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MUTAGENIC COMPONENTS AND DOSAGE EFFECTS OF ETHYL METHANESULPHONATE ON *ARACHIS HYPOGEA* (SAMNUT 24 VR.)

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ABSTRACT

Determination of the optimum concentrations of a specific mutagen for crop improvement has always been a challenge. The present study assessed the mutagenic effect of Ethyl methanesulphonate (EMS) concentrations on growth, yield and nutrient composition of *Arachis hypogea* (Samnut 24 vr.). Seeds treated with varying concentrations (0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 % v/v) of EMS for 12 hours were sown in randomized complete block design with three replicates. Alkylating effects of the EMS was studied on growth and yield parameters while nutritional quality was determined by proximate analysis at M_1 and M_2 generations. Concentrations of 0.75% and 1.00% increased growth, biological yield and seed related parameters significantly (at $P< 0.05$). Maturity time was significantly shortened by EMS application across the generations. Higher percentage seed crude fat and protein were induced by the treatments with the optimal performance at 0.75-1.00% concentrations. Mineral content indicated by the percentage ash was marginally increased. The trend in response to the EMS was similar across the generations, however, decreased vigour, reduced growth, less nut and biological yields were observed at M_2. In most traits evaluated, performance of 0.00, 0.25 and 0.50% treatments were similar, 0.75-1.00% treatments were optimal while 1.25% v/v of EMS was detrimental. Correlation analysis revealed poor linkage of characters in the groundnut; nevertheless, positive significant associations exist between number of leaves and branches, pod length and seed number, seed dimension (length and circumference) and seed weight. The study concluded that since groundnut is basically cultivated for biomass yield a well as source of plant vegetable oil and fat, EMS application at optimal concentration could be used for the crop improvement.

Keywords: Alkylating effect, Ethyl methanesulphonate, Mutagenesis, Proximate analysis, Samnut 24.

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is an important member of the family Fabaceae. It is an economically important crop not only for vegetable oil but as source of proteins, minerals and vitamins. Groundnut is the thirteenth most important food crop in the world, fourth most important source of edible oil and third most important source of vegetable protein. Groundnut is cultivated in many countries of the world on approximately 26.5 million hectares, with an average annual production of 35.7 million tons (FAO, 2007). China accounts for 37.5% of the global production, India (19%), Nigeria (11%), and about 5% from Central and South America. However, there was increase in global production which rose to 38 million tons in 2010 (FAOSTAT, 2015). Nigeria accounts for 51% of the total groundnut production in West Africa, but there was significant decline in the early 1980s due to disease incidence and drought. However, few years later, production increased at estimated 8% growth rate with area expansion of 6% and increased productivity (Ndjeunga and Ibro, 2010). Groundnut seed contain high quality edible oil, high amount of protein (Taru *et al.,* 2010) and it is a source of raw material for many products (Reddy *et al*., 2003).

Groundnut improvement is important to the economy, food security and reduction of malnutrition, particularly in sub-Sahara Africa and humid regions. Difference in ploidy status is a common reproductive isolation in groundnut, thus making the crop's genetic base very narrow (Stalker and Simpson, 1995; Alabi *et al.,* 2013). Consequently, introgression of wild genes into groundnut is only possible through complex crosses or genetic transformation (Proite *et al*., 2007; Singh and Nigam, 2016). Presently in Nigeria, available cultivars of groundnut have narrow genetic base and the allelic combinations available from working with elite germplasm are limited (Kamara, 2011), hence the need to broaden and improve the genetic base through breeding programme for yield and quality improvement (Okello *et al*., 2010). Meanwhile, the preference for certain varieties to others by local farmer is due to differences in flavour, oil content, size, shape and colour, yield and disease resistance. Primarily, the value and utilization of groundnut for both nutritional and industrial purposes depend on seed/nut quality (Alabi *et al*., 2013).

Mutagenesis is a tool in plant breeding for creation of genetic variability for further selection, hybridization and production of raw materials for genetic improvement of economically important crops (Adamu and Aliyu, 2007). The roles of chemical mutagens in enhancing genetic variability in vegetative and sexually propagated plants have been demonstrated (Kleinhofs *et al.,* 1978; Bhat *et al.,* 2005; Animasaun *et al*., 2014) and induced mutations could serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001). The main advantage of mutational breeding is the possibility of improving one or two characters without changing the entire genotype. Mutation breeding has been used to generate a number of desirable cultivars of different crops. Mutation generates variability which is a precursor for successful breeding programme (Adamu *et al*., 2005). Since natural mutation rate is usually low and may be difficult to exploit for breeding, induced mutations using physical and chemical mutagen are imperative, due to their potential to enhance mutation frequency and create mutants that could be screened for genetic and agronomic improvements (Ahloowalia and Muluszyski, 2001; Proite *et al*., 2007; Kharade *et al*., 2015).

Ethyl methanesulphonate (EMS) is one of the most commonly used chemical mutagens in plants due to its potency (Benjavad *et al*., 2012), however, determination of appropriate doses still remains a challenge. Selection of an efficient mutagenic concentration for optimum growth and yield is essential in crop improvement by mutagenesis (Wani, 2009; Arisha *et al*., 2014). Much of the variability generated by mutagenesis still remains poorly used in improvement programmes. Therefore, there is need for determination of optimum concentration for development of high biological, agronomic and nutritional quality mutants of groundnut to enhance its cultivation and utility in combating the problems of food insecurity, shortage of agro- and industrial raw materials.

Groundnut's narrow genetic base due to its monophyletic origin, lack of gene flow, ploidy barrier and self-pollination (Halward *et al*., 1991) are limitations to development of new varieties, in particular through conventional method. To this end, the present study assessed the mutagenic efficiency of different ethyl methanesulphonate (EMS) concentrations on biological and agronomic yield as well as nutritional qualities of Samnut 24 vr cultivar (followed by determination of the stability of the identified desirable traits in the mutants for two successive generations.

MATERIALS AND METHODS

Experiment Site

The experiment was conducted at University of Ilorin Botanical Gardens (N 08° 28' 53.3", E 04° 40' 28.9”), Ilorin, which lies in Southern guinea savanna belt of Nigeria. The annual rainfall of the area is about 1200 mm and temperature varies between 33 °C and 34 °C. The experiment was carried out in 2016/2017 cropping seasons.

Chemicals and Reagents Preparation

Ethyl methanesulphonate (EMS) purchased from Sigma Aldrich (USA) was used as mutagen for the study. Different concentrations of EMS (0.00%, 0.25%, 0.50%, 0.75%, 1.00% and 1.25% v/v) were prepared using phosphate buffer and distilled water.

Seed Material Preparation and Planting

Groundnut seeds (Samnut 24 vr.) were obtained from Institute of Agricultural Research, Ahmadu Bello University, Zamaru, Zaria, Kaduna State, Nigeria. Dry and healthy seeds were soaked in varying EMS concentrations for 12 hr at room temperature. Then, the treated seeds were removed and rinsed in different batches of distilled water to remove excess chemical residue prior to sowing. The seeds were sown directly into labelled planting pots (three pots per treatment) filled with mixture of garden soil (clay sand in ratio 2:1), laid out at 0.5 x 0.5 m spacing in randomized complete block design (RCBD) with three replicates.

Morphometric, Biological Yield and Seed Related Parameter Studies

Alkylating effects of different concentrations of EMS were studied on growth, biological and nut yield parameters of the plant. The fresh weight of the treated plants harvested at maturity was obtained by direct weighing using Metler balance (PL 300) while dry weight was determined by oven drying in hot air oven (Singula-Ambulacatt 1957 model) at 80-100 °C for 24 hours, then the dried plant was weighted to obtain dry weight. Mature seeds were air-dried at room temperature for two weeks; weight of 100-seeds was measured in three replicates for each treatment.

Proximate Analysis

Determination of proximate components was carried out following standard method (AOAC, 2000). The percentage dry matter (moisture) was determined by drying the nuts in an oven at 103-105 °C for 24 hours and the amount of moisture was determined as described (AOAC, 2000). Micro-Kjeldahl's method was employed to determine the total nitrogen and crude protein (N x 5.95) by standard protocol (AOAC, 2000). Crude lipids were extracted with petroleum ether using Soxhlet apparatus HT-extraction technique, while percentage ash (% minerals) and crude fibre were determined based on established method (AOAC, 2000). Total carbohydrate was estimated by difference method.

Data Analysis

Data collected from the growth and yield related traits were subjected to analysis of variance (ANOVA) using SPSS statistical software for Microsoft window operation system version 17. The level of significance was defined at $p < 0.05$. Growth and yield parameters were correlated, the difference in means was compared and separated by Duncan's multiple range test (DMRT).

RESULTS

Number of days to seeding emergence differed for the treated plants. Seedling emergence was between 4th to 7th days after sowing (DAS) for the 0.00% ethyl methanesulphonate (EMS) concentration but delayed in other treatment concentrations which ranged from 6-11 DAS. Highest percentage mean germination (95.50%) was obtained with 0.00% EMS treatment. The percentage germination was in order of 0.00% > 0.25% > 0.50% > 0.75% > 1.00% > 1.25% (Figure 1). Similar trend was also observed in the M_2 generation, but percentage germination was generally lower compared to M_1 seeds.

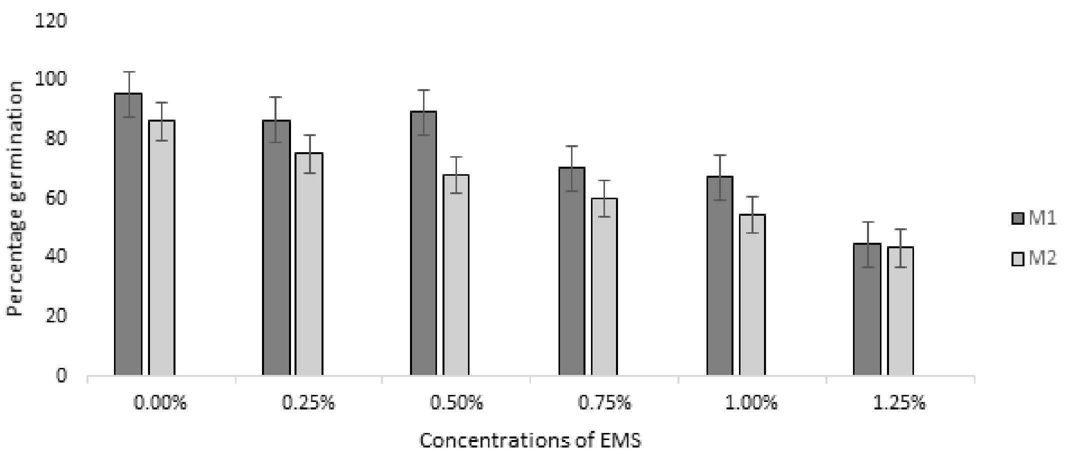

Figure 1: Percentage Germination of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate

Plant heights varied for the treatments, the 1.00% EMS treated plant had optimal effect on plant height across the weeks of evaluation for M_1 generation of groundnut (Figure 2). At 10 weeks after sowing (WAS), plant height of the 0.00% and 0.50% treated plants were at par while 1.25% treated plants had the least height. However, the trend was different in the M_2 generation where the highest plant height was achieved with 0.75%, followed by 1.00% EMS treatments. Also, the number of leaves per plant in response to different concentrations of EMS varied across the weeks of evaluation. Highest number of leaves per plant occurred in 1% EMS plants both at M_1 (40.00) and M_2 (48.33) generations, followed by 0.75% EMS plants (Figure 3). Leaf formation was poor in the groundnut variety (SAMNUT 24) in response to 1.25% EMS treatment.

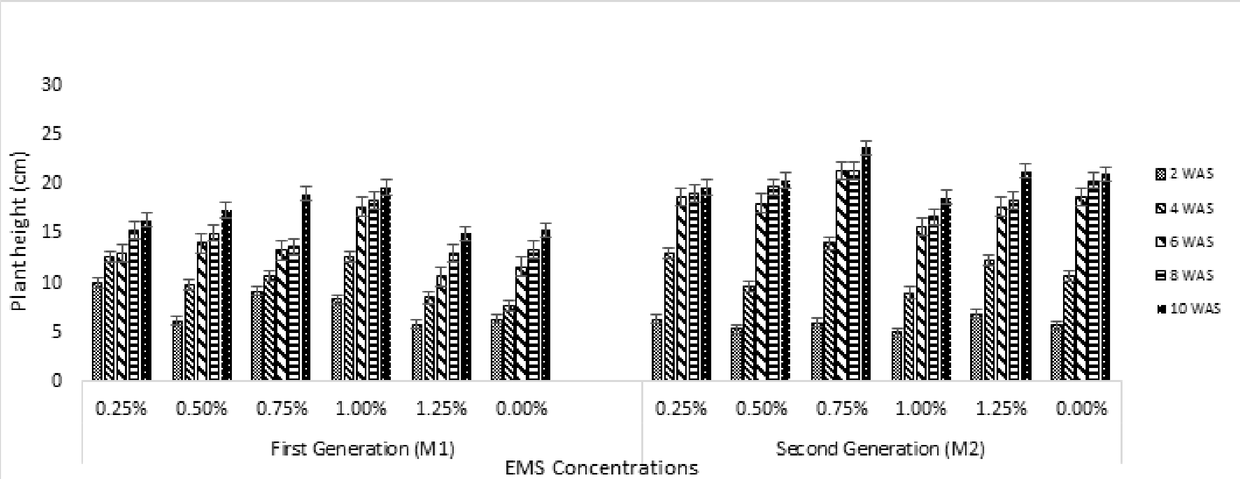

Figure 2: Plant Height (cm) of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate

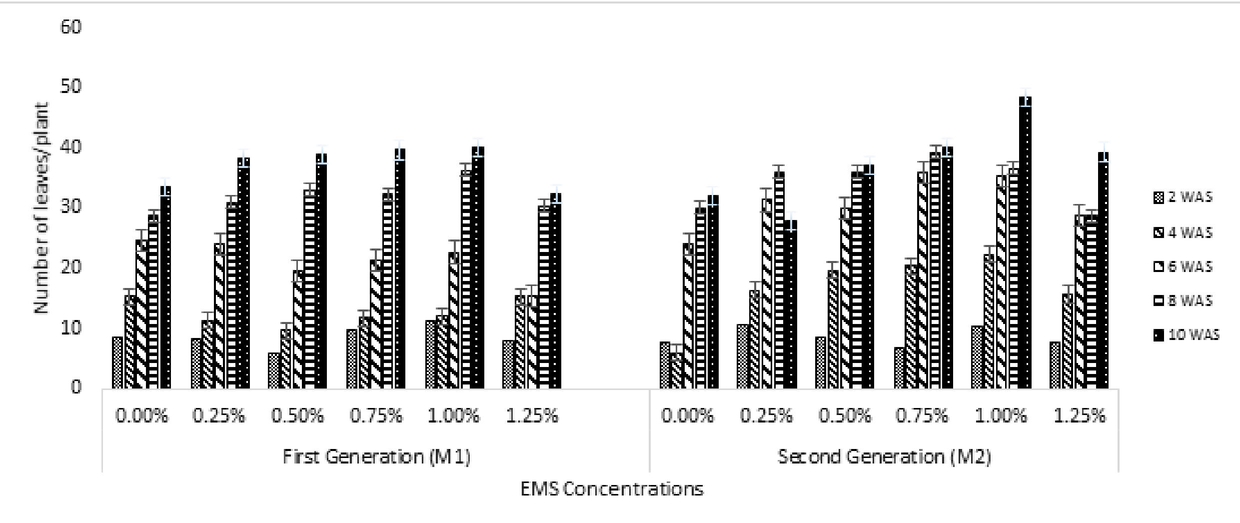

Figure 3: Number of Leaves in Groundnut Var. (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate.

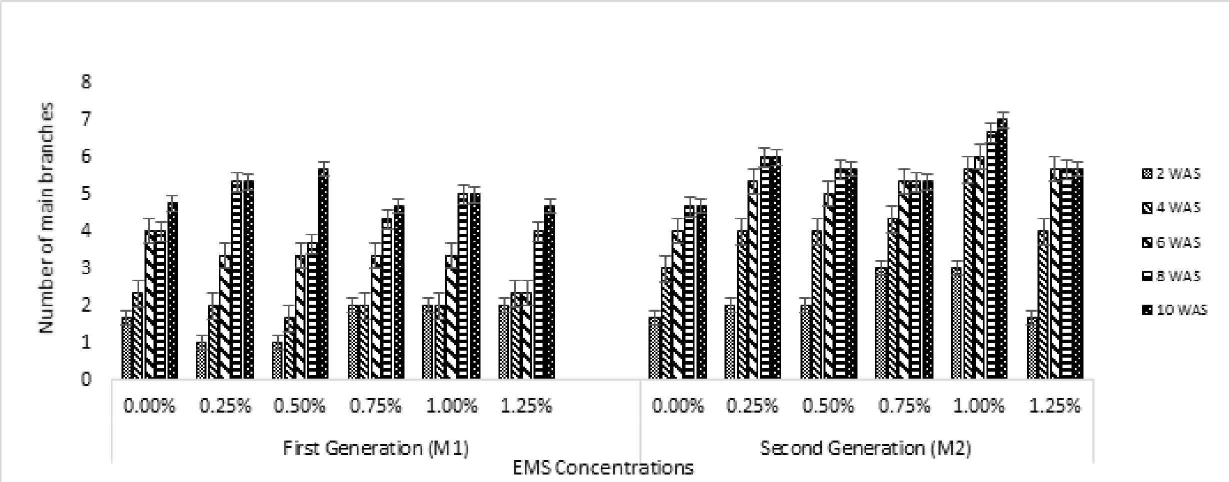

Figure 4: Number of Branches Per Plant in Groundnut (Samnut 24 Vr.) Treated with Different Concentrations of Ethyl Methanesulphonate.

At 2-8 WAS, 0.00 and 0.25% EMS induced higher number of branches among the M_1 generation of plants, but at 10 WAS, 0.50% treated plants were the most branched with an average of 5.67 branches per plant (Figure 4). The branching pattern of the M_2 plant differed from the M_1. Generally, M_2 plants were more branched, while the highest number of branches per plant (7.00) was found in 1.00% treated plant. Leaf length and breadth of groundnut (Samnut 24 vr.) in response to different concentrations of EMS varied across the weeks of evaluation for both M_1 and M_2 generations (Figure 5). EMS treatment of 0.50% produced highest mean leaf length (5.67 cm), followed by 0.25% treatment, while the least leaf length (4.67 cm) was obtained in 1.25% treated plants. Leaf blade length was similar for both generations for each of the applied concentrations of EMS. The 1.00% EMS induced broader leaves with mean values of 6.90 and 6.72 cm for the M_1 and M_2 generations of the treated groundnut plants respectively (Figure 6). Leaf breadth increased progressively with WAS, but decreased slightly from 8-10 WAS for 0.00% and 0.25% concentrations at M_1 generation.

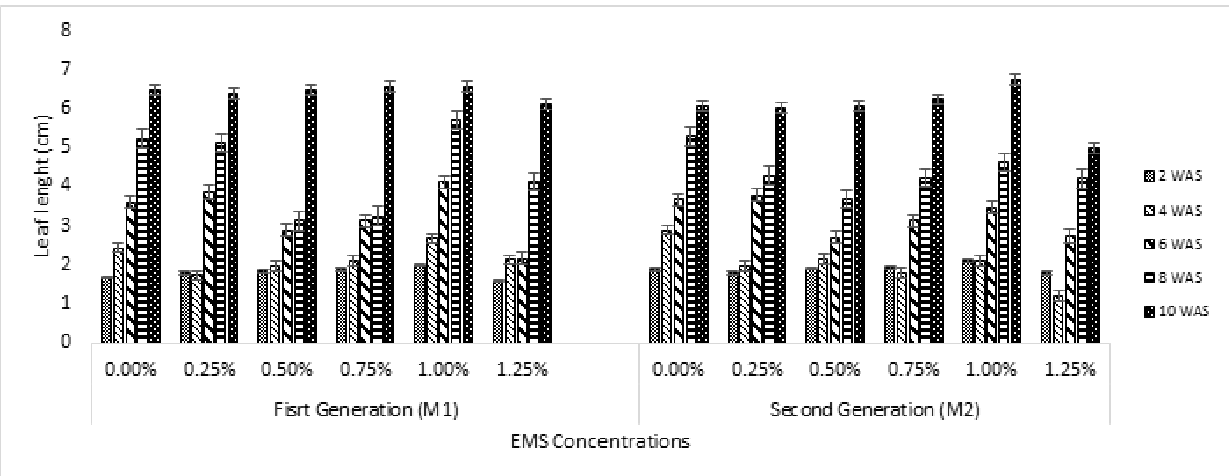

Figure 5: Leaf Length (cm) of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate.

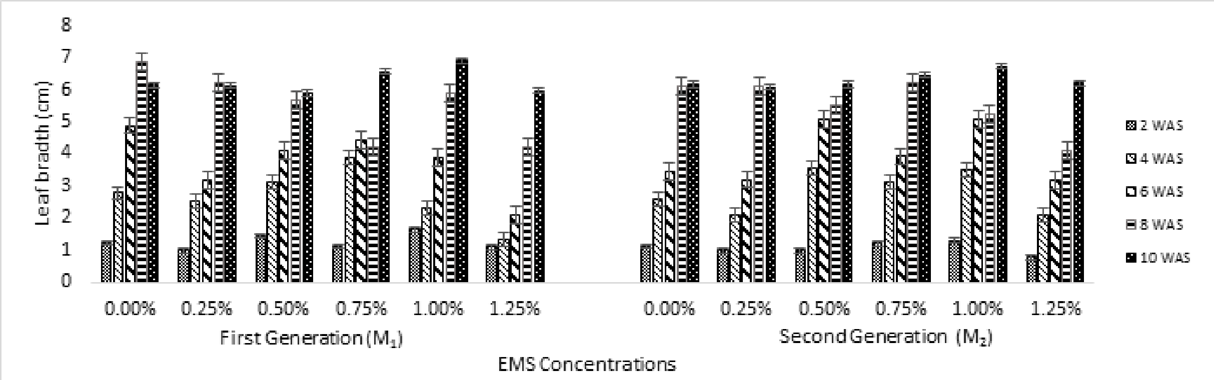

Figure 6: Leaf Breadth (cm) of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate.

At maturity (12 WAS), the M_1 plants treated with 0.75% and 1.0% EMS had highest mean plant height of 24.00 and 22.35 cm, respectively which were not significantly different (Table 1). Shortest plants were produced by 0.00% and 1.25% treatments, while the effects of 0.25 and 0.50% of EMS on the plant height were similar. Similar trend was obtained for the M_2 plants. Also, number of leaf per plant was highest for 0.75 and 1.00% treatments but similar for the rest of the treatments. However, leaf production was poor among the M_2 plants, but nevertheless, significantly highest mean number of leaf per plant was found in 1.25% treated plants. For all the treatments across the generations, number of branches per plant, length and breadth of the leaves were not significantly affected by the treatment concentrations.

Table 1: Growth Parameters of Groundnut (Samnut 24 vr.) in Response to Different Concentrations of Ethyl Methanesulphonate

	EMS Con. (%)	PH (cm)	NLP	NBP	LL (cm)	LB (cm)
M_1	0.00	16.00^{d}	42.67^{ab}	4.67^{bc}	6.60^{a}	6.60^{a}
	0.25	17.33^{cd}	42.33^{ab}	5.67^{a}	6.47^{ab}	6.47^{ab}
	0.50	21.00^{bc}	42.33^{ab}	5.00^{ab}	6.40^{ab}	6.40^{ab}
	0.75	24.00^{a}	49.33^{a}	5.00^{ab}	6.47^{ab}	6.47^{ab}
	1.00	22.53^{ab}	47.33^{a}	5.33^{ab}	6.57^{a}	6.85^{a}
	1.25	16.33^{d}	45.00^{ab}	4.57^{bc}	6.57^{a}	6.57^{a}
M_2	0.00	21.00^{bc}	19.33^{de}	4.67^{bc}	6.46^{ab}	6.46^{ab}
	0.25	20.67^{cd}	20.00^{de}	6.25^{a}	6.07^{ab}	6.07^{ab}
	0.50	20.33^{cd}	15.00^{f}	5.15^{abc}	6.03^{ab}	6.03^{ab}
	0.75	22.67^{a}	21.33^{d}	5.00^{abc}	6.07^{ab}	6.07^{ab}
	1.00	23.78^{a}	18.00^{e}	5.33^{ab}	6.23^{ab}	6.23^{ab}
	1.25	22.00^{abc}	25.33^{c}	5.67^{ab}	6.36^{ab}	6.36^{ab}

Means with the same superscripts along the same column are significantly not different at $p \leqslant 0.05$. M_1: First generation; M_2: second generation; PH: Plant height; NLP: Number of leaves per plant; NB: Number of branches per plant; LL: Leaf length; LB: Leaf breadth

The summary of nut and seed related attributes of the groundnut in response to different concentrations of EMS is shown in table 2. Across the generations, high concentrations of EMS (1.00, 1.25%) caused early flowering while low concentrations (0.00, 0.25 and 0.50%) treated plants flowered late. Number of pod per plant was adversely affected by higher concentrations of EMS for both M_1 and M_2, but favoured by low concentrations (0.00-0.50%). Although, high concentrations resulted in reduced number of pods per plant, pod length and circumference were significantly increased by concentrations above 0.50%. The least average number of seeds per pod (2.10) was obtained in plant treated with 1.25% EMS at the M_2. In term of seed dimensions, larger seeds were produced by plants treated with higher concentrations and the trend was similar for both M_1 and M_2 plants. In addition, higher concentration treatments (1.00, 1.25%) produced statistically heavier seeds than the low concentrations.

Table 2: Nut Yield and Nut Related Parameters of M_1 and M_2 Generations of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate

	Conc.(%)	NDF	NPP	PL (cm)	PC (cm)	NSP	SL (cm)	SC (cm)	100 SW (g)
M_1	0.00	47.23^{bc}	29.00^{bc}	3.87^{bc}	3.28^{d}	2.45^{abc}	1.33^{bc}	3.11^{fg}	42.93^{cd}
	0.25	49.15^{c}	31.67^{bc}	3.67^{c}	3.23^{d}	2.68^{a}	1.25^{bcd}	3.08^{gh}	40.33^{d}
	0.50	46.34^{bc}	28.33^{bc}	3.96^{bc}	3.42^{cd}	2.58^{ab}	1.42^{ab}	3.32^{de}	41.33^{d}
	0.75	42.25^{ab}	29.00^{bc}	4.86^{a}	3.90^{bc}	2.14^{bc}	1.49^{a}	3.56^{abc}	50.20^{ab}
	1.00	39.21^{a}	21.00^{de}	4.92^{a}	4.12^{ab}	2.15^{cd}	1.58^{a}	3.41^{cd}	47.35^{bc}
	1.25	39.65^{a}	19.33^{e}	4.89^{a}	4.37^{ab}	2.14^{d}	1.54^{a}	3.44^{cd}	55.00^{a}
	0.00	50.25^{c}	38.33^{a}	3.78^{c}	3.47^{cd}	2.47^{abc}	1.19^{de}	3.19^{f}	39.83^{de}
M_2	0.25	48.33^{c}	30.67^{b}	3.98^{bc}	4.02^{ab}	2.67^{a}	1.22^{de}	3.13^{fg}	42.07^{cd}
	0.50	48.10^{c}	36.33^{a}	4.91^{a}	3.73^{c}	2.51^{abc}	1.28^{abc}	3.75^{ab}	38.13^{abc}
	0.75	40.50^{ab}	29.33^{bc}	4.93^{a}	4.36^{ab}	2.58^{abc}	1.35^{ab}	3.89^{a}	40.38^{ab}
	1.00	41.20^{a}	25.67^{cd}	4.97^{a}	4.93^{a}	2.13^{d}	1.45^{a}	3.77^{ab}	47.30^{bc}
	1.25	40.25^{a}	21.33^{cde}	4.86^{a}	4.96^{a}	2.10^{d}	1.41^{ab}	3.92^{a}	51.13^{ab}

Key: M_1: First generation; M_2: Second generation; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; PC: Pod circumference; NSP: Number of seed per pod; SL: Seed length; SC: Seed circumference; 100 SW: 100-seed weight.
Means with the same superscript(s) along the same column are not significantly different at $p \leq 0.05$.

The biological yield in terms of fresh and dry weights of the plant at maturity varied across the treatments and generations. The fresh weight of the plants increased with concentrations at both M_1 and M_2 generations (Figure 7). However, this was not true for dry weight as 1.00% treatment produced plants with the least (8.07 g) average dry matter, while 1.25% EMS treated plants recorded the highest (11.06 g). Both fresh and dry matters were higher across the treatment concentrations for the M_1 than the M_2 plants. The mean dry weight per plant for the M_2 generation ranged from 3.45 - 4.39 g, and the dry matter was proportional to the applied concentrations at M_2.

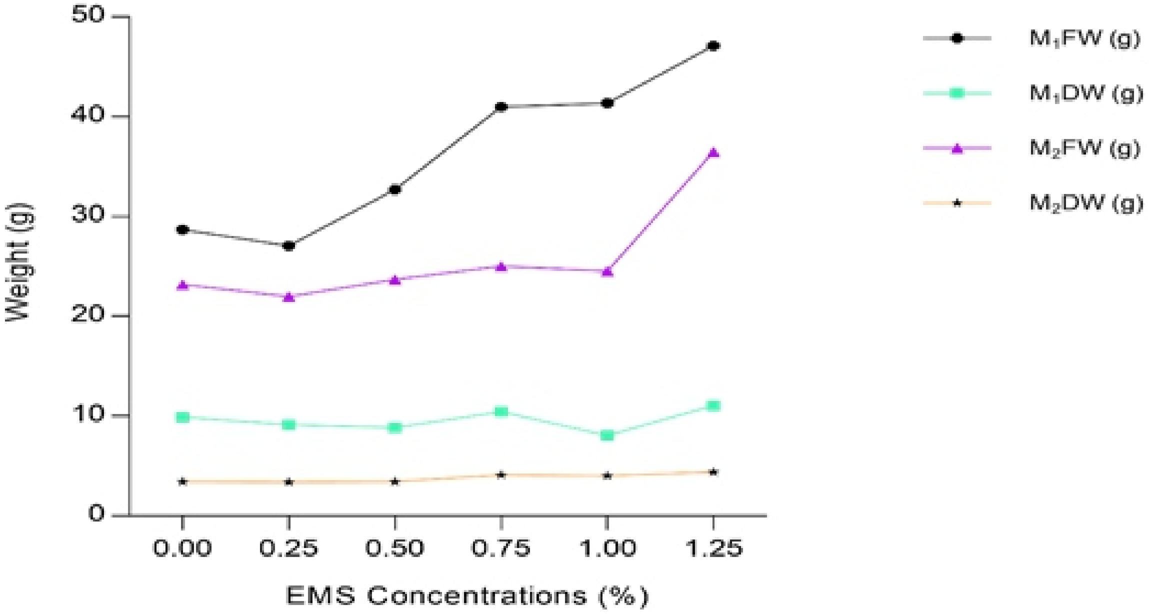

Figure 7: Fresh and Dry Matters of First (M_1) and Second (M_2) Generation of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate (EMS)

The result of the proximate analysis of the M_1 and M_2 seeds of groundnut (Samnut 24 vr.) treated with varying concentrations of EMS is shown in table 3. Higher concentrations (1.25%) produced seeds with higher moisture contents while other tested concentrations were not significantly different. The percentage ash compositions of the seeds were similar for all the treatments. The crude fat and protein content of seeds ranged from 43.73 - 45.85% and 34.44 - 37.71%, respectively with 0.75% and 1.00% EMS concentrations significantly favouring crude fat and protein in the groundnut at both M_1 and M_2. In term of fibre content, the responses of the groundnut to EMS concentrations for both generations were not statistically different. The percentage carbohydrate present in the seeds varied with higher concentrations producing significantly low amount of carbohydrate at 0.00% and mild dose of 0.25% EMS.

Table 3: Proximate Analysis of M_1 and M_2 Seeds of Groundnut (Samnut 24 vr.) Treated with Different Concentrations of Ethyl Methanesulphonate (EMS)

	Conc. (%)	%Moisture	%Ash	%Crude lipid	%Crude protein	%Crude fiber	%Carbohydrate
M_1	0.00	6.82^{bc}	3.43^{ab}	43.72^{bc}	34.44^{cd}	3.15^{ab}	7.44^{b}
	0.25	6.44^{bc}	3.45^{ab}	42.89^{cd}	34.57^{bc}	3.10^{ab}	9.55^{a}
	0.50	6.18^{c}	3.40^{ab}	43.79^{bc}	35.41^{b}	3.13^{ab}	8.09^{ab}
	0.75	7.10^{ab}	3.43^{ab}	44.72^{ab}	37.47^{a}	3.15^{ab}	4.13^{d}
	1.00	6.85^{bc}	3.49^{ab}	45.75^{a}	37.97^{a}	3.05^{abc}	3.49^{de}
	1.25	7.18^{ab}	3.49^{ab}	44.43^{ab}	35.45^{b}	3.19^{ab}	6.26^{c}
M_2	0.00	6.99^{bc}	3.27^{ab}	43.67^{bc}	35.01^{b}	3.42^{a}	7.64^{b}
	0.25	6.23^{c}	3.97^{a}	44.76$_{ab}$	34.72^{bc}	3.46^{a}	6.86^{bc}
	0.50	6.01^{cd}	3.94^{a}	44.85^{ab}	35.63^{b}	3.01^{abc}	3.31^{e}
	0.75	6.43^{bc}	3.41^{ab}	45.86^{a}	37.53^{a}	3.16^{ab}	3.43^{e}
	1.00	7.63^{ab}	3.92^{a}	45.38^{a}	37.71^{a}	3.04^{ab}	3.32
	1.25	8.08^{a}	3.42^{ab}	43.97^{bc}	34.92^{bc}	3.12^{ab}	6.49^{bc}

M1: First generation; M2: second generation. Means with the same superscripts along the same column are significantly the same at $p \leqslant 0.05$.

Table 4: Correlations and Association of Characters in Groundnut Treated with Different Concentrations of Ethyl Methanesulphonate

	PH	NL	NB	LL	LB	NDF	NPP	NSP	PL	PC	SL	SC	100-SW
PH	1												
NL	0.486*	1											
NB	0.529*	0.881*	1										
LL	-0.559	0.353	-0.485	1									
LB	-0.559	0.353	-0.485	0.864**	1								
NDF	-0.200	-0.029	0.177	0.294	0.294	1							
NPP	0.086	0.828*	-0.412	0.471	0.471	-0.314	1						
NSP	0.143	0.600	0.147	0.647	0.647	0.543	0.257	1					
PL	-0.086	-0.714	0.647	-0.441	-0.441	-0.142	-0.657	0.371**	1				
PC	-0.943	-0.429	-0.530	0.471	0.471	-0.086	0.086	-0.314	0.143	1			
SL	-0.086	-0.429	-0.088	-0.088	-0.088	-0.600	-0.314	-0.257	0.429	0.143	1		
SC	0.086	-0.200	-0.235	-0.088	-0.088	-0.657	-0.143	-0.200	0.143	-0.029	0.943**	1	
100-SW	-0.086	0.543	-0.471	0.765	0.765	0.314	0.371	0.771*	-0.714	-0.086	0.629*	0.743*	1

PH: Plant height; NL: Number of leaves; NB: Number of branches; LL: Leaf length; LB: Leaf breadth; NDF: Number of days to flowering; NPP: Number of pod per plant; NSP: Number of seed per pod; PL: Pod length; PC: Pod circumference; SL: Seed length; SC: seed circumference; 100-SW: 100-seed weight

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Table 4 showed the associations that exist between the vegetative and yield characters of groundnut treated with different concentrations of EMS. Correlations of characters evaluated were poor and only few traits were in positive association. At $p < 0.05$, there was significant correlation between plant height and number of leaves, number of branches and number of leaves. Number of leaves also associated positively with number of pod per plant just as number of pod per plant and 100-seed weight had positive significant relationship. Furthermore, leaf length and leaf breath, pod length and number of seed per pod, seed length and seed circumference had significant associations at $p < 0.01$ while linkage and associations were poor among other traits.

DISCUSSIONS

Assessment of mutagenic component and determination of dosage effect of ethyl methanesulphonate (EMS) on growth, yield and nutrient fidelity of an elite groundnut variety (Samnut 24) was studied to underscore the possibility of the utility of the chemical for the crop improvement. The variety is preferred by farmers for its oil yield and protein content (Kamara, 2011). Germination percentage and speed of germination were adversely affected by increase in the concentrations of EMS. This present result corroborated earlier reports (Salim *et al.*, 2009; Benjavad *et al.*, 2012; Aparna *et al.*, 2013; Animasaun *et al.*, 2014) which elucidated that germination percentage and index are negatively proportional to higher mutagenic dose application. Kharade *et al.* (2015) reported that increase in the doses of gamma rays and EMS led to decrease in seedling dry weight, and the authors concluded that negative relationship existed between increased mutagenic doses, percentage germination and germination index. The reduction in germination index of seeds treated with high doses or concentrations of mutagen could be due to delay or inhibition of physiological and biological processes necessary for seed germination including enzyme activities (Birara *et al.* 2014). In addition, high concentration especially 1.25% EMS in the present study might have caused damage to cell constituents, molecules (Chowdhury and Tah, 2011) and growth regulators (Salim *et al.*, 2009). Krishnaswamy and Seshu (1989) opined that the rate of germination was positively correlated with oxygen uptake, dehydrogenase activity by providing energy to the germinating embryo and interfering with integrity and overall capacity of the metabolic machinery of the young germinating primordia of rice. It was inferred that, if such pathway is disrupted by mutation from mutagenic agents, germination and seedling vigour will be negatively affected.

Reduced seedling vigour due to impaired shoot formation has been reported for higher doses of mutagenic agents in crop plants (Borzouei *et al*., 2010; Lukanda *et al*., 2012; Aparna *et al*., 2013). In the opinion of Talame *et al*. (2008), mutagenic chemicals affects mitochondrial membrane potentials of plant, as such it has the ability to induce mutation of any kind in plant cells and organs, and even the amino acid sequence. However, in the present study, ability of concentration range of 0.75-1.00% EMS to induce better growth and higher biological yield compared to the 0.00% treatment showed the concentrations were able to activate optimally the phyto-hormones and growth regulators in the plant. The larger leaf area induced by the treatment translated to higher photosynthetic surface and ultimately better growth and higher biomass yield. Growth enhancement by application of moderate concentrations of EMS was demonstrated in tomatoes (Jabeen and Mirza, 2002), *Vigna* spp (Khan *et al*, 2004) and sodium azide in groundnut - Samnut 10 & 20 (Animasaun *et al*.. 2014). In another study, Bashir *et al*. (2013) showed that EMS improved the biological process and growth performance of *Moschatus* species and the present results concurred with the earlier reports.

In term of seed characters, EMS concentrations favoured early flowering with concentrations between 0.75 - 1.00% having optimal effect on early maturity across the generations. In a similar study, Mensah and Obadoni (2007) observed that certain concentration of sodium azide induced early maturity in groundnut while Adamu *et al*. (2005) demonstrated the utility of chemical mutagen in the development of early maturing mutants in tomato. Onemli (2005) opined that EMS induced early flowering by activation of flowering hormones. This suggests that application of chemical mutagen at optimum concentration positively affect the gene switch mechanism from vegetative to reproductive phases of development. Early maturity is a desirable trait in plant breeding, more so in the recent time when the world climate trend is of concern. There is dire need for development of early maturing varieties if food security must be achieved. Therefore, further screening and selection of early maturing mutants would be of tremendous advantage. It is suffice to say that mutagenesis could bring about development of early maturing varieties of crop. But unfortunately, early maturity may not necessarily translate to higher number of pod per plant as observed in the present result. In comparison with the control (0.00% EMS treatment), other tested concentrations produced fewer number of pods per plant. In fact, the number of pods per plant was indirectly proportional to EMS concentration. Interestingly, heavier seeds were associated with higher concentrations across the generations suggesting that though fewer numbers of pods per plant were obtained with higher concentrations, seed sizes were larger. Nevertheless, pod and seed characters are inheritable in groundnut (Mensah and Obadoni, 2007) indicating the possibility of combining the two traits in a single variety through crossbreeding experiment for improvement pod and seed yield.

The proximate analysis revealed that crude fat and protein which are the most valuable nutritional components of groundnut for both human and animal were improved by EMS in Samnut 24 vr. These two nutrition components are major consideration for varietal adoption (Musa *et al*., 2010). Although, Samnut 24 vr. was developed for higher protein content (Janila *et al*., 2013), application of EMS in concentrations range of 0.75 -1.00% v/v further increased the nutrient components. While this is true for crude fat and protein, the ash content which indicated the proportion of mineral elements and crude fibre were not significantly affected in the mutant seeds. While all applied concentrations of EMS improved crude fat and protein composition of the groundnut, the result of the nutritional analysis indicated the trends for other parameters were concentration-dependent, which concurred with the view of Adamu *et al*. (2005). Consequently, there is need for screening the corresponding concentration for optimal result on a desired character. The present findings revealed that EMS concentrations between 0.75-1.00% v/v could be utilized for improvement of most of the studied parameters.

Positive linkage of characters was poor in the studied groundnut. The characters that correlated at $p < 0.05$ or $p < 0.01$ probability levels signify the existence of linkage, an indication that they could be improved together. Only few characters among the evaluated traits, in particular, the vegetative characters are linked. This implies the vegetative characters are independent and are not connected with other characters in the segregated progenies (Aliero and Morakinyo, 2002; Jabeen and Mirza, 2002). Hence, improvement of the unlinked traits can only be individually achieved while the synergistic relationship among the linked traits will ensure their joint improvement. Similar pattern of correlation was recorded in soybean (Malek *et al*., 2013). Poor correlations of phenotypic characters have been observed to occur in plants (Gohil *et al*., 2006; Micco *et al*., 2011; Ojo *et al*., 2012; Malek *et al*., 2013) and molecular techniques of hybridization may come to rescue in their breeding programme. The significant positive correlation between pod and seed size traits is consistent with earlier findings (Mensah *et al*., 2007; Bolbhat and Dhumal, 2012) and the trait can be simultaneously improved.

CONCLUSIONS

The present work showed the utility of 0.75-1.00% v/v concentrations of EMS to significantly improve growth performance, induce early maturity, production of heavier seeds and increase seed crude fat and protein contents. The response of the groundnut revealed that EMS is an effective chemical mutagen for improvement of Samnut 24, and that the optimal concentration lies between 0.75 and 1.00% v/v. Increase in nut size obtained in this study could be a good selling trait to the farmers who are still skeptical of adopting the variety due to its small nut size (compare to most other available elite varieties) despite the higher nutritional content. The revealed correlations between nut size related traits could also be explored for nut size improvement.

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AUTHORS' CONTRIBUTIONS

All the authors contributed equally to the study

CONFLICT OF INTEREST

The authors declare no conflict of interest whatsoever.

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