

MUTAGENIC EFFECTS OF EMS ON AGRONOMIC TRAITS AND PHYSICO-CHEMICAL OIL PROPERTIES OF GROUNDNUT (*Arachis hypogaea* L.)



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ABSTRACT

The monophyletic origin, self-pollination status, and reproductive isolation in groundnut have narrowed its genetic potential and limited new variety development. This research aimed to access variability in morphology, yield, and physicochemical properties of groundnut seed-oil using ethyl methane sulfonate-induced mutagenesis (EMS). An EMS solution was prepared in 100 ml of distilled water at concentrations of 0.10, 0.20, 0.30, and 0.40 %. Three groundnut varieties (SAMNUT 24, SAMNUT 25, and SAMNUT 26) were treated with the different concentrations of EMS and were evaluated following a Complete Randomized Design (CRD) with five replications. Agro-morphological parameters, as well as the physico-chemical properties of oil, were determined following standard procedures. Data collected were subjected to Analysis of Variance and Duncan's Multiple Range Test was used to separate means with significant difference at $P < 0.05$. The results reveal significant ($p < 0.05$) variations, with the 0.1 % EMS concentration producing the highest number of leaves per plant (426.00), leaf area (21.91 cm²), branches per plant (16.00), and pods per plant (24.67). Additionally, it induced mutants with indented leaf morphology in SAMNUT 24. In SAMNUT 25, 0.1 % revealed a significant increase in the number of leaves per plant (337.00), 0.2 % produced mutants with stipule leaf morphology and a striata chlorophyll mutant, while a concentration of 0.3 % revealed xantha leaf mutants. 0.4 % revealed a significant increase in the leaf area (25.01 cm²). Generally, EMS treatment demonstrated a significant increase in all the yield parameters examined, except the number of seeds per plant. In SAMNUT 26, leaf area and hundred-seed weight were increased in EMS-treated plants. EMS treatment also significantly increased the number of pods per plant, except for 0.4 %. A significant decrease was observed in the oil yield of the three varieties, except in SAMNUT 25 treated with 0.2 %. Significant variations were also observed in other physico-chemical properties of the oil. Although some of the variabilities noted are uneconomical, some of the qualities can be used as a source of many beneficial genes in cross-breeding programs.

Keywords: Breeding, crop improvement, groundnut, mutagenesis, mutants.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an essential food crop that serves as a major source of protein and vegetable oil (Nilahayati *et al.*, 2024). It underpins agriculture-dependent livelihood strategies and contributes to food, nutrition, and income security (Vu *et al.*, 2022; Ojiewo *et al.*, 2020). However, most African countries do not meet their production potential and domestic demand due to the use of old, poor-performing cultivars (Daudi *et al.*, 2021), biotic and abiotic stresses (Kalarani *et al.*, 2023), and limited genetic variability for breeding improved varieties (Tadesse and Nepir, 2024).

Groundnut oil obtained from groundnut seeds is a type of vegetable oil consumed in almost every household in Nigeria (Sulaiman *et al.*, 2022). It is primarily used for cooking, but has numerous applications in the soap and cosmetic industry (Sharma, 2018). Numerous studies are concerned with the nutritional attributes of the oil, rather than its physicochemical qualities, and because of this, little attention is given to the physicochemical properties of groundnut oil, which are very useful as a prime index for quality control processes for both industrial and domestic applications (Shwatsu, 2022).

Globally, groundnut breeding is faced with numerous challenges, due to its monophyletic origin, differences in ploidy status and lack of gene flow that has posed significant reproductive isolation (Olorunmaiye *et al.*, 2019; Alabi *et al.*, 2013). This has narrowed its genetic potential and has limited the development of new groundnut varieties. Adegoke *et al.* (2025) noted that currently in Nigeria, the genetic diversity of elite groundnut germplasm is quite limited, and most of the existing cultivars possess a narrow genetic base. This genetic constraint poses a significant challenge to its improvement efforts.

The infusion of genetic variability through routine conventional breeding methods is not suitable and efficient due to its self-pollinated status (Manjunath *et al.*, 2020). As such, innovative breeding methods through induced mutagenesis have become the most popular method for creating variability in groundnut germplasm (Gunasekaran and Pavadai, 2015). Inducing mutagenesis has played a significant role in crop enhancement methodologies and has brought about desired genetic variabilities by providing new alleles to the genome and preventing gene erosion (Penna *et al.*, 2023).

Only one groundnut variety (SAMNUT-24) has been officially released in Nigeria following gamma irradiation, while two other mutant lines obtained through gamma irradiation are currently undergoing pre-release trials. Notably, no groundnut variety has been developed in the country through EMS-induced mutagenesis. Consequently, this research was designed to create genetic variability and enhance the genetic diversity of groundnut by exploring different concentrations of EMS on three groundnut varieties at M₁ generation. The objectives of the research were to determine the effect of the different concentrations of EMS on (i) vegetative and yield attributes of groundnut (ii) physicochemical properties of groundnut oil. The outcome of this research might broaden the genetic base of groundnut and provide breeding materials to support future improvement programs aimed at improving adaptability and productivity.

MATERIALS AND METHODS

Experimental site

The field experiment was conducted at the research field of the Department of Plant Biology, Federal University of Technology Minna, Niger State, Nigeria, while the laboratory analyses were performed at the Centre for Genetic Engineering and Biotechnology, Federal University of Technology Minna. Minna is located in the North Central agroecological zone of Nigeria and lies between latitude 9°65' N, longitude 6°34' E. The monthly mean temperatures, relative and specific humidities, UV index, dew and total precipitation of the experimental site during the study duration are presented in Figure 1.

Source of seeds

Seeds of three groundnut varieties, viz, SAMNUT 24, SAMNUT 25, and SAMNUT 26 were obtained from the Institute for Agricultural Research (IAR), Zaria, Kaduna State, Nigeria. The description and characteristics of the seeds are presented in Table 1.

Preparation of stock solution and treatment of seeds

A stock solution of EMS was prepared following the method of Talebi *et al.* (2012) with slight modifications. An aliquot of 1 ml of EMS was measured using a glass pipette and mixed with 99 ml of distilled water by swirling gently in a conical flask. Using a beaker, 10 ml of the stock solution was measured and diluted with 90 ml of distilled water to make a 100 ml solution, which gave a 0.1 % EMS solution. For a 0.2 % EMS solution, 20 ml of the stock solution was diluted with 80 ml of distilled water. An aliquot of 30 ml of the stock solution was diluted with exactly 70 ml of distilled water to produce a 0.3 % EMS solution, while 40 ml of the stock solution was diluted with 60 ml of distilled water to make a 0.4 % EMS solution. The concentration of 0.0 % served as the control and was purely 100 % distilled water.

The seeds were washed under running tap water, stirred in 70 % ethyl alcohol (EtOH) for 3 minutes, and rinsed three times with sterile water. Afterwards, the seeds were sterilized in 20 % sodium hypochlorite. After surface sterilization, the seeds were soaked in distilled water for four (4) hours according to Turkoglu *et al.* (2023). The seeds were then soaked for 4 hours in the different concentrations of Ethyl Methane Sulfonate (0.0, 0.1, 0.2, 0.3 and 0.4 %). Afterwards, the treated seeds were thoroughly rinsed in running tap water

to remove the residual effects of the mutagen according to Mba *et al.* (2010). In this study, a total of 50 seeds were used for each variety, and 10 seeds of each variety were exposed to each of the treatment levels.

Experimental design

The experiment was carried out following a Complete Randomised Design (CRD) with five replicates using experimental planting bags equivalent to a 20-litre bucket size. The planting bags were filled with sandy-loam soil obtained from the experimental garden, each weighing exactly 25 kg. Two seeds were sown per bag at a depth of 2 centimetres and were later thinned to one plant per bag after the plants were properly established. The experiment consisted of twenty-five (25) planting bags per variety and a total of seventy-five (75) planting bags, with a bag-to-bag spacing of 0.5 metres. The experiment was conducted between the months of May and August 2021.

Data collection

Vegetative and yield parameters

The following agronomic parameters were recorded at different developmental stages of the plant growth following the method of Olorunmaiye *et al.* (2019): Plant height, number of leaves, leaf area, number of branches, weight of pod, number of pods per plant, pod length, number of seeds per pod, and hundred seed weight.

Determination of physicochemical properties

For the determination of the physicochemical properties of the oil, the groundnut oil was first extracted from the seed samples following the Soxhlet extraction method using n-hexane as solvent according to the method of the Association of Official Analytical Chemists (AOAC, 2012). 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 a rotary evaporator and later oven dried at 105 °C for 1 hour and stored in bottles. The free fatty acid (FFA), acid value (AV), refractive index (RI), peroxide value (PV), and iodine value (IV) were determined following the standard protocols of AOAC (2012).

Data analysis

Data obtained were subjected to a One-way Analysis of Variance (ANOVA) using SPSS

version 23.0 (2015), and Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was used to separate means with significant differences. All results were considered statistically significant at $p < 0.05$.

RESULTS

Vegetative Parameters of M1 EMS-Induced Mutants of Groundnut

In SAMNUT 24, results demonstrated no significant ($p > 0.05$) variability in the seedling height and plant height at maturity across the five treatment levels (Table 2). Contrastingly, significant ($p < 0.05$) variations were observed in the number of leaves per plant, leaf area, and number of branches per plant across the treatment levels. A significant increase was recorded in the number of leaves per plant at 0.1, 0.2, and 0.3 % EMS concentrations, with the 0.1 % EMS concentration revealing the highest number of leaves per plant. EMS concentration of 0.4 % recorded the least number of leaves but was not significantly different from the control. The highest leaf area was observed at 0.1 % while the least was recorded at 0.4 % treatment level. The highest number of branches per plant was recorded at 0.1 % (16.00), but was not significantly different from that of 0.3 % EMS concentration (14.00), while the lowest value of number of branches per plant was recorded in the control plants (6.33) (Table 2). EMS concentration of 0.1 % also demonstrated notable variability in the leaf morphology of SAMNUT 24 by producing mutants with indented leaf forms (Figure 2).

SAMNUT 25 demonstrated a significant ($p < 0.05$) decrease in seedling height, plant height, number of leaves per plant and leaf area at 0.2 % EMS concentration. EMS concentration of 0.1 % revealed a significant increase in the number of leaves per plant, and the concentration of 0.4 % revealed a significant increase in the leaf area. Results show that the concentrations of EMS used in this study have no significant impact on the number of branches per plant (Table 2). Notable variabilities were observed in leaf morphology of SAMNUT 25 following EMS-induced mutagenesis, a concentration of 0.2 % produced mutants with stipule leaf morphology and a straita chlorophyll mutant, while a concentration of 0.3 % revealed a xantha leaf mutant (Figure 2). The significantly ($p < 0.05$) highest seedling height and plant height at

maturity in SAMNUT 26 were recorded at an EMS concentration of 0.1 % (24.43 cm and 51.17 cm, respectively). EMS concentration of 0.3 % revealed the highest number of leaves per plant (262.00) and number of branches per plant (17.67). A significant increase was also observed in the leaf area of SAMNUT 26 treated with EMS over their control group (Table 2).

Yield Parameters of M1 EMS-Induced Mutants of Groundnut

The 0.1 % EMS concentration revealed a significant ($p < 0.05$) increase in the number of pods per plant of SAMNUT 24, while 0.2 and 0.4 % showed a significant decrease in the number of pods per plant relative to the control plant (Table 3). Results reveal no significant differences in the pod length, pod weight and number of seeds per pod of SAMNUT 24 across the five treatment levels. However, a significant increase was recorded in the hundred-seed weight of seeds treated with 0.1, 0.3, and 0.4 % concentrations (Table 3).

In SAMNUT 25, EMS treatment demonstrated a significant ($p < 0.05$) increase in all the yield parameters examined, except for the number of seeds per plant, where there were no significant ($p > 0.05$) differences across the treatment groups (Table 3). A significant ($p < 0.05$) increase was observed in the number of pods per plant of SAMNUT 26 treated with EMS of varying concentrations, except for 0.4 %, which recorded a lower number of pods per plant when compared with the control (Table 3). Results reveal no significant differences in the pod length and number of seeds per pod across all the treatment levels. Significantly ($p < 0.05$) higher hundred-seed weights were observed in the EMS-treated plants (Table 3).

Oil Physicochemical Properties of M1 EMS-Induced Mutants of Groundnut

Significant decrease ($p < 0.05$) was observed in the oil yield of SAMNUT 24 following treatment with EMS, with the least oil content (34.56 %) recorded at 0.1 % EMS concentration. The refractive index and iodine value were not affected by treatment with EMS. Contrastingly, 0.1 % EMS revealed a significant ($p < 0.05$) increase in the acid value and free fatty acid content. EMS concentration of 0.3 and 0.4 % shows a significant decrease in peroxide

value, while 0.1 and 0.2 % significantly increase peroxide value (Table 4). In SAMNUT 25, 0.2 % EMS concentration demonstrated a significant increase in oil yield (39.34 %). Contrastingly, a significant decrease was recorded in oil yield across other treatment groups. A significant decrease was recorded in the acid value and free fatty acid content of oil obtained from plants treated with EMS, with the significantly least value recorded at 0.1 % EMS concentration. EMS concentration of 0.4 % revealed the significantly ($p < 0.05$) highest iodine value and the significantly ($p < 0.05$) least peroxide value (Table 4).

SAMNUT 26 revealed a significant decrease in oil yield, acid value, free fatty acid content and iodine value following treatment with EMS. However, no significant difference was observed in the refractive index value across all the treatment levels (Table 4).

DISCUSSION

Numerous studies have established that EMS is a potent mutagen and causes a high density of random irreversible point mutations (Chen *et al.*, 2020; Greene *et al.*, 2003), depending on factors like EMS concentration, plant species, treatment duration and temperature (Asif and Ansari, 2019). The exposure to different concentrations of EMS resulted in the exhibition of variability in the morphological and yield attributes in the mutagen-treated plants. The variability observed in the seedling and plant height in this study conforms with the reports of Jadhav *et al.* (2018), who have reported significant variability in the plant heights of groundnut following treatment with different concentrations of EMS. The authors reported that lower doses of EMS are more potent in inducing variability than the higher doses. Janila *et al.* (2016) noted that plant height is a critical trait in groundnut breeding and is often associated with vegetative vigour, biomass production, and high yield potential. It also exhibits moderate to high heritability, making it a reliable trait for early-generation selection.

The significant variability demonstrated in the number of branches and leaves per plant is consistent with the report of Gunasekaran and Pavadai (2015). This variability may be attributed to the chromosomal alterations induced by EMS mutagenesis. The emergence of

a higher number of branches in certain treatment groups, which consistently led to a higher number of leaves and leaf area, suggests that EMS treatment stimulated enhanced vegetative growth. John (2019) noted that a higher number of branches per plant and a greater leaf area index are positively associated with enhanced biomass production and yield.

Raina and Khan (2020) opined that chlorophyll mutants are indicators of the mutagenic potential of a treatment in creating genetic variability and, ultimately, genetic changes within a breeding population. The creation of xantha and straita chlorophyll mutants following treatment with 0.2 % and 0.3 % EMS, along with the exhibition of stipule and indented leaf forms, indicates the presence of genetic variability induced by EMS mutagenesis. Similar findings involving chlorophyll mutants have been reported by Manjunath *et al.* (2020).

The variabilities observed in the yield attributes demonstrate that EMS is a beneficial agent in inducing genetic changes in groundnut. Although some of the variabilities noted are uneconomical, some of the qualities can be used as a source of many beneficial genes in cross-breeding programs. The results of this study are in line with reports of previous studies of EMS mutagenesis in groundnut by Nadaf (2017), Gangadhara *et al.* (2018), and Gangadhara *et al.* (2021), who have all reported variability in the number of pods per plant, pod weight, and hundred seed weight. The presence of high genetic diversity in these traits allows for the selection of high-yielding mutants, which is vital for every crop improvement program. Bharatkumar (2015) suggests that these variabilities could either be a result of pleiotropic effects of mutated genes or chromosomal aberrations or gene mutations following induced mutagenesis.

The differential response in vegetative and yield characters among groundnut varieties treated with EMS can be attributed to their genotypic difference in their sensitivity and responsiveness to mutagenic exposure (Goudar *et al.*, 2016) or relative differences in the mutability of genes for the different traits.

In this study, most EMS treatments led to a reduction in oil yield, consistent with the

findings of earlier studies, which reported that chemical mutagenesis often disrupts complex metabolic pathways, including those involved in oil accumulation (Bado *et al.*, 2015). According to Demian (1990), acid value is an important indicator of vegetable oil quality and is used to measure the extent to which glycerides in the oil have been decomposed.

The significant decrease observed in the acid value of EMS-treated plants is a highly desirable trait in plant breeding programs as it reflects minimal free fatty acid (FFA) contents, which is indicative of superior oil quality. Pandurangan *et al.* (2014) noted that a reduction in acid values indicates good lubricating properties and prevents the oxidation of oil, which prevents gum and sludge formation. Some of the EMS-treated groundnut lines recorded acid values that fell within the acceptable limits (<0.6 mgKOH/g) for refined vegetable oils (Codex, 1999). This suggests that EMS mutagenesis may have induced favourable alterations in lipid metabolism or storage stability of the oil. Similar findings of alterations in seed oil properties and fatty acid composition have been reported in *Brassica napus* by Tang *et al.* (2020). Pandurangan (2014) opined that iodine value is a measure of unsaturation of vegetable oils and an indication of their susceptibility to oxidation. The significant decrease observed in iodine value in EMS-treated plants indicates lipid oxidation, which could be attributed to the presence of metallic ions. According to Nkafamiya *et al.* (2010), this will enhance oxidation after the formation of hydrogen peroxide. Okechalu *et al.* (2011) noted that peroxide value determines the degree of oxidation of oil and gives an indication of the level of deterioration of oil and fats, with higher values indicating higher susceptibility to oxidation (Atasie *et al.*, 2009). The notable reductions recorded in peroxide values in some of the EMS-treated plants could be explored for improving groundnut seed oil quality, as it indicates low levels of oxidative rancidity and suggests high levels of antioxidants.

CONCLUSION

The findings of this study reveal EMS as a potent mutagen in the creation of valuable

genetic variability in groundnut. It generated a wide range of morphological and yield-related mutations and also led to the emergence of chlorophyll mutants across different concentrations. Additionally, this study reveals the creation of variability in the physicochemical properties of groundnut oil, with some EMS-treated lines producing oil with improved qualities. Although not all of the variability created was agronomically desirable, some of these variations can be utilized as a source of many beneficial genes in cross-breeding programs.

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Table 1: Description of research materials

Variety name	Original name	National code	Outstanding characteristics	Potential yield
SAMNUT 24	ICIAR 19BT	NGAH-11-24	Extra early maturing, rosette resistant, high oil content	2 t/ha
SAMNUT 25	ICGX-SM-00020/P5/P10	NGAH-13-25	High rosette resistance, high yield and early maturing	3.8 t/ha
SAMNUT 26	ICGX-SM-00018-P5/P15/P2	NGAH-13-26	Early maturing, high yield and high rosette resistance	3.8t/ha

Table 2: Vegetative parameters of EMS-induced Mutant Lines (M₁) of Groundnut

Variety/Conc.	SH (cm)	PH (cm)	NL	LA (cm ²)	NBP
SAMNUT 24					
0.0 %	16.43±2.66 ^a	45.20±1.46 ^a	130.33±15.60 ^a	18.63±1.02 ^{ab}	6.33±1.33 ^a
0.1 %	14.87±1.41 ^a	44.80±3.60 ^a	426.00±31.26 ^c	21.91±1.16 ^b	16.00±2.00 ^b
0.2 %	11.60±2.20 ^a	39.90±1.93 ^a	276.00±52.12 ^b	19.89±1.02 ^{ab}	10.33±1.76 ^{ab}
0.3 %	15.27±4.72 ^a	45.63±4.43 ^a	366.00±25.01 ^{bc}	21.22±1.26 ^{ab}	14.00±3.06 ^b
0.4 %	11.40±4.25 ^a	43.17±2.19 ^a	115.33±2.91 ^a	17.71±0.74 ^a	9.67±0.88 ^{ab}

SAMNUT 25					
0.0 %	18.37±2.62 ^b	40.47±1.74 ^b	224.67±7.86 ^{ab}	19.45±0.31 ^b	9.33±0.67 ^a
0.1 %	16.47±1.99 ^b	41.07±5.74 ^b	337.33±48.89 ^b	21.33±0.99 ^b	10.67±3.18 ^a
0.2 %	4.97±1.17 ^a	23.17±1.42 ^a	157.33±29.07 ^a	14.29±0.91 ^a	8.33±1.45 ^a
0.3 %	22.07±2.23 ^b	44.43±0.46 ^b	208.00±66.04 ^{ab}	21.95±1.18 ^b	10.67±2.73 ^a
0.4 %	18.43±3.16 ^b	42.93±1.49 ^b	227.33±5.46 ^{ab}	25.01±1.34 ^c	6.67±0.88 ^a
SAMNUT 26					
0.0 %	18.10±0.79 ^a	37.43±0.32 ^a	119.33±5.21 ^a	20.15±2.28 ^a	10.33±0.33 ^a
0.1 %	24.43±2.24 ^b	51.17±3.85 ^c	112.00±2.31 ^a	26.64±1.01 ^b	13.00±1.15 ^a
0.2 %	19.77±2.24 ^{ab}	48.47±1.42 ^{bc}	171.33±37.71 ^a	26.10±0.74 ^b	10.67±1.45 ^a
0.3 %	16.03±1.96 ^a	40.53±1.49 ^{ab}	262.00±13.32 ^b	26.79±0.86 ^b	17.67±2.19 ^b
0.4 %	20.73±0.94 ^{ab}	42.90±3.16 ^{ab}	130.00±12.49 ^a	23.71±1.27 ^{ab}	10.67±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. SH; seedling height; PH; Plant Height, NL; number of leaves per plant, LA; leaf area; NBP; number of branches per plant.

Table 3: Yield parameters of EMS-induced Mutant Lines (M₁) of Groundnut

Variety/Conc.	NPP	LP (cm)	WP (g)	NSP	HSW (g)
SAMNUT 24					
0.0 %	17.33±5.49 ^c	2.21±0.15 ^a	1.44±0.81 ^a	1.90±0.10 ^a	45.96±0.09 ^b
0.1 %	24.67±0.33 ^d	2.04±0.16 ^a	1.80±0.15 ^a	1.70±0.15 ^a	49.76±0.11 ^c
0.2 %	9.67±1.67 ^a	2.05±0.14 ^a	1.77±0.16 ^a	1.80±0.13 ^a	37.80±0.09 ^a
0.3 %	18.67±3.76 ^c	2.05±0.14 ^a	1.77±0.16 ^a	1.80±0.13 ^a	50.52±0.12 ^d
0.4 %	13.00±4.73 ^b	2.24±0.09 ^a	1.70±0.09 ^a	1.90±0.10 ^a	50.52±0.12 ^d
SAMNUT 25					
0.0 %	11.67±2.03 ^a	1.92±0.12 ^a	1.44±0.09 ^a	1.80±0.13 ^a	30.00±0.15 ^a
0.1 %	26.33±2.03 ^c	2.07±0.17 ^{ab}	1.89±0.92 ^b	2.00±0.00 ^a	51.06±0.21 ^c
0.2 %	20.33±2.03 ^b	2.39±0.13 ^b	1.64±0.11 ^{ab}	1.80±0.13 ^a	54.34±0.16 ^d
0.3 %	19.00±1.73 ^b	2.06±0.13 ^{ab}	1.75±0.16 ^{ab}	1.70±0.15 ^a	53.86±0.10 ^d
0.4 %	20.00±3.61 ^b	2.09±0.12 ^{ab}	1.72±0.16 ^{ab}	1.90±0.10 ^a	50.50±0.28 ^b
SAMNUT 26					
0.0 %	13.33±0.88 ^b	2.21±0.11 ^a	1.64±0.72 ^a	1.90±0.10 ^a	37.56±1.39 ^a
0.1 %	21.67±2.33 ^d	2.18±0.13 ^a	1.86±0.12 ^{ab}	1.80±0.13 ^a	50.40±0.46 ^c
0.2 %	18.67±3.84 ^c	2.19±0.16 ^a	1.74±0.16 ^a	1.80±0.13 ^a	49.64±0.15 ^c
0.3 %	22.00±1.00 ^d	2.43±0.78 ^a	2.08±0.49 ^b	2.00±0.00 ^a	49.34±0.27 ^c
0.4 %	10.33±1.45 ^a	2.21±0.11 ^a	1.64±0.72 ^a	2.00±0.00 ^a	44.64±0.15 ^b

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

Table 4: Oil Properties of EMS Mutant Lines (M₁) of Groundnut Oils

Treatments	Oil Yield (%)	Refractive Index	Acid Value mgKOH/g	FreeFatty Acid (%)	Iodine Value g/100g	Peroxide meqO ₂ /kg
SAMNUT 24						
0.0 %	39.27±0.05 ^d	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
0.1 %	34.56±0.04 ^a	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}
0.2 %	39.01±0.01 ^c	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
0.3 %	39.06±0.00 ^c	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
0.4 %	38.66±0.05 ^b	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
SAMNUT 25						
0.0 %	36.51±0.03 ^c	1.47±0.00 ^a	1.59±0.06 ^e	0.80±0.03 ^c	8.84±0.01 ^a	7.50±0.17 ^b
0.1 %	32.34±0.03 ^b	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
0.2 %	39.34±0.60 ^d	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

0.3 %	31.07±0.08 ^a	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
0.4 %	35.70±0.07 ^c	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
SAMNUT 26						
0.0 %	33.43±0.01 ^e	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
0.1 %	23.81±0.03 ^a	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
0.2 %	27.12±0.06 ^c	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
0.3 %	31.13±0.00 ^d	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
0.4 %	24.70±0.14 ^b	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

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

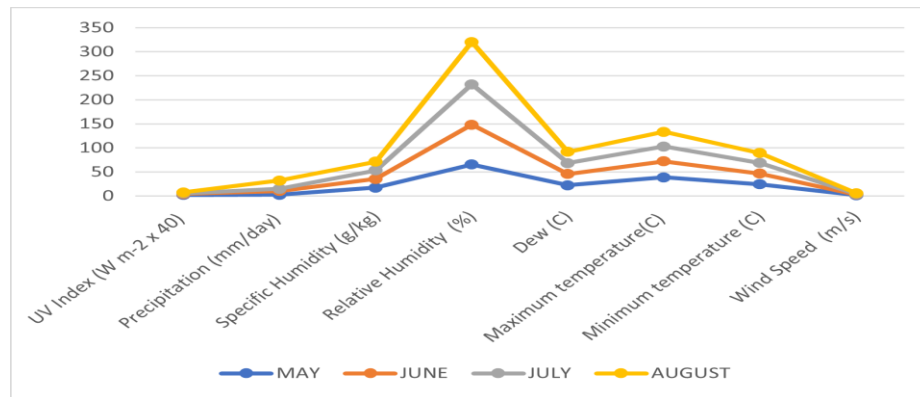


Figure 1: Monthly Mean Temperature, Humidity, UV Index, Dew Point, and Total Precipitation at the Experimental Site During the Study Period. Source: <https://power.larc.nasa.gov/data-access-viewer/>

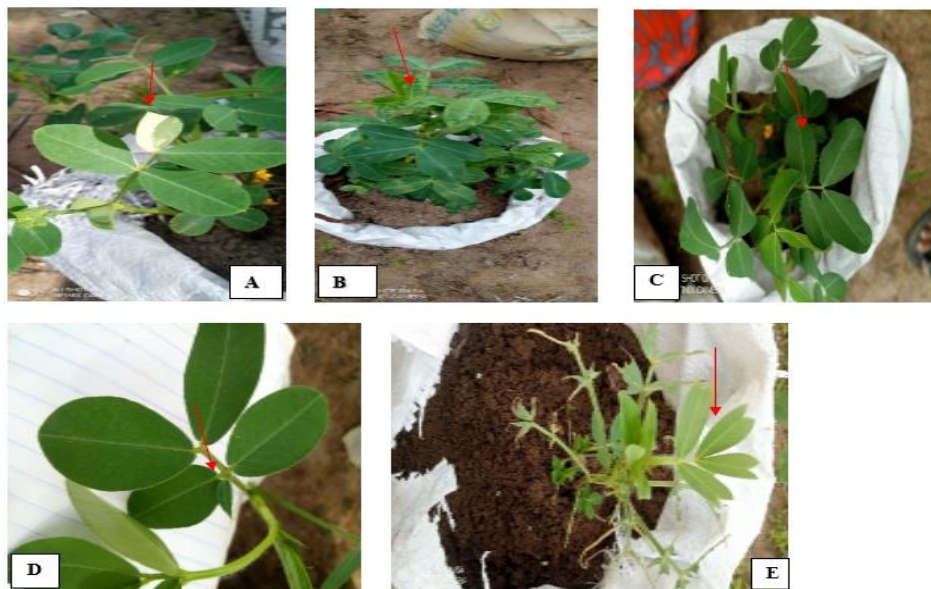


Figure 2: Chlorophyll and Leaf Mutant of M_1 EMS mutagenized *Arachis hypogea* L. A. Striata B. Xantha. C. control of the mutant plants. D. stipule leaflet morphology E. indented leaf morphology