EVALUATION OF INSECT PEST TOLERANCE AND OIL QUALITY IN ETHYL METHANE SULFONATE (EMS) EXPOSED GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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A THESIS SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF TECHNOLOGY (MTECH) IN PLANT BIOLOGY (APPLIED PLANT BREEDING AND GENETICS).

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is a multipurpose legume crop widely cultivated in sub-Saharan Africa. However, there are several species of groundnut field insect pest which are responsible for substantial yield losses. Hence, the study aimed at evaluating EMS induced groundnut genotypes for insect pest tolerance and oil quality. The seeds of four (4) groundnut genotypes, SAMNUT 26, SAMNUT 25, SAMNUT 24 and ICG 4412 collected from the institute for Agricultural Research (IAR), Samaru, Zaria, Kaduna state were treated with various concentrations of EMS Viz (0.0 % (control), 0.1 %, 0.2 %, 0.3 % and 0.4 %) for 6 hours and sown in well labeled planting bags along side with their respective controls. The experimental bags were laid out in Complete Randomized Design (CRD) with three (3) replicates each. Data were collected on Agromorphological parameters and insect infestation rate of each genotype. Seeds from the genotypes were further characterised for oil yield and oil attributed parameters at NCRI, Badegi. The result of plant height at maturity showed that the tallest plant for SAMNUT 25 and SAMNUT 26 was recorded in 0.30 % concentration with the value of 44.43 and 45.63 cm, respectively while for SANUT 24 and ICG4412 was obtained in 0.10 % with the value of 51.17 and 50.13 cm, respectively. With the exception of SAMNUT 24 in which the control had the least height (37.43 cm), the least height 39.90, 23.17 and 29.60 for SAMNUT 26, SAMNUT 25 and ICG4412 were recorded in 0.20 % EMS treated plants. Concentration 0.1 % of SAMNUT 24 had the highest number of leaves and leaf area among the genotypes with the value 426 leaves and 26.78 cm^2, respectively. Yield parameters assessment revealed that SAMNUT 24 had the highest average number of pod per plant (26.33 pods) and seed per plant (2.39 seeds) at 0.10 % and 0.20 % concentration, respectively. Highest weight of pod (2.08 g) and 100 seeds (54.34 g) were recorded at 0.30 % of SAMNUT 26 and at 0.10 % of SAMNUT 24, respectively. Significantly ($p < 0.05$) least weight of 100 seeds per plant was recorded at the control of ICG4412 with the value of 6.00 g. In terms of oil yield, 0.20 % treated seeds of SAMNUT 25 had the maximum oil yield (39.34 %) among the genotypes while 0.30 % treated seeds of ICG 4412 plants had the minimum of 21.78 %. Significant variations were observed in number of insects among the genotypes and concentrations at different weeks. Lower insect populations among the genotypes were observed at 14th weeks of evaluation, with different genotype having least insect infestation as different concentration. The positive changes in pod characteristics of SAMNUT 24 (0.1 % an 0.2 %, EMS treatments) could be exploited for increasing the productivity of groundnut and for further improvement of the crop.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Groundnut (*Arachis hypogaea* L.), also known as earthnut, monkeynut and peanut is a native of South America belonging to the family Leguminosea (Fabaceae). It is an annual herb that is distinguished from most other species by producing aerial flowers, but fruiting below the soil level (Tillman and Stalker, 2009). Peanut is one of the most important oil and protein crops in the world (Tingting *et al*., 2020). In 2017, global peanut production reached 47.10 M tons in a total cultivated area of 27.95 M ha (Food and Agricultural Organisation of the United Nation, FAOSTAT, 2020). Groundnut is grown in diverse environments throughout the world between 40 °N and 40 °S (Food and Agriculture Organization, 2013). Groundnut seeds are a rich source of oil (35 – 56 %), protein (25 – 30 %), carbohydrates (9.5 – 19 %), minerals (P, Ca, Mg and K) and vitamins (E, K and B) (Gulluoglu *et al*., 2016). The shells are also used for fuel by some local oil factories or they are sometimes spread on the field as a soil amendment (Ahmed *et al*., 2010).

According to Taru *et al*. (2010), Groundnut is the 13th most important food crop of the world, 4th most important source of edible oil and 3rd most important source of vegetable protein. FAO (2017) estimates that groundnut production stands at about 47 million metric tons cultivated on a total of 28 million hectares worldwide, with an average productivity of 1.6 tons/ha. Groundnut production is concentrated in Asia and Africa, where it is mostly grown under rain-fed conditions with limited external inputs (Ibrahim *et al*., 2012). Edible quality and export worthiness of groundnut is mainly determined by physical factors vi

z., consistency of seed mass and shape, absence of immature seeds, integrity of seed testa, larger seed size, integrity of the seed at the time of processing, and blanching efficiency (Dwivedi and Nigam, 2005).

Studies indicated that consuming groundnut at least four times a week showed a 37% reduced risk of coronary heart disease (Suchoszek-Lukaniuk *et al*., 2011) and anticancer activity with 50 % inhibition of the proliferation of related leukemia cells (Hwang *et al*., 2008). Groundnut is a popular crop in developing countries, Nigeria inclusive (Girei *et al*., 2013). It is commonly grown in intercrops due to its nitrogen fixing ability thereby enriching the soil for other crops (Konlan *et al*., 2013). Studies have shown that groundnut could fix between 40 and 80 kg N per hectare in one year (Janila *et al*., 2013).

Dabhade *et al*. (2012) reported that avoidable yield loss due to major insect pests of groundnut was recorded to the tune of 48.57 % in pod and 42.11 % in fodder. Studies reveal that 15 to 20 % of the total oilseed production is lost directly or indirectly by the attack of insect and mite pests every year (Biswas and Das, 2011). Induced mutagenesis is one of the most important approaches for broadening crop genetic variability to overcome the limitations associated with a narrow genetic basis (Asif *et al*., 2019). Induced mutants not only serve as an important functional genomics tool, but additionally, as intermediate material in crop breeding (Henry *et al*., 2014). The heritability of important traits such as resistance against pest and diseases, production and quality can be analyzed through analysis of the induced mutation (Fawad *et al*., 2015; Kanwal *et al*., 2015; Mumtaz *et al*., 2015; Naseer *et al*., 2015; Naseem *et al*., 2015; Masood *et al*., 2015). Induced mutagenesis have been used in peanut (Wang *et al*., 2015) to generate mutants with resistance to biotic stress (Gowda *et al*., 2010), color of the testa, salinity resistance (Sui *et al*., 2016) pod development (Wan *et al*., 2017) and high-oleate content

(Tang *et al*., 2013; Bera *et al*., 2018).

A number of chemical and physical mutagens are widely employed to induce genetic variability in plants. Ethyl methyl sulfonate (EMS) is a potent and popular chemical mutagen that has been effectively used to induce a high-density of random irreversible point mutations uniformly distributed in the genome (Mahto *et al*., 2018). Yield contributing traits such as, days to flowering, days to maturity, plant height, primary branches per plant, pods per plant, seeds per pod, 100 Seed weight and seed yield per plant) are the metric traits which are quantitatively inherited (Mahto *et al*., 2018).

1.2 Statement of the Research Problem

Field pests are one of the major challenges affecting groundnut production since they cause quality and yield losses. There are several species of groundnut field insect pests which are responsible for substantial yield losses (Biswas, 2014). Studies reveal that 15 - 20 percent of the total oilseed production is lost directly or indirectly by the attack of insect pests every year (Biswas and Das, 2011). Farmers mainly depend on the conventional synthetic chemical insecticides to protect their plants against insect pests, although, conventional synthetic insecticides usually provide quick and adequate control for the time being, they are usually expensive and not environmental friendly. The continuous usage of synthetic insecticides caused health hazards, development of pest genotyperesistance to pesticides, resurgence and upset by pests and environmental pollution (Nas, 2004). Unfortunately, application of mutation breeding using EMS is yet to be exploited for inducing insect pest tolerance in the groundnut genotypes, especially in the study area.

1.3 Aim and Objectives of the Study

The aim of this study is to evaluate for insect pest tolerance and oil quality in Ethyl methyl sulfonate (EMS) exposed groundnut (*Arachis hypogaea* L.) genotypes.

The objectives of this research were to determine the:

i. effects of . Ethyl methyl sulfonate (EMS) on selected agro-morphological and yield traits of the first mutant (M_1) lines of the groundnut genotypes.
ii. common insect pests associated with the groundnut genotypes on the field.
iii. optimum concentration of EMS that induces resistance on the groundnut genotypes against insect pest.
iv. oil properties of the first mutant (M_1) line of the groundnut genotypes

1.4 Justification for the Study

Groundnut is a source of income and food security for rural households in sub-Saharan Africa. The crop has various industrial uses including products such as food, feed, paints, lubricants and insecticides (Variath and Janila, 2017). It is also an ideal crop in rotational systems to improve soil fertility due to its natural ability to fix atmospheric nitrogen (Jaiswal *et al*., 2017).

Chemical mutagenesis is a simple approach and is regarded as an effective and central tool for the improvement of yield and quality characters of crops (Sheikh *et al*., 2012). Ethyl methyl sulfonate (EMS), is one of the potent alkylating chemical mutagen for chemical mutagenesis. It is more effective have been observed in bell pepper (Manzila and Pryato 2020); groundnut (Motagi *et al*., 2009). than physical mutagens (Bhat *et al*., 2005). Mutants with disease resistance have been observed in bell pepper (Manzila and Pryato 2020); groundnut (Motagi *et al*., 2009).

Studies revealed that 15 to 20 % of the total oilseed production is lost directly or indirectly by the attack of insect and mite pests every year (Biswas and Das, 2011). Approximately, 3400 groundnut mutants have been developed using EMS delivering useful genetic variation in groundnut breeding (Knoll *et al*., 2011). All these studies suggested that EMS is an effective mutagen which can be used to generate mutants in a variety of plants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Domestication of Groundnut

Groundnut (*Arachis hypogaea* L.) is used in Africa, Asia, Europe and Australia, while in North and South America it is commonly referred to as 'peanut'. It originated from central part of Brazil or Northeastern Paraguay, while the centre of diversity of the genus includes Western Brazil, Bolivia, Paraguay, and Northern Argentina (Simpson *et al*., 2001). The earliest archaeological records of groundnuts in development are from Peru, 3750-3900 years before present (BP). Groundnuts were generally spread through South and Central America when Europeans arrived at the continent, likely by the Arawak Indians. There is likewise archaeological affirmation of their reality from Mexico, dated 1300-2200 preceding present (PP). After European contact, groundnuts were scattered around the world. The Peruvian runner type was taken toward the Western Pacific, Southeast Asia, China and Madagascar. The Spanish acquainted the Virginia type to Mexico, through the Philippines, in the sixteenth century. The Portuguese at that point took it to Africa, and later to India, through Brazil. Virginia types evidently arrived at the South east United State (US) with the slave trade (Prasad *et al*., 2009; Chandran *et al*., 2016; Audu *et al*., 2017).

2.2 Botanical Description of Groundnut

Groundnut (*Arachis hypogaea* L.) belongs to the family Fabaceae subfamily Papilionidae, tribe Aeschnomeneae, subtribe Stylosanthinae, grnus *Arachis* and species *A. hypogaea*. It is a self pollinating, indeterminate, annual herbaceous legume of genus and species *Arachis*

hypogea which is derived from these two Greek words 'arachos' meaning 'weed' and 'hypogea' meaning 'underground chamber' (Adinya *et al*., 2010). Three different root categories in germinating groundnut were observed; the thick primary seminal root with extensive secondary thickening; the first-order lateral roots which were long and thin with limited secondary thickening; and second and higher-order lateral roots, which were anatomically simple and thin, with little or no secondary growth (Tajima *et al*., 2008).

Groundnuts leaves are tetrafoliate and they occur alternately on the main stem and lateral branches and the exception to this general leaf forms are the three trifoliate species (*A. guaranitica*, *A. tuberosa*, and *Arachis sesquijuga*) from section Trierectoides. The leaves are subtended by a partially adnate stipule. The leaflets are usually oblong to lanceolate and occur in two opposite pairs. The stems can be pubescent or glabrous, angular, and are usually green but can be pigmented as in Valencia-types which are dark purple. Pubescence and pigmentation on stems and leaves have been shown to limit damage from leaf feeding insect pests (Sharma *et al*., 2003).

Morphological variations in branching and flowering patterns, pod and seed traits are used to characterize different botanical varieties (Krapovickas *et al*., 2013). The varieties are further distinguishable into a number of market types or cultivars like Runner (small seeded), Virginia (large seeded), Peruvian runner, Valencia and Spanish (Li *et al*., 2014). According to Krapovickas *et al*. (2007), groundnut can reach the height of 30-50 cm tall, leaves are opposite, and pinnate with four leaflets; each leaflet is 1-7 cm long and 1-3 cm across (wide), the flowers are yellowish 9 orange with reddish veining, it grew underground to produced "pegs" which later develops to a matured groundnut pod; the pods are 3-7cm long containing 1-4 seeds, placed to a depth of 7 - 10 cm this zone is referred to as pod zone (Ademiluyi *et al*., 2011).

2.2.1 Botanical Classification

Kingdom Plantae

Division Tracheophyta

Class Magnoliophyta

Order Fabales

Family Fabaceae

Subfamily Fabiodeae

Tribe Aeschynomenaceae

Genus *Arachis*

Species *A. hypogaea*

Source: Bertioli *et al*. (2011)

2.3 Global Production of Groundnut

The report of the Food and Agriculture Organization (FAO) indicates that the three key groundnut producing countries in the world are China (45%), India (16%), and Nigeria (11%) with Ghana (0.7%) being the least (FAO 2019). According to the report of the United States Department of Agriculture (USDA), a global preliminary estimate of 27.8 million hectares of land was dedicated to groundnut production in 2018/2019 with a projection of 26.50 million hectares of land for the 2019/2020 production season. Actual groundnut production was 27.15 million hectares of land for the 2017/2018 production period with a yield of 1.72 metric tons per hectare. Global preliminary and projected yields of groundnut production for the 2018/2019 and 2019/2020 production year are 1.68 and 1.71 metric tons per hectare respectively (United State Department of Agriculture; USDA, 2020).

The global rankings of area, yield, and production of groundnut (in selected countries) are presented in Table 2.1. The largest area of groundnut production in the world is India with 4.89 million hectares, the lowest being Ghana with 0.34 million hectares of land. Countries recording the highest and lowest yields are the United States and Sudan with 4.49 and 0.74 metric tons per hectare respectively. Nigeria tops Africa as far as production output and area allocation is concerned. A cursory look at the USDA report reveals that India, United States and Myanmar are producing above the projections for the 2019/2020 production year. Although Nigeria and Senegal are doing the same, it is recommended that African countries expand production as a way of realizing a major boost in output (Chakuri, 2018).

Table 2. 1: Global Rankings of Area, Yield, and Production of Groundnut

S/N	Country	Average Area (Million Hectares)	Average Production (Millions Metric Tons)	Average Yield (metric Tons Per Hectare)
1	China	17.09	4.62	3.71
2	India	4.89	6.65	1.36
3	Nigeria	2.82	4.25	1.51
4	United States	0.72	3.23	4.49
5	Sudan	2.22	1.65	0.74
6	Myanmar	0.99	1.57	1.59
7	Senegal	1.25	1.41	1.13
8	Indonesia	0.58	1.08	1.85
9	Argentina	0.38	0.87	2.27
10	Cameroun	0.43	0.60	1.40
11	Vietnam	0.19	0.45	2.37
12	Ghana	0.34	0.43	1.2

Source: (USDA, 2020).

2.4 Biochemical Composition of Groundnut Seeds

Groundnut is accepted as a potential source of food grade protein and an energy dense food. The seed typically contains 36% to 54% oil, 16% to 36% protein, and 10% to 20% carbohydrates (Apekshita *et al*., 2021). It is also known as poor man's nut and being seen as potential functional food. A 100 g of groundnut kernels provide 567 kcal of energy and 8.5 g of dietary fiber (Arya *et al*., 2016) Consumption of peanuts can reduce risk of inflammation and diseases like diabetes, cancer, gallstone and alzheimer's (Arya *et al*., 2016; Toomer, 2018).

Tsai *et al*. (2004) reported that those who consume groundnut and groundnut butter five times a week or more have a reduced risk in gallbladder disease as much as 25 %. Evidences shows that adding groundnut and groundnut butter into diet does not lead to weight gain or higher bodyweight (Mattes *et al*., 2008). Johnston *et al*. (2007) researched exclusively on school children and found that there was weight loss in groundnut fed group whereas the control group gained weight in a span of 2 years. Evidence is also showing that the type of healthy monounsaturated fat in groundnut seed may stimulate a hormone that helps to feel satisfied after consumption (Schwartz *et al*., 2008).

Groundnut have been recognized as a valuable protein source since the 1800s. Protein in groundnut is plant-based, it carries additional components promoting positive health benefits like fibre and unique bioactive, unlike animal protein. Groundnut are high in arginine, an amino acid, which is one of the building blocks of protein. It contains about 100-120grams of fibre per kg (Kris-Etherton *et al*., 2011). The defatted protein flour after oil extraction in groundnut, has immense uses and has been exploited in meat-like products that can be used to formulate cholesterol free vegetarian alternatives (Jani and Devani 2020). They are replete

with polyphenols, oligomeric, and polymeric procyanidins; with the oligomeric fractions predominated by dimers, trimers, and tetramers (Dudek *et al*., 2017; Levy *et al*., 2017).

2.5 Economic Importance of Groundnut

Groundnut is one of the most widely consumed legumes globally due to its nutrient content, affordability and taste. It is rich in protein and energy and has been utilized worldwide to address nutritional needs (Nankya *et al*., 2021). In many countries, groundnut seeds provide a significant nutritious contribution to the diet due to their rich protein, lipid, and fatty acid content (Toomer, 2017), and in sub-Saharan African countries (Okello *et al*.,2010), due to the high nutrient content of groundnut, they have been used to combat malnutrition in most developing countries (Bonku, and Yu, 2019). Improved knowledge of the nutritional chemistry of groundnut has enabled improved peanut products within the food industry (Toomer, 2017).

Groundnut is an important nutritional supplement to the main cereal diets of maize, millet and sorghum and also a significant source of income that contributes significantly to livelihoods and food security (Okello *et al*., 2013). In the Northern part of Nigeria, apart from being consumed whole, groundnuts are processed in a wide range of other products which includes; groundnut paste which is fried to obtain groundnut oil (*man gyada*), groundnut cake (*kulikuli*), salted groundnut (*gyada maigishiri*), a gruel or porridge made with millet (*kunun gyada*), groundnut candy (*kantun gyada*) and groundnut soup (*miyar gyada*) (Hamidu *et al*., 2006). Apart from eating groundnut (raw or roasted) as a food diet, they are processed into groundnut confections, groundnut butter, groundnut flour, groundnut paste for cooking, and manufacturing of oils (Arya *et al*., 2016; Kyei *et al*., 2020). In the United States groundnuts are consumed in a wide variety of forms such as raw, boiled or roasted, and are widely used to prepare a variety of packaged

foods (peanut butter, candies, confections, and snack products (Apekshita *et al*., 2021).

2.6 Climatic and Soil Requirements of Groundnut

Light, sandy loam soil is favoured for the production of groundnut. The soil should also be light colored which shows that it is relatively low in organic matter. The pH should be 5.5 to 7.0 (slightly acidic to neutral). Groundnut cannot tolerate saline soils (Desmae and Sones, 2017). Temperature of 30 °C is regarded as ideal for fast germination and development of pods (Chandran *et al*., 2016). The crop requires between 250 and 1,000 mm of rain during the developing time frame: very early maturing groundnut varieties need 250-400 mm; early varieties 300 - 500 mm; late maturing varieties 500 - 1,000 mm. when rainfall is above 1,000 mm groundnut should be grown on ridges unless the soil is very well drained (Desmae and Sones, 2017).

In addition, the ideal temperatures for growing groundnut should be between 25 – 30 °C as temperatures above 35 °C are not favourable to groundnut production. Under lower temperatures, germination is delayed and the delay in germination exposes the seeds to soil pathogen attack for a longer period. At temperature below 17 °C, crop growth almost ceases with the limit temperature for groundnut germination being 18 °C. However, temperatures between 20 – 30 °C results in ninety-five percent (95 %) germination. Cooler temperature, particularly at night has been reported to also delay harvesting (Meena *et al*., 2015). According to the report of (Ajeigbe *et al*., 2014; Desmae and Sones, 2017) groundnut should not be grown in territories of excess of 1,500 metres above sea level as the temperature is probably going to be low for groundnut and it will influence its production.

2.7 Groundnut Production in Nigeria

In Nigeria, groundnut is produced in all the agro-ecological zones of the country, though cultivation is predominant in nineteen (19) States located within the Sahel, Sudan and Guinea agro ecological zones. These States are: Federal Capital Territory (Federal Capital Territory/F CT Abuja), Kano, Katsina, Kaduna, Jigawa, Sokoto, Zamfara, Kebbi, Adamawa, Bauchi, Yobe, Taraba, Borno, Benue, Plateau, Nasarawa, Kogi, Niger and Kwara (National Agricultural Extension and Research Liaison Service; NAERLS, 2017). Ibrahim *et al*. (2012) reported that developing countries constitute 97 % of the global area cultivated. It production is concentrated in Asia and Africa, where it is mostly grown under rain-fed conditions with limited external inputs.

According to (FAOSTAT, 2018), groundnut is grown on 26.4 million ha worldwide with a total production of 47.1 million metric tons, and an average productivity of 1.4 metric tons/ha. Nigeria is considered the 4th largest producer of groundnut in the world after China, India and united state with an output of 17,150,121 9,179,000 321,110 and 2,420,000 million metric ton nes respectively in 2017.

2.8 Groundnut Harvest

Based on the report of Saxena *et al*. (2014), groundnut is an indeterminate plant, so the pod maturity is not homogeneous. They added that, in choosing the best harvest date, a farmer must explore his/her crops all the time, as the groundnut plant usually gives an indication of when to harvest. Groundnut matured between 80 - 120 days; some of the indications of maturity according to Ajeigbe *et al*. (2014) includes;

a. Seed colour: the colour of seeds in the pods can likewise be utilized as a sign. Young, immature seed is usually white in colour and changes to pink and dull pink as the seed matures
b. Pod colour: inner walls display a dark-brown colour as a result of darkening of the inner tissue of the hull. At the point when 75 % of the pods of the selected number of plants have reached maturity by showing the dark discoloration, harvesting can begin. The external wall of the pods should show different shades on the inner cell layer when scraped with a blade. The colours are white on the immature and yellow pods, and orange, light brown or black on mature pods. Harvesting can be done when 70 % of the pods show the other colours except white.
c. Leaves: the leaves develop a yellow colour and are dry at the tips.
d. Prevailing weather conditions: these can impact the assurance of the harvest date since they influence quality. Drought decides the harvest date when the soil is desiccated to such a degree that the plant withers and the seeds in the pods begin to shrivel and take on a ripe appearance. Such groundnuts must be harvested immediately.

Groundnut can be harvested either by using a hoe or ox-drawn plow (usually used for spreading groundnut varieties on heavy soils and during dry conditions) or by hand pulling the whole plant (this is conceivable when there is sufficient dampness in the soil). This strategy is powerful in lifting the whole plant from soils, with low pod disease (Saxena *et al*., 2014).

2.9 Pests and Diseases of Groundnut

Pests and diseases are a major constraint to groundnut production since they cause

quality and yield losses. The relative economic importance of pests (insect pests, diseases, weeds, birds, nematodes and rodents) varies from one region to other region depending upon the environment, the cropping patterns and the local cultivation practices. The pests which cause significant damage at one place are considered as minor pests in other places. Some pests are restricted in distribution and are confined to few areas while some are widely distributed and cause economic reduction. Moreover, the pest scenario of the crops is changing year after year and more and more new pests are being added to the existing list. Studies revealed that 15 - 20 % of the total oilseed production is lost directly or indirectly by the attack of insect and mite pests every year (Biswas and Das, 2011).

Insect pests damage almost every part of the plant. They can be classified as foliage feeders, intracellular feeders, root and pod feeders and stored product feeders. Foliage feeders include groundnut leaf miner (*Aproaerema modicella*), red necked peanut worm (*Stegasta hosqueella*), army worms, velvet bean caterpillar (*Anticarsia gemmatalis*) and hairy caterpillas (*Amsacta sp p*.) (Hagan *et al*., 2005). Intracellular feeders include leafhoppers (*Empoasca spp*), tobacco thrips (*Franklineilla fiusca, Thrips palmi, Scirtothrips dorsalis*), groundnut aphid (*Aphis craccivora*), two-spotted spider mite (*Tetranychus urticae*) and white flies (*Bemicia tabaci*). Aphids, thrips, jassids and leaf miners are the most important pre- and postharvest insect pests that cause significant economic losses in groundnut worldwide. Poor control of weeds early in the season can cause great yield reduction (Hagan *et al*., 2005).

2.9.1 Army worm

Many insect species live and feed on the groundnut crop, but only few causes significant damage that result in large reductions in pod and haulm yields (Ranga and Rameshwar, 2013).

Armyworm is a Lepidopteran pest native to tropical and subtropical America that attacks over 80 different crop species, but with a preference for graminaceous crops. Similarly, Ranga and Rameshwar (2013) reported over 100 plant species attacked by this insect pest among which groundnuts are included. In January 2016, *S. frugiperda* invaded the western Africa and rapidly attained outbreak population levels in some crops (Goergen and Tam, 2016; Midega *et al*. 2018), then quickly invaded most African countries, India, Myanmar, Thailand and other Asian countries (Common wealth Agricultural Bureaux Iternational; CABI 2017; Early *et al*. 2018; FAO 2018; Nakweta 2018). *Spodoptera frugiperda* larvae feed on the stems, leaves and reproductive parts of more than 350 plant species, causing major damage to economically important cultivated grasses such as maize, rice, groundnuts, sugarcane, sorghum and wheat but also other vegetable crops and cotton (Midega *et al*., 2018; Montezano *et al*., 2018; Jiang *et al*., 2019).

2.9.2 Aphids

Anuj *et al*. (2021) reported that, among the insect pests of groundnut, aphid, *Aphis craccivora* Koch is a serious sucking pest with worldwide distribution and known to attack several leguminous and non-leguminous plants. Aphids suck the sap from tender shoots and twigs and sometimes severely infest the plant and they are vectors of rosette disease. It settles on green plants and trees of the leguminasae family. Weather parameters play an important role on the population dynamics and distribution of groundnut aphids. It is considered to be one of the most important pests of crops causing great losses to yield.

2.9.3 Thrips

Thrips are small insects that live in the flowers and folded leaflets of groundnut. They are only

about 2 mm long, pale cream in color and are usually hidden from view, and are therefore not very conspicuous. The most important ones on groundnut are *T. palmi, S. dorsalis* and *F. schultzei*. It is virtually impossible to distinguish between species with the naked eye under field conditions, although their damage symptoms vary slightly. Nymphs and adults suck sap from the surface of the leaflets with their sucking and rasping mouthparts. This initially results in white patches on the upper and necrotic patches on the lower surface of the leaves. Distortions of the young leaflets and patchy areas of necrotic tissue get punctured and split as the leaflets grow. Injury is normally seen in seedlings (Ranga and Rameshwar, 2013).

Table 2.2: List of some Insect Pests Attacking Groundnut with their Common Name, Scientific Name, Family and Order.

Common name	Scientific name	Family	Order
Hairy caterpillar (walker)	*Spilarctia oblique*	Arctiidae	Lepidoptera
Common cutworm	*Spodoptera litura* F	Noctuidae	Lepidoptera
Defoliator	*Spodoptera* Hub.	Noctuidae	Lepidoptera
Jassids	*Empoasca terminalis* Dist.	Jassidae	Homoptera
Pod borer	*Helcoverpa armigera* Hub.	Noctuidae	Lepidoptera
Hairy caterpillar	*Spilosoma nydia* Butl.	Arctiidae	Lepidoptera
Leaf roller	*Anarsia ephippias* (Meyr.)	Noctuidae	Lepidoptera
Black cutworm	*Agrotis ipsilon* (Hufn.)	Noctuidae	Lepidoptera
Black weevil	*Cyrtozemia cognate* marshall	Curculionidae	Coleopteran
Aphid	*Aphis craccivora* (Koch)	Aphididae	Homoptera

Source: (Biswas, 2014)

2.10 Mutation Breeding

According to Roychowdhury and Tah (2013), Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic recombination or genetic segregation, but induced by chemical, physical or biological agents. Mutation breeding employs three types of mutagenesis. These are induced mutagenesis, in which mutations occur as a result of treatment with chemical mutagens; site-directed mutagenesis, which is the process of creating a mutation at a defined site in a DNA molecule; and insertion mutagenesis, which is due to DNA insertions, either through genetic transformation and insertion of T-DNA or activation of transposable elements or irradiation (gamma rays, X - rays, ion beam, etc.) (Kharkwal and Shu 2009; Forster and Shu 2012). The key point in mutation breeding is the process of identifying individuals with a target mutation, which involves two major steps: mutant screening and mutant confirmation. Mutant screening is a process involving selection of individuals from a large mutated population that meet specific selection criteria, e.g. disease resistance, early flowering as compared to the parent. However, these selections are often regarded as putative mutants or false mutants (Forster and Shu, 2012). Mutant confirmation, on the other hand, is the process of reevaluating the putative mutants under a controlled and replicated environment using large samples. Through this process, many putative mutants are revealed to be false mutants. In general, the mutations that are important in crop improvement usually involve single bases and may or may not affect protein synthesis (Mba, 2013).

2.10.1 Chemical mutagens

The effect of chemical mutagens on plant materials is generally considered milder (Acquaah,

2006). An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. Acquaah, (2006) reviewed that the ratio of mutational to undesirable modifications is generally higher for chemical mutagens than for physical mutagens. Despite the large number of mutagenic compounds, only a small number has been tested in plants (Wani *et al*., 2014). Among them, only a very restricted group of alkylating agents has found large application in plant experimental mutagenesis and plant mutation breeding. Over 80 % of the registered new mutant plant varieties reported in the International Atomic Energy Association (IAEA) database (IAEA, 2015) obtained through chemical mutagenesis were induced by alkylating agents. Of these, three compounds are significant: ethyl methyl sulfonate (EMS), 1-methyl-1-nitrosourea and 1–ethyl–1-nitrosourea, which account for 64 % of these varieties. Chemical mutagens have been successfully employed in mutation breeding programmes to artificially generate variations for development of new varieties with improved traits, such as increased yield, reduced plant height and resistance to disease (Goyal & khan, 2009; Khursheed *et al*., 2015; Tantray *et al*., 2017).

2.10.2 Physical mutagens

Physical mutagens are electromagnetic radiations such as gamma rays, UV rays, α-rays, X- rays, β-rays and fast neutrons. They are highly penetrating. However, UV rays are non-ionising radiation with low penetration capacity generally induce dimer formation and deamination of DNA bases (Aamir *et al*., 2018).

2.11 Mutation Breeding in Groundnut

Previous researchers revealed that through injection of 0.3 % EMS into flowers of Huayu 16 at 9:00 - 9:30 a.m. and subsequent selection, it was able to develop a high-yielding groundnut

cultivar - Huayu 40 (Wang *et al*., 2011). The improved variety, Huayu 40 has an erect growth habit and sequential branching pattern. As compared with its wild type (Huayu 16), Huayu 40 possesses faster growing and darker green foliage (Wang *et al*., 2011). In addition, Wang *et al*. (2011) also reported that leaf water content, chlorophyll a and b content of Huayu 40 were significantly higher than those of Huayu 16. Suradkar and satpute (2011) conducted a study to check the EMS sensitivity on groundnut *A. hypogaea* and to find suitable concentration of EMS (0.05 %, 0.10 %, 0.15 % and 0.20 %) on two varieties of groundnuts TAG-24 and AK159. It was concluded that 0.05 % concentration of EMS induces good genetic variability in both varieties.

Gunasekaran and Pavadai (2015) induced physical and chemical mutagenesis in groundnut. They Treated groundnut cultivar VRI-2 with EMS at different concentration (0.1 %, 0.2 %, 0.3 %, 0.4 %, 0.5 % and 0.6 %) and reported that, the increasing concentration of EMS decreases phenotypic (Plant height, number of branches) and yield characters (number of pods per plant and seed weight) in M_1 generation. In M_2 generation, plant height, number of leaves per plant, 100 seed weight and number of pod per plant increases with increase in mutagenic doses.

Muniappan *et al*. (2016) investigated the effect of EMS on groundnut cultivar TMV-7 particularly for M_1 generation with special reference to amino acid. The healthy and viable seeds of groundnut cultivar were exposed to different concentration of EMS (10 – 50 mM). Decrease in growth, morphological parameters like plant height, survival percentage, number of branches, number of pods per plant and 100 seed weight with increasing concentration of EMS was recorded. The study was useful to understand the morphological and physiological changes induced by chemical mutagen particularly in M_1 generation.

Mayur *et al*. (2018) studied induced chemical mutagenesis in groundnut (*A hypogaea*).

Groundnut variety (LGN-1) was treated with EMS at different concentrations (0.2 %, 0.4 %, 0.6 %, 0.8 % and0.1 2 %) to study it effect on various morphological traits when compared with control. Decrease was observed in number of leaves per plant, number of branches and plant height with increase in concentration of EMS. Ethyl methane sulfonate at 0.2 % of EMS showed adverse effect in growth parameters such as number of leaves (19.86), plant height (12.13 cm), number of branches (5.26) as compared with the control (20.60, 12.53 cm,5.13 respectively). It was found that lower concentration appeared to be better effective treatment for inducing variability as compared with other concentrations (0.4 - 0.12 %). Similarly, an investigation was carried out by Gangadhara *et al*. (2018) on the effects of various doses (0.2 - 0.6 %) of EMS on physical and quality traits of groundnut (*Arachis hypogaea* L.) in M_4 families of the groundnut variety TPG 41. Among the different concentration of EMS treated, TPG41 population, 0.2 % EMS concentration was found to be effective in inducing variability for pod yield per plant, kernel length, hundred kernel weight and shelling out turn in M_4 families. It was concluded that, the promising mutants' identities in M_4 generation with respect to physical and quality traits need further confirmation through large scale evaluation.

Abady *et al*. (2019) reviewed Groundnut (*Arachis hypogaea* L.) improvement in sub-Saharan Africa. The role of new tools in breeding such as, high-throughput and automated phenotyping techniques, rapid generation advancement, single seed descent approach, marker-assisted selection, genomic selection, next-generation sequencing, genetic engineering and genome editing for accelerated breeding and cultivar development of groundnut were analyzed. It was concluded that Groundnut breeding in SSA is mainly dependent on limited phenotypic selection in segregating generations resulting in low selection efficiencies. Consequently, a limited number of improved groundnut genotypes were developed and deployed. To develop climate

resilient, improved varieties with resistance to biotic and abiotic stress tolerance and quality attributes there is need to employ advanced techniques in the breeding processes.

Olorunmaiye *et al*. (2019) assessed the mutagenic effects of EMS concentration on yield, growth and nutrient composition of *Arachis hypogaea* (SAMNUT 24 variety) with the following concentrations (0.0, 0.25, 0.50, 0.75, 1.00 and 1.25 % v/v) of EMS. Higher percentage seed crude fat and protein were induced by treatments with the optimal performance at 0.75 - 1.00 % concentration. However, decreased viguor, less nut, reduced growth and biological yield were observed at M_2. It was therefore concluded that, since groundnut is basically cultivated for biomass yield as well as fat and sources of plant vegetable, EMS application at optimal concentration could be used for the crop improvement.

Tingting *et al*. (2020) investigated Ethyl methyl sulfonates induced mutagenesis and its effect on peanut yield, agronomy and quality traits, treated two widely cultivated peanut genotypes Huayu 22 and Yueyou 45 with different concentrations of EMS. The study identified potentially useful mutants associated with dwarfism, leaf shapes and colours, high oil /or protein content, seed size and testa colour among individual of M_2 generation. Stable inheritance in M_3 generation individuals was reported. The mutant line selected in the study may be used as germplasm resources and breeding material in peanut breeding programmes.

2.11.1 Mutation breeding for tolerance against insects and diseases

Manzila and Pryato (2020) investigated the variations in germinin virus resistant mutant lines of chili pepper developed through EMS mutagenesis using 0.3 % EMS concentration. It was concluded that EMS mutagenesis successfully induced genetic variability in the cultivar of pepper. Motagi *et al*. (2009) studied induced groundnut mutant line for resistance against foliar

diseases through EMS mutagenesis. It was reported that, the groundnut genotype yielded high frequency of foliar disease resistant. Mutant 28 - 2, 45 and 100 combined multiple resistance and early maturity. The mutant lines were also resistant to thrips, tobacco cut worm and tolerant to Aspergillus infection. The mutant can serve as a superior germplasm in improving groundnut resistant to diseases and also its productivity.

Motagi *et al*. (2009) treated Dharwad early runner (DER) with EMS (0.5 %). The mutant line developed resistant to rust disease. Similarly, Prasad *et al*. (2000) developed a groundnut mutant 28 - 2 for resistant to tobacco cut worm (*Spodoptera litura*) and thrip through EMS mutagenesis. Harshbabu *et al*. (2004) also developed 28-2 mutant line of groundnut for resistant against thrip, tobacco cut worm and tolerant to Aspergillus infection through EMS mutagenesis.

2.12 Importance of Mutation in Creating Variation

The main advantage of mutational breeding is the possibility of improving one or two characters without changing the entire genotype. Mutation generates variability which is a predecessor for successful breeding programme (Adamu *et al*., 2005). Induced mutations using physical and chemical mutagen have the potential to enhance mutation frequency and create mutants that could be screened for genetic and agronomic improvements (Ahloowalia and Maluszynski, 2001; Proite *et al*., 2007; Kharade *et al*., 2015). Through breeding and selection, plants have been improved in yield, quality, taste, size and resistance to disease and plants adapt to diverse climates and conditions (William, 2007).

2.13 Physico-chemical Characterization of Groundnut Oil

Shashikant (2017) studied the physiochemical characteristics of four different groundnut varieties Ak-12-24, Tag-1 (Tg-1), Tag-17 (Tg-17) and Kopargoan-1 (k-1) reported, the extracted

oil and refractive index were in the range of 39.81 - 42.19 % and 1.4627 - 1.4632 respectively. It was concluded that the groundnut oil can be a source of edible oil. Satpute and Suradkar (2018) conducted a study on groundnut (TAG-24 and AK-159) enhancement through induced mutation by gamma ray and EMS for the purpose of oil percentage, saponification, iodine value and protein percentage. They concluded that 0.15 and 0.1 % concentration of EMS can be used to improve oil quality of groundnut. Also, induced mutation can enhance the oil yield, nutritional value and stability of groundnut (*Arachis hypogaea* L.).

Table 2. 3: Compositional Requirements of Groundnuts

S/N	Characteristics	Requirements
1	Refractive index 40°C	1.460-1.465 °C
2	Saponification value mg	187-196 mg KOH/g
3	Iodine value(wij's)	80-107 I_2/100g
4	Acid value	Non virgin; 0.6 %, virgin oil; 4.0 %
5	Peroxide value	10 mEq/kg

Source: (Biswas and Das 2011)

2.14 Iodine Value

The iodine value (IV) indicates the degree of unsaturation of the oil. It is defined as the number of grams of iodine absorbed by 100 g of oil (Sadoudi and Ali, 2017). The iodine value or iodine number is a generally accepted parameter expressing the degree of unsaturation, the number of carbon-carbon double bonds in fats or oils (William and Vida, 2015). This value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of oil

to oxidation. The determination of the iodine value is also important in classifying oils and fats (Kapila *et al*., 2005). The higher the amount of unsaturation, the more iodine is absorbed: therefore, the higher the iodine value the greater the degree of unsaturation. High iodine value indicates high unsaturation of fats and oils and low iodine value oils are more saturated with fewer double-bonds (Atsu-Barku *et al*., 2012). Also higher iodine values are evidence that the oils could be used in the manufacture of cosmetics, oil paints and vanish, as well as nutritional purposes (Knothe, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at the experimental 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 488, 788 Temperature ranges between 35 °C and 37.5 °C while relative humidity varies from 40 - 80 % (Adeboye *et al*., 2011). The area has two seasons: raining season between May to October and dry season between November and April each year. It has a low humid soil type with favorable climatic condition for planting which make it easy for groundnut crop to grow successfully and express all its traits.

3.2 Source of Groundnut Seed

The groundnut varieties used for the experiment were collected from the Institute for Agricultural Research (IAR), Samaru, Zaria, Kaduna State, Nigeria.

The varieties collected were:

(i) SAMNUT 24

(ii) SAMNUT 25

(iii) SAMNUT 26

(iv) ICG 4412

3.3 Mutagenic Treatments

Mutagenic treatment was conducted in the laboratory of Department of Plant Biology, Federal University of Technology Minna. Groundnut seeds were presoaked in distilled water for 4 hours. This allows the mutagen to diffuse more rapidly to the tissues of interest (Forster and Shu, 2012). The seeds were soaked for 4 hours in different concentrations of Ethyl Methane Sulfonate (0.0, 0.1, 0.2, 0.3 and 0.4 %) (Mba *et al*., 2010). The treated seeds were thoroughly washed in running tap water to remove the residual effects of the mutagen.

3.4 Preparation of Stock Solution

Stock solution of EMS was prepared by diluting 1 ml of EMS in 99 ml of water. Using a beaker, 20 ml of the stock solution was measured and diluted with 80 ml of distilled water to make a 100 ml solution this gave 0.2 % all other appropriate concentrations of EMS were diluted in distilled water to have the concentration required for the experiment.

3.5 Experimental Design

The experiment was designed in a Complete Randomized Design (RCBD) with three replicates. The planting was done using plant bags. The plant bags were filled with light sandy to sandy-loam soil, then water was added to moisten the soil. Both treated and control seeds were planted. The control seeds were planted in four (4) plant bags in a row for each of the varieties while the treated seeds were also planted per concentration, each concentration having four bags in a row. Three (3) seed each were planted per bag.

3.6 Data Collection

3.6.1 Observation of insect pest of groundnut

Observations on the population of different insect pests were recorded from germination to maturity stages of the crop. Data on different species of insects were recorded from 3 plants in each concentrations of the groundnut genotypes. Sequential appearance of the insect pests, their nature and quantity of damage were carefully observed and recorded. Records were taken by visual observations on the standing crop during 7:00 - 10:00 am at 2 days intervals.

The insects were identified following the method of Biswas (2014).

3.6.2 Vegetative parameters

The Vegetative parameters were taken using the method of Olorunmaiye *et al*. (2019).

i. Number of days to seed emergence: the number of days in which each seed emerge were taken and recorded.
ii. Plant height (cm) at 2weekly interval: the height of each plant was taken using meter rule from base to the plantlet and the average length was expressed in centimeters
iii. Number of Leaves: the numbers of leave were counted manually at maturity
iv. Leaf length: The length of leaves in each genotype were measured using a meter rule
v. Leaf Width: The width of leaves were measured using a meter rule,

3.6.3 Yield parameters

i. Number of pod per pod: the number of pod per plant were counted manually and recorded.

ii. Number of seeds per pod: the number of seed per each plant were counted and recorded.

iii. Length of pods: the length of pod in each genotype were measured using meter rule.

iv. Weight of pod: the weight of pods was recorded using weighing balance.

v. Weight of 100 seed; the weight of 100 seed per mutant line was recorded using weighing balance.

3.6.4 Analysis of physico-chemical parameters of groundnut oil

3.6.4.1 Oil extraction

Groundnut oil was extracted from its flour using n-hexane (anon-polar solvent) according to the method of Association of official Analytical Chemists; AOAC (2012**).** Flour samples were used for extraction using a soxhlet extractor. The lipid was extracted for 5 hours with a 500 ml volumetric flask containing the solvent, which was heated with an electric heater at 70 °C. Oil/solvent extracts were evaporated off using rotary evaporator and later oven dried at 105 °C for 1 hour and stored in bottles to be analyzed later.

3.6.4.2 Peroxide value

Peroxide value (PV) is a measure of the concentration of a substance that can oxidize potassium iodide to iodine (Sadoudi and Ali, 2017). It is a mili equivalents of oxygen (hydro peroxides) per 1000 gram of oil. This was done by the AOAC (2012).

Oil sample (2.0 g) was accurately weighed into a conical flask, and dissolved in solvent mixture containing 12 ml chloroform and 18 ml glacial acetic acid. To the solution 0.5 ml of a saturated aqueous potassium iodide solution was added. The flask was stoppered and allowed to stand for 1 min. Thirty milliliters of water was added and the solution was titrated with 0.1 M sodium thiosulphate solution until the yellow color had almost gone. About 0.5 ml of starch solution was introduced and titration continued with the reagent added slowly until the blue-black color

disappeared. During titration, the flask was continuously and vigorously shaken to transfer the liberated iodine from the chloroform layer to the aqueous layer. A blank titration was also performed, and the peroxide value was obtained from the formula (Sadoudi and Ali 2017).

$$\text{Peroxide value}\% = \frac{V \times T}{M} \times 100 \qquad 3.1$$

Where,

V= Amount in ml of standardized sodium thiosulphate used for the test corrected to take into account the blank test

T= Exact normality of the Sodium thiosulphate solution used

M= Mass in grams of the test portion

3.6.4.3 Free fatty acid (FFA) content

A clean dry beaker was weighed and 2 g of pre-heated oil (heated to about 50 °C) was added and reweighed Aliquots of ethanol was added to the oil to completely free the fatty acids and the ethanol-oil mixture was then titrated with 0.1N NaOH using phenolphthalein indicator. The volume (V) of NaOH required to produce the first permanent pink colour was recorded and the free fatty acid content of the oil was determined from the formula:

$$\%\text{FFA} = \frac{M \times V \times N}{10 \times M} \qquad 3.2$$

Where;

M: Relative molecular mass of Palmitic acid =256,

V: Volume of NaOH used

N: Normality (concentration) of NaOH used

M: Weight of oil used

10: constant.

3.6.4.4 Iodine value

Nadeem *et al*. (2013) method was used to determine the iodine value. Some 0.2 g of oil sample was weighed and placed in a 250 mL flask and 20 mL of chloroform was then added to the sample. Wijs reagent (25 mL) was added with the aid of a pipette and the resulting mixture stirred and stored in a dark place at 25 °C for 30 minutes before 10 mL of 30 % potassium iodide and 100 ml of distilled water was added to the mixture. The mixture was then titrated with 0.1 N sodium thiosulphate until the yellow colour almost disappeared. One milliliter of starch solution was then added and the mixture titrated further until the blue starch-iodine colour disappeared. A blank titration was also carried out and the Iodine value calculated using the formula below:

$$\text{Iodine value} = \frac{\text{TD}}{\text{M}} \times 1.269 \qquad 3.3$$

Where;

TD: Titre difference

M: mass of sample (g) and 1.269= constant

3.6.4.5 Acid value

Five (5) g of sample was weighed and placed in a 250 mL flask and fifty (50) milliliter of a mixture of equal volumes of ethanol and ether, which has been neutralized by 0.5 N of potassium hydroxide, was then added. The resulting mixture was heated for 10 minutes to allow for complete dissolution of the sample and then cooled. One milliliter of phenolphthalein indicator was then added while shaking the contents vigorously. The mixture was then titrated with 0.5 N

potassium hydroxide until a pink colour was obtained as described by AOAC (2010). The acid value was then calculated using the formula:

$$\text{Acid value} = \frac{\text{TD}\times\text{N}}{\text{M}} \times 56.1 \qquad 3.4$$

3.6.4.6 Determination of refractive index

The refractive indices, η40 D, (RI), of the oils and fat samples were measured using the Abbe refractometer connected to a thermostatically controlled water bath that maintained the temperature of the refractometer at 40 ± 0.1ºC used. A drop of the oil was placed on the surface of the refractometer and the reading was taken (AOAC 2010).

3.7 Data Analysis

Data collected for insect population was analyzed using descriptive statistics. Average number of insects per verities was calculated and means level of infestation scores of each verities was determined. The morphological data was subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20 to determine the level of significance among the treatment. Duncan Multiple Range Test (DMRT) was used to separate the means where there are differences. All analysis was carried out at 5 % ($P<0.05$) level of significance**.**

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1 Vegetative parameters of M_1 line of EMS treated groundnut

4.1.1.1 Plant height

The results of analysis of variance (ANOVA) for morphological parameters are presented as follows. For SAMNUT 26, no significant difference ($p>0.05$) was observed in all the treated groundnut varieties including the control. The control had the highest value at week 2 (16.43 cm) however, EMS concentration 0.1 % had the highest value for plant height at week 4 and 6 (27.47 cm and 31.80 cm) respectively while at week 10, 0.1 % concentration had a height of 39.73 cm this value is not significantly different ($p>0.05$) from 0.3 % (39.73 cm). The highest plant height at maturity was recorded at 0.3 % concentration (45.63 cm). The lowest plant height across the weeks were recorded at 0.2 % (11.60 cm, 16.73 cm, 25.17 cm, 31. 73 cm, 35. 07 cm and 39.90 cm) as well as 0.4 % (11.40 cm, 18.53 cm,26.13 cm, 33.33 cm, 37.77 cm and 43.17 cm) respectively. However, there were no significant difference ($p>0.05$) among all the treatments.

In SAMNUT 25, the highest plant height at week 2,4,6,8,10 and 12 (22.07 cm, 30.77 cm, 32.90 cm, 36.93 cm, 40.30 cm and 44.48 cm) was due to 0.3 % Ethyl methyl sulfonate (EMS) treated plants. These values were not significantly different ($p>0.05$) from that of 0.1 % EMS treated plants (18.43 cm, 24.53 cm, 28.63 cm, 32.60 cm, 37.10 cm and 40.47 cm). The least values of plant height for SAMNUT 25 for all the weeks were recorded at 0.2 % with the

value of 4.97 cm, 7.70 cm, 12.23 cm, 17.10 cm, 20.26 cm and 23.17 cm at week 2, 4, 6, 8, 10 and 12 respectively (Table 4.1). Notable variations were observed in plant height of SAMNUT 24 across the EMS treated plants and the controls. The highest value for plant height was recorded at 0.1 % EMS treated plant at 2,4,6,8,10 and 12 weeks after planting (24.43 cm, 30.77 cm,35.87 cm, 40.23 cm, 44.57 cm and 51.17 cm) respectively. The least value (18.10 cm) at 2 weeks after planting was due to 0.3 % treated plant while at 4, 6, 8, 10 and 12 WAP, the control plant had the least height with the value of 21.30 cm, 26.73 cm, 29.63 cm, 33.80 cm and 37.43 cm respectively. The lowest values were not significantly different ($p>0.05$) from the height obtained from 0.3 % and 0.4 % EMS treated plant but significantly different ($p<0.05$) from the highest values (Table 4.1).

In ICG4412 variety, the highest plant height at week 2,4,6 and 8 (22.23 cm, 28.53 cm, 33.40 cm and 37.20 cm) was due to 0.4 % EMS treated plant and the least value was due to 0.2 % treated plants with the height of 12.20 cm, 15.97 cm, 19.00 cm 23.47 cm, 25.90 cm and 29.60 cm respectively. These values were not significantly different ($p>0.05$) from the height of all other plants at week 2 and 4. However at week 6, the highest plant height (33.40 cm) due to 0.4 % treatment was significantly different from that of 0.2 % (19.00 cm) but not significantly different from those of the control (24.57 cm), 0.1 % (31.07 cm) and 0.3 % (22.90 cm). Similar trend was also observed at week 8. Meanwhile at week 10, the highest plant height (42.20 cm) was due to 0.1 % EMS, the value was significantly different from that of the control (32.93 cm), 0.2 % (25.90 cm) and 0.3 % (35.53 cm) but significantly the same with that of 0.4 % (41.03 cm).

Table 4.1: Plant Height of EMS Mutant Lines (M_1) of the Groundnut Genotypes 2WAP

Treatment	PH2 (cm)	PH4 (cm)	PH6 (cm)	PH8 (cm)	PH10 (cm)	PH12 (cm)
SAMNUT 26						
Control	16.43±2.66^{a}	25.23±2.74^{a}	30.67±2.32^{a}	35.53±2.54^{a}	38.97±2.02^{a}	45.20±1.46^{a}
0.1	14.87±1.41^{a}	27.47±2.66^{a}	31.80±1.82^{a}	34.17±1.47^{a}	39.73±2.70^{a}	44.80±3.60^{a}
0.2	11.60±2.20^{a}	16.73±2.47^{a}	25.17±1.59^{a}	31.73±1.63^{a}	35.07±2.06^{a}	39.90±1.93^{a}
0.3	15.27±4.72^{a}	20.43±5.29^{a}	27.23±6.63^{a}	35.43±5.55^{a}	39.53±4.81^{a}	45.63±4.43^{a}
0.4	11.40±4.25^{a}	18.53±4.12^{a}	26.13±2.98^{a}	33.33±2.40^{a}	37.77±2.34^{a}	43.17±2.19^{a}
SAMNUT 25						
Control	18.37±2.62^{b}	25.33±2.54^{b}	30.17±1.92^{b}	34.30±1.91^{b}	37.43±1.29^{b}	40.47±1.74^{b}
0.1	16.47±1.99^{b}	23.33±2.52^{b}	27.17±3.28^{b}	31.33±3.39^{b}	35.93±4.56^{b}	41.07±5.74^{b}
0.2	4.97±1.17^{a}	7.70±2.17^{a}	12.23±1.40^{a}	17.10±0.75^{a}	20.26±0.94^{a}	23.17±1.42^{a}
0.3	22.07±2.23^{b}	27.77±1.83^{b}	32.90±1.20^{b}	36.93±1.47^{b}	40.30±0.40^{b}	44.43±0.46^{b}
0.4	18.43±3.16^{b}	24.53±3.21^{b}	28.63±2.44^{b}	32.60±2.46^{b}	37.10±1.43^{b}	42.93±1.49^{b}
SAMNUT 24						
Control	18.10±0.79^{a}	21.30±1.78^{a}	26.73±0.74^{a}	29.63±0.67^{a}	33.80±0.32^{a}	37.43±0.32^{a}
0.1	24.43±2.24^{b}	30.77±2.91^{b}	35.87±2.89^{c}	40.23±2.56^{b}	44.57±2.72^{b}	51.17±3.85^{c}
0.2	19.77±2.24^{ab}	27.03±1.27^{ab}	33.57±2.18^{bc}	39.20±2.01^{b}	43.33±1.73^{b}	48.47±1.42^{bc}
0.3	16.03±1.96^{a}	21.43±1.91^{a}	27.57±1.78^{ab}	31.97±1.36^{a}	36.57±1.56^{a}	40.53±1.49^{ab}
0.4	20.73±0.94^{ab}	26.80±1.68^{ab}	30.10±1.18^{abc}	33.67±1.43^{a}	37.30±1.27^{a}	42.90±3.16^{ab}
ICG 4412						
Control	15.47±1.53^{a}	18.83±1.76^{a}	24.57±2.36^{ab}	28.17±2.28^{ab}	32.93±3.15^{b}	37.53±3.76^{ab}
0.1	20.33±3.22^{a}	25.33±3.95^{a}	31.07±2.98^{ab}	36.33±2.07^{ab}	42.20±0.96 ^{c}	50.13±2.11^{c}
0.2	12.20±2.08^{a}	15.97±2.44^{a}	19.00±2.18^{a}	23.47±1.61^{a}	25.90±1.45^{a}	29.60±1.33^{a}
0.3	15.57±4.65^{a}	19.00±5.03^{a}	22.90±5.35^{ab}	29.00±6.47^{ab}	33.53±5.84^{ab}	42.87±2.55^{bc}
0.4	22.23±3.39^{a}	28.53±4.65^{a}	33.40±4.70^{a}	37.20±4.40^{b}	41.03±2.87 ^{c}	44.10±2.60^{bc}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$. PH = Plant Height

4.1.1.2 Number of leaves

The result obtained for number of leaves in SAMNUT 26 showed that no significant difference ($p>0.05$) exist in all the treatments at week 2 and 4. At week 6 and 8 the maximum average number of leaves was due to 0.3 % (60.67 and 117.33) EMS concentration. While the minimum values at week at week 6 and 8 was recorded in 0.1 % (37.33 and 52.67) EMS concentration. These values were not significantly different ($p>0.05$) from that of 0.2 %, 0.4 % and the control (Table 4.2). The highest average number of leave at week 10 and 12 were recorded in 0.3 % (165.33 and 262) EMS concentration while the least was recorded in 0.1 % (71.33 and 112.00) EMS concentration. These values were significantly different ($p>0.05$) from 0.4 % (100.67 and 130) control (85.33 and 119.33) and all the other treatments at 8 and 12 weeks. The highest value for number of leaves at week 10 and 12 was significantly different ($p<0.05$) from the values recorded from all other treatments (Table 4.2).

In SAMNUT 25, the least value recorded at week 2, 4 and 6 was in the control (12.67, 25.33 and 50.00) respectively. These values were not significantly different ($p>0.05$) from 0.2 % EMS treated plants (12.67, 25.33 and 51.33) respectively, but significantly different ($p<0.05$) from 0.1 % (20.00 and 29.33) 0.3 % (14.00 and 33.33) 0.4 % (16.00 and 34.67) at week 2 and 4. However, at week 6, the highest value was due to 0.3 % (62.00) EMS treated plants while the least was recorded at the control (50.00). The highest number of leaves were obtained at 0.1 % EMS treated plants at week 8, 10 and 12 (143.33, 259.33 and 337.33). Meanwhile, at week 12, the highest number of leaves (337.33) was due to 0.1 % EMS. This value was not significantly different ($p>0.05$) from 0.2 % (157.33).

Lowest number of leaves per plant for SAMNUT 24 was obtained in control at 2, 4, 6, 8 and 10 weeks with the value of 12.67, 19.33, 34.67, 70.00 and 101.33, respectively. . This values

were significantly different ($p<0.05$) from other concentration, but not different from 0.4 % at week 2 (13.33 leaves per plant), 8 (86.00 leaves per plant) and 10 (105.33 leaves per plant) (Table 4.2). At week 2, 6, and 8 the highest number of leaves per plant was recorded in 0.3 % concentration with the values of 26.67, 118.00 and 178.07, respectively while at week 4, 10 and 12 the highest number of leaves per plant was recorded at 0.1 % with the values of 56.65, 326.00 and 426.00. These values were significantly different ($p <0.05$) from the values of all other concentration except that of 0.3 % at week 2 and 6 that were not significant to 0.1 (25.33) and 02 (23.33) as well as 0.1 (113.33) and 0.2 (98.00), respectively. (Table 4.2).

No significant difference ($p> 0.05$) was observed in number of leaves for ICG 4412 at week 2 and 4 across EMS concentration and their control. The highest value for number of leaves recorded at week 2 and 4 were 18.00 and 52.00, respectively while the least number of leaves per plant at these weeks were 13.33 and 51.33, respectively. Similarly, the least number of leaves recorded at week 6 was due to 0.3 % (117.33) EMS concentration. This value was not significantly different ($p>0.05$) from 0.1 %, 0.2 % and 0.4 % with the value of (162, 156, 141, respectively). The highest number of leaves per plant for ICG 4412 was obtained in the control at 4, 6, 8, 10 and 12 weeks with the value of 56.67, 210.67, 270.67, 334.00 and 390.00 respectively, this values were significantly different ($p< 0.05$) from other concentration, but not significantly different from 0.1 at week 6 (162 leaves per plant), 8 (214.67 leaves per plant) and 10 (270 leaves per plant). At week 6, the highest number of leaves per plant was recorded in concentration 0.2 % and 0.4 % with the value 156.00 and 141. 33 respectively, followed by concentration 0. 3 % with the value 284.33 at week 10.

Table 4.2 Number of Leaves of EMS Mutant Lines (M_1) of the Groundnut Genotypes

Treatments	NL2	NL4	NL6	NL8	NL10	NL12
SAMNUT 26						
Control	13.33±0.67^{a}	25.33±1.33^{a}	38.67±1.76^{a}	58.00±3.46^{a}	85.33±6.96^{ab}	119.33±5.21^{a}
0.1	17.33±3.53^{a}	26.67±1.33^{a}	37.33±5.33^{a}	52.67±7.86^{a}	71.33±5.21^{a}	112.00±2.31^{a}
0.2	17.33±3.53^{a}	25.33±1.33^{a}	40.67±4.67^{a}	70.00±11.37^{a}	110.00±19.43^{b}	171.33±37.71^{a}
0.3	19.33±2.40^{a}	28.67±1.76^{a}	60.67±7.06^{b}	117.33±13.78^{b}	165.33±10.4^{c}	262.00±13.32^{b}
0.4	13.33±1.33^{a}	25.33±1.33^{a}	38.00±0.00^{a}	63.33±2.91^{a}	100.67±3.71^{ab}	130.00±12.49^{a}
SAMNUT 25						
Control	12.67±0.67^{a}	25.33±1.33^{a}	50.00±2.00^{a}	114.00±3.06^{a}	178.00±5.77^{ab}	224.67±7.86^{ab}
0.1	20.00±4.00^{b}	29.33±2.96^{ab}	52.67±3.53^{a}	143.33±10.09^{a}	259.33±23.84^{b}	337.33±48.89^{b}
0.2	12.67±0.67^{a}	25.33±1.33^{a}	51.33±1.76^{a}	90.67±12.67^{a}	126.67±30.69^{a}	157.33±29.07^{a}
0.3	14.00±1.15^{ab}	33.33±0.67^{b}	62.00±11.14^{a}	103.33±32.58^{a}	156.00±39.34^{a}	208.00±66.04^{ab}
0.4	16.00±1.15^{ab}	34.67±1.76^{b}	58.67±0.67^{a}	127.33±11.10^{a}	181.33±8.82^{ab}	227.33±5.46^{ab}
SAMNUT 24						
Control	12.67±0.67^{a}	19.33±1.33^{a}	34.67±1.76^{a}	70.00±2.00^{a}	101.33±8.74^{a}	130.33±15.60^{a}
0.1	25.33±1.33^{b}	56.67±4.67^{d}	113.33±4.81^{c}	172.00±6.11^{bc}	326.00±32.08^{c}	426.00±31.26^{c}
0.2	23.33±3.71^{b}	42.00±2.31^{c}	98.00±15.28^{c}	136.00±25.06^{b}	224.00±50.12^{b}	276.00±52.12^{b}
0.3	26.67±1.33^{b}	48.67±1.76^{cd}	118.00±3.46^{c}	178.67±8.35^{c}	302.67±28.85^{bc}	366.00±25.01^{bc}
0.4	13.33±1.33^{a}	29.33±2.67^{b}	60.00±6.11^{b}	86.00±1.15^{a}	105.33±1.76^{a}	115.33±2.91^{a}
ICG4412						
Control	13.33±0.67^{a}	56.67±5.93^{a}	210.67±37.78^{b}	270.67±46.79^{b}	334.00±33.31^{b}	390.00±16.29^{c}
0.1	18.00±3.06^{a}	52.67±3.53^{a}	162.00±15.62^{ab}	214.67±13.78^{ab}	270.00± 7.57^{ab}	314.00±5.03^{b}
0.2	15.33±0.67^{a}	51.33±1.76^{a}	156.00±31.01^{ab}	190.67±26.49^{ab}	210.00±31.89^{a}	222.33±29.58^{a}
0.3	17.33±0.67^{a}	62.00±11.14^{a}	117.33±9.68^{a}	174.00±13.01^{a}	233.33±24.00^{a}	284.33±30.56^{ab}
0.4	14.67±1.76^{a}	58.67±0.67^{a}	141.33±20.28^{ab}	175.33±10.48^{a}	211.33±12.02^{a}	235.33±10.97^{a}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$. NL = Number of leaves. 2

4.1.1.3 Leaf area

No significant difference ($p>0.05$) was observed in leaf area of SAMNUT 26 in week 2 and 4. The highest leave area was due to 0.3 % (8.53, 11.26 cm^2) EMS concentration in both weeks while the lowest value observed was due to 0.4 % (5.04 and 7.90 cm^2) and control (4.41 and 7.90 cm^2). Significant difference ($p<0.05$) was observed in leave area of SAMNUT 26 at week 6, 8, 10 and 12. EMS concentration 0.1 (13.90, 16.92, 19 and 21.91 cm^2) had the highest values at this weeks followed by EMS concentration 0.3 having the highest values at week 8, 10 and 12 (17.01, 19.08 and 21.22 cm^2). These values were not significantly different ($p>0.05$) from other treatments except for 0.4 % (10.36, 13.24, 15.31 and 17.71 cm^2) which has the lowest values at week 6, 8, and 12 respectively (Table 4.3).

For SAMNUT 25, significant difference ($p<0.05$) was found in leave area across the treated groundnut genotypes. The lowest value recorded at week 2 was due to 0.2 % (3.63 cm^2) EMS concentration. This value was not significantly different ($p>0.05$) from 0.1 % (6.25 cm^2), 0. 3 % (4.39 $cm^{2)}$ and control (5.91 cm^2) but significantly different from the highest value recorded in 0.4 % (7.95 cm^2) EMS concentration. Similarly, at week 4, the highest leave area was observed at 4 % (17.62 cm^2) EMS concentration, while the lowest was recorded at 0.2 % (7.68 cm^2) EMS concentration. These values were significantly different ($p<0.05$) from each other and from other treatments except 0.1 % (17.09 cm^2) which was significantly the same ($p>0.05$) with 0.2 % (7.68 cm^2) (Table 4.1). The highest value for leave area at week 6 was due to 0.4 % (19.24 cm^2) EMS concentration. This value was not significantly different ($p>0.05$) from 0.1 % and 0.3 % (16.54 and 15.98 cm^2) EMS concentration respectively (Table 4.3). At week 8, EMS concentration 0.4 % had the highest leaf area (21.88 cm^2) followed by 0.1 % and 0.3 % (18.51 and 17.93 cm^2) respectively, while the least value was recorded at

0.2 % (10.61 cm^2) EMS concentration. These values were significantly different ($p<0.05$) from one other. Similarly, at week 10 and 12, the least value for leaf areas were recorded respectively at concentration 0.2 % (12.43 and 14.29 cm^2) while the highest was recorded at 0.4 % (23.90 and 25.01 cm^2) EMS concentration. These values were significantly different ($p<0.05$) from all the treatments including the control (Table 4.3).

In SAMNUT 24, at week 2, no significant difference ($p>0.05$) was observed in leaf Area for all the treatments. The lowest leaf Area at week 2 was recorded at the control (4.71 cm^2) while the highest value (6.96 cm^2) was due to 0.3 % EMS treatment. The highest value recorded at week 4, 6, 8 and 10 (18.17 cm^2, 20.11 cm^2 22.06 cm^2 and 24.29 cm^2) was from 0.1 % EMS treated plants. These values were not significantly different ($p>0.05$) from 0.2 % (17.09) and 0.3 % (17.28) at week 4. Similar trend was also observed at week 6,8 and 10 of 0.2 % and 0.3 % EMS treated plants. The highest leaf Area recorded at week 12 was due to 0.3 % EMS concentration. This value is significantly the same ($p<0.05$) with 0.1 % (26.64 cm^2), 0.2 % (26.10 cm^2) but not significantly different ($p>0.05$) from the control (20.15 cm^2) (Table 4.3).

For ICG4412, the control had the highest value for leaf area (6.54 cm^2) at week 2 while the highest at week 4,6,8,10 and 12 was obtained at 0.1 % (17.25 cm^2, 19.69cm^2, 22.32 cm^2, 24.36 cm^3 and 26.58 cm^2) EMS treated plants. However, the least value for leaf area at week 2, 4, 6, 8, 10 and 12 was due to 0.2 % (4.57 cm^2, 9.47 cm^2, 11.80 cm^2, 14.29 cm^2, 17.05 cm^2 and 19.54 cm^2) EMS treated plants. These values were significantly different ($p<0.05$) from all other treatments but not significantly different ($p>0.05$) from 0.1 % (4.69 cm^2) 0.3 % (4.78 cm^2) at week 2. (Table 4.3).

4.1.1.4 *Effect of EMS on chlorophyll and leaf morphology of the groundnut genotypes*

Chlorophyll mutants where identified in M_1 line of the groundnut genotypes of SAMNUT 25 at 0.2 % and 0. 3 % treated plants (Plate I). Leaf mutants were also observed in SAMNUT 24 at 0.1 % an SAMNUT 25 at 0.2 % EMS treated plants. In SAMNUT 25, the leaf was observed to have a stipule protrusion while in SAMNUT 24 an indented leaf was observed (Plate I).

Plate I: Chlorophyll and Leaf Mutant of M_1 EMS mutagenized *Arachis hypogea* L. A. and B. chlorophyll mutants. C. control of the mutant plants. D**.** stipule leaflet. E. indented leaf morphology

Table 4.3 Leaf Area of EMS Mutant Lines (M_1) of the Groundnut Genotypes

Treatment	LA2 (cm^2)	LA4 (cm^2)	LA6 (cm^2)	LA8 (cm^2)	LA10 (cm^2)	LA12 (cm^2)
SAMNUT 26						
Control	4.41±0.34^{a}	7.90±0.73^{a}	11.90±0.80^{ab}	14.88±1.04^{ab}	16.72±1.39^{ab}	18.63±1.02^{ab}
0.1	6.75±0.77^{a}	10.34±0.85^{a}	13.90±1.01^{b}	16.92±1.00^{b}	19.00±0.66^{b}	21.91±1.16^{b}
0.2	6.82±0.99^{a}	9.44±0.35^{a}	12.29±0.61^{ab}	15.27±0.58^{ab}	17.77±0.78^{ab}	19.89±1.02^{ab}
0.3	8.53±1.99^{a}	11.26±1.74^{a}	13.53±1.37^{ab}	17.01±1.09^{b}	19.08±1.24^{b}	21.22±1.26^{ab}
0.4	5.04±1.33^{a}	7.90±1.32^{a}	10.36±1.32^{a}	13.24±0.96^{a}	15.31±0.88^{a}	17.71±0.74^{a}
SAMNT25						
Control	5.91±0.59^{ab}	11.44±0.29^{b}	13.38±0.37^{b}	14.50±0.59^{b}	16.60±0.79^{b}	19.45±0.31^{b}
0.1	6.25±0.85^{ab}	17.09±0.72^{c}	16.54±2.02^{ab}	18.51±0.84^{c}	19.37±0.88^{bc}	21.33±0.99^{b}
0.2	3.63±0.35^{a}	7.68±0.53^{a}	9.20±0.95^{a}	10.61±1.02^{a}	12.43±0.92^{a}	14.29±0.91^{a}
0.3	4.39±1.84^{a}	12.24±1.58^{b}	15.98±1.25^{ab}	17.93±1.12^{c}	20.41±1.12^{c}	21.95±1.18^{b}
0.4	7.95±0.83^{b}	17.62±1.53^{c}	19.24±1.23^{b}	21.88±1.29^{d}	23.90±1.13^{d}	25.01±1.34^{c}
SAMNUT 24						
Control	4.71±0.25^{a}	11.84±1.61^{a}	13.03±1.63^{a}	15.14±1.57^{a}	17.18±1.99^{a}	20.15±2.28^{a}
0.1	6.85±0.67^{a}	18.17±0.84^{c}	20.11±0.87^{c}	22.06±0.82^{b}	24.29±0.70^{b}	26.64±1.01^{b}
0.2	5.16±1.88^{a}	17.09±0.50^{bc}	19.06±0.32^{bc}	20.67±0.28^{b}	23.63±0.71^{b}	26.10±0.74^{b}
0.3	6.96±0.38^{a}	17.28±0.58^{bc}	19.01±0.56^{bc}	21.24±1.02^{b}	24.08±0.66^{b}	26.79±0.86^{b}
0.4	6.56±1.69^{a}	14.00±1.29^{ab}	16.55±1.24^{b}	18.89±1.22^{b}	21.19±0.90^{b}	23.71±1.27^{ab}
ICG4412						
Control	6.54±0.28^{b}	11.20±0.80^{ab}	14.00±0.88^{ab}	15/91±0.87^{ab}	17.95±0.69^{ab}	20.34±0.55^{ab}
0.1	4.69±0.35^{a}	17.25±1.13^{c}	19.69±0.83^{c}	22.32±0.60^{c}	24.37±0.49^{c}	26.58±0.38^{c}
0.2	4.57±0.54^{a}	9.47±0.67^{a}	11.80±0.92^{a}	14.29±1.04^{a}	17.05±0.99^{a}	19.54±0.82^{a}
0.3	4.78±0.47^{a}	14.21±0.78^{bc}	16.30±0.50^{bc}	18.60±0.77^{b}	20.78±1.01^{b}	23.23±0.97^{b}
0.4	5.56±0.32^{ab}	10.70±1.79^{ab}	12.89±1.89^{ab}	15.33±1.49^{ab}	18.27±1.55^{ab}	20.67±1.76^{ab}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$. LA = Leaf area

4.1.1.5 Number of branches at maturity

The number of branches recorded at maturity of SAMNUT 26 shows that, EMS treated plants at 0.3 % concentration had the highest number of branches (17.67) whereby, the control had the least number of branches (10.33). This value is not significantly different ($p>0.05$) from 0.2 % (10.67) and 0.4 % (10.67) treatments. In SAMNUT 25, the highest number of branches was obtained at 0.1 % (10.67) treated plants. No significant difference was observed in the highest number of branches at maturity in 0.1 % (10.67) and (10.67) EMS treated plants. However, the lowest number of branches obtained in this variety was due to 0.4 % EMS treated plants.

In SAMNUT 24, the minimum number of branches recorded at maturity was obtained at the control (6.33). This value was not significantly different ($p>0.05$) from 0.4 % (9.67) but significantly different ($p<0.05$) from all other treatments. The maximum number of branches recorded at maturity of this variety was observed in 0.1 % (16.00) EMS treated plants. This value is not significantly different ($p>0.05$) from 0.3 % (14.00) EMS treatments. The number of branches observed in ICG4412 at maturity shows that ICG4412 variety had the highest value (16.33). This value is significantly different ($p<0.05$) from 0.3 % (10.33) and 0.4 % (8.67) EMS treated plants but not significantly different ($p>0.05$) from 0.1 % (13.33) and 0.2 % (11.67) EMS treated plant. The least value for number of branches was obtained at 0.4 % (8.67) EMS treated plants.

Table 4.5 Number of Branches of EMS Mutant Lines (M_1) of the Groundnut Genotypes

Treatments	SAMNUT 26 NB	SAMNUT 25 NB	SAMNUT 24 NB	ICG4412 NB
Control	10.33±0.33^{a}	9.33±0.67^{a}	6.33±1.33^{a}	16.33±1.33^{b}
0.1	13.00±1.15^{a}	10.67±3.18^{a}	16.00±2.00^{b}	13.33±0.33^{ab}
0.2	10.67±1.45^{a}	8.33±1.45^{a}	10.33±1.76^{ab}	11.67±2.85^{ab}
0.3	17.67±2.19^{b}	10.67±2.73^{a}	14.00±3.06^{b}	10.33±0.88^{a}
0.4	10.67±0.67^{a}	6.67±0.88^{a}	9.67±0.88^{ab}	8.67±0.67^{a}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$

4.1.2 Yield parameters of M1 lines of EMS treated groundnut line

4.1.2.1 Weight of pod

No significant difference ($p>0.05$) was observed in weight of pod in SAMNUT 26 at the control, 0.2 % and 0.4 % (1.64 g, 1.74 g and 1.64 g) EMS concentration respectively. These values were not significantly different ($p>0.05$) from 0.1 % (1.86 g) EMS concentration but significantly different ($p<0.05$) from 0.3 % (2.08 g) EMS concentration. No significant difference ($p>0.05$) was observed in weight of pod for SAMNUT 24 at 0.2 %, 0.3 % and 0.4 % (1.64 g, 1.75 g and 1.72 g) EMS concentration (Table 4.5). EMS concentration 0.1 % had the highest value for weight of pod (1.89 g). This value was significantly different from the control (1.44 g). The least value for weight of pod was observed in the control (Table 4.5).

No significant difference ($p>0.05$) was observed in weight of pod of SAMNUT 25 across all treatments. Concentration 0.1 % (1.80 g) had the highest weight of pod while the lowest number of pod was recorded in control (1.44 g) (Table 4.5).

The maximum weight of pod in ICG4412 was recorded at 0.2 % (1.77 g) EMS concentration which was not significantly different ($p>0.05$) from control, 0.1 % and 0.3 % (1.47 g, 1.58 g and 1.46 g). The minimum value was recorded at 0.4 % (1.36 g) EMS concentration (Table 4.5).

4.1.2.2 Length of pod

No significant difference ($p>0.05$) exist in length of pod of SAMNUT 26 across all the treatments. The highest length of pod was recorded at 0.3 % (2.43 cm) treatment while the

least was recorded at 0.1 % treatment (2.18 cm). Significant variations was observed in length of pod of SAMNUT 24. The minimum length of pod was recorded at the control (1.92 cm), this value was not significantly different ($p>0.05$) from length of pod recorded at 0.4 % EMS concentration (1.92 cm). The highest length of pod was recorded at 0.1 % treated plants (2.39 cm). This value was not significantly different ($p>0.05$) from that recorded at 0.2 % and 0.3 % treatments (2.07 cm and 2.06 cm) respectively (Table 4.5). For SAMNUT 25, no significant difference ($p>0.05$) was observed in length of pod. The highest length of pod was recorded at 0.4 % EMS treated plants (2.24 cm) while the least was recorded at 0.1 % treated plants (2.04 cm). This value was not significantly different from 0.2 % and 0.3 % (2.05 cm and 2.05 cm) respectively (Table 4.5). In ICG4412, the highest length of pod was recorded at 0.2 % EMS concentration (2.11 cm) this value was not significantly different ($p>0.05$) from 0.3 % (2.04 cm) EMS treated plants. The least value for length of pod was recorded at 0.1 % (1.72 cm). This value was not significantly different ($p>0.05$) from that recorded at the control (1.79 cm) (Table 4.5).

4.1.2.3 Number of seed per pod

The highest number of seed in SAMNUT 26 was observed at 0.3 % and 0.4 % (2.00 and 2.00) EMS concentration. While the lowest value was recorded at 0.1 % and 0.2 % (1.80 and 1.80) EMS concentration. This values are not significantly different ($p>0.05$) from each other. Similarly, for SAMNUT 24, no significant different ($p>0.05$) was observed in number of seed across the treatments. The highest number of seed was recorded at 0.1 % (2.00) EMS concentration while the lowest was recorded at 0.3 % (1.70) EMS concentration (Table 4.5). The responses of the SAMNUT 25 to EMS concentrations were not statistically different for all treatments. The highest value was recorded in control and 0.4 % (1.90 and 1.90) while the

least value was recorded at 0.1 % (1.70) EMS concentration. All these values are not significantly different ($p>0.05$) from each other (Table 4.5).

In ICG4412, the highest number of seed was recorded at 0.1 %, 0.2 %, 0.3 % and 0.4 % (1.60, 1.90, 1.90 and 1.50) EMS concentration respectively while the least was recorded in the control (0.50) (Table 4.5). This value (1.90) was significantly different ($p<0.05$) from all the other values.

4.1.2.4 Number of pods per plant

The number of pods per plant recorded in SAMNUT 26 was obtained at 0.4 % treated plants with the value (10.33). This value was significantly different from all other treatments including the control. The highest number of pods per plant recorded was obtained at 0.3 % EMS treated plants (22.00). This value was significantly the same with the value recorded at 0.1 % concentration (21.67) (Table 4.5). For SAMNUT 24, the least number of pods per plant was observed at the control (11.67). This value was significantly different ($p<0.05$) from all values recorded at the treatments. The highest number of pod per plant was recorded at 0.1 % EMS concentration (26.33). This value was not significantly different from all treatments (Table 4.5).

The number of pod per plant recorded for SAMNUT 25 shows that concentration 0.2 % had the least value (9.67). This value was significantly different ($p<0.05$) from all other treatments. The highest number of pods per plant in this variety was recorded at 0.1 % EMS treated plants (24.67). This value was significantly different ($p<0.05$) from the control, 0.3 % and 0.4 % (17.33, 18.67 and 13.00 respectively) (Table 4.5). For ICG4412, the highest number of pods per plant was recorded at 0.1 % EMS treated plants with the value (17.33)

while the least number of pods per plant was recorded at the control (11.67). This value was not significantly different ($p>0.05$) from that recorded at 0.2 % and 0.3 % (12.00 and 12.33) but significantly different from 0.4 % (15.33) (Table 4.5).

4.1.2.5 Weight of hundred seeds

The maximum weight of Hundred Seeds recorded in SAMNUT 26 was at 0.1 % EMS concentration (50.40 g). This value is not significantly different ($p>0.05$) from 0.2 % (49.64 g) and 0.3 % (49.34 g) but significantly different ($p<0.05$) from the control (37.56 g). This value is significantly different from all the treatments. The highest weight of Hundred Seeds obtained in SAMNUT 24 was due to 0.2 % (54.34 g) this value is not significantly different ($p>0.05$) from 0.3 % (53.86 g) but significantly different from control (30.00 g), 0.1 % (51.06 g) and 0.4 % (50.50 g). The least hundred seeds weight was obtained at the control 30.00 g) (Table 4.5)

The highest value for Weight of hundred seeds in SAMNUT 25 was recorded at 0.3 % (50.52 g) and 0.4 % (50.52 g) followed by 0.1 % (49.76 g) then the control (45.96 g) while the least value was recorded at 0.2 % (37.80 g) EMS concentration (Table 4.5). Significant difference ($p<0.05$) exist in Weight of thousand seeds of ICG4412 across the treatments. The least value was observed in the control (6.00 g) while the highest value was due to 0.2 % (51.90 g). This value is significantly different ($p<0.05$) from all other treatments. The highest value which was recorded in 0.2 % EMS concentration was followed by 0.1 % and 0.4 % (47.76 and 47.96 g) EMS concentration and then 0.3 % (43.86 g) EMS concentration (Table 4.5).

4.1.2.6 Effect of EMS on pods and seeds of the groundnut genotypes

Notable increase in Pods and seeds size were observed in SAMNUT 24 at 0.1 %, 0.2 % and

0. 3 % treated plants while in ICG4412 it was observed at 0.2 %, 0. 3 % and 0.4 % treated plants. No significant difference ($p>0.05$) were observed in Pods and seed sizes in other verities at other concentrations of EMS (Plate III, IV, V and VI).

Table 4.5 Weight of pod, Length of pod, Number of seeds per pod and weight of hundred seeds of EMS Mutant Lines (M_1) of the Groundnut Genotypes

Treatments	WP (g)	LP (cm)	NSP	WHS (g)	NPP
SAMNUT 26					
Control	1.64±0.72^{a}	2.21±0.11^{a}	1.90±0.10^{a}	37.56±1.39^{a}	13.33±0.88^{b}
0.1	1.86±0.12^{ab}	2.18±0.13^{a}	1.80±0.13^{a}	50.40±0.46^{c}	21.67±2.33^{d}
0.2	1.74±0.16^{a}	2.19±0.16^{a}	1.80±0.13^{a}	49.64±0.15^{c}	18.67±3.84^{c}
0.3	2.08±0.49^{b}	2.43±0.78^{a}	2.00±0.00^{a}	49.34±0.27^{c}	22.00±1.00^{d}
0.4	1.64±0.72^{a}	2.21±0.11^{a}	2.00±0.00^{a}	44.64±0.15^{b}	10.33±1.45^{a}
SAMNUT 24					
Control	1.44±0.09^{a}	1.92±0.12^{a}	1.80±0.13^{a}	30.00±0.15^{a}	11.67±2.03^{a}
0.1	1.89±0.92^{b}	2.07±0.17^{ab}	2.00±0.00^{a}	51.06±0.21^{c}	26.33±2.03^{c}
0.2	1.64±0.11^{ab}	2.39±0.13^{b}	1.80±0.13^{a}	54.34±0.16^{d}	20.33±2.03^{b}
0.3	1.75±0.16^{ab}	2.06±0.13^{ab}	1.70±0.15^{a}	53.86±0.10^{d}	19.00±1.73^{b}
0.4	1.72±0.16^{ab}	1.92±0.12^{a}	1.90±0.10^{a}	50.50±0.28^{b}	20.00±3.61^{b}
SAMNUT 25					
Control	1.44±0.81^{a}	2.21±0.15^{a}	1.90±0.10^{a}	45.96±0.09^{b}	17.33±5.49^{c}
0.1	1.80±0.15^{a}	2.04±0.16^{a}	1.70±0.15^{a}	49.76±0.11^{c}	24.67±0.33^{d}
0.2	1.77±0.16^{a}	2.05±0.14^{a}	1.80±0.13^{a}	37.80±0.09^{a}	9.67±1.67^{a}
0.3	1.77±0.16^{a}	2.05±0.14^{a}	1.80±0.13^{a}	50.52±0.12^{d}	18.67±3.76^{c}
0.4	1.70±0.09^{a}	2.24±0.09^{a}	1.90±0.10^{a}	50.52±0.12^{d}	13.00±4.73^{b}
ICG4412					
Control	1.47±0.16^{ab}	1.79±0.17^{a}	0.50±0.22^{a}	6.00±0.04^{a}	11.67±3.28^{a}
0.1	1.58±0.09^{ab}	1.72±0.17^{a}	1.60±0.16^{b}	47.76±0.07^{c}	17.33±0.88 ^{c}
0.2	1.77±0.13^{b}	2.11±0.12^{a}	1.90±0.10^{b}	51.90±0.12^{d}	12.00±6.08^{a}
0.3	1.46±0.09^{ab}	2.04±0.12^{a}	1.90±0.10^{b}	43.86±0.10^{b}	12.33±1.76^{a}
0.4	1.36±0.12^{a}	1.89±0.16^{a}	1.50±0.67^{b}	47.96±0.08^{c}	15.33±4.33^{b}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P < 0.05$. WP= Weight of pod, LP= Length of pod, NSP= Number of seeds per pod, HSW= weight of hundred seeds, NPP= number of pod per plant.

Plate II: Pod of SAMNUT 24 EMS mutagenized M_1 line. A. control. B. 0.1 % EMS concentration. C. 0.2 % EMS concentration. D. 0.3 % EMS concentration. E. 0.4 % EMS concentration.

Plate III: Seed of SAMNUT 24 EMS mutagenized M_1 line. A. control. B. 0.1 % EMS concentration. C. 0.2 % EMS concentration. D. 0.3 % EMS concentration. E. 0.4 % EMS concentration.

Plate IV: Pod of ICG 4412 EMS mutagenized M_1 line. A. control. B. 0.1 % EMS concentration. C. 0.2 % EMS concentration. D. 0.3 % EMS concentration. E. 0.4 % EMS concentration.

Plate V: Seeds of ICG 4412 EMS mutagenized M_1 line. A. control. B. 0.1 % EMS concentration. C. 0.2 % EMS concentration. D. 0.3 % EMS concentration. E. 0.4 % EMS concentration.

4.1.3 Fatty acid profile of EMS mutant lines (M_1) of groundnut oils

4.1.3.1 Refractive index

The results of refractive index of the extracted oil from the samples showed that there were no significant difference ($p>0.05$) across the various genotypes and treatments. For SAMNUT 26, the highest refractive index was recorded at the control, 0.2 %, 0. 3 % and 0.4 % (1.48) while the least was in 0.1 % treatment with the value of 1.46 (Table 4.6).

4.1.3 **2** *Acid value*

The lowest acid value for SAMNUT 26 was recorded at 0.2 % (0.65 %) EMS concentration while the highest was recorded at the control (1.41 %) followed by 0.4 % (1.26 %) then 0.1 % (1.06 %) EMS concentration these values were significantly different from each other (Table 4.6). The least acid value for SAMNUT 25 was recorded at 0.1 % (0.54 %) EMS concentration followed by 0.2 % (0.73 %) EMS concentration while the highest was recorded at the control (1.59 %) followed by 0.4 % (1.14 %) EMS concentration then 0.3 % (1.03 %) EMS concentration. The least acid value for SAMNUT 24 was due to 0.2 % and 0.4 % (0.65 and 0.64 %) EMS concentration respectively while the highest value was due to 0.1 % (1.46 %) EMS concentration followed by the control (1.32 %). The least acid value for ICG4412 was observed at the control (0.00 %) while the highest was observed at 0.3 % (2.10 %) EMS concentration, the values were significantly different from each other (Table 4.6).

4.1.3.3 Free fatty acid

The highest free fatty acid for SAMNUT 26 was observed in the control (0.70 %) followed

by 0.4 % (0.63) EMS concentration then 0.1 % (0.53 %) EMS concentration (Table 4.6). These values are significantly different ($p<0.05$) from 0.3 % (0.43 %) EMS concentration. The least value was recorded at 0.2 % (0.32 %) EMS concentration. Similarly, the highest free fatty acid was recorded at the control (0.80 %) this value is followed by 0.4 % (0.57 %) EMS concentration and then 0.3 % (0.52 %) EMS concentration. Concentration 0.2 % (0.34 %) is significantly different ($p<0.05$) from all other concentration (Table 4.6).

No significant difference ($p<0.05$) was observed in free fatty acid of SAMNUT 24 at 0.2 % and 0.4 % (0.32 and 0.32 %) EMS concentration. The highest free fatty acid value was recorded at 0.1 % (0.73 %) EMS concentration. This value is significantly different ($p<0.05$) from the control (0.66 %) and 0.3 % (0.63 %) EMS concentration. The least free fatty acid for ICG4412 was recorded at the control (0.00 %) while the highest was recorded at 0.3 % (1.05 %) EMS concentration (Table 4.6).

4.1.3.4 Iodine value

The highest iodine value for SAMNUT 26 was recorded at the control (8.19 I_2/100g) this value was significantly different ($p<0.05$) from all the treatments (Table 4.6). The least iodine value for SAMNUT 25 was recorded at the control (8.84 I_2/100g) while the highest was recorded at 0.4 % (12.06 I_2/100g) EMS concentration. This highest value was not significantly different ($p>0.05$) from 0.1 % (11.62 I_2/100g) EMS concentration. No significant difference ($p<0.05$) was observed for iodine value at 0.2 % (11.56 I_2/100g) and 0.3 % (11.28 I_2/100g) EMS concentration. Also, no significant difference ($p>0.05$) was observed for iodine value of SAMNUT 24 across EMS concentration. The highest value was recorded at 0.4 % (11.31 I_2/100g) EMS concentration while the least was recorded at 0.3 % (11.05 I_2/100g) EMS concentration (Table 4.6). For ICG4412, the least iodine value was

observed at the control (0.00 I_2/100g) while the highest was recorded at 0.1 % (9.65 I_2/100g) EMS concentration followed by 0.4 % (8.92 I_2/100g) EMS concentration (Table 4.6). This value is not statistically different from 0.3 % (8.84 I_2/100g) EMS concentration and 0.2 % (8.73 I_2/100g) EMS concentration (Table 4.6).

4.1.3.5 Peroxide value

Significant difference was observed in peroxide value of SAMNUT 26 across the treatments. The least peroxide value was due to 0.3 % (6.73 6.73 meq O_2/kg) EMS concentration while the highest peroxide value was recorded at the control (Table 4.6). No significant difference ($p>0.05$) was observed in peroxide value of SAMNUT 26 at 0.1 % and 0.2 % (7.37 and 7.37 6.73 meq O_2/kg) EMS concentration. The peroxide value for SAMNUT 25 shows that significant difference exist between EMS concentrations. 0.4 % (4.53 6.73 meq O_2/kg) EMS concentration had the lowest value while the highest value was due to 0.2 % (8.70 6.73 meq O_2/kg) EMS concentration. This highest value was not significantly different ($p>0.05$) from the control, 0.1 % and 0.3 % (7.50, 8.27 and 7.95 6.73 meq O_2/kg respectively) EMS concentration (Table 4.6).

The least peroxide value for SAMNUT 24 was recorded at 0.3 % (3.97 mEq/kg) EMS concentration while the highest was recorded at 0.2 % (8.40 6.73 meq O_2/kg) EMS concentration (Table 4.6). This value was significantly different ($p<0.05$) from 0.1 % (8.17 6.73 meq O_2/kg) EMS concentration. The value recorded at the control (8.03 6.73 meq O_2/kg) was significantly different ($p<0.05$) from all the treatments except for 0.1 % EMS treatment. The highest peroxide value for ICG4412 was recorded at 0.3 % EMS concentration with the value (25.63 meq O_2/kg). This value is significantly different ($p<0.05$) from all other treatments including the control. The least peroxide value was recorded at the control (0.00).

Concentration 0.1 %, 0.2 % and 0.4 % (13.50, 15.77 and 8.63 meq O_2/kg) are statistically different from the least value recorded. (Table 4.6).

4.1.3.6 Oil yield

The least oil yield in SAMNUT 26 was recorded at 0.1 % (23.81 %) EMS concentration, while the highest was recorded at the control (33.43 %) (Table 4.6). Concentration 0.3 % (31.13 %) was significantly different ($p<0.05$) from 0.4 % (24.70 %) EMS concentration which was also significantly different ($p<0.05$) from 0.2 % (27.12 %) EMS concentration. In SAMNUT 25, the highest oil yield was recorded at 0.2 % (39.34 %). No significant difference ($p>0.05$) was observed in oil yield at control (36.51 %) and 0.4 % (35.70 %) EMS concentration (Table 4.6). The least value for oil yield of SAMNUT 25 was due to 0.3 % (31.07 %) EMS concentration. This value was significantly different ($p<0.05$) from that obtained at 0.1 % (32.34 %) EMS concentration (Table 4.6).

For SAMNUT 24, the control (39.27 %) had the highest oil yield followed by 0.3 % and 0.2 % (39.06 and 39.01 %) EMS concentration, while the least value was due to 0.1 % (34.56 %) EMS concentration 0.4 % (38.66 %) is significantly different ($p<0.05$) from all other concentrations. The highest oil yield for ICG4412 was due to 0.4 % (37.27 %) EMS concentration followed by 0.1 % (32.74 %) EMS concentration. No significant difference ($p<0.05$) was observed in oil yield for ICG4412 at 0.2 % (22.76 %) and 0.3 % (21.78 %) EMS concentration (Table 4.6).

Table 4.6: Oil Properties of EMS Mutant Lines (M_1) of Groundnut Oils

Treatments	Refractive Index	Acid Value (%)	FreeFatty Acid (%)	Iodine Value I_2/100g	Peroxide meqO_2/kg	Oil Yield (%)
SAMNUT 26						
Control	1.48±0.00^{a}	1.41±0.02^{e}	0.70±0.01^{e}	8.19±0.00^{b}	7.75±0.08^{d}	33.43±0.01^{e}
0.1	1.46±0.00^{a}	1.06±0.03^{c}	0.53±0.01^{c}	7.10±0.01^{a}	7.37±0.03^{c}	23.81±0.03^{a}
0.2	1.48±0.00^{a}	0.65±0.01^{a}	0.32±0.01^{a}	7.33±0.01^{a}	7.37±0.03^{c}	27.12±0.06^{c}
0.3	1.48±0.00^{a}	0.85±0.03^{b}	0.43±0.01^{b}	7.15±0.01^{a}	6.73±0.07^{a}	31.13±0.00^{d}
0.4	1.48±0.00^{a}	1.26±0.02^{d}	0.63±0.01^{d}	7.31±0.16^{a}	7.03±0.03^{b}	24.70±0.14^{b}
SAMNUT 24						
Control	1.47±0.00^{a}	1.32±0.01^{c}	0.66±0.00^{c}	11.17±0.12^{a}	8.03±0.03^{b}	39.27±0.05^{d}
0.1	1.47±0.00^{a}	1.46±0.00^{d}	0.73±0.00^{d}	11.16±0.16^{a}	8.17±0.17^{bc}	34.56±0.04^{a}
0.2	1.47±0.00^{a}	0.65±0.01^{a}	0.32±0.01^{a}	11.08±0.24^{a}	8.40±0.00^{c}	39.01±0.01^{c}
0.3	1.47±0.00^{a}	1.26±0.02^{b}	0.63±0.01^{b}	11.05±0.24^{a}	3.97±0.03^{a}	39.06±0.00^{c}
0.4	1.47±0.00^{a}	0.64±0.00^{a}	0.32±0.00^{a}	11.31±0.01^{a}	4.09±0.02^{a}	38.66±0.05^{b}
SAMNUT 25						
Control	1.47±0.00^{a}	1.59±0.06^{e}	0.80±0.03^{e}	8.84±0.01^{a}	7.50±0.17^{b}	36.51±0.03^{c}
0.1	1.47±0.00^{a}	0.54±0.02^{a}	0.27±0.01^{a}	11.62±0.01^{bc}	8.25±0.08^{b}	32.34±0.03^{b}
0.2	1.47±0.00^{a}	0.73±0.02^{b}	0.34±0.01^{b}	11.56±0.01^{b}	8.70±0.03^{b}	39.34±0.60^{d}
0.3	1.47±0.00^{a}	1.03±0.02^{c}	0.52±0.01^{c}	11.28±0.28^{b}	7.95±1.72^{b}	31.07±0.08^{a}
0.4	1.47±0.00^{a}	1.14±0.02^{d}	0.57±0.01^{d}	12.06±0.01^{c}	4.53±0.13^{a}	35.70±0.07^{c}
ICG4412						
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.1	1.47±0.00^{b}	1.27±0.04^{b}	0.64±0.02^{d}	9.65±0.03^{c}	13.50±0.17^{c}	32.74±0.09^{c}
0.2	1.47±0.00^{b}	0.05±0.01^{b}	0.50±0.01^{c}	8.73±0.01^{b}	15.77±0.10^{d}	22.76±0. 37^{b}
0.3	1.47±0.00^{b}	1.05±0.82^{b}	1.05±0.82^{e}	8.84±0.02^{b}	25.63±0.03^{e}	21.78±0. 39^{b}
0.4	1.47±0.00^{b}	0. 38±0.00^{b}	0.38±0.00^{b}	8.92±0.06^{b}	8.63±0.03^{b}	37.27±2.04^{d}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$

4.1.4 Insect population

The highest insect pest population at 0.00 % was recorded in ICG4412. This population is significantly different from that recorded in SAMNUT 25 and SAMNUT 26, but not significantly different ($p>0.05$) from SAMNUT 24. The least number of insects were recorded in SAMNUT 25 variety. This population is significantly different ($p<0.05$) from all other varieties.

At 0.1 % EMS concentration, the highest insect population was recorded in SAMNUT 24. This insect population is significantly different ($p<0.05$) from other varieties. The least insect population at this concentration was obtained in SAMNUT 25. This population was not significantly different ($p>0.05$) from that obtained in SAMNUT 26 and ICG4412 varieties.

At 0.2 % EMS concentration, the least insect population were recorded in SAMNUT 25, while no significant difference was observed in insect population of SAMNUT 24 and ICG4412 at this concentration. The highest insect population were recorded in SAMNUT 26. This population is significantly different from population of all other varieties.

At 0.3 %, the least number of insects were recorded from SAMNUT 25 while the highest were recorded from SAMNUT 26. No significant difference ($p>0.05$) was observed in number of insects in SAMNUT 26 and all other varieties.

At 0.4 % EMS concentration, SAMNUT 25 recorded the least insect population while SAMNUT 26 recorded the highest. Significant difference ($p<0.05$) exist in number of insects among all varieties at this concentration.

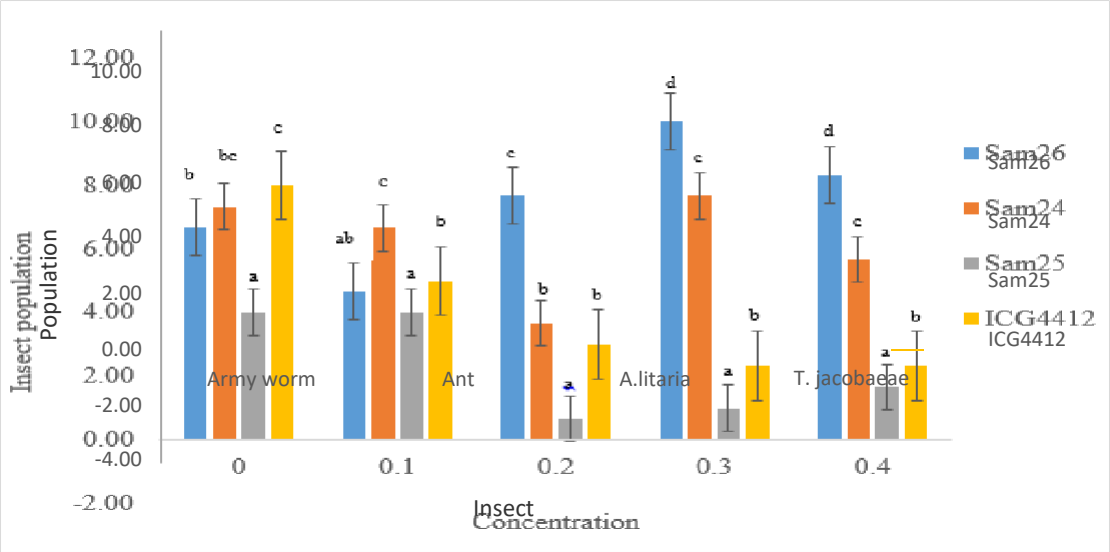

Figure 4.1: Insect population based on EMS concentrations and genotypes

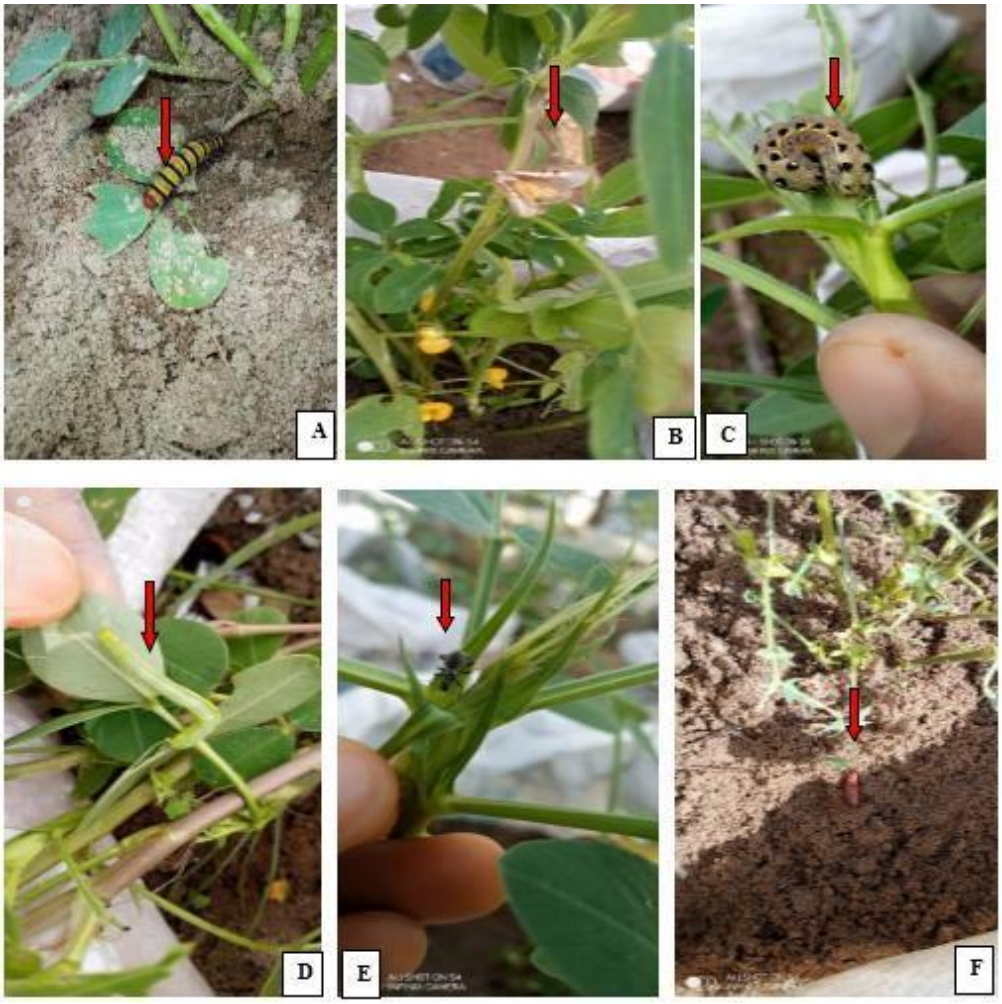

Plate VI. Different insects pest found on the field.

4.2 Discussion

4.2.1 Vegetative parameters of M_1 line of EMS treated groundnut

4.2.1.1 Plant height

Efficient mutagenesis had been reported to be vital for breeding programs. The success of Ethyl methyl sulfonate (EMS) mutagenesis depend on many factors such as the treatment duration, concentration and temperature. (Arisha *et al*., 2014; Asif *et al*., 2019). The maximum plant height (51.17 cm) obtained at maturity of this study exceeded those reported by earlier authors; Tingting *et al*. (2020) 44.99 cm, Olorunmaiye *et al*. (2019) 24.00 cm, and 38.50 cm by Gunasekaran and Pavadai (2015); who worked on EMS treated groundnut genotypes. The differences observed might be due to concentration used and temperature variation. High concentration of EMS had also been reported to result in damage cell constituents, molecules (Chowdhury and Tah 2011) and growth regulators (Salim *et al*., 2009); thereby resulting in low height. Thus the concentration used in this research seems to be moderate and effective for this traits.

4.2.1.2 Number of leaves and leaf area

The number of leaves in a crop plays important roles in the yield of a plant, as the leaves are photosynthetic site of the plant. Number of leaves per plants and leaf area is an essential trait as they translate a higher photosynthetic surface and ultimately better growth and higher biomass yield of the crop. The ranged number of leaves obtained in this study (112.00 – 426.00) exceeded the range 15.60 - 20.60 earlier reported by Mayur *et al*. (2018), 42.33 - 49.33 by Olorunmaiye *et al*. (2019) and 33.75 - 50.12 by Gunasekaran and Pavadai (2015).

Variation in number of leaves had earlier been reported by Aslam *et al*. (2018). Increase in leaf area was observed in most of the mutant lines over the control in all varieties. Similar finding was reported by Rajib and Jagatpati (2011) who observed increase in leaf area in all the treatments over the control in M_1 generation of Dianthus caryohyllus. This might be attributed to the physiological disturbance or chromosomal inbalance caused to the cells of the plant by the mutagen. Janila *et al*. (2013) reported that, high number of leaves in groundnut suggests them for inclusion for future groundnut breeding program aiming at improving the number of leaves for a high yield.

4.2.1.3 Number of branches

The range of value in terms of number of branches 10.33 - 17.67 in SAMNUT 26; 6.67 - 10.67 in SAMNUT 25; 6.33 - 16.00 in SAMNUT 24 and 8.67 - 16.33 in ICG4412 exceeded those reported by Tingting *et al*. (2020), they reported a range of 7.4 - 8.5 in HY22 and YY45 EMS treated groundnuts. As well as those reported by Olorunmaiye *et al*. (2019) who even reported lower range. The 0.1 % and 0.4 % EMS concentration seems to be the optimum dose for enhancing higher number of branches in SAMNUT 24, 25 and 26 but not in ICG4412. The difference could indicate variation in the genetic makeup of each of them. Different varieties or genotypes of crop tend to respond differently to doses of mutagen since some may be dosage tolerant while others may be not.

The maximum number of branches (16.33) obtained in this study exceeded those reported by the above authors. This observation might be due to differences in groundnut genotypes and mutagenic doses. Similarly, previous studies reported that sensitivity to chemical mutagens differs with genotype (Kumar *et al*., 2015; Ali *et al*., 2010).

4.2.1.4 Number of seed per pod

The number of seeds observed in this study (0.50 - 2.00) is contrary to the report of Olorunmaiye *et al*. (2019) who reported higher values (2.14 - 2.68) in M_1 generation of groundnut (SAMNUT 24 Vr.) treated with different concentration of EMS. Mutagenic treatments differs with variaties. High values of number of seeds obtained in most of the treatments 0.3 % and 0.4 % (2.00) in SAMNUT 26, 0.1 %(2.00) in SAMNUT 24, 0.3 % and 0.4 % in ICG4412 shows that EMS is effective at certain concentration (0.1 - 0.4 %). This result is contrary to the report of Thilagavathi and Mullainathan (2011) who observed decrease in number of seed per pod in treatments than in control of M1 generation of *Vigna mungo* and *Vigna unguiculata* by Rizwana *et al*. (2005) which were also treated with EMS. This differences might be attributed to the physiological disturbance or chromosomal damage of the plant cell (Ramya *et al*., 2014).

4.2.1.5 Number of pod per plant and length of pod

The range number of pod per plant recorded in this study (9.67 - 26.33) is below the range (25.39 - 29.50) reported by Gunasekaran and Pavadai (2015). The difference observed might be attributed to differences in the genotypes. Increase in some mutant line in number of pod per plant of some varieties (SAMNUT 24 and SAMNUT 26) shows the Mutagenic effect of EMS on the plant. Similar trend was also observed in number of pod per plant in the treatment than in control by (Gunasekaran and Pavadai 2015; Gangadhara *et al*., 2018 in EMS mutagenised groundnut genotypes.

The range length of pod recorded in this study (1.72 - 2.43 cm) is below the range (3.87 - 4.92 cm) reported by Olorunmaiye *et al*. (2019). High concentration of EMS results in increase in length of pod by the author. This differences observed might be due to

environmental factors and genotypes used.

4.2.1.6 Weight of hundred seed

Similar to increase in weight of hundred seed observed in the treatments as compare to corresponding control in some of the genotypes (SAMNUT 26, SAMNUT 24 and ICG4412); Tingting *et al*. (2020) reported increase in hundred seed weight of EMS treated groundnut genotype (HY22 and YY45). The increase in the treatment over the control might be attributed to increase in surface area of the leave for photosynthesis in the treated plants leading to increase in photosynthesis and yield (Devi and Mullainathan 2011; Borovsky *et al*., 2013). The minimum hundred seed weight (6.00 g – 37.56 g) recorded in this study fall within the range reported by earlier authors, 24.73 g – 38.14 g by Gangadhara *et al*. (2018), 38.13 g – 39.83 g by Olorunmaiye *et al*. (2019) who worked on EMS mutagenized groundnuts. Also, the maximum weight of hundred seeds (43. 86 g – 54. 34 g) fall within the range reported by the authors (Gangadhara *et al*., 2018 and Olorunmaiye *et al*., 2019). The close agreements of these results could be attributed to similarity in the doses used for the treatment which might cause similar alteration in the enzyme activity.

4.2.1.7 Weight of pod

The increase observed in weight of pods of some mutant line over the control may be attributed to the beneficial effect of EMS on the pods. This result is in conformity with report of Muhammad and Seyed (2015) who reported increase in pod weight of okra mutants due to EMS over the control. Contrarily, Sonone *et al*. (2010) reported a decrease in pod weight of EMS mutagenized groundnut genotype (AK - 280). This differences observed might be due to differences in mutagenic doses and genotype used.

4.2.2: Fatty acid profile of EMS mutant lines (M_1) of groundnut oils

4.2.2.1 Oil content

The composition of groundnut oil is influenced by several sets of factors consisting of genet ic factors, environmental factors and interaction between environmental and genetic factors (Andersen and Gorbet, 2002; Isleib *et al*., 2008 and Chaiyadee *et al*., 2013).

The minimum oil content (0.00 - 8.63 %) recorded in IG4412 of this study is below the values reported by earlier authors who worked on groundnut. 45.25 % by Gunasekaran and pavadai (2015), 48.10 % by Satpute and Suradkar (2018) and 29.27 % by Tingting *et al*. (2020). The maximum value 39.34 % in SAMNUT 25, 33.43 in SAMNUT 26 and 39.27 in SAMNUT 24 obtained this study exceeded the maximum values reported by Tingting *et al*. (2020). Differences observed in oil content of the groundnut genotypes might be attributed to differences in genetic constituents of the genotype used. These findings were supported by Mzimbiri *et al*., (2014) and Escobedo *et al*., (2015).

4.2.2.2 Peroxide value

Peroxide value is an indicator of oxidative rancidity, therefore high peroxide value in oil indicates a weak oil resistance to peroxidation during storage and indicates a deterioration level (Adebayo *et al*., 2012 and Mohammed and Hamza, 2008). The peroxide values obtained in this study (4.53 - 9.65 00 meq O_2/kg) fall within Codex acceptable range for oil (≤ 10.00 meq O_2/kg). The increase in peroxide values in some of the mutant lines over the control might be due to oxidation and preferential cleavage of bonds in the oil. Also, this could be attributed to interaction of EMS with fat molecules which triggered dehydration, polymerization and oxidation reactions. Lower peroxide values observed in this study were

mostly at higher concentration of EMS. SAMNUT 26 variety had it lowest peroxide value at 0.3 % treatment (6.73 00 meq O_2/kg), SAMNUT 24 at 0.3 % (3.97 6.73 00 meq O_2/kg), SAMNUT 25 at 0.4 % (4.53 6.73 00 meq O_2/kg) and ICG4412 at 0.4 % (8.63 6.73 00 meq O_2/kg). This results shows that higher concentration of EMS (0.3 and 0.4 %) can be used to improve the crop for low peroxide property.

4.2.2.3 Refractive index

High refractive index of oil explained that the fatty acids in the oil contain a large number of carbon atoms (Bello and Olawore, 2012). The highest refractive index obtained in this study (1.47 - 1.48) within the range of those obtained from the report of Reda and Nassaar (2018) (1.45 - 1.56 °C). The refractive index in this study fall within the range (1.460 - 1.465) reported by (Biswas and Das 2011).

4.2.2.4 Iodine value

Significant variation observed in iodine value among the varieties is in conformity with reports of satpute and suradkar (2018), Reda and Nassaar (2018) and Shashikant (2019). The highest value (12.06) obtained in SAMNUT 25 is lower than that reported by these authors. The differences observed might be due to variation in the groundnut genotypes. High iodine values are mostly required by oil processors and it indicates the presence of unsaturated fatty acid and can also be used to quantify the amounts of double bonds present in the oil which signifies the susceptibility of the oil to oxidation (Paul, 2013). The high iodine value obtained in some of the mutant line might be due to mutagenic effect on the genetic makeup of the plant.

4.2.2.5 Free fatty acid

Free fatty acid is the percentage by weight of specific fatty acid. The quantity of free fatty acid in oil is an indicator of its overall quality therefore, high quality oils are low in free fatty acids. The ranged of free fatty acid observed in this study (0.32-1.05 %) were within acceptable level and healthy for human consumption. Kratz *et al*. (2002) reported that free fatty acid exceeding 5 % makes it unhealthy for human consumption, therefore, groundnut oil with free fatty acid less than 5 % is valuable for food as observed in this study.

High Free fatty acid indicates poor quality of oil as it gives a bad taste (Dayrit *et al*., 2007). The low free fatty acid obtained at 0.1 % (0.32 %) of SAMNUT 26, 0.2 % (0.32 %) of SAMNUT 24, 0.1 % of SAMNUT 25 (0.27 %) and 0.4 % (0.38 %) in ICG 4412 revealed that EMS is an effective mutagen in reducing FFA content of groundnut oil, and the oils are suitable for consumption.

4.2.2.6 Acid value

The acid value obtained in this study (0.54-2.10 mg KOH/g) is lower that the report of Shashikant (2019) who obtained the range of 3.17-4.29 mg KOH/g in the following groundnut genotypes UF70 130 (Uf70-130), Kopargaon-1(K-1), Kopargaon-3(K-3) (Maharashtra) and RSB - 103-87(Rajasthan) (Rsb-103 - 87). The low acid values obtained in this study shows that the oil can be a good source of edible oil and can be use in industries for soap production.

4.2.3 Insect Population

The difference observed in insect population among various concentrations of EMS might be attributed to mutagenic effect of EMS on the plant. Similarly, Baguma *et al*. (2021) reported variation in number of whitefly in cassava plant among EMS concentrations and also among the genotypes. Decrease in insect pest population among the concentrations might be attributed to EMS treatments which could have induced cell wall fortification or release of chemicals such as antioxidants, and phytoalexine or enhancement of the activity of pest resistance related enzymes (Daayf *et al*., 2012). The reduction in insect pest population observed in this study among the treatments over the control in some varieties indicate their ability to resist infestation and tolerate the insects in some varieties. This findings is contrary to the report of Baguma *et al*. (2021) who reported higher infestation in the treatments plants than in the control.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The variations observed in vegetative and yield traits especially in pod characteristics of these mutants suggested that they are not yet stable and are still segregating. The positive changes in pod characteristics of SAMNUT 24 (0.1 % an 0.2 %, EMS treatments) could be exploited for increasing the productivity of groundnut and for further improvement of the crop.

Variability in oil reflects the existence of genetic diversity and suggests potential genetic improvements. Mutants with significantly higher oil contents than the control viz SAMNUT 25 (39.34 %) IG4412 (37.27 %) could be selected for breeding for improved oil contents in the groundnut genotypes.

Results of insect population revealed that, EMS is an effective mutagen in reducing insect pest of groundnut especially in SAMNUT 25 (0.2 % EMS treatment).

5.2 Recommendations

i. Genotypes with larger pods, seed size and high oil contents such as SAMNUT 24 and ICG 4412 should be recommended for multi-locational trial to determine the stability of the genotypes.

ii. Studies should be carried out on the amino acid composition of the groundnut genotypes for proper selection of elite genotype(s) with high protein value.

iii. Further studies should be carried out to assess the genetic diversity of the genotypes using molecular markers.

iv. Further studies should be carried out on the effect of EMS on insect pest resistance of these groundnut genotypes.

5.3 Contribution to Knowledge

The thesis established that Ethyl Methyl Sulfonate induced beneficial effect on agro-morphological parameters of the groundnut genotypes. In SAMNUT 24, 0.1 % EMS, produced the highest plant height (51.17 cm) at maturity. Yield parameter assessment revealed that SAMNUT 24, had the highest average number of pod per plant (26.33 pods) and seed per plant (2.39 seeds) at 0.1 % and 0.2 % concentrations respectively. The thesis further revealed that 0.2 % EMS concentration induced insect pest tolerance in SAMNUT 25 by drastically reducing the population of the insect. Similarly, SAMNUT 25 exposed to 0.2 % concentration produced mutants with the highest oil yield (39.34 %). In SAMNUT 24, iodine value was highest (11.31 I_2 / 100 g) in 0.4 % EMS concentration. Similarly, in SAMNUT 25, 0.4 % EMS concentration produced the highest iodine value (12.06 I_2 / 100 g).

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