.05$ to determine variation among the storage materials. Pearson correlation analysis was carried out to establish the relationship among the variables used to measure seed damage.

5.4 Results

Visual observations indicated that there were no eggs present on seeds stored in the grain storage bags in experiment 1 and glass bottle in the 1st and 2nd experiments. Eggs were present on seed stored in the grain storage bags in the second experiment; in the thin polyethylene bags, cellulose paper bag, and gourd in both experiments (Table 10).

The greatest damage score 5 was observed on seeds stored in cellulose bags (5), and gourd (1) compared to grain storage bags(2), polyethylene bags(2) and glass bottles(1) as measured by weight loss (0.01), number of holes per seed, number of seeds with holes, and damage score (Table 10). Seeds stored in glass bottle showed the least damage for the traits measured. Seed Germination rate was higher for seeds stored in the glass bottles 92%. Germination was generally less than 52% for the other treatments.

Table 10: Influence of packaging material on cowpea seed damage caused by *Callosobruchus maculatus*

	Packaging material	Experiment 1					Experiment 2					
		Weight Loss (g)	**No of holes /10 seeds**	**Seeds with holes in 100 sample**	**Damage score**	**Eggs present**	**Weight Loss (g)**	**No of holes /10 seeds**	**Seeds with holes in 100 sample**	**Damage score**	**Germination rate (%) P=0.01**	**Eggs Presence**
1.	**Cellulose paper bag**	38^{a}	23^{a}	99^{a}	5	Yes	17.6^{b}	12^{b}	70^{c}	5	49.3^{b}	Yes
2.	**Gourd**	30^{a}	25^{a}	66^{a}	4	Yes	18.5^{b}	11^{b}	72^{c}	5	40.7^{ab}	Yes
3.	**Grain storage bags**	3^{b}	1^{b}	14^{b}	2	No	7.5^{a}	5^{a}	41^{b}	2	29.3^{a}	Yes
4.	**Polyethylene bag**	0.25^{b}	0^{b}	0^{b}	2	Yes	7.5^{a}	5^{a}	42^{b}	2	51.3^{b}	Yes
5.	**Glass bottle**	0.01^{b}	0^{b}	0^{b}	1	No	0.04^{a}	0.2^{a}	2^{a}	1	92.7^{c}	no

Five different packaging materials were evaluated for efficacy in storage of cowpea seed. Damage on the stored cowpea was determined by weight loss of the initial seed, number of holes on 10 seeds, proportion of seeds that had holes, general damage score and presence of eggs. The experiment was repeated twice. Germination tests were carried out in experiment two.

Although grain storage bags and polyethylene bags showed similar seed damage, seed germination was significantly lower for the grain storage bag treatment $p \leq 0.05$; seed stored in glass bottles also showed more rapid germination compared to other treatments. In terms of germination speed and mean germination time glass bottle was significantly different ($p \leq 0.05$) from gourds and cellulose bags (Figure 6).

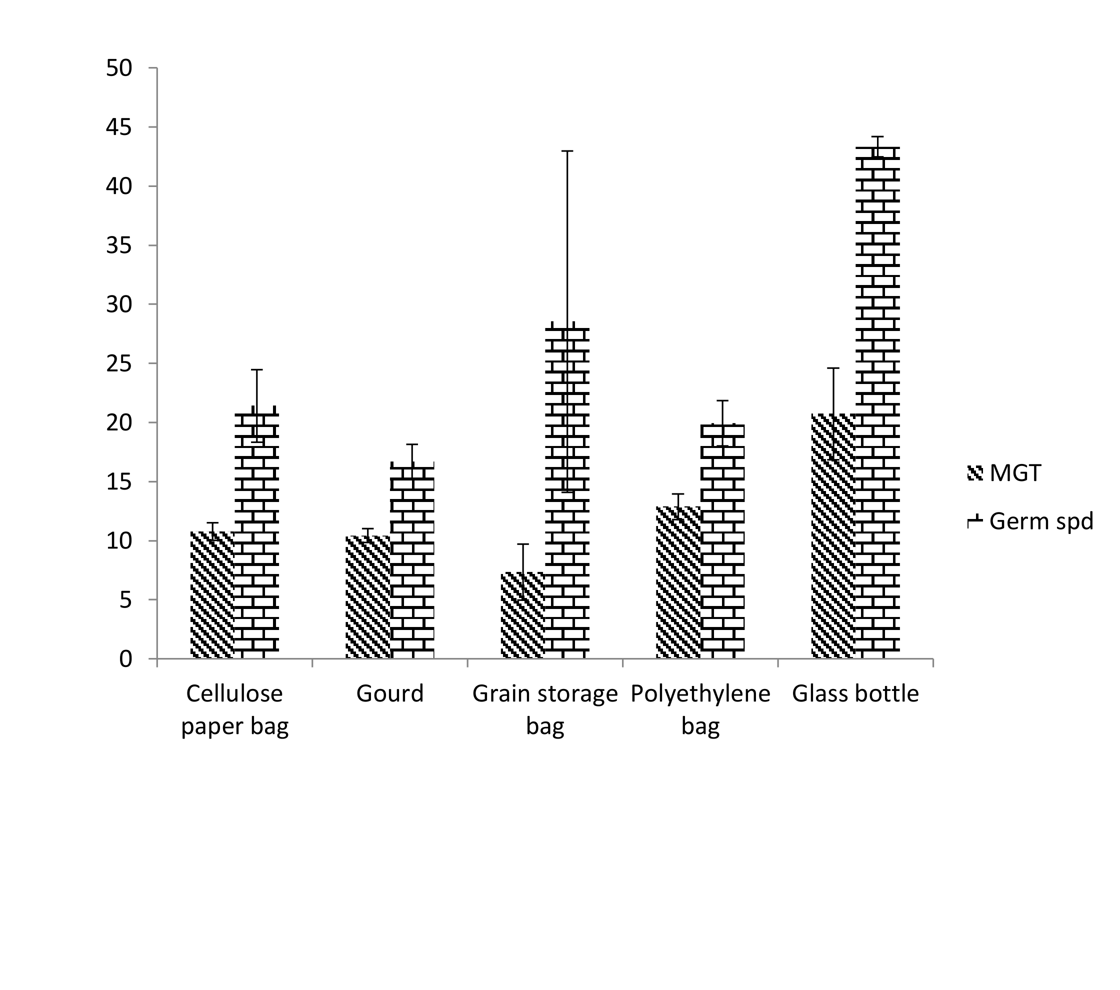

Figure 6: Variation in germination attributes of stored cowpea seed in different packaging materials.

MGT- mean germination time p= 0.029, Germ spd- Germination speed p=0.142.

There was strong correlation between weight loss and number of holes per seed, proportion of seeds with holes, germination rate and germination speed. Significant positive correlation was observed between weight loss, number of holes per seed, damage score and number of seeds with holes ($p \leq 0.05$); however, the germination traits were not as highly correlated with seed damage (Table 11) traits.

Table 11: Correlation between seed damage parameters as caused by *C. maculatus*

	Weight loss	No. of holes/seed	Seeds with holes	Damage Score	Germination	Germ. Speed	MGT
Weight loss(g)	-						
No. holes/seed	0.934**						
Seeds with holes	0.878**	0.969**					
Damage Score	0.933**	0.842**	0.811**				
Germination rate	-0.508*	-0.498	-0.575*	-0.457			
Germ spd	-0.562*	-0.604*	-0.694*	-0.580*	0.4235		
MGT	-0.471	-0.438	-0.489	-0.429	0.8763**	0.299	-

Germ spd- Germination speed, MGT- Mean Germination Time, *-significant **- highly significant.

5.5 Discussion

The low weight loss and least number of emergence holes are attributed to the glass bottle being an airtight container which prevents gaseous exchange. Similarly, the grain storage bags with double heavy gauge polyethylene do not allow exchange of gases. Insects present during the storage period will survive on limited oxygen in the early stages of seed storage but later on suffocate due to lack of oxygen (Divekar *et al*., 2016). Storage bags which are multilayerd reduce cowpea weevil infestation by causing insulation against the weevil; when the grain and insects respire, the inter-granular oxygen levels are greatly reduced from 21 % to 5% resulting in reduced insect activity and eventually causing their mortality (Groote *et al*., 2013). This explains the high rate of insect mortality in the glass bottle for both experiments and for the grain storage bags in the first set of experiment. Use of PICS in storage of mung beans and pigeon peas in Kenya is reported to have retained grain weight and kept pest infestation low as opposed to polypropylene bags (Mutungi *et al*., 2014). The grain storage bags infestation was higher in the second set of experiment compared to the first set of experiment. This is attributed to the cowpea line used for the second set of experiment that was procured from the farmer. The reason could be the seeds did not go through a thorough and proper seed handling procedure like drying and sorting which can lead to faster seed deterioration (Houssou *et al*., 2010). The polyethylene bag, on the other hand, ranked second in keeping the seeds from damage. This is because the polyethylene bag is thin walled and partially hermetically sealed or could allow some gaseous exchange. Even though the polythene bag allows some gaseous exchange it prevents direct entry and infestation by the weevils. The effectiveness of the thin polyethylene is dependent on the environment of storage and inherent insect pest in the seed lot (Malarkodi and Ananthi, 2017); storage in this material can be accompanied by seed treatment to increase efficiency. The use of polyethylene material in storage of cowpea is better than unpackaged seeds or storage in cloth bags (Susmithar and Rai, 2017). In East Africa many small holder farmers prefer traditional methods of seed storage (Francis and Waithaka, 2015) like gourds. The gourd is a traditional seed storage technique used by farmers in Eastern and Western Kenya (Njonjo *et al*., 2019); it provides a physical barrier to weevil infestation but does not provide sustained storage conditions due to the inherent pests already present on the seed. The lid which is made from animal skin has avenues for gaseous exchange and there are other secondary holes on the gourd itself. Presence of air circulation and moisture allows weevils inherent in the seed lot to proliferate and lay eggs continuously as it is the case with non-hermetic material (Malarkodi and Ananthi., 2017). To improve utilization of gourd in seed storage, farmers have to dress the seeds with an insecticide, a biological agent or products that kill any inherent weevils. It is also important to use gourds that do not allow entry of the weevil (Nyamandi and Maphosa, 2013). The cellulose paper bag which represents minimum or no packaging at all showed highest level of seed damage. With uncontrolled moisture and air circulation the inherent weevils are able to aerate and reproduce at a very high rate. The infestation within the cellulose paper bag was continuous throughout the entire storage period. The seed lot or grain stored under these conditions is not suitable for consumption because it has holes, flour dust; it's broken, and loses its aesthetic value. The germination rate similarly declines hence not suitable for commercial planting (Cruz *et al*., 2015).

Seed packaging material in storage affect weight retention and germination in cowpea seed (Yakubu , 2014). Weight retention is important because the loss in weight directly translates to the returns for retailers and farmers. In the current study, it was evident that poor storage of 10kg seed would result in loss of 1kg therefore for every 90 kilograms' sack that would result to a total loss of 18kg. This loss to a farmer translates to loss in seed and monetary value. On the contrary hermetic storage like glass bottles retains seed weight up to 99%. Use of clay pots a non-hermetic material in cowpea seed storage is reported to have recorded 13% weight loss which is similar to the current study for gourd and cellulose paper bag (Yakubu, 2014). The germination rate recorded was however 73% which is different from the gourd and cellulose bags. Mean germination time and germination speed are important measures of germination. In this study it took a shorter time for all the seeds in the glass bottle to germinate compared to the other packaging materials. In a related study, seed stored in glass bottles and polythene bags recorded higher germination rate and the seeds were stored for longer periods as opposed to those stored in the cotton cloth (Bourtey *et al*,. 2016). The low germination over storage time is because there is deterioration that occurs during this time, which may be due to weevil damage, nutrient exhaustion and deterioration (Susmithar and Rai., 2017). Seeds that are well stored retain higher germination capacity like the case of the glass bottle. Cowpea weevil infestation has a direct impact on the germination of seeds during storage since the seeds that were less infested also expressed higher percentage germination and vice versa. Germination capacity for physiologically mature cowpea seed was found to range from 66- 92 % (Olasoji *et al*.,(2013). Seed that is poorly packaged for storage has potential to reduce its germination capacity as the case in gourds and cellulose bags. Poor quality seed will lead to a rapid loss of viability and this could cause poor crop establishment and eventually low productivity (Morad, 2013). Ilesanmi and Gungula (2013) also established that cowpea germination reduced to 35% after some period of storage even with *Moringa oleifera* treatment.

The seed in gourd and cellulose paper bag packaging materials were more damaged and exhibited a low germination rate compared to those that were minimally damaged like in the glass bottle in seven days. Significant positive correlation between seed damage parameters means that any of the parameters could be used for determination of seed damage by the cowpea weevil (Augustine *et al*., 2018). For example loss in weight was directly related to degree seed damage thus leading to poor germination in the field (Morad, 2013). When the level of damage increased the germination rate reduced. This implies that seed damage has a direct influence on germination rate and mean germination time (Olasoji *et al*., 2013).

5.6 Conclusions and recommendations

The glass bottle was the best packaging material for cowpea seed storage followed by polyethylene and grain storage bags. Airtight packaging materials had least weight loss, lower number of holes per seed and had higher seed germination rates.

Inappropriate and inadequate packaging of cowpea seed for storage will lead to loss of seed weight, germination capacity and aesthetic value.

Therefore in order to better manage cowpea weevil infestation, application of hermetic storage techniques is recommended for packaging of seed for storage and therefore, farmers are encouraged to modify the storage conditions in order to provide for hermetic storage conditions in cowpea weevil management.

Use of the grain storage bags is equally recommended because appropriate and proper sealing is done seeds can be kept for a long time without being damaged by cowpea weevil because of the achieved air tight conditions.

CHAPTER SIX

GENERAL DISCUSSION

Cowpea lines used in the study differed significantly in both plant and seed characteristics. Significant differences among the lines were observed for biochemical composition. Plant and seed characteristics are important for identification, selection and production of cowpea (Abadassi, 2015). Hutchinson, *et al*.,(2017) reported variation in maturity, leaf and pod charateristics among twenty eight Kenyan accessions collected in the coastal region. Genetic variation is an important component for cowpea classification and selection (Menssena *et al*., 2017) and breeding. Seed coat color is one of the seed quality parameters for cowpea selection (Aysun and Erkut 2013). Seed color, seed size, relative resistance to diseases and seed taste influences farmers' and consumers selection in cowpea lines (Kumar *et al*., 2015).

Biochemical composition differed among the cowpea lines and had influence on cowpea weevil infestation. Mogbo *et al*., (2014) reported higher number of emergence holes and weight loss in a cowpea variety with 21.8 % protein content. The protein content has a positive correlation with the cowpea weevil infestation. Similarly, Fawki *et al*., (2012) observed a negative correlation between cowpea seed protein content with susceptibility to cowpea weevil infestation. Tannin is one of the phenolic compounds present in most colored cowpea genotypes (Sombier *et al*., 2018). Cowpea seed differ in seed coat color with dark color in seed coat being associated with high tannin content (Oyeyinka *et al*, 2013). The mode of action of tannins leading to less infestation is antixenosis whereby cowpea lines with high tannin content are less prefered as hosts by cowpea weevil; as tannins deter egg oviposition and proliferation (Smith and Clement, 2012)

Legumes are known to be rich in amino acids. Dominant amino acids in cowpea, chick pea, green pea and lentil were lysine, leucine and arginine (Iqbal *et al*., 2006). Fenugreek a popular legume in the Mediterranean countries and Asia is rich in asparagine and glutamine more so in hydrophobic amino acids (Feyzi *et al*., 2015).

Lentils are rich in most of the essential amino acids and having tryptophan as a limiting amino acid (Kahraman, 2016). Black soybean seed is abundant in acidic amino acids and low in hydrophobic amino acids (Chen *et al*., 2017). The positive correlation between total amino acid content and damage by the beetles like cowpea weevil is due to their ability to detect nutrient concentrations within grains and thus infest. This is facilitated by the presence of neurosensory ducts (Chapman and Bernays, 1995). Amino acid derivatives are examples of plant volatiles which can be utilized by pests to locate hosts for feeding and oviposition (Schoonhoven *et al*., 2005)

Quality of seed packaging material is as important as the quality of the seed at storage. Lack of proper packaging of seed predisposes them to complete loss due to the cowpea weevil infestation and colonization. Cowpea farmers are still forced to use plant products against the cowpea weevil because they are better than synthetic pesticides (Asawalam and Anaeto, 2014). Lack of proper seed handling or presence of infested seed at storage time, limits the effectiveness of the grain storage bags as it happened in the second experiment. Controlled atmosphere around the seed has been suggested to provide a solution to long-term storage of food grain which involves modified atmosphere by controlling gaseous exchange, humidity and temperatures (Divya *et al*., 2016).

In the current study eggs and live weevils were observed at the end of the experiment in gourds and cellulose bags meaning that continued infestation is likely to proceed even after 5 months of storage. To achieve prolonged storage then farmers and other seed consumers are encouraged to use seed treatment procedures (Behera *et al*., 2016); care has to be taken so that they do not apply pesticides beyond the recommended levels that could end up in food products (Muhammad, 2015) or harm the environment.

Germination rate is a measure of seed quality. Storage of seed under hermetic conditions aids in retaining weight and maintain the seed quality in terms of germination capacity. The glass bottle registered 92% germination rate after a storage period of 5 months which is above the acceptable germination rate which stands at 75% (ISTA 2000). The type of the material used for packaging and storage of cowpea will affect seed quality at the end of the storage period. Seed that is stored in hermetic environment will retain its weight, aesthetic value, and also the germination capacity (Bourtey *et al*., 2016). Proper storage of cowpea using hermetic methods will lead to minimal losses, ensure prices of cowpea are uniform and also ensure consistency in production and supply of cowpea grains (Yakubu, 2012).

CHAPTER SEVEN

GENERAL CONCLUSION AND RECOMMENDATION

7.1 General conclusion

Cowpea lines studied represent a rich germplasm based on the variation in plant and seed characteristics. Plant and seed characteristics of cowpea lines differed among the cowpea lines and they had an influence on *Callosobruchus maculatus* infestation during storage. Cowpea weevils preferred late maturing lines, light colored seed and those with high 100 seed weight.

Biochemical composition (protein, amino acids and tannin content) differed among the cowpea lines. Cowpea lines with higher protein and amino acid content were more infested as opposed to those with high tannin content.

For better management of cowpea weevil infestation, the hermetic packaging technique in storage sustains cowpea seed quality. Identification and selection of resistant cowpea lines and proper packaging material for seed storage will reduce losses in cowpea seed and avail quality seed.

7.2 General recommendation

The information on plant and seed characteristics and their influence on weevil infestation should be incorporated in plant breeding programs and it's important for farmers in selection of lines that are resistant to weevil infestation .The lines that have been identified to showing lower damage can be used for breeding for resistance against cowpea weevil.

Farmers should ensure that the seed is well packaged for storage; and provide conditions that strive to achieve hermetic storage conditions in cowpea weevil management.

7. 3 Future research

It is recommended that for future research, cowpea packaging material should be assessed by monitoring dynamics in the conditions within the storage materials to explain the mechanisms controlling the weevil infestation.

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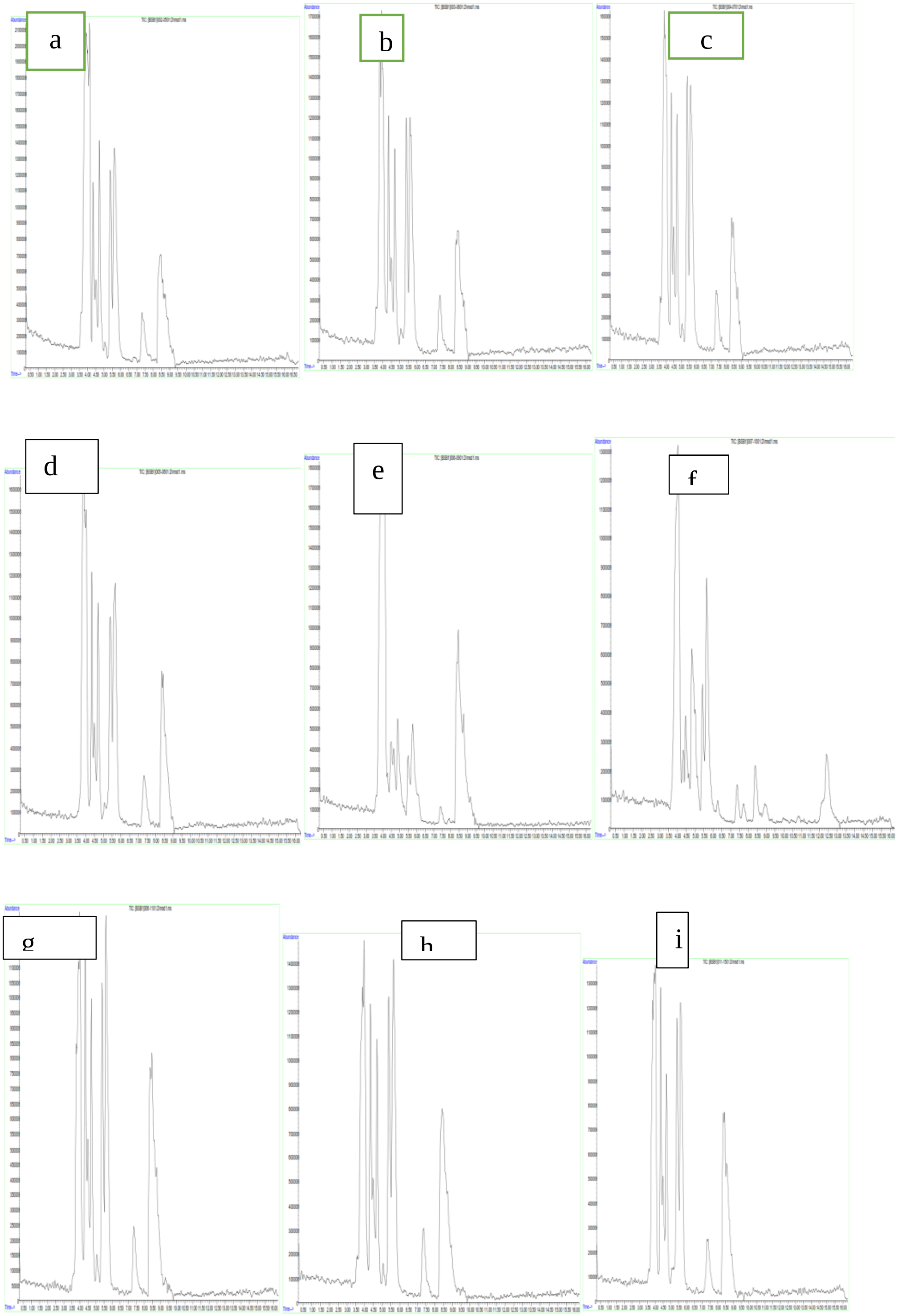
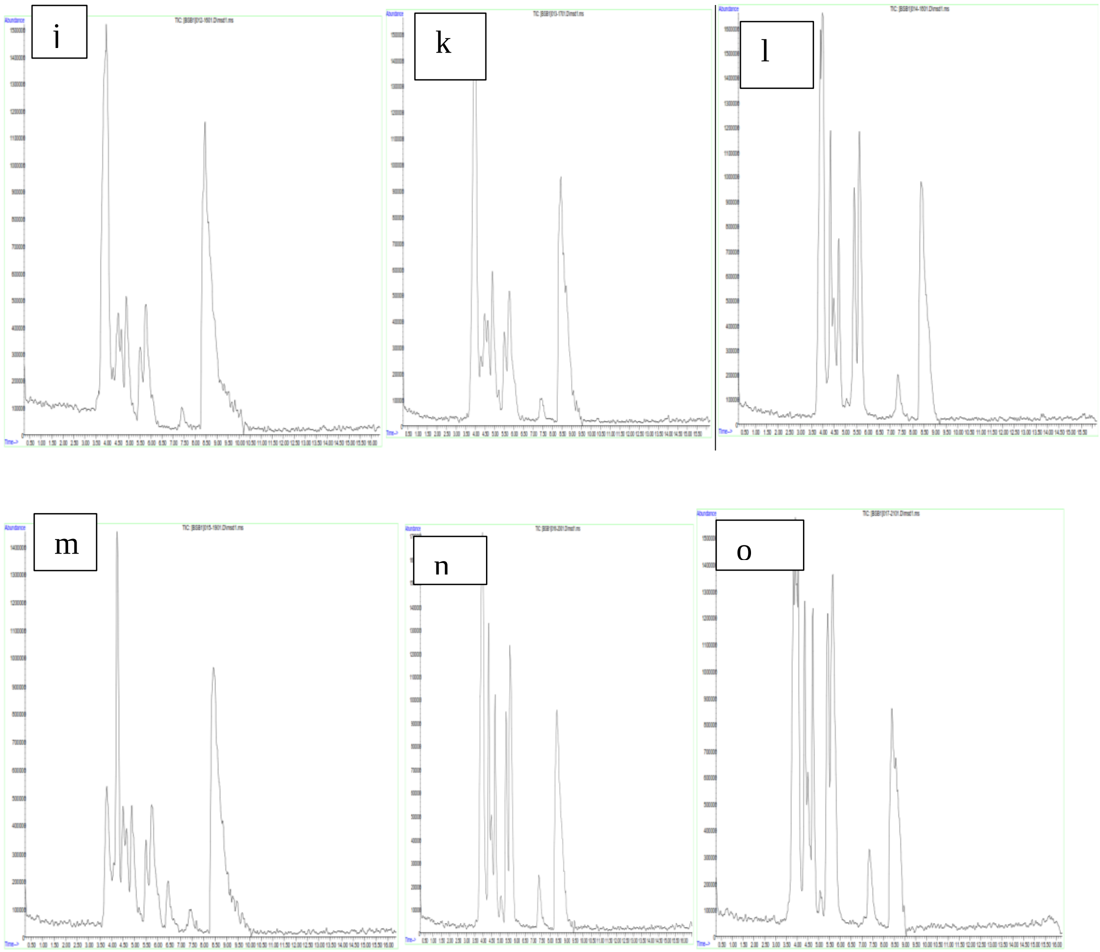
APPENDICES

Appendix I: Chemical profile of liquid chromatography mass spectroscopy for amino acids



Chemical profile of LC-MS amino acid analysis for a) GBK003645, b)GBK003659 c) ACC 25, d) GBK003814, e) KAB 1, f) GBK003660R, g) 9334, h) GBK00369 and i) LAM 4



Chemical profile of Liquid Chromatography Mass Spectroscopy (LC-MS) amino acid analysis for j) GBK005173 k) DAKAWA l) KENKUNDE m) GBK003695 n) GBK003689 o) GBK003656