EVALUATION OF INSECT PEST TOLERANCE IN ETHYL METHANE SULPOHONATE (EMS) EXPOSED COWPEA (*VIGNA UNGUICULATA* L.)

BY

MUHAMMAD, Salamatu
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ABSTRACT

Cowpea [*Vigna unguiculata* (L.) Walp.] is a widely cultivated legume in the semiarid tropics of Africa and the United States. Low yield of cowpea is often associated with insect pest attacks. This study aimed at evaluating the insect pest tolerance in Ethyl methane sulphonate (EMS) induced cowpea. The seeds were collected at National Centre for Genetic Resources and Biotechnology (NACGRAB). The seeds were planted in 25 kg planting bags at the botanical garden of the Department of Plant Biology, Federal University of Technology, Minna using a Complete Randomized Design (CRD) with 4 replicates. Four cowpea genotypes (Early white, Local variety, IT90K-76 and IT97-556-4) were treated with different concentrations of EMS (0.00%, 0.10%, 0.20%, 0.30% and 0.40%). Control of IT97K-556-4 had the highest plant height at week 20 (70.73 cm), 0.40 % of IT97K-556-4 had the highest number of leaves, it also had the highest leaf width at 0.10 % and highest leaf length at 0.20 % concentration of EMS. The accessions varied considerably in terms of morphological parameters. For insect infestations, the associated insects are Cowpea foliage weevil *(Callosobruchus maculatus)* and Armyworm *(Spodoptera frugiperda)*. Low insect infestation of 0.20 % concentration of EMS for IT97K-556-4 and Local variety compared to other treatments is an indication of tolerance to the genotypes. For yield parameters, the accessions and concentrations varied considerably. Accession Early white had the heaviest weight of pod and weight of 100 seeds in concentration 0.30 % (2.10 g) and concentration 0.20 % (20.03 g) respectively. Accession Local variety had the best in length of pod (14.70 cm), number of seed per pod (12.33), number of pod per plant (12.33) in control. For proximate composition, control of IT90K-76 genotype had the highest moisture content at concentration 0.30 % (10.78), it had the highest Ash content (5.88), crude fat (4.48) and crude fibre (0.39). Local variety had the highest dry matter and carbohydrate at concentration 0.10 % (94.00) and 0.40 % (70.15). For mineral composition, IT97K-556-4 had the highest sodium, potassium and magnesium at 0.10 % (270 mg /100 g), 0.40 % (710 mg /100 g) and 0.10 % (470 mg /100 g. it can be concluded that there is an indication of induced variability in the crop and has a potential of being selected in crop breeding programs. It is therefore recommended that further studies should be carried out to ascertain the effect of EMS on cytological content and molecular compositions of the genotypes.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Cowpea [*Vigna unguiculata* (L.) Walp.] is a widely cultivated legume in the semiarid tropics of Africa and the United States (Huynh *et al.,* 2015). It can be grown in regions with an average annual rainfall of 2.5 to 8 inches (Cook *et al.*, 2005). Cowpea feeds millions of people in the developing world with an annual worldwide production estimated around 4.5 million metric tons on 12 to 14 million ha (Diouf, 2011). The bulk of cowpea production and consumption is in sub-Saharan Africa (SSA), particularly West and Central Africa. Nigeria is the highest producer of cowpea grains, with annual production of about 2.14 million metric tonnes and consumes more than 3.0 million metric tonnes. The other major producers are Niger Republic and Burkina Faso with an average of 1.59 and 0.57 million metric tonnes, respectively (Food and Agriculture Organization Statistics-FAOSTAT, 2017).

Cowpea production is constrained by many factors that are both biotic and abiotic (Hall *et al*., 1997). These factors are responsible for the low grain yield of cowpea across Sub-Saharan Africa in particular. The low cowpea yields have been attributed to several factors which include the use of unimproved varieties, poor soil conditions, inadequate management practices, poor cultural practices and heavy biotic stresses, particularly from insects, diseases and parasitic weeds which often attack in the field (Horn *et al.,* 2015).

Cowpea has often been referred to as "poor man's meat" due to the high levels of protein found in the seeds and leaves (Hamid *et al.,* 2016). It is important because it serves as a source of nutrients for human and as fodder for animals and its ability to fix atmospheric

nitrogen enhances soil fertility. Cowpea is mainly cultivated by small-holder farmers because of the low cost of production (Belay *et al.,* 2017). In these regions a high proportion of the rural population depends on cowpea for their nutritional and economic subsistence, as this crop is rich in carbohydrates and proteins. Its cultivation serves as a source of employment and income (Silva and Neves, 2011; Freitas *et al.,* 2014).

Cowpea seeds provide a rich source of proteins and calories, as well as minerals and vitamins (Gonçalves *et al.,* 2016). This complements the main cereal diet in countries that grow cowpeas as a major food crop (Phillips *et al.,* 2003). A seed can consist of 25 % protein and has very low fat content (Rangel *et al.,* 2003). Cowpea starch is digested more slowly than the starch from cereals, which is more beneficial to human health (Gonçalves *et al.,* 2016). The grain is a rich source of folic acid, an important vitamin that helps prevent neural tube defects in unborn babies (Witthöft and Hefni, 2016). However, it does contain some anti-nutritional elements, notable phytic acid and protase inhibitors, which reduce the nutritional value of the crop (Gonçalves *et al.,* 2016).

Insect pests attack cowpea both in field and in storages. Several studies have reported major field pests of cowpea including *Aphis craccivora* (Koch), bruchids (*Callosobruchus maculatus*), beetles, foliage beetles and leafhoppers. The pest occurs throughout the vegetative stages of the plant, feeding on leaves and act as virus vectors. Farmers have described predominant field pests including aphids causing yield losses (Horn *et al.,* 2015). Various national and international research programs notably the International Institute of Tropical Agriculture (IITA) are actively developing improved cowpea cultivars with high yields, early maturity, pest and disease resistance (Dugje *et al.,* 2009). Most breeding

programs use conventional and molecular breeding tools to harness cowpea genetic variation for breeding (Hall, 2012).

The International atomic energy agency (IAEA) has been supporting in genetic improvement of various crops including cowpea through the use of artificial mutagenesis such as x-rays, Ethyl methane sulphonate (EMS) and gamma rays (Mba *et al.,* 2010). This has led to development and release of improved cowpea cultivars in Africa, Asia, and Latin America (Reddy *et al.,* 2013). Most cowpea breeding initiatives have led to broadening genetic bases of the crop to adapt to various cropping systems and in the development of consumer preferred varieties with enhanced nutritional quality (Lima *et al.,* 2011). The following breeding methods have been used in cowpea improvement programs: Pure-line selection, pedigree breeding, backcross breeding, single seed descent selection method, bulk population breeding and mutation breeding (Singh *et al.,* 2016).

Mutation- breeding programme has proved to be a successful tool in bringing amelioration in self- pollinated crops. Future research on induced mutations would also be important in the functional genomics of many food crops including cowpea. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement and an efficient mean to supplement existing germplasm for cultivar improvement in breeding programmes (Kozgar *et al.,* 2012).

Chemical mutagen generally produce induced mutations, which lead to base pair substitution especially Guanine and Cytosine to Adenine and Thymine resulting in amino acid changes, which changes the function of proteins, but do not abolish their functions. These chemo mutagens also induce a broad variation of morphological and yield structure change in comparison to normal plants (Greene *et al.,* 2003). Alkylating agents such as

Ethyl methane sulphonate (EMS) induce chemical modification of nucleotides, which result in mispairing and base changes. Strong biased alkylation of guanine (G) residue results, forming O6-ethyl guanine, which can pair with thymine (T) but not with cytosine (C). Through subsequent DNA repair, the original G/C pair can then be replaced with A/T. Ninety-nine percent of mutations from alkylation of guanine induced by EMS are reported as G/C-to-A/T transitions (Greene *et al.,* 2003).

1.2 Statement of the Research Problem

The major constraint that negatively influence cowpea productivity are insect pests (Karungi *et al.,* 2000). Studies have indicated that insect pests are the major production constraint (Oyewale and Bamaiyi, 2013). They further reported that aphids (*Aphis craccivora* Koch), thrips (*Megalurothrips sjostedti* Trybom), legume pod borers (*Maruca vitrata* Fab. Syn. *Maruca testulalis* Geyer) and a complex of pod sucking bugs are the most important. Traditionally, Nigerian farmers have been relying heavily on pesticides for the control of various weeds, insect pests and diseases, such agricultural inputs have become so expensive and almost unaffordable. In addition, the continuous usage of these pesticides pose several health challenges on the farmers that use them. There has also been reports of residual effects of the application of insecticides on the environment as well as agrotoxicity. Therefore, there is need for alternative to these synthetic pesticides.

1.3 Aim and Objectives

The aim of this study is to evaluate Insect pest tolerance in Ethyl Methane Sulphonate (EMS) induced cowpea (*Vigna unguiculata*) genotypes.

The objectives of this study are to determine the;

i. effect of insect pest tolerance in Ethyl Methane Sulphonate (EMS) induced cowpea genotypes.
ii. optimum concentration of EMS that induces resistance to insect pest on the cowpea genotypes
iii. effect of EMS on selected agro-morphological and yield traits on the first mutant generation (M1) lines of the cowpea genotypes.
iv. effects of EMS on proximate and mineral composition of the M1 lines of the cowpea genotypes.

1.4 Justification for the Study

Cowpea is an important grain legume in sub- Saharan Africa with Nigeria inclusive. It is one crop that can meet the dietary needs of the poor masses. This is because of high nutritional composition of the crop and it contains 24.8% protein, fat 1.9%, fiber 6.3%, carbohydrate 63.6%, thiamine 0.00074%, Riboflavin 0.00042% and Niacin 0.00281%. Induced mutagenesis has the potential to create genetic variation for genetic enhancement and breeding in a relatively shorter time unlike natural mutation (Tulmann Neto *et al.,* 2011). Induced mutation have been successfully used in breeding of seed propagated crops since 1940s (Gnanamurhy *et al.,* 2012). Ethyl methane sulphonate (EMS) which is a chemical mutagen produce a range of novel traits and broadening of genetic diversity of plants (Lagoda, 2007). With development of new techniques such as targeting induces local lesions in genomes, EMS mutagenesis can be used for both forward and reverse genetic studies. Ethyl methane sulphonate (EMS) has been long considered as a potential chemical mutagen for inducing beneficial genetic variability in crop plants and has been used for generating breeding lines.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of Cowpea

The precise origin of cultivated cowpea has been a matter of speculation and discussion for many years because of a lack of archaeological evidence. Cowpea is believed to have originated from West Africa by some workers, because both wild and cultivated species thrive in the region (Jackai and Daoust, 1986). Some authorities felt that cowpeas originated either in the southern Sahel of north-central Africa or in Ethiopia, and then spread to Asia and the Mediterranean by way of Egypt (Alayande *et al.,* 2012). Another view is that they originated in India and were introduced into Africa some 2,000 to 3,500 years ago (Alayande *et al.,* 2012). The major centre of diversity of cultivated cowpea is found in West Africa, in an area including the savanna region of Nigeria, southern Niger, part of Burkina Faso, Northern Benin, Togo, and the Northwestern part of Cameroon (Ng and Marechal, 1985). The name cowpea probably originated from the fact that the plant is an important source of hay for cows in the south-eastern United States and in other parts of the world (Timko *et al.,* 2007). Today cowpea is grown throughout the tropic and subtropic areas around the whole world. It is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al*., 2002; Tarawali *et al*., 2002; Sanginga *et al*., 2003)

2.2 Taxonomy and Botanical Classification of Cowpea

Vigna unguiculata is a member of the *Vigna* (peas and beans) genus. *unguiculata* is a Latin for "with a small claw", which reflects the small stalks on the flower petals (Small, 2009). All cultivated cowpeas are found within the universally accepted *V. unguiculata* subspecies

unguiculata classification, which is then commonly divided into four cultivar groups: *unguiculata*, *biflora*, *sesquipedalis*, and *textilis* (Padulosil and Ng 1997). Some well-known common names for cultivated cowpeas include black-eye pea, southern pea, yardlong bean, *catjang*, and crowder pea (Timko *et al.*, 2007). The classification of the wild relatives within *V. unguiculata* is more complicated, with over 20 different names having been used and between 3 and 10 subgroups described (Pasquet, 1999). The original sub-groups of *stenophylla*, *dekindtiana*, and *tenuis* appear to be common in all taxonomic treatments, while the variations *pubescens* and *protractor* were raised to subspecies level by a 1993 characterization (Padulosil and Ng 1997). The name was most likely acquired due to their use as a fodder crop for cows (Timko *et al.*, 2007).

Kingdom- Plantae
Sub-kingdom- Tracheobionta
Super-division- Spermatophyta
Division- Magnoliophyta
Class- Magnoliopsida
Sub-class- Rosidae
Order- Fabales
Family- Fabaceace
Genus- *Vigna*
Species- *V. unguiculata*

Source: Timko *et al.* (2007).

2.3 Botanical Description

The cowpea [*Vigna unguiculata* (L.) Walp.] is an annual herbaceous legume cultivated for its edible seeds or for fodder. Cultivated cowpeas are herbaceous annuals that are either erect, prostrate or climbing annuals with a tap root and virtually all are glabrous. They are mostly grown for grain but a small proportion (about 10%) are grown as green leafy vegetables and fodder in Africa or as fresh pods in eastern Asia (Boukar *et al.*, 2011). Cowpea *V. unguiculata* can grow up to 80 cm and up to 200 cm for climbing cultivars. Germination is epigeal with the first pair of true leaves being simple and opposite and subsequent leaves being trifoliate with oval leaflets (6-15 cm long and 4-11 cm broad) and alternate.

The papillonaceous flowers are born on racemose inflorescences at the ends of peduncles that arise from leaf axils and can be white, yellowish, pale blue or violet. Peduncles are stout and grooved and usually much longer than the leaves (2-20 cm long). The leaf petiole is 5 to 25 cm long. For each inflorescence, flowers are sequentially produced in alternating pairs on thickened nodes at the tip with cushion-like extra-floral nectaries between each pair of flowers. The flower is large (standard is 2-3 cm in diameter), with a straight keel, diadelphous stamens (one free and nine fused), a sessile ovary with many ovules, and a style that is bearded along the inside and ends in an oblique stigma. Flowers are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in colour. Pods occur in pairs forming a V-shape, vary in size, shape, colour, texture, mostly pending and vertical, but they can be erect. Usually yellow when ripe, but may also be brown or purple in colour. They are cylindrical, 2-6 cm long and 3-12 mm broad and contain 8-20 seeds. The seeds are relatively large (2 to 12 mm

long) and weigh 5 to 30 g/100 seeds. Seeds can be white, pink brown or black (Heuzé *et al.,* 2013).

2.4 Cultivation and Agronomy of Cowpea

Cowpeas thrive in poor dry conditions, growing well in soils up to 85 % sand (Obatolu, 2003). This makes them a particularly important crop in arid, semi-desert regions where not many other crops will grow. Its nitrogen-fixing ability means that as well as functioning as a sole crop, cowpea can be effectively intercropped with sorghum, millet, maize, cassava, or cotton (Blade *et al.,* 1997). The optimum temperature for cowpea growth is 30 °C (86 °F), making it only available as a summer crop for most of the world. It grows best in regions with an annual rainfall between 400 and 700 mm (16 and 28 in). The ideal soils are sandy and it has better tolerance for infertile and acid soil than most other crops. Generally, 133,000 seeds are planted per hectare (54,000/acre) for the erect varieties and 60,000 per hectare (24,000/acre) for the climbing and trailing varieties. The seeds can be harvested after about 100 days or the whole plant used as forage after about 120 days. Leaves can be picked from 4 weeks after planting.

These characteristics, along with its low fertilization requirements, make the cowpea an ideal crop for resource-poor farmers living in the Sahel region of West Africa. Early-maturing varieties of the crop can thrive in the semi-arid climate, where rainfall is often less than 500 mm (20 in). The timing of planting is crucial, as the plant must mature during the seasonal rains (Dugje *et al.,* 2009). The crop is mostly intercropped with pearl millet, and plants are selected that provide both food and fodder value instead of the more specialized varieties *(*Matsunaga *et al.,* 2006).

Storage of the seeds can be problematic in Africa due to potential infestation by postharvest pests. Traditional methods of protecting stored grain include using the insecticidal properties of Neem extracts, mixing the grain with ash or sand, using vegetable oils, combining ash and oil into a soap solution or treating the cowpea pods with smoke or heat (Poswal and Akpa, 1991). More modern methods include storage in airtight containers, using gamma irradiation, or heating or freezing the seeds. Temperatures of 60 °C (140 °F) kill the weevil larvae, leading to a recent push to develop cheap forms of solar heating that can be used to treat stored grain (Murdock and Shade 1991). One of the more recent developments is the use a cheap, reusable double-bagging system (called PICs) that asphyxiates the cowpea weevils (Baributsa *et al.,* 2010).

2.5 Uses of Cowpea

Cowpea is one of the most important sources of protein in the diet of animals and man. It is one of the most critical human food and was been used as a crop plant since Neolithic times. Cowpea plays a major role in the family diet as it is utilized in different ways. It supplies more than half the plant protein in the diets in many developing countries (Aliyu and Wachap, 2014). Green seeds, young fresh leaves and immature green pods are eaten as a vegetable (Gerrano *et al.,* 2015, „2017, „2019). The seeds can be consumed fresh along with the pods and leaves as a vegetable. Dried seeds are consumed after cooking. The plant can be used as a forage or for hay or silage. The roots are eaten in Sudan and Ethiopia, and the peduncles and stems are used as fibres in Nigeria (Adeyemo, 2012). The haulms utilised for livestock fodder during the dry season when food is scarce (Asiwe, 2009). Fortified cereals with legumes has been used as weaning diets. It is a multifunctional crop

that provides food to human being and feed to livestock, it fixes nitrogen, is a protein rich, drought tolerant and early maturing crop (Sariah, 2010).

2.6 Nutritional Contents of Cowpea

Cowpea seeds provide a rich source of proteins and calories, as well as minerals and vitamins (Gonçalves *et al.,* 2016). This complements the mainly cereal diet in countries that grow cowpeas as a major food crop (Phillips *et al.,* 2003). A seed of cowpea can consist of 25% protein and has very low fat content (Rangel *et al.,* 2003). Cowpea starch is digested more slowly than the starch from cereals, which is more beneficial to human health (Gonçalves *et al.,* 2016). The grain is a rich source of folic acid, an important vitamin that helps prevent neural tube defects in unborn babies Witthöft and Hefni (2016). The cowpea has often been referred to as "poor man's meat" due to the high levels of protein found in the seeds and leaves (Hamid *et al.,* 2016). Cowpea is an important sources of energy (calories), proteins, vitamins (A, B-complex, C and K); and minerals such as iron, copper, magnesium, manganese and phosphorus in human diets (Abbas and Shah 2007; Oghbaei and Prakash 2016).

Sequel to the indispensable use of cowpea as a nutritive food substance in human and animal diets, various researchers have explored the proximate and mineral compositions of local varieties of this grain as an alternative to other dietary food substances (Singh *et al.,* 2002). Due to its strategic significance in Nigeria and the world at large, research on the proximate composition and long term genetic improvement are going on within various laboratories. However, it does contain some anti-nutritional elements, notable phytic acid

and protase inhibitors, which reduce the nutritional value of the crop (Gonçalves *et al.*, 2016). Although little research has been conducted on the nutritional value of the leaves and immature pods, what is available suggests that the leaves have a similar nutritional value to black nightshade and sweet potato leaves, while the green pods have less anti-nutritional factors than the dried seeds (Gonçalves *et al.*, 2016).

2.7 Cowpea Production

The bulk of cowpea production and consumption is in sub-Saharan Africa (SSA) particularly West and Central Africa. Nigeria has the highest production output, followed by Niger and Burkina Faso. Nigeria is the largest producer of cowpea in the world (FAO, 2020). The production of cowpea has spread to East and Central Africa, India, Asia, South and Central America (Sariah, 2010). Most cowpeas are grown on the African continent, particularly in Nigeria and Niger, which account for 66% of world cowpea production. The Sahel region also contains other major producers such as Burkina Faso, Ghana, Senegal, and Mali. Niger is the main exporter of cowpeas and Nigeria the main importer. Exact figures for cowpea production are hard to come up with as it is not a major export crop. While they play a key role in subsistence farming and livestock fodder, the cowpea is also seen as a major cash crop by Central and West African farmers, with an estimated 200 million people consuming cowpea on a daily basis (Langyintuo *et al.*, 2003). According to the Food and Agriculture Organization of the United Nations, as of 2012, the average cowpea yield in Western Africa was an estimated to be 483 kilograms per hectare (0.195 t/acre), which is still 50% below the estimated potential production yield.

2.8 Mutation Breeding

Mutation is considered as a tool to study molecular nature and functions of genes. Under *in vitro* conditions, mutations prepare the ground for breeding plants by expanding their range of genetic diversity (Adamu and Aliyu, 2007). Use of mutagens is a rapid and new method employed for improving qualitative and quantitative traits in many plants. Mutagens can influence cytological, biochemical, physiological, and morphological properties of plant tissues and cells. Success in any mutagenesis program under *in vitro* condition depends on developing repeatable procedures for regeneration of plants. Mutagenic treatments and efficient screening of mutated populations are optimised to achieve desirable changes (Jain, 2006).

There are four common mutagenesis methods

(1) Physical agents such as UV, X-ray radiation and fast neutron (FN),

(2) Chemical mutagens such as ethyl methane sulfonate (EMS), N-nitroso-N-methylurea (NMU), ethyl nitrosourea (ENU), 1,2:3,4-diepoxybutane (DEB),

(3) Biological agents such as T-DNA and transposons (Hancock *et al.*, 2011), and

(4) Transgenic technologies such as CRISPR-Cas9, TALENs, gene knockdown using RNAi (Lu *et al.*,2015).

Physical and chemical mutagens have been successfully used in plant breeding programs to artificially generate genetic variation for the development of new varieties with improved traits such as increased yield, earliness, reduced plant height, and resistance to disease (Maluszynksi *et al.*, 2000). Induced mutation can rapidly create variability in quantity and quality of crops. According to Ahloowalia *et al.* (2004) he reported that induced

mutagenesis has been used to obtain direct mutants or by using these mutants in hybridization to generate desirable horticultural traits. Mutation breeding has contributed significantly to plant improvement, resulting in release of at least 2250 varieties of different crops.

2.8.1 Physical mutagens

Physical mutagens include various types of radiation, *viz*., X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra violet rays. A brief description of these mutagens is presented below:

Table 2.1: Commonly used physical mutagens (radiations), their properties and mode of action.

Type of Radiation	Main Properties
X-rays	S.I (Sparsely ionizing), penetrating and non-particulate
Alpha Particles	D.I (Densely ionizing), particulate, less penetrating and positively charged
Beta Ray Particles	S.I (Sparsely ionizing), particulate, less penetrating, and positvely charged
Fast and Thermal Neutrons	D.I (Densely ionizing), particulate, neutral particles, highly penetrating
Ultra Violet Rays	Non-ionizing, low penetrating

Source: (Singh, 2004)

2.8.2 Chemical mutagens

The chemical mutagens can be divided into four groups, viz. 1) alkylating agents, 2) base analogues, 3) acridine dyes, and 4) others. A brief description of some commonly used chemicals of these groups is presented below.

Table 2.2: Some commonly used chemical mutagens and their mode of action

Group of mutagen	Name of chemical	Mode of action
Alkayting agents	Ethyl methane sulphonate (EMS)	AT-GC Transitions
	Methyl methane sulphonate (MMS)	Transitions
	Ethyl ethane sulphonate (EES)	GC-AT Transitions
	Ethyl imines	Transitions
Base Analogue	5 Bromo Uracil	AT-GC Transitions
	2 Amino Purine	AT-GC Transitions
Acridine Dyes	Acriflavin	Deletion,addition and frame shifts
Ohers	Nitrous acid	AT-GC Transitions
	Hydroxylamine	GC-AT Transitions
	Sodium Azide	

Source: Singh *et al.* 2006

Singh *et al.* (2006) carried out a mutagenesis programme using three chemical mutagens viz. Ethyl methane sulfonate (EMS), Methyl methane sulfonate (MMS) and Sodium azide (SA) on two varieties of cowpea. In M2 generation, a wide spectrum of macro-mutations was observed in the progenies of both the varieties including few seed color mutants. Several M2 progenies of the two cowpea varieties were significantly superior to their respective parents for seed yield per plant.

2.8.3 Ethyl methane sulphonate (EMS)

Ethyl methane sulphonate is a mutagenic, teratogenic, and carcinogenic organic compound. It produces random mutation in genetic material by nucleotide substitution. It produces only point mutation. The mutagenic agent Ethyl methanesulphonate (EMS) can be used to make mutations at a higher frequency and generate genetic variation from which desired mutants may be selected (Asbah, 2007, „Ibrahim, 2008 „Talebi *et al.,* 2012). Ethyl methane sulfonate (EMS) is more effective than physical mutagens (Bhat *et al.,* 2006). EMS induces

Cytosine-to-Thiamine changes resulting in Cytosine/Guanine to Thiamine/Adenine substitutions (Kim *et al*., 2007). It is a colourless liquid compound with a molecular weight of 124 and is 8% soluble in water. EMS belongs to the group of the alkylating agents. These compounds have one or more reactive alkyl groups, which are capable of being transferred to other molecules at a position of higher electron density. According to their number of functional groups, they are mono-, bi-, or poly-functional alkylating agents. Bi- and poly-functional alkylating agents are generally more toxic than a mono-functional agent. EMS is a mono-functional alkylating agent.

Decrease in quantitative traits at higher concentrations of EMS has been reported by Kozgar *et al.* (2011) in *Vigna radiata* and *Vigna mungo*. Wani (2012) also reported the increase of various quantitative characters like number of pods per plant and 100 seeds weight at lower concentrations of EMS in M3 generation in chickpea (*Cicer arietinum* L.). Decrease in seed germination has also been reported by (Khan and Wani, 2004) in mungbean. A similar result was also observed by (Watto, 2012), after studying the mutagenic variability by EMS in Basmati rice. Selvaraj and Jaykumar (2004) observed decrease in seed germination, pollen fertility and general variation in quantitative traits with increasing concentrations of EMS on sunflower. Basu *et al.* (2008) observed improvement of different quantitative traits like pod length and number of pods in M3 plants of Fenugreek (*Trigonella foenum-graecum* L.) after treating with different concentrations of EMS.

Selvaraj (2012) studied different quantitative characters like plant height, number of days for first flowering, after treatment of EMS in *Jatropha curcas* L. They observed that height decreased with increasing concentrations of EMS while the number of days for first

flowering considerably reduced at lower concentrations while as the same increased at higher concentrations.

2.9 Overview of Mutation Breeding in Cowpea

Mutations are the ultimate source of genetic variation, a raw material for plant breeding programs. Induced mutation derived through the use of gamma rays, x-rays, or EMS is a powerful tool for crop genetic enhancement and breeding. Appropriate dose of radiation should be established on target genotypes before large scale mutagenesis is undertaken (Tshilenge-Lukanda *et al.,* 2012). Induced mutations provides considerable genetic variation within a reasonably short period of time when natural genetic variation of the crop is limiting for breeding. Parry *et al.* (2009) reported that mutation breeding process is fast forward in developing diverse germplasm and it may take only up to 6 generations (M6). This can be followed by further generations by single seed descent to generate near-homozygous material as opposed to the conventional breeding techniques. It is however recommended to have a very large populations of induced mutations in order to ensure that gene of interest carries sufficient significant mutations. The size required is dependent on the dosage of mutagen and the level of gene duplication created by recent or ancient polyploidization events. Studies indicate that induced mutagenesis has successfully modified several plant traits such as plant height, maturity, seed shattering resistance, disease resistance, oil quality and quantity, malting quality, size and quality of starch granules of cowpea (Goyal and Khan, 2010; Singh *et al.,* 2013).

Despite its importance and significant contribution to plant breeding and genetics, there is limited information that induced mutation could have negative impact on the environment or on organisms. Furthermore, it was found that most research papers only discussed the

importance without reporting the possible negative impact (Mba *et al.,* 2010; Tulmann Neto *et al.,* 2011). Chopra (2005) and Slabbert *et al.* (2004) gave details on varieties and the techniques to induce mutation from different countries including USA, China and India. In general induced mutation technique has been in use for over 100 years (Shu, 2008). This gave a clear indication that the method have been used and accepted for over 100 years without harmful effects resulting from its use or application. Suprasanna *et al.* (2015) reported that the mutant varieties developed and released in major crops have been cultivated by farmers in large areas and have resulted in increased food production, thus contributing to food security. Most of these breeding programs use conventional and molecular breeding tools to harness cowpea genetic variation for breeding. Furthermore, the international atomic energy agency (IAEA) has been supporting member states in genetic improvement of various crops including cowpea through the use of artificial mutagenesis such as gamma rays, x-rays, and Ethyl methanesulphonate (EMS) (Mba *et al.*,2009). This has led to development and release of improved cowpea cultivars in Africa, Asia, and Latin America (Viswanatha *et al.*, 2011; Reddy *et al.*, 2013). Further, most cowpea breeding initiatives lead in broadening the genetic bases of the crop to adapt various cropping systems and agro-ecologies, and in the development of consumer-preferred varieties with enhanced nutritional quality (Singh *et al.,* 2002; Lima *et al.,* 2011).

2.10 Insect Pest of Cowpea

Insect pest pose great threat to cowpea production in Nigeria. The crop is severely attacked at every stage of its growth by insects. There are many different types of insect pests that affect cowpea at many locations with complete loss of grain yield due to heavy infestations if no control measures are taken. The most damaging insect pest are aphids, flower thrips,

maruca pod borer, leaf weevil, armyworm and pod-sucking bugs, leaf hoppers, foliage beetles leaf hoppers, cowpea beetles among others Oluwafemi *et al*. (2013). Fall armyworm *(Spodoptera frugiperda)* affect crop at different stages of growth, from early age to maturity. Fall armyworm cut down young plant and damages leaves giving it a torn appearance.

2.11 Mutation Breeding for Insect Pest and Disease Resistance

Induced mutagenesis has been used to create plant varieties that show resistance to insect pest and diseases. Transgenic method is a powerful means of development of insect-pest resistant varieties for sustainable crop improvement. Transfer of insect resistance genes by transgenic technology hold a key to the development of resistance varieties for improved yield (Sahoo and Jaiwal 2008). Transfer of insect resistance genes by transgenic technology hold a key to the development of resistance varieties for improved yield. It is now possible to develop transgenic Bt cowpea cultivars with resistance to the pod borer *Maruca vitrata*. Some modifications have also been made to improve the genetic transformation systems, which have led to the development of new transgenic cowpea with resistance to bruchids and caterpillars (Higgins *et al.,* 2012). Sodium azide has been used in plant breeding for several biotic and abiotic stress such as *Zea mays* resistant against pathogen Striga (Kiruki *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was conducted at the departmental garden of the Department of Plant Biology, Federal University of Technology Minna, Niger State, Nigeria. Minna is located in the North central geopolitical zone of Nigeria found within latitude 9˚36‟ north and longitude 6˚34‟ east. Minna covers a land area of 88 square kilometers with an estimated human population of 348,788 (Niger state MAAH Bulletine, 2008). Minna has annual temperature of 20˚ C to 30˚ C and relative humidity of 61 %. The area has two seasons; raining season between May to October and dry season between November to April each year. It has a low humid soil type with favourable climatic condition for planting which make it easy for cowpea to grow successfully and express all its traits.

3.2 Collection of Seeds

Four (4) cowpea genotypes were collected from National Centre for Genetic Resources and Biotechnology (NACGRAB), Oyo State. The collected samples are Early white, Local variety, IT90K-76 and IT97K-556-4.

3.3 Collection and Labelling of Planting Bags

Twenty planting bags were collected with openings at the base to allow passage of water. Each planting bags were filled with 25 kg sandy-loamy soil.

3.4 Mutagenic Treatment

Mutagenic treatment were conducted at the laboratory of the Department of Plant Biology, Federal University of Technology, Minna. Cowpea seeds were presoaked in distilled water for 4 hours. This allows the mutagen to diffuse more rapidly to the tissues of interest (Foster and Shu 2012). The seeds were then soaked for 4 hours in different concentrations

of Ehtyl Methane Sulphonate (0.00 % control, 0.10 %, 0.20 %, 0.30 % and 0.40 %). The treated seeds were thoroughly washed in running tap water to remove the residual effects of the mutagen if any.

3.5 Experimental Design

The experiment were designed in a complete randomized design (CRD) with four (4) replicates. The planting bags were filled with sandy to sandy-loamy soil, little water were added to moisten the soil. Cowpea seeds were then planted indicating their accession numbers and their treatment/concentrations, five (5) seeds were planted per pot. The seedlings were then thinned to two (2) seedlings per pot at two (2) weeks after sowing (Daudu and Falusi, 2011).

3.6 Insect Pest Observation

Insect pest observation on different population was recorded from germination to maturity stages of the crop. No insecticide or pesticide was added to reduce the population of the insects. They were allowed to multiply naturally. Data on different species of insect were recorded from the plants in all cowpea accessions. Records were taken by physical observation of the insect(s) population at 2 days intervals. The insect were graded on the basis of their population density per plant, nature and extent of crop damage and yield reduction of the crop. The time of severe attack was noted on the basis of degree of infestation observed at two days interval according to the method of (Biswas, 2014).

3.7 Morphological and Yield Parameters

The morphological parameters such as plant height, length of leaf blade, length of internode, width and girth of the plants were measured using the method of (Falusi *et al.*, 2012).

3.7.1 Plant height

The height of the plant was taken from the stem at soil level to the last node using a tape rule. This was taken at two weeks interval.

3.7.2 Length of leaf

Length of leaf was taken using a tape rule. This was taken at two weeks interval.

3.7.3 Width of leaf

The width of the leaf was also measured using a tape rule using a tape rule. This was taken at two weeks interval.

3.7.4 Number of leaves

Number of leaves was counted at maturity manually by physical observation.

3.7.5 Number of seeds per pod

Number of seeds per pod were counted visually

3.7.6 Number of pods per plant

Number of pods per plants were counted visually

3.7.7 Length of pod

Length of pod was measured using a tape rule.

3.7.8 Weight of pod

Weight of pod was taken using a balance scale.

3.7.9 Weight of 100 seeds per mutant line

Weight of 100 seeds per mutant line was also taken using balance scale.

3.8 Proximate Composition Analysis

3.8.1 Moisture content: Moisture content was determined using Association of Official Analytical Chemist (AOAC) 2019. An aliquot two gram (2 g) of the samples were weighed

into separate Petri dish of known weight. They were oven-dried at 105 ± 1 °C for four (4) hours. The samples were placed in a desiccator for cooling and were later weighed. The moisture content was calculated as follows:

$$\text{Moisture} = \frac{\text{W1} - \text{W2}}{\text{W2}} \times 100 \qquad 3.1$$

W1= Weight of sample before drying

W2= Weight of sample after drying

3.8.2 Ash content: Ash contents were determined following the procedure of AOAC (2019). Exactly 2 g of each sample were weighed into separate crucibles and were burned in a furnace at 550 °C until a light grey ash was observed. The samples were transferred to the desiccators and were allowed to cool and were weighed to obtain ash content.

$$Ash\ content\ \% = \frac{change\ in\ weight}{initial\ weight\ of\ food\ before\ drying} \times 100 \qquad 3.2$$

3.8.3 Crude fat: crude fat were determined following the procedure of AOAC (2019) which is evaluated through soxhlet extraction with petroleum ether. Some grams of the sample are extracted in a soxhlet apparatus with solvent under reflux for certain period. The extraction solvent is collected in a round flask and removed by vacuum evaporation. The flask with the extracted fat fraction is dried in oven at 150^0 C to remove moisture, cooled down in desiccator and weighted.

The total fat content will be calculated with the following formula.

$$Total\ fat = \frac{(M_2 - M_1)}{M_0} \times 100 \qquad 3.3$$

3.8.4 Crude protein: The protein content was determined using a micro-Kjedhal method AOAC, (2019) which involves wet digestion, distillation, and titration. An aliquot 3 g of each sample was weighed into separate boiling tubes that contained one catalyst tablet (0.15 g $CuSO_4$, 5 g K_2SO_4, and 0.1 g TiO_2) and 25 ml of concentrated sulfuric acid. The tubes were gradually heated for digestion to occur. The digest was diluted with 10 ml of 40 % NaOH, 100 ml distilled water, and 5 ml $Na_2S_2O_3$ anti-bumping agent was added. Exactly 10 ml of boric acid was then added to the sample. The NH_4 content in the distillate was determined by titrating with 0.1 N standard HCl using a 25 ml burette. A blank was prepared without the sample. The protein value obtained was multiplied by a conversion factor, and the results were expressed as the amount of crude protein. The percentage crude protein was calculated as:

$$Crude\ protien = \frac{actual\ titre\ value - titre\ value\ of\ blank\ \times 0.1\ \times 0.014\ \times conversion\ factor}{Weight\ of\ food\ sample} \times 100 \qquad 3.4$$

3.8.5 Crude fibre: Crude fibre was determined using the method of AOAC (2019). A measure of 5 g of each sample was placed into separate 500 ml Erlenmeyer flask and 100 ml of TCA digestion reagent was added. It was then brought to boiling point and refluxed for exactly 40 minutes counting from the start of boiling. The flask was removed from the heater, cooled for about 10 minutes, and then filtered with a Whatman paper. The residue was rinsed with hot water and was stirred continuously using a spatula. The sample was dried overnight at 105 °C. After drying, it was transferred to a desiccator and was allowed to cool. The sample was then weighed as W1. It was then burnt in a furnace at 500 °C for six (6) hours and allowed to cool, and reweighed as W2. The crude fibre content was calculated as:

$$Crude\ fibre\ \% = \frac{W1 - W2}{W_0} \times 100 \qquad 3.5$$

W_1= Weight of crucible + fibre + ash

W_2= Weight of crucible + ash

W_0= Dry weight of food

3.8.6 Carbohydrate content: Carbohydrate content was determined by subtracting the total sum of the percentage of moisture, ash, crude fibre, and crude protein from hundred (100).

Total carbohydrate (%) = 100 – (% Moisture + % Ash + % Fat + % Protein + % Fibre) 3.6

3.9 Mineral Composition

The following mineral elements: Na, K, Ca, Mg and P were detected by using the Atomic Absorption Spectrophotometer (AAS) as in (Karpiuk *et al.*, 2016). The Atomic absorption (AA) spectrometer is used to analyze metals at very low concentrations, typically in the parts per million (ppm) or parts per billion (ppb) ranges. A liquid sample containing dissolved material whose concentration is to be measured is aspirated into a thin, wide AA flame, or is introduced into a small carbon furnace which is heated to a high temperature. The principle of AAS is the measurement of absorption of radiation by free atoms. The total amount of absorption depends on the number of free atoms present and the degree to which the free atoms absorb the radiation. At the high temperature of the AA flame, the sample is broken down into atoms and it is the concentration of these atoms that is measured.

3.10 Data Analysis

Data collected for insect population were analyzed using descriptive statistics. Average number of insect per treatment were calculated and mean level of infestation scores of each

accession was determined by the use of a histogram. The quantitative data collected was transformed using logarithm transformation where it was subjected to analysis of variance (ANOVA) using SAS 9.2. The morphological data were subjected to ANOVA to determine the significant difference among the mean. Duncan Multiple Range Test (DMRT) was used to separate the mean where there were differences. Other data analysis were carried out using statistical package for social science at 5 % level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Survival percentage (%)

A total number of four (4) genotypes where planted in this study namely Early white, Local variety, IT90K-76 and IT97K-556-4. All the treatments first mutant generation M1 as well as their controls across the accessions exhibited 100 % survival percentage. The survival percentage showed that all the planting bags grew cowpea plants. Thus, the EMS concentrations did not affect the survival percentage of the seeds (Table 4.1)

Table 4.1: Survival percentage of M1 Generation of the different cowpea accessions as Different concentrations of EMS

Accession Name	Concentration (%)	Survival Percentage (%)
Early White	0.00	100
Early White	0.10	100
Early White	0.20	100
Early White	0.30	100
Early White	0.40	100
Local Variety	0.00	100
Local Variety	0.10	100
Local Variety	0.20	100
Local Variety	0.30	100
Local Variety	0.40	100
IT90K-76	0.00	100
IT90K-76	0.10	100
IT90K-76	0.20	100
IT90K-76	0.30	100
IT90K-76	0.40	100
IT97K-556-4	0.00	100
IT97K-556-4	0.10	100
IT97K-556-4	0.20	100
IT97K-556-4	0.30	100
IT97K-556-4	0.40	100

4.1.2 Morphological Parameters

4.1.2.1 Plant height at seedling stage

The analysis of variance (ANOVA) for morphological parameters showed that significant difference ($p < 0.05$) exist among the genotypes collected. The effect of EMS on selected cowpea genotypes varied succinctly from one genotype to another (Table 4.2). In Early white, the highest seedling height (22.97 cm) was due to 0.40 % concentration of EMS. This was followed by 0.30 % concentration (22.53 cm) then the control (22.20 cm). The least seedling height for Early white (16.27 cm) was due to 0.20 % concentrations; this value was not significantly different ($p < 0.05$) from the highest value (Table 4.2).

Meanwhile, in the local variety genotype, 0.30 % produced the highest seedling height (25.87 cm) while the control produced the least seedling height (15.13 cm). These values were significantly different ($p < 0.05$) from each other. The 0.20 % concentration produced cowpea with seedling height of 24.77 cm; this value was significantly the same ($p > 0.05$) with the highest value (25.87 cm). (Table 4.2)

Similarly, in IT90K-76 genotype, the least seedling height (14.33 cm) was found in the control. This value was significantly different ($p < 0.05$) from those produced by 0.20 % concentration (27.93 cm) and 0.30 % (28.73 cm), but significantly the same with those produced by 0.10 % concentration (18.67 cm) and 0.40 % concentration (23.63 cm). (Table 4.2).

In genotype IT97K-556-4, the highest seedling height (31.67 cm) was produced by cowpea seeds treated with 0.20 % concentration of EMS. This was followed by 27.33 cm produced by 0.40 % concentration treatment and then the control (26.17 cm). These values however, were not significantly different from one another. (Table 4.2).

The highest seedling plant height among the whole genotypes was found in genotype IT97K-556-4 concentration 0.20 % (31.67 cm). This was followed by genotype IT90K-76 concentration 0.30 % (28.73 cm), this was followed by genotype IT90K-76 concentration 0.20 % (27.93 cm) and then genotype IT97K-556-4 concentration 0.40 % (27.33 cm). The least was produced by genotype IT90K-76 concentration 0.00 % (14.33 cm) of EMS. (Table 4.2).

In Early white genotype, the best concentration for seedling height was found in concentration 0.40 % (22.97 cm). In Local variety genotype, the best concentration for seedling height was seen in concentration 0.30 % (25.87 cm). The best concentration for seedling height in genotype IT90K-76 was concentration 0.30 % (28.73 cm). The best concentration for seedling height in genotype IT97K-556-4 was concentration 0.20 % (31.67 cm).

4.1.2.2 Plant height at week (8) eight after planting

In Early white, the highest plant height at week 8 (32.70 cm) was due to 0.00 % (control) concentration of EMS. This was followed by 0.30 % concentration (32.10 cm) and then concentration 0.40 % (30.20 cm). The least plant height at week 8 for Early white genotype (25.37 cm) was due to 0.20 % concentration. These values were not significantly different ($p > 0.05$) from the highest value (Table 4.2).

In Local variety genotype, the highest plant height at week 8 (33.20 cm) was produced by cowpea seeds treated with 0.30 % concentration of EMS. This was followed by 32.97 cm produced by 0.20 % concentration treatment and then concentration 0.10 % (27.43 cm). These values were however, not significantly different from one another (Table 4.2).

Similarly, in IT90K-76 genotype, the least plant height at week 8 (23.57 cm) was found in the control. This value was significantly different ($p < 0.05$) from those produced by 0.20 % concentration (37.93 cm) and 0.30 % concentration (37.57 cm) (Table 4.2).

In genotype IT97K-556-4, the highest plant height at week 8 (64.77 cm) was produced by cowpea seeds treated with 0.10 % concentration of EMS. This was followed by 40.50 cm produced by 0.20% concentration treatment then the control (43.00 cm). These values were however, not significantly different from one another (Table 4.2).

For plant height among the whole genotypes at week 8, the least was produced by genotype Local variety concentration control (23.43 cm). This value was significantly different ($p<0.05$) from those produced by genotype Early white concentration 0.30 % (32.10 cm), genotype IT90K-76 concentration 0.20 % (7.93 cm) and genotype IT97K-556-4 concentration 0.10 % (64.77 cm) and Early white concentration 0.20% (25.37 cm) (Table 4.2).

The best concentration for plant height at week 8 of genotype Early white was in concentration 0.00 % (32.70 cm). The best in genotype Local variety was in concentration 0.30 % (33.20 cm). In genotype IT90K-76, the best concentration for young plant height was in concentration 0.20 % (37.93 cm). The best in IT97K-556-4 was in concentration 0.10 % (64.77 cm).

4.1.2.3 Plant height at week (20) twenty

In Early white genotype, 0.00 % produced the highest plant height at week 20 (67.80 cm) while 0.30 % had the least plant height at week 20 (43.77 cm). These values were significantly different ($p < 0.05$) from each other. The 0.20 % concentration produced cowpea with plant height at week 20 (44.43 cm), this value was significantly the same ($p > 0.05$) with 0.30 % concentration of EMS (43.77 cm) (Table 4.2).

In Local variety, the highest plant height at week 20 (58.37 cm) was due to 0.00 % (control) and 0.10 % concentration of EMS while 0.40 % produced the least plant height (42.97 cm), this value was significantly different ($p < 0.05$) from the highest value. The 0.30 % concentration produced cowpea with 56.60 cm plant height at week 20, this value was significantly the same ($p > 0.05$) with the highest value (58.37 cm) (Table 4.2).

Meanwhile, in IT90K-76 genotype, 0.10 % produced the highest plant height at week 20 (51.33 cm) while 0.40 % produced the least plant height at week 20 (46.60 cm). The 0.20 % concentration produced cowpea with plant height of 50.93 cm, this value was significantly the same ($p > 0.05$) with the highest value (51.33 cm) (Table 4.2).

Similarly, in IT97K-556-4, 0.00 % produced the highest plant height at week 20 (70.73 cm) while 0.30 % produced the least plant height (38.50 cm). These values were significantly different ($p < 0.05$) from each other and from all other concentrations (Table 4.2).

In genotype Early white, the best concentration for plant height at week 20 was found in control (67.80 cm). The best matured plant height of genotype local variety was in control (58.37cm) and concentration 0.10% of EMS (58.37 cm). The best concentration for matured plant height of genotype IT90K-76 was in concentration 0.10% (51.33 cm). In

genotype IT97K-556-4, the best concentration for plant height was in concentration 0.10 % (53.13 cm).

Table 4.2: Impacts of EMS on the Morphological Parameters of M1 Generation of the different cowpea accessions.

Concentrations	Seedling Height (cm)	8 weeks Plant Height (cm)	20 weeks Plant Height (cm)
Early white			
Control	22.20±1.97^{a}	32.70±3.82^{a}	67.80±0.89^{b}
0.1	20.17±3.34^{a}	27.47±3.06^{a}	45.17±7.58^{a}
0.2	16.27±0.55^{a}	25.37±1.09^{a}	44.43±1.54^{a}
0.3	22.53±1.35^{a}	32.10±1.63^{a}	43.77±2.92^{a}
0.4	22.97±5.91^{a}	30.20±5.38^{a}	44.57±4.72^{a}
Local variety			
Control	15.13±3.59^{a}	23.43±4.95^{a}	58.37±1.92^{b}
0.1	19.60±0.59^{ab}	27.43±1.21^{a}	58.37±0.85^{b}
0.2	24.77±0.54^{b}	32.97±1.33^{a}	46.60±4.88^{a}
0.3	25.87±1.07^{b}	33.20±2.07^{a}	56.60±0.99^{b}
0.4	15.37±3.85^{a}	23.37±3.85^{a}	42.97±10.41^{a}
IT90K-76			
Control	14.33±3.24^{a}	23.57±4.2^{a}	48.00±3.03^{a}
0.1	18.67±4.82^{ab}	26.60±5.76^{a}	51.33±3.94^{a}
0.2	27.93±3.73^{b}	37.93±4.11^{b}	50.93±1.09^{a}
0.3	28.73±2.47^{b}	37.57±2.79^{b}	49.53±0.90^{a}
0.4	23.63±4.95^{ab}	31.43±5.45^{a}	46.60±7.65^{a}
IT97K-556-4			
Control	26.17±5.52^{a}	43.00±6.41^{a}	70.73±10.45^{b}
0.1	22.87±7.68^{a}	64.77±39.83^{a}	53.13±11.87^{ab}
0.2	31.67±2.17^{a}	40.50±1.72^{a}	51.80±5.84^{ab}
0.3	17.97±4.59^{a}	24.27±4.76^{a}	38.50±1.11^{a}
0.4	27.33±1.44^{a}	34.77±1.79^{a}	49.97±6.80^{ab}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

4.1.2.4 Leaf length at week (20) twenty

In Early white, the highest leaf length (17.73 cm) was due to 0.10 % concentration of EMS. This was followed by 0.30 % concentration (17.00 cm) then the control (16.93 cm). The least leaf length for Early white (16.37 cm) was due to 0.40 % concentration. This value was not significantly different ($p > 0.05$) from the highest value (Table 4.3). Meanwhile, in the Local variety genotype, 0.20 % produced the highest leaf length (15.97 cm) and then 0.3 % concentration of EMS (15.13 cm). The least leaf length was 0.10 % (13.07 cm). This value was not significantly different ($p > 0.05$) from the highest value (Table 4.3). Similarly in IT90K-76, the least leaf length (11.17 cm) was found in the control while the highest leaf length was found in 0.20 % (14.00 cm). The values are significantly the same with those produced by 0.10 % (13.20 cm), 0.30 % (13.07 cm) and 0.40 % (13.37 cm) concentration (Table 4.3).

In genotype IT97K-556-4, the highest leaf length (21.97 cm) was produced by cowpea seeds treated with 0.20 % concentration of EMS. This was followed by 21.87 cm produced by 0.30 % concentration treatment and then 0.40 % (21.57 cm). These values were significantly different from one another (Table 4.3). Leaf length among the whole genotypes showed that genotype IT90K-76 at Concentration 0.00% (11.17 cm) was recorded the shortest while genotype IT97K-556-4 at concentration 0.20% (21.97 cm) was recorded the highest in terms of leaf length. These values are significantly different from one another and from other genotype. There was no significant difference between genotype Local variety at concentration 0.40% (13.67 cm) and genotype IT90K-76 concentration 0.40% (13.37 cm) (Table 4.3).

In genotype Early white, the best concentration for leaf length was in concentration 0.10% (17.73 cm). The best leaf length in genotype Local variety was in concentration 0.20% (15.97 cm). Concentration 0.20% of genotype IT90K-76 had the best leaf length (14.00 cm). The best leaf length for genotype IT97K-556-4 was found in concentration 0.20% (21.97 cm).

4.1.2.5 Leaf width at week (20) twenty

In Early white, the highest leaf width (11.80 cm) was due to 0.20 % concentration of EMS. This was followed by 0.30 % concentration (11.27 cm) then 0.10 % (10.30 cm). The least leaf width for Early white (9.90 cm) was due to 0.00 % concentration, this value was not significantly different ($p > 0.05$) from the highest value (Table 4.3). Meanwhile in the Local variety genotype 0.30 % produced the highest leaf width (12.07 cm) while 0.10 % produced the lowest leaf width (9.00 cm). These values were significantly different ($p > 0.05$) from each other. The 0.20 % concentration produced cowpea with leaf width of 11.8 cm, this value were significantly the same with the highest value (12.07 cm) (Table 4.3).

Similarly in IT90K-76 genotype, the highest Leaf width (10.73 cm) was due to 0.00 % concentration of EMS. This was followed by 0.40 % concentration (10.60 cm) and then 0.30 % (9.97 cm). This value were not significantly different ($p > 0.05$) from the highest value (Table 4.3). In genotype IT97-556-4, the highest leaf width (14.83 cm) was produced by cowpea seeds treated with 0.10 % concentration of EMS. The least leaf width (11.60 cm) was produced by the control. The 0.40 % concentration produced with leaf width of (14.13 cm) was significantly the same with the highest value (14.83 cm) (Table 4.3).

For leaf width among all the genotypes, the highest was produced by genotype IT97K-556-4 concentration 0.10 % (14.83 cm). These value was significantly different ($p<0.05$) from

those produced by genotype IT90K-76 concentration 0.00 % (10.73 cm), genotype Early white concentration 0.00 % (9.90 cm), genotype Local variety concentration 0.10 % (9.00 cm) and genotype Early white concentration 0.10 % (10.30 cm) (Table 4.3). In genotype Early white, the concentration that had the highest leaf width was 0.20% (11.80 cm). The highest concentration of leaf width in genotype Local variety was 0.30% (12.07 cm). Concentration 0.00% of genotype IT90K-76 had the highest leaf width (10.73 cm). The highest leaf width of genotype IT97K-556-4 was found in concentration 0.10% (14.83 cm).

4.1.2.6 Number of leaves at week (20) twenty

In Early white, the highest number of leaves (93.00) was produced due to 0.10 % concentration of EMS. This was followed by 0.20 % concentration (86.00) then 0.30 % concentration 76.33. The least Number of leaves for Early white (57.33) was in concentration 0.00 %. These values were not significantly different ($p > 0.05$) from one another (Table 4.3). Meanwhile, in Local variety the highest number of leaves (82.67) was produced in the control. This was followed by 0.10 % concentration 74.67. The least number of leaves was in concentration 0.3 % (57.00). The 0.20 % & 0.40 % value (63.00) was significantly the same with that produced by 0.10 % concentration of EMS (74.67) (Table 4.3).

In genotype IT90K-76, the highest number of leaves (76.00) was produced by 0.10 % concentration of EMS. This was followed by concentration 0.30% (68.33) and then the control 54.33. The least number of leaves was in 0.40 % concentration of EMS (47.00). The 0.30 % concentration of EMS (68.33) value was significantly the same with the highest value (76.00 cm) (Table 4.3). In genotype IT97K-556-76, the highest number of leaves (98.67) was produced by cowpea seeds treated with 0.20 % concentration of EMS. This

was followed by 86.00 produced by 0.40 % concentration treatment and then 0.30 % produced 69.33. These values however, were not significantly different from one another (Table 4.3).

For number of leaves among the whole genotypes, it showed that genotype IT97K-556-4 concentration 0.20 % (98.67) produced the highest number of leaves. This was followed by genotype Early white concentration 0.10 % (93.00). This was also followed by genotype Early white concentration 0.20 % (86.00). These values were however, significantly different from the lowest value of number of leaves produced by genotype IT97K-556-4 concentration 0.00 % (53.00) (Table 4.3).

In genotype Early white, the highest concentration that had the highest number of leaves was in concentration 0.10 % (93.00). The highest number of leaves in genotype Local variety was in concentration 0.00 % (82.67). Concentration 0.10 % had the highest number of leaves in genotype IT90K-76 (76.00). The concentration with the best number of leaves in genotype IT97K-556-4 was found in concentration 0.20 % (98.67).

Table 4.3: Impacts of EMS on the Leaf Parameters of M1 Generation of the different cowpea accessions

Concentrations	Leaf Length week 20 (cm)	Leaf Width week 20 (cm)	Number of Leaves week 20
Early white			
Control	16.93±0.43[a]	9.90±0.43[a]	57.33±9.91[a]
0.1	17.73±0.50[a]	10.30±0.81[a]	93.00±14.00[b]
0.2	16.83±1.51[a]	11.80±0.85[a]	86.00±35.17[a]
0.3	17.00±1.01[a]	11.27±0.19[a]	76.33±21.84[a]
0.4	16.37±0.32[a]	10.27±0.07[a]	70.00±1.53[a]
Local variety			
Control	15.17±0.62[a]	10.80±0.06[ab]	82.67±23.79[a]
0.1	13.07±0.64[a]	9.00±0.35[a]	74.67±8.84[a]
0.2	15.97±0.46[a]	11.83±0.64[b]	63.00±11.59[a]
0.3	15.13±1.73[a]	12.07±0.68[b]	57.00±9.45[a]
0.4	13.67±1.02[a]	11.07±1.43[ab]	63.00±14.42[a]
IT90K-76			
Control	11.17±0.61[a]	10.73±0.27[a]	54.33±7.69[a]
0.1	13.20±0.38[ab]	9.87±0.47[a]	76.00±6.56[a]
0.2	14.00±0.85[b]	9.30±0.15[a]	53.00±0.58[a]
0.3	13.07±0.43[ab]	9.97±0.23[a]	68.33±24.13[a]
0.4	13.37±1.16[ab]	10.60±1.64[a]	47.00±10.26[a]
IT97K-556-4			
Control	18.20±0.06[a]	11.60±0.61[a]	53.00±14.42[a]
0.1	18.47±2.31[ab]	14.83±1.35[a]	55.33±1.76[a]
0.2	21.97±0.52[b]	14.83±1.33[a]	98.67±47.94[a]
0.3	21.87±0.09[b]	13.40±0.17[a]	69.33±5.78[a]
0.4	21.57±0.20[ab]	14.13±0.84[a]	86.00±18.34[a]

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

4.1.3 Insect observation

4.1.3.1 Insect population in each accession

In this study, two insect species were seen associated with the crops; these were Cowpea foliage weevil (*Callosobruchus maculatus*) (plate I A & B) and Armyworm (*Spodoptera frugiperda*) (plate I C & D). In Early white genotype, fifty-eight (58) leaf weevil were

observed while no armyworm recorded. In Local variety genotype, armyworm had a value of two (2) while leaf weevil had eighteen (18). In IT90K-76 genotype, leaf weevil recorded a value of sixty-seven (67) while armyworm had only one (1). In IT97K-556-4 genotype, leaf weevil had a value of one hundred and eighty (180) while armyworm had one (1) (Figure 1).

4.1.3.2 Insect population in each concentration of ems

In 0.00 % concentration of EMS (control), genotype IT97K-556-4 had the highest number of insect population with a value of sixty-three (63). Genotype Early white and IT90K-76 both had the same value of ten (10). The lowest value was in genotype Local variety with a value of four (4) (Figure 2).

In 0.10 % concentration of EMS, genotype IT97K-556-4 had the highest number of insect population with a value of thirty-one (31). This was followed by genotype Early white with a value of twelve (12) and then genotype IT90K-76 with a value of eight (8). The lowest insect population was in genotype Local variety and recorded a value of one (1) (Figure 2).

In 0.20 % concentration of EMS, genotype IT97K-556-4 had the highest number of insect population with a value of twenty-three (23). This was followed by genotype IT90K-76 with a value of nineteen (19) and Early white had a value of eleven (11). Genotype Local variety recorded no value hence the lowest for this concentration (Figure 2).

A similar trend was observed in 0.30 % concentration of EMS where genotype IT97K-556-4 had the highest number of insect population with a value of thirty (30). This was followed by Early white with a value of sixteen (16) and then IT90K-76 with a value of eleven (11). The least insect population for 0.20 % concentration was produced by genotype Local variety with a value of eight (8) (Figure 2).

In 0.40 % concentration of EMS, genotype IT97K-556-4 had the highest number of insect population with a value of thirty-four (34). This was followed by Early white with a value of twenty-five (25) and then IT90K-76 with a value of twenty (20). The least insect population in 0.40 % concentration was in genotype Local variety with a value of seven (7) (Figure 2).

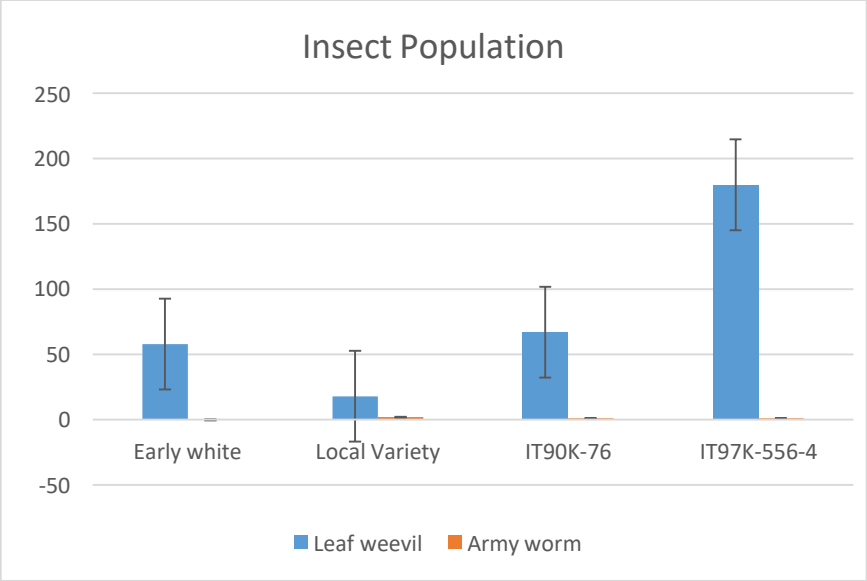

Figure 1: Insect Population Distribution among the Mutant Genotypes.

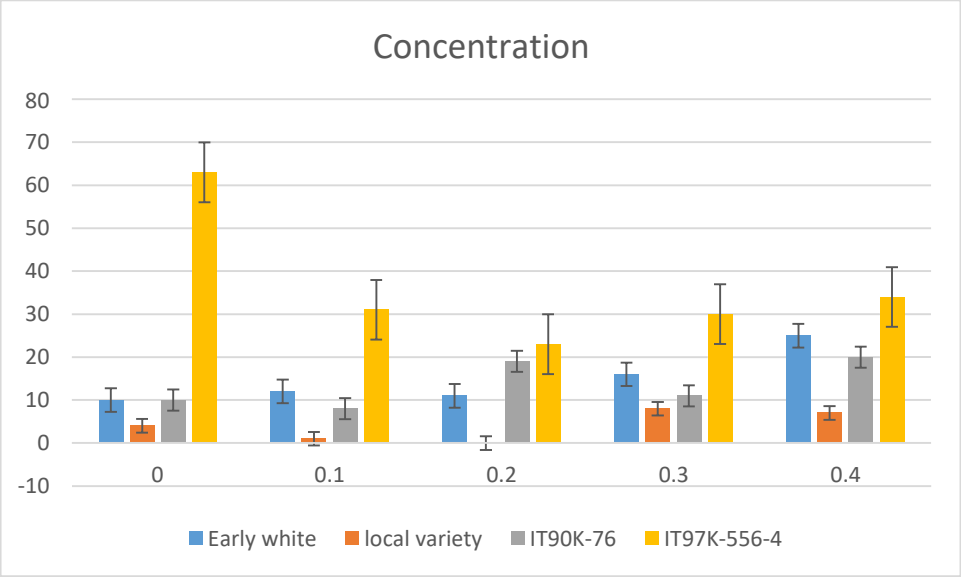

Figure 2: Insect Population Distribution Based on different Concentrations.

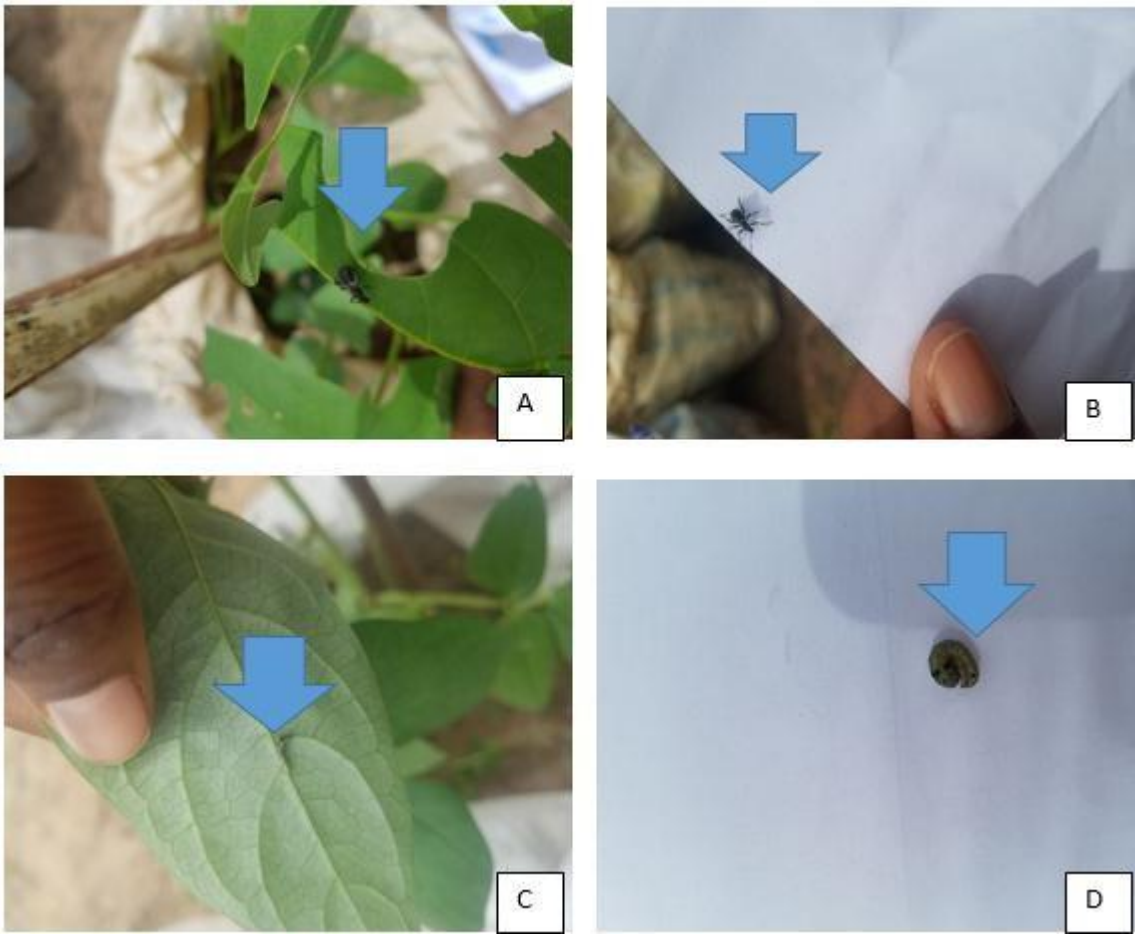

Plate I: A & B Leaf weevil C & D Army worm

Source: Field photograph

4.1.4 Yield parameters

The analysis of variance for yield parameters showed that significant difference ($P<0.05$) exist among the genotype examined.

4.1.4.1 Length of pod

In Early white genotype, the highest pod length was 10.00 cm produced by 0.20 %, this value was significantly different ($p < 0.05$) from all the other treatments. The least pod length was 6.20 cm produced by the control, this value were also significantly different from all other treatments except in 0.30 % concentration treatment (7.73 cm) (Table 4.4).

Different trend was observed in the local variety genotype where the highest pod length was found in the control (14.70 cm) while the lowest value (9.90 cm) was produced by 0.40 % concentration treatment. These values (control and 0.40 % concentration) were significantly different from each other but were significantly the same with all other treatments (Table 4.4).

Meanwhile, in IT90K-76 genotype, the least pod length was produced by 0.20 % concentration (9.13 cm) while the highest pod length was produced by 0.30 % (11.53 cm). However, these values were not different significantly ($P> 0.05$) from each other and from other treatments (Table 4.4).

In genotype IT97K-556-4, the highest length of pod was produced by 0.10 % (14.20 cm). This was followed by the control (13.53 cm) and then 0.40 % (12.70 cm). The least pod length was produced by 0.20 % (12.03 cm). However, there was no significant difference among all the treatments (Table 4.4).

Among the different genotypes, the response to EMS tend to vary, the highest length of pod was found in genotype Local variety concentration (control) 0.00 % (14.70 cm), this was followed by genotype IT97K-556-4 concentration 0.10 % (14.20 cm). This was also followed by genotype Local variety concentration 0.20 % (13.90 cm). The least was produced by genotype Early white concentration (control) 0.00 % (6.20 cm) (Table 4.4).

4.1.4.2 Weight of pod

For weight of pod of genotype Early white, the highest was found in 0.30 % (2.10 g), this was followed by 0.40 % (2.00g) while the lowest value (1.30 g) was found in the control (0.00). The values obtained by 0.30 % and 0.40 % were not significantly different from all the other treatments but were significantly different from that of the control (Table 4.4). Meanwhile, in the Local variety genotype the highest weight of pod was found in the control (1.60 g) while the lowest value (0.80 g) was produced by 0.10 % concentration treatment. These values were significantly different from each other, but were significantly the same with all other treatments (Table 4.4).

In IT90K-76, the highest weight of pod was found in 0.40 % (1.90 g) concentration of EMS. This was followed by 0.30 % (1.57 g) and then 0.20 % (1.10 g). The least weight of pod was produced by the control (0.83 g). These values were significantly different from each other (Table 4.4). Similarly, in genotype IT97K-556-4, the least weight of pod was produced by 0.20 % (0.67 g). The highest value 1.03 g was found in 0.40 % concentration of EMS. The 0.10 % value 0.97 g was significantly the same with the highest value (1.03 g) (Table 4.4).

For weight of pod among the whole genotypes, the lowest weight was produced by genotype IT97K-556-4 concentration 0.20 % (0.67 g) while the highest was produced by

genotype Early white concentration 0.30 % (2.10 g). The value produced by genotype Local variety concentration 0.10 % (0.80 g), that of genotype IT90K-76 concentration control (0.83 g) and that of genotype IT97K-556-4 concentration o.10 % (0.97 g) are significantly the same but significantly different from the value obtained by weight of pod of genotype Early white concentration 0.30 % (2.10 g) (Table 4.4).

4.1.4.3 Number of seeds per pod

In Early white genotype, the highest number of seed per pod was produced by 0.30 % (9.67) concentration of EMS. This was followed by 0.10 % (9.33) and then 0.40 % (9.00). The least number of seed per pod was produced by 0.20 % concentration of EMS (6.33). These values were not significantly different from one another (Table 4.4). Similarly, in local variety accession, the highest number of seed per pod was found in the control (12.33) followed by 0.20 % concentration (12.00) while the lowest value (5.33) was produced by 0.3 % concentration treatment. These values were significantly different from each other but were significantly the same with other treatment (Table 4.4).

Meanwhile, in genotype IT90K-76, the highest number of seed per pod (9.67) was produced by 0.30 % concentration of EMS. This was followed by (9.33) produced by 0.40 % and then the control (8.00). The least number of seed per pod was produced by 0.20 % (6.33). These values were not significantly different from one another (Table 4.4). Different trend was observed in IT97K-556-4 accession where the highest number of seed per pod was found in the control (11.33) while the lowest value was produced by 0.20 % concentration treatment (6.67). These values were significantly different from each other but were significantly the same with all other treatments (Table 4.4).

The Local variety genotype concentration 0.00 % (control) produced the highest number of seed per pod (12.33), this was followed by accession Local variety concentration 0.20 % (12.00), this was also followed by accession IT97K-556-4 concentration control (11.33) and then accession IT97K-556-4 concentration 0.30 %. The least was obtained in accession Early white concentration 0.20 % (6.33).

4.1.4.4 Number of pod per plant

In Early white genotype, the highest number of pod per plant was found in the control (8.00) while the lowest value (2.33) was produced by 0.20 % concentration treatment. These values were significantly different from each other, but were significantly the same with all other treatments (Table 4.4). Similarly, in local variety, the lowest number of pod per plant was produced by 0.30 % concentration of EMS (2.00) while the highest value (12.33) was produced by the control. These values were significantly different from each other but were significantly the same with all other treatment (Table 4.4).

Meanwhile, in IT90K-76, the highest number of pod per plant was produced by the control (11.67) while the lowest value (8.00) was produced by 0.10 %. These values were significantly different from each other, but were significantly the same with all other treatments (Table 4.4). In IT97K-556-4, the highest number of pod per plant was produced by 0.40 % concentration of EMS (2.67), this was followed by the control (2.33) and then 0.10 % (2.00). The least value (1.00) was produced by 0.20 % concentration of EMS. (Table 4.4) Different trend was observed in number of pod per plant where genotype Local variety concentration control produced the highest (12.33) among the whole genotypes while genotype IT97K-556-4 concentration 0.20 % had the lowest (1.00). However, the

values produced by accession Early white concentration 0.20 % (2.33) and that of accession Local variety concentration 0.30 % (2.00) is significantly the same. (Table 4.4).

4.1.4.5 Weight of hundred seeds

In Early white, the highest weight of 100 seeds was 20.03 g produced by 0.20 %, this value was significantly different ($p < 0.05$) from all other treatments. The least weight of 100 seeds was 13.63 g produced by the control, this value was also significantly different from all the other treatment except in 0.40 % concentration treatment (14.00g) (Table 4.4). Similarly, different trend was observed in the Local variety genotype where the highest weight of 100 seeds was produced by 0.40 % (10.60 g) while the lowest was produced by 0.30 % (8.00 g). These values are significantly different from all other treatments (Table 4.4).

In IT90K-76 genotype, the highest weight of 100 seeds was produced by 0.20 % (11.50 g). This was followed by 0.40 % treatment (11.20 g) and then 0.10 % (11.00 g). The least weight of 100 seeds was produced by 0.30 % (10.40 g) (Table 4.4). Meanwhile, in genotype IT97K-556-4, the least weight of 100 seeds was produced by 0.30 % (8.53 g). The highest weight of 100 seeds was produced by 0.10 % (17.00 g). The 0.40 % value (16.97 g) was significantly the same with the highest value (Table 4.4).

The highest weight of 100 seed was found in genotype Early white concentration 0.20 % (20.03 g) among the whole genotypes, this was followed by genotype IT97K-556-4 concentration 0.10 % (17.00 g), this was followed by genotype IT97K-556-4 concentration 0.40 % (16.97 g) and then followed by genotype Early white concentration 0.10 % (16.10 g). The lowest weight of 100 seeds was found in genotype Local variety concentration 0.30 % (8.00 g) (Table 4.4).

Table 4.4: Effects of EMS on the Yield Parameters of M1 Generation of the different cowpea accessions.

Concentrations	Length of pod (cm)	Weight of pod (g)	Number of seeds per pod	Number of pods per plant	weight of 100 seeds (g)
Early white					
Control	6.20±0.70^{a}	1.30±0.21^{a}	6.67±0.67^{a}	8.00±0.58^{b}	13.63±0.12^{a}
0.1	8.83±0.44^{b}	1.93±0.13^{ab}	9.33±1.86^{a}	4.67±1.20^{ab}	16.10±0.06^{b}
0.2	10.00±0.72^{c}	1.67±0.18^{ab}	6.33±0.88^{a}	2.33±0.33^{a}	20.03±0.55^{c}
0.3	7.73±0.50^{a}	2.10±0.29^{b}	9.67±1.20^{a}	6.33±1.86^{b}	15.80±0.06^{b}
0.4	9.57±0.49^{b}	2.00±0.12^{b}	9.00±0.58^{a}	4.67±0.33^{ab}	14.00±0.06^{a}
Local variety					
Control	14.70±0.42^{b}	1.60±0.12^{b}	12.33±0.33^{b}	12.33±4.37^{b}	9.80±0.06^{d}
0.1	13.50±0.52^{ab}	0.80±0.12^{a}	7.33±0.88^{ab}	3.00±0.00^{a}	9.50±0.06^{c}
0.2	13.90±0.29^{ab}	1.27±0.18^{ab}	12.00±2.08^{b}	11.00±1.53^{b}	8.60±0.06^{b}
0.3	11.70±1.27^{ab}	1.17±0.13^{ab}	5.33±1.86^{a}	2.00±0.00^{a}	8.00±0.06^{a}
0.4	9.90±2.30^{a}	1.13±0.35^{ab}	9.00±3.06^{ab}	9.00±2.00^{ab}	10.60±0.06^{e}
IT90K-76					
Control	9.67±1.09^{a}	0.83±0.07^{a}	8.00±1.00^{a}	11.67±0.88^{b}	10.60±0.06^{b}
0.1	10.03±0.96^{a}	1.00±0.10^{a}	7.33±1.20^{a}	8.00±1.15^{a}	11.00±0.06^{c}
0.2	9.13±0.68^{a}	1.10±0.10^{a}	6.33±1.33^{a}	10.00±0.58^{ab}	11.50±0.06^{e}
0.3	11.53±0.42^{a}	1.57±0.27^{b}	9.67±1.20^{a}	11.33±0.33^{ab}	10.40±0.06^{a}
0.4	11.17±0.69^{a}	1.90±0.06^{b}	9.33±0.88^{a}	11.00±1.53^{ab}	11.20±0.06^{d}
IT97K-566-4					
Control	13.53±0.53^{a}	1.00±0.06^{a}	11.33±0.67^{b}	2.33±0.88^{b}	13.00±0.06^{c}
0.1	14.20±0.31^{a}	0.97±0.07^{a}	10.33±1.20^{b}	2.00±0.58^{b}	17.00±0.06^{d}
0.2	12.03±1.33^{a}	0.67±0.07^{a}	6.67±0.33^{a}	1.00±0.00^{a}	12.00±0.12^{b}
0.3	12.60±1.45^{a}	0.97±0.19^{a}	10.67±1.20^{b}	1.33±0.33^{a}	8.53±0.09^{a}
0.4	12.70±1.29^{a}	1.03±0.13^{a}	9.00±1.53^{ab}	2.67±0.67^{c}	16.97±0.09^{d}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

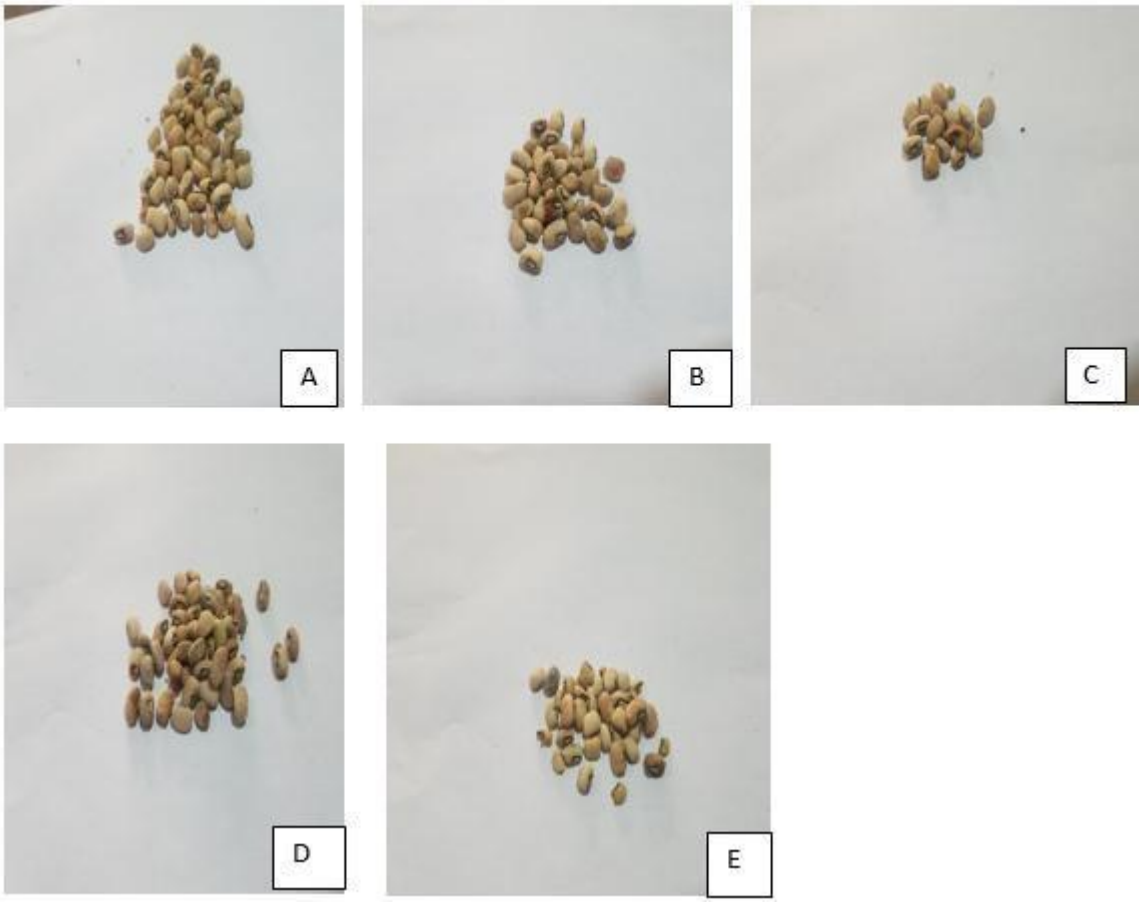

Plate II: Yield of Accession Early white A. control (0.00 %) B. 0.10 % EMS concentration C. 0.20 % EMS concentration D 0.30 % EMS concentration E. 0.40 % EMS concentration.

Source: Field Photograph.

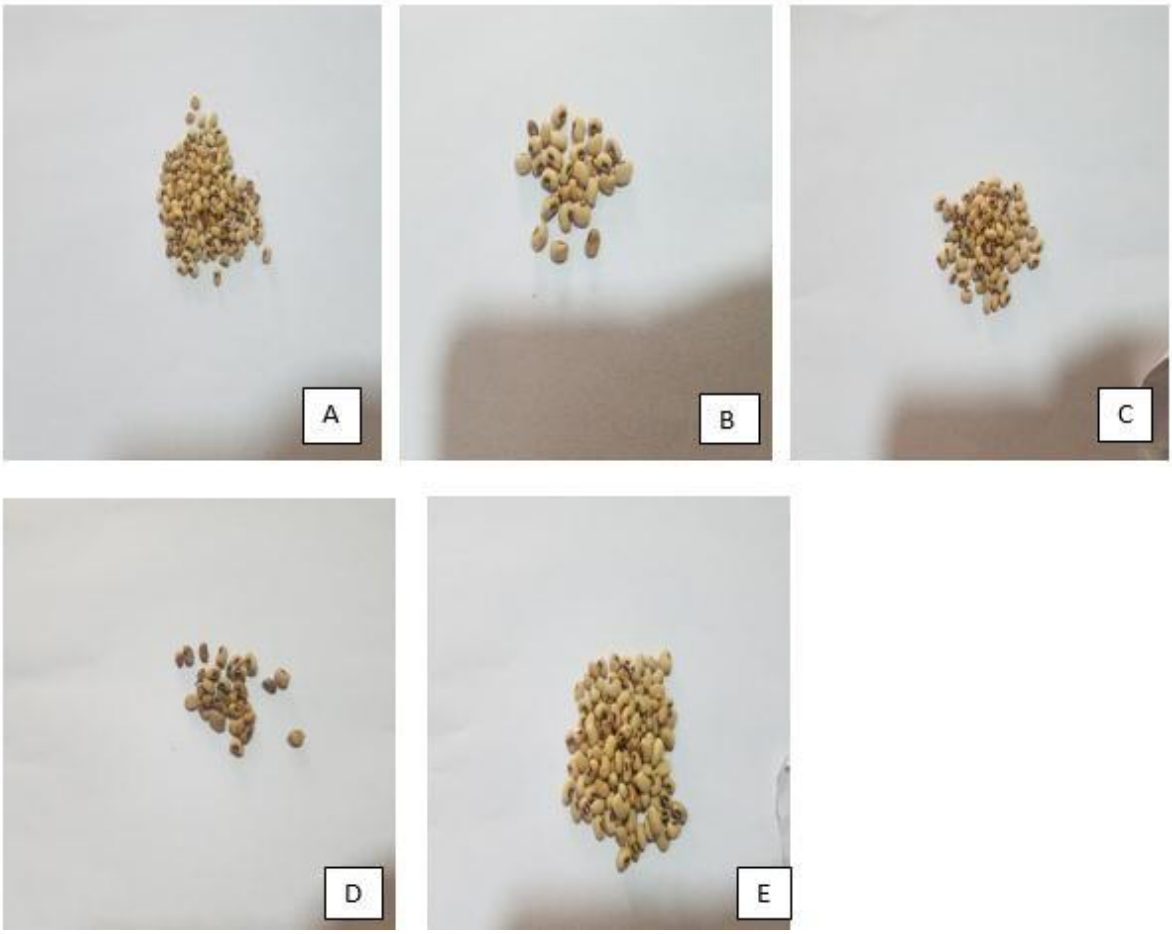

Plate III: Yield Accession of Local variety A. control (0.00 %) B. 0.10 % EMS concentration C. 0.20 % EMS concentration D. 0.30 % EMS concentration E. 0.40 % EMS concentration.

Source: Field Photograph

Plate IV: Yield of Accession IT90K-76 A. control (0.00 %) B. 0.10 % EMS concentration C. 0.20 % EMS concentration D. 0.30 % EMS concentration E. 0.40 % EMS concentration.

Source: Field Photograph

Plate V: Yield of Accession IT97K-556-4 A. control (0.00 %) B. 0.10 % EMS concentration C. 0.20 % EMS concentration D. 0.30 % EMS concentration E. 0.40 % EMS concentration.

Source: Filed Photograph

4.1.5 Proximate analysis

The analysis of variance (ANOVA) for proximate analysis showed that significant difference ($p<0.05$) exist among the genotypes and their respective treatments.

4.1.5.1 Dry matter

In Early white, the highest dry matter was produced by 0.10 % (93.07 %) while the least dry matter was obtained by 0.30 % concentration of EMS (90.20 %). These values were significantly different from one another. However, the value obtained by 0.20 % (92.08 %) was significantly the same with that of the control (92.08 %) (Table 4.5). Similarly, in local variety the lowest dry matter was produced by 0.20 % (89.32 %) while 0.10 % produced the highest dry matter (94.00 %). These values are significantly different from each other, but the value produced by 0.30 % (91.78 %) was significantly the same with that produced by 0.40 % (91.18 %) (Table 4.5).

Meanwhile, in IT90K-76 the highest dry matter was produced by 0.20 % (92.08 %). The lowest was produced by 0.30 % (89.22 %). These values are significantly different from one another. However, the value produced by 0.10 % concentration of EMS (90.10 %) is the same with that produced by 0.40 % concentration of EMS significantly (Table 4.5). In IT97K-556-4, the highest dry matter was produced by 0.10 % concentration of EMS (93.65 %). This was followed by 0.40 % (93.65 %). This was followed by 0.4 % (93.38 %) and then 0.30 % (92.88 %). These values were significantly different from one another (Table 4.5).

The highest dry matter content in the whole genotypes and concentration was seen in genotype Local variety concentration 0.10% (94.00 %) while the lowest dry matter content

was seen in genotype IT90K-76 concentration 0.30 % (89.22 %). These values were significantly different from one another and from other genotypes and concentrations. However, there is no significant difference between genotype Early white concentration 0.00 % (92.08 %), and genotype IT90K-76 concentration 0.20 % (92.08 %) and between genotype Local variety concentration 0.00 % (92.16 %) and accession IT97K-556-4 concentration 0.20 % (92.16 %). The highest dry matter content of genotype Early white was concentration 0.10 % (93.07 %). The highest dry matter content of accession Local variety was concentration 0.10 % (94.00 %). The highest dry matter content of accession IT90K-76 was concentration 0.00 % (90.38 %). The highest dry matter content of accession IT97K-556-4 was concentration 0.40 % (93.38 %).

4.1.5.2 Moisture content

In Early white, the lowest moisture was obtained in 0.10 % (6.92 %) while the highest value was obtained by 0.30 % concentration of EMS (9.80 %). These values are significantly different from one another but the value produced by the control (7.92 %) is significantly the same with what was produced by 0.20 % (7.92 %) (Table 4.5). Different trend was observed in Local variety where the highest moisture content was found in 0.20 % concentration of EMS (10.68 %) while the lowest was produced by 0.10 % (6.00 %). These values where significantly different from each other (Table 4.5).

Similarly, in IT90K-76 the lowest moisture was produced by 0.20 % (7.92 %) while the highest was produced by 0.30 % concentration of EMS (10.78 %). These values are significantly different from one another. (Table 4.5). Meanwhile, in IT97K-556-4, the highest moisture content was produced by 0.20 % concentration of EMS (7.88 %). This was followed by the control with a value of (7.36 %) and then 0.30 % concentration of

EMS (7.12 %). The least was produced by 0.10 % (6.35 %). These values are different from each other significantly. (Table 4.5).

The lowest moisture content in the whole genotypes and concentration was seen in genotype Local variety concentration 0.10 % (6.00 %) while the highest moisture content was seen in genotype IT90K-76 concentration 0.30 % (10.78 %). These values are significantly different from one another and from other genotypes and concentrations. However, there is no significant difference between genotype Early white concentration 0.00 % (7.92 %) accession Local variety concentration 0.00 % (7.84 %), genotype IT90K-76 concentration 0.20 % (7.92 %) and genotype IT97K-556-4 concentration 0.30 % (7.12 %).

The highest moisture content of genotype Early white was concentration 0.30 % (9.80 %). The highest moisture content of genotype Local variety was concentration 0.20 % (10.68 %). The highest moisture content of genotype IT90K-76 was concentration 0.30 % (10.78 %). The highest moisture content of genotype IT97K-556-4 was concentration 0.20 % (7.88 %).

4.1.5.3 Ash percentage

In genotype Early white, the least ash content was produced by 0.10 % concentration and 0.30 % concentration with a value of 1.96 % for both while the highest was produced by 0.40 % concentration of EMS (2.97 %). These values are significantly different from one another. However, the 0.20 % concentration value (2.94 %) is significantly the same with that of the control (2.94 %) (Table 4.5). Different trend was observed in Local variety, the highest value (3.96 %) of ash content is in 0.40 % concentration of EMS. This was followed by 0.30 % concentration of EMS (3.77 %) and then control (2.94 %). The lowest

ash content was seen in 0.10 % and 0.20 % 2.00 % for both values. These values are significantly different from one another (Table 4.5). Meanwhile, in IT90K-76 highest ash content was found in the control 5.88 %. This was followed by 0.30 % with a value (5.00 %) and then 0.10 % concentration of EMS. These values were however, not significantly different from one another (Table 4.5).

In IT97K-556-4, the lowest ash content was produced by the control (1.98 %) while the highest ash content was produced by 0.30 % concentration of EMS (3.92 %). These values were significantly different from each other (Table 4.5). The highest ash content in all genotypes and concentration is seen in genotype IT90K-76 concentration 0.00 % (5.88 %) while the lowest ash content was seen in genotype Early white concentration 0.10 % and concentration 0.30 % (1.96 %) for both. These values are significantly different from one another and from other genotypes and concentrations. However, there is no significant difference between genotype Early white concentration 0.00 % (2.94 %), genotype Local variety concentration 0.00 % (2.94 %), genotype IT90K-76 concentration 0.20 % (2.97 %) and genotype IT97K-556-4 concentration 0.10 % (2.94 %) and concentration 0.40 % (2.94 %).

The highest ash content of genotype Early white was concentration 0.40 % (2.97 %). The highest ash content of genotype Local variety was concentration 0.40 % (3.96 %). The highest ash content of genotype IT90K-76 was concentration 0.00 % (5.88 %). The highest ash content of genotype IT97K-556-4 is concentration 0.30 % (3.92 %).

4.1.5.4 Crude fat

In Early white genotype, the highest crude fat was produced by 0.40 % (3.98 %). This was followed by 3.61 % produced by 0.10 % and then 3.55 % produced by 0.20 %. The least content was produced by the control (2.15 %). These values are significantly different from each other (Table 4.5). Similarly, in local variety genotype, the highest crude fat content was produced by 0.30 % 3.00 % while the lowest crude fat was produced by 0.40 % 1.48 %. These values are significantly different from each other. However, the 0.10 % value (2.45 %) is significantly the same with 0.20 % value (2.46 %) (Table 4.5).

Different trend was observed in IT90K-76 genotype where the highest value was of crude fat was produced by the control (4.48 %) while the lowest crude fat was produced by 0.40 % (1.99 %). These values are significantly different from one another. The 0.10 % value (2.30 %) is significantly the same with 0.20 % value (2.35 %) (Table 4.5). The lowest crude fat content in the whole genotypes and concentration was seen in genotype Local variety concentration 0.40 % (1.48 %) while the highest crude fat content was seen in accession IT90K-76 concentration 0.00 % (4.48 %). These values are significantly different from one another and from other genotypes and concentrations. However, there is no significant difference between genotype Early white concentration 0.30 % (3.26 %) and genotype Local variety concentration 0.30 % (3.00 %).

The highest crude fat content of genotype Early white was concentration 0.40 % (3.98 %). The highest crude fat content of genotype Local variety was concentration 0.30 % (3.00 %). The best crude fat content of genotype IT90K-76 was concentration 0.00 % (4.48 %).

Table 4.5: Proximate composition of M1 Generation of the different cowpea accessions as well as different concentrations of EMS

Parameters	Dry matter (%)	Moisture (%)	Ash (%)	Crude fat (%)
Early white				
Control	92.08±0.50^{c}	7.92±0.50^{b}	2.94±0.50^{b}	2.15±0.25^{a}
0.1	93.07±0.50^{d}	6.93±0.50^{a}	1.96±0.50^{a}	3.61±0.10^{d}
0.2	92.08±0.50^{c}	7.92±0.50^{b}	2.94±0.50^{b}	3.55±0.10^{c}
0.3	90.20±0.20^{a}	9.80±0.20^{d}	1.96±0.50^{a}	3.26±0.10^{b}
0.4	91.26±0.10^{b}	8.74±0.10^{c}	2.97±0.00^{c}	3.98±0.00^{e}
Local variety				
Control	92.16±0.15^{c}	7.84±0.15^{b}	2.94±0.50^{b}	2.76±0.10^{c}
0.1	94.00±0.00^{d}	6.00±0.00^{a}	2.00±0.00^{a}	2.45±0.50^{b}
0.2	89.32±0.00^{a}	10.68±0.00^{d}	2.00±0.00^{a}	2.46±0.15^{b}
0.3	91.78±0.25^{b}	8.23±0.25^{c}	3.77±0.50^{c}	3.00±0.00^{d}
0.4	91.18±0.20^{b}	8.82±0.20^{c}	3.96±0.00^{d}	1.48±0.10^{a}
IT90K-76				
Control	90.38±0.25^{c}	9.63±0.25^{b}	5.88±0.10^{e}	4.48±0.10^{d}
0.1	90.10±0.50^{b}	9.90±0.50^{c}	4.90±0.10^{c}	2.30±0.10^{c}
0.2	92.08±0.50^{d}	7.92±0.50^{a}	2.97±0.00^{a}	2.35±0.10^{c}
0.3	89.22±0.20^{a}	10.78±0.20^{d}	5.00±0.00^{d}	2.00±0.00^{b}
0.4	90.10±0.50^{b}	9.90±0.00^{c}	3.96±0.00^{b}	1.99±0.00^{a}
IT97K-556-4				
Control	92.64±0.00^{b}	7.36±0.00^{d}	1.98±0.00^{a}	
0.1	93.65±0.00^{d}	6.35±0.00^{a}	2.94±0.50^{b}	
0.2	92.12±0.00^{a}	7.88±0.00^{e}	3.00±0.00^{c}	
0.3	92.88±0.00^{c}	7.12±0.00^{c}	3.92±0.10^{d}	
0.4	93.38±0.00^{d}	6.62±0.00^{b}	2.94±0.50^{b}	

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

4.1.5.5 Crude protein

For crude protein of Early white, the lowest value was produced by 0.20 % (16.11 %) while the highest value was produced by the control (21.28 %). These values were significantly different from one another (Table 4.6).

Meanwhile, in Local variety, the highest crude protein (21.02 %) was produced by 0.20 % concentration of EMS. This was followed by 0.10 % (19.70 %) and then the control (18.83 %). The least crude protein was produced by 0.40 % (15.32 %). These values were significantly different from one another (Table 4.6).

Similarly, in genotype IT90K-76, the highest crude protein was produced by 0.20 % (23.82 %) while the least was produced by 0.30 % (15.06 %). These values were significantly different from each other (Table 4.6).

Different trend was observed in IT97K-556-4, where the highest value of crude protein (27.41 %) was produced by 0.10 % concentration of EMS while the lowest value (14.36 %) was produced by 0.20 % concentration of EMS. These values were significantly different from each other. However, the 0.40 % value (19.96 %) and that of the control (19.70 %) are significantly the same (Table 4.6).

The highest crude protein content in the whole genotypes and concentration is seen in genotype IT97K-556-4 concentration 0.10 % (27.41 %) while the lowest crude protein content was seen in genotype IT97K-556-4 concentration 0.20 % (14.36 %). These values were significantly different from one another and from other genotypes and concentrations. However, there is no significant difference between genotype Early white concentration 0.00 % (21.28 %), genotype Local variety concentration 0.20 % (21.02 %).

The highest crude protein content in genotype Early white was concentration 0.00 % (21.28 %), whereas, the highest crude protein content in genotype Local variety was concentration 0.20 % (21.02 %). Similarly, the highest crude protein content in genotype IT90K-76 was concentration 0.20 % (23.82 %). The highest crude protein content in genotype IT97K-556-4 was concentration 0.10 % (27.41 %).

4.1.5.6 Crude fibre

In Early white, the lowest crude fibre was produced by 0.10 % and 0.30 % 0.13 % for both concentrations) while the highest value of crude fibre was produced by 0.40 % (0.20 %). These values are significantly different from each other. However, the 0.20 % value and that of the control 0.19 % for both are significantly the same (Table 4.6). Similarly, in Local variety, the lowest crude fibre value obtained was produced by 0.10 % and 0.20 % 0.13 % for both, while the highest value of crude fibre was produced by 0.40 % (0.26 %). These values were significantly different from each other (Table 4.6).

A different trend was observed in IT90K-76 where the highest value of crude fibre was obtained in the control (0.39 %). This was followed by 0.30 % concentration of EMS (0.33 %) and then 0.10 % (0.32 %). These values were significantly different from each other (Table 4.6). The highest crude fibre content in the whole genotypes and concentration is seen in genotype IT90K-76 concentration 0.00 % (0.39 %) while the lowest fibre content was seen in genotype Local variety concentration 0.10 % and concentration 0.20 % (0.13 %) for both. These values were significantly different from one another and from other genotypes and concentrations. However, there was no significant difference between genotype Early white concentration 0.00 % (0.19 %) and concentration 0.20 % (0.19 %),

genotype Local variety concentration 0.00 % (0.19 %) and genotype IT90K-76 concentration 0.20 % (0.19 %).

The highest crude fibre content for genotype Early white is concentration 0.40 % (0.20 %). The highest crde fibre content for genotype Local variety is concentration 0.40 % (0.26 %). The highest crude fibre content for genotype IT90K-76 is concentration 0.00 % (0.39 %).

4.1.5.7 Carbohydrate

In Early white genotype, the lowest carbohydrates content was produced by the control (65.51 %) while the highest carbohydrate value was produced by 0.20 % concentration of EMS (69.29 %). These values are significantly from each other. However, the 0.10 % value (68.45 %) and that of 0.30 % concentration of EMS (68.11 %) is significantly the same (Table 4.6). Meanwhile, in Local variety genotype, the highest carbohydrates content was produced by 0.40 % concentration of EMS (70.15 %). This was followed by 0.10 % concentration of EMS (69.72 %) and then 0.30 % (68.56 %). The lowest carbohydrates content was produced by 0.20 % (63.71 %). These values were however, significantly different from each other (Table 4.6).

Similarly, in IT90K-76 genotype, the lowest carbohydrates content was produced by the control (60.28 %) while the highest was produced by 0.30 % concentration of EMS (66.83 %). However, these values were significantly different from each other. The 0.10 % concentration value (62.56 %) and that of 0.20 % concentration value (62.74 %) are significantly the same (Table 4.6). The lowest carbohydrate content in all genotypes and concentration was observed in genotype IT90K-76 concentration 0.00 % (60.28 %) while the highest carbohydrate content was seen in genotype Local variety concentration 0.40 % (70.15 %). These values were significantly different from one another and from other

genotypes and concentrations. However, there was no significant difference between genotype Early white, concentration 0.10 % (68.45 %) and genotype Local variety, concentration 0.30 % (68.56 %). The highest carbohydrate content for genotype Early white is concentration 0.20 % (69.29 %). The highest carbohydrate content for genotype Local variety is concentration 0.40 % (70.15 %). The highest carbohydrate content for genotype IT90K-76 is concentration 0.30 % (66.83 %).

4.1.5.8 Energy value

In Early white genotype, the highest value was obtained by 0.10 % concentration of EMS (381.98 Kcal/g). This was followed by 0.20 % (373.54 Kcal/g) and then followed by 0.40 % (372.28 Kcal/g). The lowest energy value was obtained by the control 366.55 Kcal/g. The values were however, significantly different from one another (Table 4.6). A different trend was observed in Local variety genotype where the lowest energy value was produced by 0.40 % (355.21 Kcal/g). The highest energy value was produced by 0.10 % concentration of EMS (379.73 Kcal/g). These values were significantly different from one another (Table 4.6).

Meanwhile, in IT90K-76, the highest energy value was produced by 0.20 % concentration of EMS (367.41 Kcal/g) while the lowest energy value was produced by 0.30 % (345.54 Kcal/g). These values were significantly different from one another (Table 4.6). The highest Energy value content in all genotypes and concentration is seen in genotype Early white concentration 0.10 % (381.98 Kcal/g) while the lowest Energy value content was seen in genotype IT90K-76 concentration 0.30 % (345.54 Kcal/g). These values were significantly different from one another and from other genotype and concentrations. However, there was no significant difference between genotype Early white concentration

0.30 % (368.73 Kcal/g), genotype Local variety concentration 0.30 % (366.10 Kcal/g) and genotype IT90K-76 concentration 0.20 % (367.41 Kcal/g).

The highest Energy value content of genotype Early white was concentration 0.10 % (381.98 Kcal/g), however, the highest Energy value content of genotype Local variety was concentration 0.10 % (379.73 Kcal/g). The highest Energy value content of genotype IT90K-76 was concentration 0.20 % (367.41 Kcal/g).

Table 4.6: Proximate composition of M1 Generation of the different cowpea accessions as well as different concentrations of EMS

Parameters	Crude protein (%)	Crude fibre (%)	Carbohydrate (%)	Energy value (Kcal/g)
Early white				
Control	21.28±0.10 e	0.19±0.20 b	65.51±0.50 a	366.55±0.50 a
0.1	18.91±0.20 d	0.13±0.50 a	68.45±0.20 c	381.98±0.20 e
0.2	16.11±0.10 a	0.19±0.20 b	69.29±0.50 d	373.54±0.20 d
0.3	16.72±0.20 b	0.13±0.50 a	68.11±0.45 c	368.73±0.20 b
0.4	16.99±0.15 c	0.20±0.20 c	67.13±0.50 b	372.28±0.20 c
Local variety				
Control	18.83±0.20 c	0.19±0.20 b	67.43±0.20 b	369.90±0.20 d
0.1	19.70±0.10 d	0.13±0.10 a	69.72±0.25 d	379.73±0.15 e
0.2	21.02±0.25 e	0.13±0.10 a	63.71±0.00 a	361.07±0.50 b
0.3	16.20±0.50 b	0.25±0.50 c	68.56±0.10 c	366.10±0.50 c
0.4	15.32±0.15 a	0.26±0.50 d	70.15±0.15 e	355.21±0.10 a
IT90K-76				
Control	19.35±0.50 b	0.39±0.10 e	60.28±0.25 a	358.80±0.30 d
0.1	20.05±0.10 c	0.32±0.20 c	62.56±0.20 c	350.78±0.20 b
0.2	23.82±0.15 e	0.19±0.30 a	62.74±0.20 c	367.41±0.50 e
0.3	15.06±0.50 a	0.33±0.00 d	66.83±0.25 d	345.54±0.15 a
0.4	21.89±0.50 d	0.26±0.50 b	61.55±0.00 b	353.46±0.50 c
IT97K-556-4				
Control	19.70±0.10 b			
0.1	27.41±0.15 d			
0.2	14.36±0.00 a			
0.3	25.66±0.70 c			
0.4	19.96±0.20 b			

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

4.1.6 Mineral composition

The analysis of variance (ANOVA) for mineral composition showed that significant difference ($p<0.05$) exist among the accessions.

4.1.6.1 Sodium (Na) content

In Early white, the highest Sodium (Na) content was obtained by the control (210 mg/100g) while the lowest Na content was obtained by 0.20 % concentration of EMS (160 mg/100g). These values were significantly different from one another. However, the 0.30 % value and that of 0.40 % concentration of EMS value are significantly the same 170 mg/100g for both concentration. (Table 4.7). Different trend was observed in Local variety where the highest Na content was produced by 0.20 % concentration of EMS (210 mg/100g). This was followed by (200 mg/100g) produced by 0.10 % concentration of EMS and then the control (190 mg/100g). The least Na content (150 mg/100g) was produced by 0.40 % concentration of EMS. These values are significantly different from one another (Table 4.7).

Meanwhile, in IT90K-76, the least Na content was produced by 0.30 % with a value of (150 mg/100g) while the highest Na content was produced by 0.20 % with a value of 240 mg/100g. These values are significantly different from one another. (Table 4.7). In IT97K-556-4 genotype, the least Na content was produced by 0.20 % (140 mg/100g). The highest was produced by 0.10 % (270 mg/100g). These values are significantly different from one another. However, the 0.40 % value (200 mg/100g) and that of the control (200 mg/100g) are significantly the same (Table 4.7). The highest Na content in all genotypes and concentration was seen in genotype IT97K-556-4 concentration 0.10 % (270 mg/100g) while the lowest Na content was seen in genotype IT97K-556-4 concentration 0.20 % (140 mg/100g). These values are significantly different from

one another and from other genotypes and concentrations. However, there is significant difference between genotype Local variety concentration 0.10 % (200 mg/100g), genotype IT90K-76 concentration 0.10 % (200mg/100g) and genotype IT97K-556-4 concentration 0.00 % (200 mg/100g) and concentration 0.40 % (200 mg/100g). The highest Na content in genotype Early white is concentration 0.00 % (210 mg/100g). The highest Na content in genotype Local variety is concentration 0.20 % (210 mg/100g). The highest Na content in genotype IT90K-76 is concentration 0.20 % (240 mg/100g). The highest Na content in genotype IT97K-556-4 is concentration 0.10 % (270 mg/100g).

4.1.6.2 Potassium (K) content

In Early white, the highest potassium K content was produced by 0.40 % (590 mg/100g) while the least K content was produced by 0.10 % (300 mg/100g). These values are significantly different from each other. However, the 0.30 % value 400 mg/100g and that of the control 400 mg/100g is significantly the same (Table 4.7). In Local variety, the highest K content was produced by 0.40 % (530 mg/100g) while the lowest K content was produced by the control (330 mg/100g). These values are significantly different from one another. However, the 0.30 % value 520 mg/100g is significantly the same with the highest value (530 mg/100g) (Table 4.7).

Similarly, in IT90K-76 genotype, the least K content was produced by 0.20 % concentration of EMS (430 mg/100g) while 0.40 % concentration of EMS produced the highest K content (490 mg/100g). The 0.10 % concentration of EMS value 450 mg/100g is significantly the same with that of the control (460 mg/100g) (Table 4.7).

Meanwhile, in IT97K-556-4, the least K content was produced by 0.20 % (430 mg/100g) concentration of EMS. The highest K content was produced by 0.40 % (710 mg/100g). These

values are significantly different from one another. However, the 0.30 % value 580 mg/100g is significantly the same with that of the control 590 mg/100g (Table 4.7). The lowest K content in all genotypes and concentration is seen in accession Early white concentration 0.10 % (300 mg/100g) while the highest K content was seen in genotype IT97K-556-4 concentration 0.40 % (710 mg/100g). These values were significantly different from one another and from other genotypes and concentrations. However, there was no significant difference between genotype Local variety concentration 0.20 % (430 mg/100g), genotype IT90K-76 concentration 0.20 % (430 mg/100g), concentration 0.30 % (430 mg/100g) and genotype IT97K-556-4 concentration 0.20 % (430 mg/100g). The highest K content of genotype Early white was concentration 0.40 % (590 mg/100g). The highest K content of genotype Local variety was concentration 0.40 % (530 mg/100g). The highest K content of genotype IT90K-76 was concentration 0.40 % (490 mg/100g). The highest K content of genotype IT97K-556-4 was concentration 0.40 % (710 mg/100g).

4.1.6.3 Calcium (Ca) content

In Early white genotype the highest Ca content was produced by 0.10 % concentration of EMS (840 mg/100g). This was followed by 0.20 % (760 mg/100g) and then 0.30 % (640 mg/100g). The least Ca content was produced by 0.40 % (80 mg/100g). These values were significantly different from one another. (Table 4.7). Meanwhile, in Local variety the least Ca content was produced by 0.10 % (320 mg/100g) concentration of EMS. The highest Ca content was produced by the control and 0.40 % (600 mg/100g for both). These values were significantly different from one another. (Table 4.7).

Different trend was observed in IT90K-76 genotype, the highest Ca content value was produced by 0.30 % concentration of EMS (1920 mg/100g). This was followed by 0.40 % concentration of EMS (1120 mg/100g) and then the control (1080 mg/100g). The least Ca content was produced by 0.10 % concentration of EMS (360 mg/100g). These values were significantly different from one another (Table 4.7). In IT97K-556-4 the least Ca content was produced by the control (360 mg/100g) while the highest Ca content was produced by 0.30 % concentration of EMS (880 mg/100g). These values were significantly different from one another. (Table 4.7).

The highest Ca content in all genotypes and concentration was seen in genotype IT90K-76 concentration 0.30% (1920 mg/100g) while the lowest Ca content was seen in genotype Early white concentration 0.40 % (80 mg/100g). These values were significantly different from one another and from other genotypes and concentrations. However, there was no significant difference between genotype Local variety concentration 0.10 % (320 mg/100g), genotype IT90K-76 concentration 0.10 % (360 mg/100g) and genotype IT97K-556-4 concentration 0.00 % (360 mg/100g). The highest Ca content of accession Early white was concentration 0.10 % (840 mg/100g). The highest Ca content of accession Local variety was concentration 0.00 % and 0.40 % (600 mg/100g) for both. The highest Ca content of accession IT90K-76 was concentration 0.30 % (1920 mg/100g). The highest Ca content of accession IT97K-556-4 was concentration 0.30 % (880 mg/100g).

4.1.6.4 Magnesium (Mg) content

In Early white, the highest magnesium (Mg) content was produced by 0.30 % concentration of EMS (340 mg/100g) while the lowest was produced by 0.20 % concentration of EMS (140 mg/100g). These values were significantly different from one another (Table 4.7).

Meanwhile, in Local variety, the lowest Mg content was produced by 0.40 % concentration of EMS (210 mg/100g). The highest Mg content was produced by the control (320 mg/100g). These values were however, significantly different from one another (Table 4.7). Similarly, in IT90K-76, the highest Mg content was produced by 0.30 % concentration of EMS (400 mg/100g) while the lowest was produced by 0.10 % concentration of EMS (100 mg/100g). These values were significantly different from one another (Table 4.7). Different trend was observed in IT97K-556-4 where the highest Mg content was produced by 0.10 % (470 mg/100g) while the lowest Mg content was produced by 0.20 % (150 mg/100g). These values were significantly different from one another. However, the 0.40 % value 290 mg/100g and that of the control 290 mg/100g was significantly the same (Table 4.7).

The lowest Mg content in all genotypes and concentration was seen in genotype IT90K-76 concentration 0.10 % (100 mg/100g) while the highest Mg content was seen in genotype IT90K-76 concentration 0.30 % (400 mg/100g). These values are significantly different from one another and from other genotypes and concentrations. However, there was no significant difference between genotype Local variety concentration 0.30 % (300 mg/100g) and genotype IT90K-76 concentration 0.20 % (300 mg/100g) and genotype Early white concentration 0.20 % (230 mg/100g) and genotype Local variety concentration 0.10 % (240 mg/100g). The highest Mg content of accession Early white was concentration 0.30 % (340 mg/100g). The highest Mg content of accession Local variety was concentration 0.00 % (320 mg/100g). The highest Mg content of accession IT90K-76 was concentration 0.30 % (400 mg/100g). The highest Mg content of accession IT97K-556-4 was concentration 0.10 % (470 mg/100g).

4.1.6.5 Phosphorous (P) content

In Early white, the lowest phosphorous (P) content was produced by 0.10 % (60 mg/100g) while the highest P content was produced by 0.20 % (120 mg/100g). These values were significantly different from one another. However, the 0.30 % value 80 mg/100g and that of the control 80 mg/100g was significantly the same. Also, the 0.40 % value 120 mg/100g and that of 0.20 % 120 mg/100g was also significantly the same. (Table 4.7). Similarly, in Local variety, the highest P content was produced by 0.20 % (990 mg/100g) while the least P content was produced by 0.10 % (90 mg/100g). These values were significantly different from one another. However, the 0.40 % value 100 mg/100g, that of 0.30 % 100 mg/100g and that of the control 100 mg/100g were significantly the same. (Table 4.7).

The same trend was observed in IT90K-76 genotype, where the highest P content was produced by 0.40 % (100 mg/100g) while the lowest was produced by 0.30 % concentration of EMS (80 mg/100g). These values were significantly different from one another. However, the 0.20 % value (90 mg/100g), that of 0.10 % (90 mg/100g) and that of the control are significantly the same. (Table 4.7). The highest P content in all genotypes and concentration was seen in genotype IT97K-556-4 concentration 0.40 % (140 mg/100g) while the lowest P content was seen in genotype Early white concentration 0.10 % (60 mg/100g). These values are significantly different from one another and from other genotype and concentrations. However, there was no significant difference between genotype Local variety concentration 0.10 % (90 mg/100g), genotype IT90K-76 concentration 0.10 %, concentration 0.00 % and concentration 0.20 % (90 mg/100g) for all and genotype IT97K-556-4 concentration 0.10 % and concentration 0.20 %(90 mg/100g) for both.

The highest P content of genotype Early white was concentration 0.20 % (120 mg/100g). The highest P content of genotype Local variety was concentration 0.40 % (100 mg/100g). The highest P content of genotype IT90K-76 was concentration 0.40 % (100 mg/100g). The highest P content of genotype IT97K-556-4 was concentration 0.40 % (140 mg/100g).

Table 4.7: Mineral compositions of M1 Generation of the different cowpea accessions as well as different concentrations of EMS

Parameters	Sodium Na(mg/100 g)	Potassium K(mg/100 g)	Calcium Ca(mg/100 g)	Magnesium Mg(mg/100 g)	Phosphorous P(mg/100g)
Early white					
Control	210±0.15^{d}	400±0.35^{b}	280±0.00^{b}	270±0.10^{d}	80±0.05^{b}
0.1	190±0.05^{c}	300±0.05^{a}	840±0.00^{e}	230±0.10^{c}	60±0.00^{a}
0.2	160±0.05^{a}	580±0.10^{c}	760±0.00^{d}	140±0.00^{a}	120±0.20^{c}
0.3	170±0.15^{b}	400±0.20^{b}	640±0.00^{c}	340±0.00^{e}	80±0.05^{b}
0.4	170±0.00^{b}	590±0.15^{d}	080±0.00^{a}	220±0.20^{b}	120±0.15^{c}
Local variety					
Control	190±0.10^{c}	330±0.18^{a}	600±0.00^{d}	320±0.00^{e}	100±0.15^{b}
0.1	200±0.15^{d}	470±0.50^{c}	320±0.00^{a}	240±0.20^{b}	90±0.20^{a}
0.2	210±0.00^{e}	430±0.00^{b}	440±0.00^{b}	260±0.20^{c}	990±0.20^{c}
0.3	160±0.10^{b}	520±0.10^{d}	560±0.00^{c}	300±0.20^{d}	100±0.20^{b}
0.4	150±0.15^{a}	530±0.20^{d}	600±0.00^{d}	210±0.10^{a}	100±0.35^{b}
IT90K-76					
Control	190±0.20^{b}	460±0.00^{b}	1080±0.00^{c}	120±0.00^{b}	90±0.10^{b}
0.1	200±0.50^{c}	450±0.50^{b}	360±0.00^{a}	100±0.00^{a}	90±0.00^{b}
0.2	240±0.10^{e}	430±0.20^{a}	640±0.00^{b}	300±0.00^{c}	90±0.15^{b}
0.3	150±0.50^{a}	430±0.50^{a}	1920±0.00^{e}	400±0.00^{e}	80±0.20^{a}
0.4	210±0.45^{d}	490±0.00^{c}	1120±0.00^{d}	360±0.20^{d}	100±0.10^{c}
IT97K-556-4					
Control	200±0.15^{b}	590±0.50^{c}	360±0.00^{a}	290±0.10^{b}	120±0.10^{b}
0.1	270±0.20^{d}	470±0.50^{b}	840±0.00^{d}	470±0.10^{d}	090±0.20^{a}
0.2	140±0.20^{a}	430±0.17^{a}	680±0.00^{c}	150±0.10^{a}	090±0.15^{a}
0.3	260±0.15^{c}	580±0.10^{c}	880±0.00^{e}	380±0.20^{c}	120±0.20^{b}
0.4	200±0.00^{b}	710±0.20^{d}	560±0.00^{b}	290±0.10^{b}	140±0.50^{c}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

4.2 Discussion

4.2.1. Morphological Parameters

The difference observed between mutant lines of cowpea collected is an indication of induction of genetic variability in the plants in terms of the morphological parameters.

4.2.1.1. Plant height

The result of plant height obtained in Early white, local variety and IT97K-556-4 shows that the control had the highest plant height. The highest plant height of the control is in line with the report of Mayur *et al.* (2018) who observed decrease in plant height with increase in concentrations of EMS. In IT90K-76, there is significant increase in treatments than in the control. Significant highest plant height obtained in IT90K-76 compared to the control could be an indication of disturbance in the chromosome of the plant due to the mutagen (Kumar and Tripathi, 2008). However, there is no significant difference in different concentration levels. It also shows that effect of mutagens and control were significant, indicating the mutagens were effective in inducing variability in cowpea and is in line with the study of (Bharati, 2010).

4.2.1.2. Number of leaves

The decrease in number of leaves with increase in concentration of EMS in Early white revealed that lower concentration was more effective than the higher concentration. This is in line with the study of Mayur *et al.* (2018) who observed decrease in number of leaves with increase in concentrations of EMS. Also, the result of number of leaves obtained in Local variety, IT90K-76 and IT97K-556-4 revealed that significant and wide variation observed in number of leaves

per plant is an indication that the genotypes differed with respect to these traits. Similar report has been made by earlier authors such as (Agbogidi and Egho, 2012; Joshua *et al*., 2015).

4.2.2 Insect infestation

Chemical mutagenesis have been used to create resistance to diseases and pathogen outbreak in crops like cabbage (Maluszynski *et al.*, 2000) and soybean (Khan and Tyagi, 2013). Cowpea foliage weevil (*Callosobruchus maculatus*) and Armyworm (*Spodoptera frugiperda*) infestation varied significantly with EMS concentration and among accessions.

In Early white, insect infestation was higher in the treatments (0.40 % concentration of EMS) than in the control. In accession Local variety, insect infestation was higher in treatments than in control also, it was higher at 0.30 % concentration of EMS. In accession IT90K-76, insect infestation was higher in treatment too than in the control and it was higher at 0.40 % concentration of EMS, while in accession IT97K-556-4, insect infestation was higher in control than in treatments.

EMS at different concentration reduced the presence of insect (insects were more on the control). In this study, the low insect infestation of 0.20 % concentration of EMS for IT97K-556-4 and Local variety compared to all other treatment and the control, is an indication of tolerance of the genotypes and it reflect the optimum concentration of the genotype. IT97K-556-4 concentration 0.40 % harbored the highest number of infestation while accession Local variety concentration 0.20 % recorded no insect infestation and hence the least in the aspect of insect infestation. The result obtained gives great possibility for inducing resistance in cowpea against the insect pests. This study also shows that EMS may be a valuable mutagen in inducing resistance and/or tolerance to insect infestation. The difference in tolerance or susceptibility to insect infestation in

EMS treatment can be explained by difference in resistant gene or genetic composition. (Baguma *et al.,* 2021). Higher concentration of EMS showed better result which is in line with this study. (Baguma *et al.,* 2021).

4.2.3 Yield parameters

Yield is an indispensable parameter in mutation breeding because ultimately the plant breeder wants to improve yield along with other beneficial traits. The result obtained from the present study indicates that EMS could be utilized to improve the yield characters of the cowpea accessions studied. The study revealed that the effect of the mutagen concentrations on the yield parameters varied among the cowpea accessions.

4.2.3.1 Number of pod per plant

Number of pod per plant obtained in Early white and Local variety showed that control had the highest value in both than in the mutagens treated in this study. The significance high number of pod per plant of 0.20 % concentration for local variety with the control plant is indication of its superiority to other treatment and a potential treatment for the crop improvement. However, number of pod per plant obtained in this study in IT90K-76 showed that there is an increase in number of pods at higher concentrations of EMS which is not line with the report of Kozgar *et al.* (2011) in *Vigna radiata* and *Vigna mungo*. Number of pod per plant obtained in this study in IT97K-556-4 showed that the highest number of pod per plant was found in accession 0.40 % concentration of EMS. The result of this study, confirms the report of Garuba *et al.* (2021) who reported an increase in number of pod per plant in EMS

4.2.3.2 Weight of 100-seeds

Weight of 100 seed of Early white showed that control had the lowest weight when compared to mutagens used. This might be due to change in genetic composition in the chromosome of the plant, which can lead to loss of a segment, translocation, inversion or deletion of a segment (Shah *et al*., 2008). However, in local variety, IT90K-76 and IT97K-556-4 the lowest was found in concentration 0.30 %, concentrations of EMS in all the genotypes. This was not in line with report of wani, (2012) who reported increase 100 seed weight at lower concentrations of EMS in M_3 generation in Chickpea.

4.2.3.3 Length of pod

Length of pod of Early white obtained in this study showed that pod with increased length were found in plants treated with EMS than in Control. The result of this study is in line with the study of Bolbhat *et al.* (2012). However, pod length obtained in the control of Local variety did better than the mutagen treated, this contradict the result of Bolbhat *et al.* (2012). Length of pod obtained in IT90K-76 and IT97K-556-4 showed that there was no significant difference in different concentration levels. It also shows that effect of mutagens and control were significant, indicating the mutagens were effective in inducing variability in cowpea this is in line with the study of (Bharati, 2010). This results indicates presence of sufficient variability for length of pod.

4.2.4 Proximate composition

Proximate compositions of legumes are reported to be influenced by genetic especially species or varietal factors and environmental parameters (Dhole and Srinivasalu, 2015).

4.2.4.1 Moisture content

Moisture content obtained in Early white and IT90K-76 had the highest value in 0.30 % concentration of EMS. Similarly, moisture content obtained in Local variety and IT97K-556-4 had the highest value in 0.20 % of EMS concentration. However, the moisture content of cowpea 10.39 g/100 g was reported by Masood and Rizwana (2010) which is in line with the result obtained in this study. This could be an indication of good keeping quality of the seeds.

4.2.4.2 Ash content

Ash content of IT90K-76 obtained in this study showed that the control did better than the treated mutagens. However, the result of ash content obtained in Early white, Local variety and IT97K-556-4 showed that the treated mutagens did better than the control. The value of ash content obtained in this study, is not within the range of values reported by Alayande *et al.* (2012) which were between 4.24 and 4.07 g/100g. Famata *et al*.2013 carried out a similar study on different varieties of *V.unguiculata* and reported the least value to be 1.93 which is in line with the least value obtained in this study (1.96%). This result shows that cowpea is low in ash content.

4.2.4.3 Crude fat

The result of crude fat in Early white and Local variety showed that treated seeds did better than the control, However, in IT90K-76, crude fat did better in the control than in the mutagen.Crude fat in ranged from 1.48g to 4.48g in this study. These values are within the range of Olopade *et al* (2017) reported a value of 1.86 % for Olo-oyin. This value shows that cowpea cannot be considered as an oil seed. Seeds are considered as oil seeds when their oil yield is greater than

17% (Adaramola *et al.*, 2016) thus cowpea is not an oil seed and cannot not be suitable for commercial production.

4.2.4.4 Crude protein

The protein content obtained in Early white was higher in the control than in mutagens. Local variety, IT90K-76 and IT97K-556-4 all showed that protein content were higher in mutagens than in the control. In local variety, it was higher in 0.20 % EMS, In IT90K-76, it was higher in 0.20 % EMS and in IT97K-556-4, it was higher in 0.10 % EMS. This shows that a significant improvement in protein content can be achieved through induced mutagenesis. This is similar to the findings of Adekola and Oluleye (2007) in cowpea. Owolabi *et al* (2012) reported a range from 19.84 to 26.61% which is closely related with the range obtained from this study.

4.2.4.5 Crude fibre

Crude fibre content obtained in Ealy white and Local variety had the highest value in 0.40 % concentration of EMS both. However, the crude fibre content of IT90K-76 showed that it better in the control than in the mutagen. The result of the fibre content in this study was not in agreement with the range of values reported by Owolabi *et al*. (2012) 3.46 to 4.88 % and Otitoju *et al*. (2015) (3.77 to 7.01 %) in their separate studies of different varieties of cowpea. This might be attributed to mutagen treatment used (Ibrahim, 2008).

4.2.4.6 Carbohydrate content

Carbohydrate content obtained in Early white ranged from 65.51 % to 69.29 %, the content obtained in local variety ranged from 63.71 % to 70. 15 %. Similarly, the carbohydrate content obtained in IT90K-76 ranged between 60. 28 % to 66.83 %. This result is not within the range of the report of Otitoju *et al.* (2015) who recorded values 45.66 to 55.74 % for different varieties of

cowpea and this may be due effect of mutagen used. Similarly, Olalekan and Bosede (2010) reported carbohydrate content of 56. 6 % which was also lower than value obtained in this study.

4.2.4.7 Energy value

Energy value obtained in Early white revealed that the mutagens did better than the control. Energy value ranged between 345.54 and 381.98. The energy value of food can be estimated from the level of crude protein, carbohydrate and crude fat present by multiplying the constituents by the factor, 4, 4 and 9 respectively. It therefore, showed that the cowpea genotypes have high energy value which is in in with the report of (Uduak, 2018).

4.2.5 Mineral composition

Cowpea seeds were analyzed for the following mineral content; sodium, potassium, calcium, magnesium and phosphorous. The higher content of potassium, Calcium and Phosphorous in some of the treated genotypes, indicates the effectiveness of the concentrations and the mutagens. Also, the variation in the effectiveness of the genotypes with respect to the minerals could be the reflection in the differences in the genetic composition of the genotypes.

4.2.5.1 Calcium content

The result of calcium obtained in Early white, IT90K-76 and IT97K-556-4 showed that it did better in 0.10 %, 0.30 % and 0.30 % concentration of EMS respectively than in their respective controls. In Local variety however, the control did better than the treated mutagens. The calcium content in this study is higher than the one produced by IT97K-499-8 (684.8 mg) and IT96D-773 (630 mg) (Mamiro *et al.,* 2011). The difference in values of calcium could be due to differences of locations of these seeds (Osunbitan *et al.,* 2016).

4.2.5.2 Potassium content

The result of potassium obtained in this study showed a similar trend were the highest potassium content in all the genotypes studied was found in 0.40 % concentration of EMS. However, the result of potassium of (Uduak, 2018) showed that potassium content was 248.53 for brown beans and 241.12 for white beans was lower than the result of potassium obtained in this study. The result of potassium in this study was in line with the reports of Alayande *et al.* (2012) that K occurring at 741 mg 100 g-1 was the highest occurring mineral in white-coated seeds of cowpea. This study and other literatures, have shown that cowpea is rich in potassium (Inobeme *et al*, 2014).

4.2.5.3 Sodium content

The result of sodium obtained showed in Early white showed that control had the highest sodium content. It also showed that with increase in mutagen does, there is a decrease of sodium content obtained. The result of sodium content obtained in Local variety and IT90K-76 showed that they had the highest sodium content at 0.20 % concentration of EMS. Similarly, in IT97K-556-4, sodium content was highest in 0.10 % concentration of EMS. These values is not in agreement with those of Osunbitan *et al.* (2016) who reported values between 5.73- 23.70 mg/kg for varieties of bean flour.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

It can be concluded that the difference observed in agro-morphological and yield parameters among the treated and the control genotypes is an indication of induced variability in the trait of the plants.

The effectiveness of 0.20 % concentration of local variety and IT97K-556-4 in both agro-morphology and nutritional composition is an indication of its superiority and it reflect the optimum concentration of the genotype.

The low susceptibility, insect population and high yield as well as improvement of nutritional content of treatment revealed an improvement in the crop which could be further explored.

5.2 Recommendations

It is therefore recommended that further studies should be carried out to

1. Ascertain the effect of EMS on cytological content of the genotypes.
2. Know the effect of EMS on the molecular compositions of the genotypes.

5.3 Contribution to Knowledge

The thesis established that ethyl methyl sulphonate (EMS) had beneficial effect on weight of 100 seeds of Early white accession; where the control had the lowest value (13.63 g), the highest value was found in 0.20 % concentration of EMS (20.03 g). In addition, 0.30 % concentration of EMS induced the highest weight of pod (2.10 g) in Early white accession. The thesis further revealed that accession IT97K-556-4 exposed to 0. 10 % EMS produced mutants with the

highest Sodium (270 mg/100 g) and Magnesium (470 mg/ 100 g); whereas, 0.40 % EMS also produced mutants with the highest Potassium (710 mg/ 100 g) in IT97K-556-4. Similarly, the 0.20 % concentration of EMS induced tolerance against insect infestation in IT97K-556-4 and Local variety. The thesis also revealed that 0.40 % concentration of EMS of Local Variety produced more yield than the control.

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