

Effect of Different Doses of Ethyl Methyl Sulfonate (EMS) on Agronomic Improvement in M₁ Generation of Three Pepper Varieties of *Capsicum chinense* Jacq. (Habanero Pepper).

Adaugo Gift Ibeh^{1*} and Kelechwu Chris Egbucha²

¹ Department of Biotechnology

Alex Ekweme Federal University Ndufu –Alike Ikwo Abakaliki, Ebonyi State, Nigeria.

² Department of plant science and Biotechnology

Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Abstract

Habanero pepper (*Capsicum chinense* Jacq.) is one of the bioactive plants, known for its pungency and pharmaceutical needs. It is a spicy fruit, used for its condiments but it plays a major role in pharmaceutical industries because of its chemical contents known as capsaicin and Dihydrocapsaicin, a phenolic compound derived from Capsaicinoid. *Capsicum chinense* research in terms of mutagenesis in Nigeria is still at a low level compared to other Solanaceae vegetables such as eggplants. Irrespective of its pharmaceutical and its numerous uses of habanero pepper. The plant has little mutagenic research attention, hence, needs for this study. Healthy and quality seeds of Habanero pepper were freshly extracted from the fruits and pre-soaked in distilled water for 3 hours. The pre-soaked seeds were soaked in freshly prepared EMS concentrations (0.0% as control, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%) for 5 hours. An experimental design was set in a screen house in a randomized complete block design (RCBD) with three replications. Data collections were made on 3, 6 and 9 weeks after transplanting (WAT). The measured parameters include; plant height, number of leaves, number of branches, stem girth, days to first flowering, number of fruits per plant, weight of fresh fruits and number of seeds per plant. The present study shows that the treatments significantly improved the growth and the yield characters of the *Capsicum chinense*. The analysis of variance showed there were significant differences at $P < 0.05$ between the means of the treated plants when compared with the control. The results of the present study have shown that EMS ranging from 0.1% to 0.5% treatments could be used by plant breeders to improve the growth and yield characters of this species. It is therefore recommended that plant breeders should utilize these techniques of applying lower concentrations of EMS on this species to create and produce more of this phenolic compound in the pharmaceutical world for the ever-increasing populations.

Keywords: Mutagenesis; ethyl methane sulphate; EMS; habanero pepper; growth; yield

Date of Submission: 06-12-2021

Date of Acceptance: 21-12-2021

I. Introduction

Habanero pepper (*Capsicum chinense* Jacq.) is annually grown as vegetable crops in tropical and subtropical regions and originated from Caribbean and Yucatan peninsula of Mexico. Habanero pepper belongs to the family of Solanaceae and genus of *Capsicum*. Chinese pepper like the datil pepper, Ajidulce (Ose Ibeku) and Habanero chile (Ose Nsukka) are typically pungent with powerful floral flavors and known for its ugliest shape with wrinkled skin and long, stinger-like bottoms¹. Its moisture content is within ranges reported for other commercial pepper varieties and is processed for its oleoresins and capsaicin for inclusion in many products¹¹. It is becoming increasingly popular among consumers, with industrial applications also rising worldwide to match with the rapid growth of population in the world, because of its nutritional values and medicinal needs around the world therefore, there is need to increase pepper production⁷. Average world production and cultivated area of dry, red, yellow and green peppers are estimated at 3.9, 4.0 and 34.5 million tons for about 60% of the global pepper production in 2016. Pepper is the most widely used spice and condiment in the world and is greatly priced for its pungency and adding special flavor to many cuisines throughout the world. It is rich in mineral quality, vitamin and antioxidant carotene lycopene content¹⁴. Cultivations of this plant have been reported in many countries like India, Portuguese, Europe, etc. down to West Africa like Nigeria especially in Northern Nigeria where datil pepper are grown and sold to other parts of the country and also to the Eastern Nigeria especially in Umuahia north, Abia State among the Ibeku people Ajidulce are known as Ose Ibeku

popularly cultivated for their native dishes because of its flavors and Habanero chile are known as OseNsukka popularly cultivated by Nsukka people as economic crop in Enugu state¹⁷. The major constraint in the development of improved varieties is the limited genetic variability results⁹. There are many techniques for breeding plants; mutation breeding is one of such technique applied for crop improvement.

Induced mutations have played a significant role in meeting challenges relating to the world food and nutritional security by way of mutant germ-plasm enhancement and utilization for the development of improved varieties in several crops.

Different mutagens are applied on seeds to enhanced its agronomic traits. Among them, are the alkylating agents known as ethyl methane sulphonate (EMS) a potent chemical mutagen used to induce mutational variability on plants to increase yield and ameliorate the malnutritional problem of the world. It is more effective than other chemical and physical mutagens¹⁹ usually induced point mutations and nucleotide substitution were nucleotide is been substitute by another nucleotide either transitional or transversional⁵. It is pertinent to mention that in spite of the numerous works that have been done on pepper plants, most of them are still understudied hence, need for this study.

II. Materials and Methods

The present study was carried out at the screen house of Michael Okpara University of Agriculture Umudike. Umudike lies on latitude 05° 29' N and longitude 07° 33' E (NRCRI Meteorological Reports¹³). In the rainforest area of the South-East agricultural zone of Nigeria. The area covers about 100,000 m² and lies about 8 to 10 kilometres East of Umuahia, the Abia State capital. It has a humid tropical climate with marked wet and dry seasons. The rainy season spans eight months (from March to October) and the dry season starts from November to February. The average annual rainfall for Umudike ranges from 1568.4 mm to 2601.3 mm within ten (10) years period. Three varieties of pepper (Datil pepper, Ajidulce locally known as Oselbeku and Habanero chile as OseNsukka) were collected locally as a fresh seed from oriugba market in umuahia north LGA, Abia State. For proper identification of the fruits at the taxonomic unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike.

Seed treatment

EMS preparation was done according to the method of Mbaet *al*¹². Healthy and quality seeds (100 seeds in each beakers) of Habanero peppers were freshly extracted from the fruits and pre-soaked in distilled water for 3 hours. The pre-soaked seeds were soaked in freshly prepared buffer 7 EMS concentrations at (0.1%, 0.3%, 0.5%, 0.7% and 0.9%) and 0.0% as control, for 5 hours with intermittent shaking. After the treatment time, the seeds were removed from the solutions and washed thoroughly in running tap water five times to remove the residual chemicals on the seeds. Sowing was done immediately to grow the M_1 generation. The experimental design was set up in a randomized complete block design (RCBD) with three replications. The plot size was 13 m x 43 m with a total of 36 pots bag. The planting space was 0.3 m x 0.7 m with a total of one plant per bag. Planting was done in May 2021 to raise the nursery plants then transplanted on June 2021. The matured plants were harvested in September 2021 as seeds of M_1 generation and was conserved for further studies. Data obtained from the growth and yield parameters were subjected to analysis of variance (ANOVA) for randomized complete block design (RCBD) using Genstat Twelfth edition software⁸. Significant differences observed were obtained at 5% level of probability.

III. Results

Effects of different concentrations of EMS on plant height (cm) : Table 1 shows the result of different EMS concentrations on the plant height at different weeks after transplanting. The result obtained in all the varieties for plant height at 3 WAT, 6 WAT and 9 WAT was significantly different between the mean of the treatments and the varieties when compared to the control differed significantly at $P < 0.05$. In Datil pepper among the treatment at 3 WAT and 6 WAT shows that 0.1% EMS treatment recorded maximum plant height (24.53 cm) and (41.83 cm) followed by 0.5% EMS treatment at 9 WAT (62.83 cm) while 0.9% EMS treatment recorded minimum plant height. In Oselbeku at 3 WAT, 6 WAT and 9 WAT among the treatments 0.1% EMS recorded maximum plant height with the mean values of 26.63 cm, 42.17 cm and 67.50 cm and the highest EMS dose (0.9%) recorded minimum plant height. Similarly data recorded in OseNsukka at 3 WAT, 6 WAT and 9 WAT 0.1% EMS treatment showed maximum plants height compared with control and highest EMS dose (0.9%) recorded the minimum plant height. The result obtained showed a concentration dependent effect in all the weeks of data. The analysis of variance showed that the treatments and varieties differs significantly ($P < 0.05$) (table 1).

Table 1: Effect of different concentrations of EMS on Plant height (cm)

Varieties	EMS conc. %	3 WAT	6 WAT	9WAT
DATIL PEPPER	0	20.33 ± 1.40	34.40 ± 1.80	50.10 ± 2.43
	0.1	24.53 ± 1.23	41.83 ± 2.17	56.50 ± 2.24
	0.3	17.80 ± 0.35	38.27 ± 1.59	55.20 ± 2.85
	0.5	20.03 ± 0.55	34.17 ± 0.19	62.83 ± 2.99
	0.7	15.30 ± 0.44	25.53 ± 0.08	49.67 ± 2.12
	0.9	14.40 ± 0.18	21.37 ± 0.06	35.73 ± 1.20
	Total	18.73	32.59	51.7
OSE IBEKU	0	22.83 ± 1.85	33.67 ± 1.29	62.17 ± 2.43
	0.1	26.63 ± 0.70	42.17 ± 2.80	67.50 ± 2.99
	0.3	23.53 ± 0.10	38.60 ± 1.18	64.00 ± 2.94
	0.5	16.63 ± 0.11	32.93 ± 1.06	56.50 ± 2.50
	0.7	16.70 ± 1.71	28.27 ± 1.47	52.67 ± 0.73
	0.9	14.40 ± 0.13	25.60 ± 1.88	39.00 ± 0.83
	Total	20.12	33.5	57.0
OSE NSUKKA	0	22.70 ± 0.95	33.70 ± 0.46	58.00 ± 1.45
	0.1	25.53 ± 0.12	35.70 ± 0.81	62.17 ± 2.83
	0.3	20.07 ± 0.25	32.37 ± 0.40	60.93 ± 2.58
	0.5	18.17 ± 0.22	27.30 ± 1.27	52.33 ± 2.14
	0.7	16.93 ± 0.30	24.50 ± 1.27	49.83 ± 2.43
	0.9	15.73 ± 0.53	22.33 ± 1.07	35.17 ± 0.75
	Total	19.86	29.32	53.1
	LSD _(0.05)	*	**	**

WAT= Weeks after transplant, *= significant, **= very significant.

Effects of different concentrations of EMS on number of leaves per plant : The mean number of leaves recorded at 3WAT, 6WAT and 9WAT on each of the three pepper varieties are shown in table 2. The result of the analysis of variance showed that there was significantly difference on the number of leaves at 3WAT and 6WAT. The mean separation using LSD (Least significant difference) showed that the EMS seeds were significantly different at $P < 0.05$. At 9WAT there was no significantly difference at $P > 0.05$. Among the treatments in Datil pepper 0.1% EMS treatment showed maximum number of leaves while 0.9% EMS treatment recorded minimum number of leaves in all the weeks after transplant. Among the treatments in OseIbeku 0.1% EMS treatment showed maximum number of leaves while 0.9% EMS treatment recorded minimum number of leaves in all the weeks after transplant. Also in OseNsukka 0.1% EMS treatment recorded maximum number of leave at 9WAT (65.67) followed by 0.3% EMS treatment (62.67) while 0.9% EMS treatment showed minimum number of leaves in all the weeks after transplant(Table 2)

Table 2: Effects of different concentrations of EMS on number of Leaves per plant.

Varieties	EMS conc. (%)	3 WAT	6 WAT	9WAT
Datil Pepper	0	11.33 ± 1.17	17.67 ± 1.84	50.00 ± 2.69
	0.1	20.00 ± 3.04	36.00 ± 3.36	69.67 ± 3.66
	0.3	17.67 ± 3.22	32.67 ± 3.07	61.67 ± 3.73
	0.5	18.00 ± 2.08	31.33 ± 3.18	64.00 ± 3.49
	0.7	14.00 ± 2.60	26.67 ± 3.02	52.00 ± 3.87
	0.9	11.00 ± 0.67	20.00 ± 1.76	44.33 ± 2.68
	Total	15.3	27.4	56.9
OseIbeku	0	12.00 ± 0.88	25.00 ± 2.31	61.33 ± 3.26
	0.1	14.67 ± 2.71	32.67 ± 4.30	73.67 ± 4.88
	0.3	16.67 ± 2.27	29.33 ± 3.98	70.67 ± 9.75
	0.5	15.00 ± 2.03	26.33 ± 4.19	58.67 ± 4.91
	0.7	11.67 ± 1.68	29.33 ± 3.86	53.00 ± 3.21
	0.9	11.67 ± 0.84	21.00 ± 1.76	41.00 ± 2.17
	Total	13.61	27.3	59.7
OseNsukka	0	16.67 ± 1.58	35.00 ± 2.79	59.33 ± 3.01
	0.1	19.00 ± 2.65	36.33 ± 3.34	65.67 ± 3.06
	0.3	21.67 ± 2.04	31.33 ± 2.29	62.67 ± 3.93
	0.5	12.67 ± 0.77	25.33 ± 3.75	48.33 ± 3.31
	0.7	11.33 ± 0.69	23.00 ± 1.07	45.67 ± 4.76
	0.9	10.67 ± 0.38	14.67 ± 1.07	27.67 ± 0.84
	Total	15.33	27.6	51.6
	LSD _(0.05)	**	**	NS

NS=not significant, **= very significant.

Effects of different concentrations of EMS on number of branches per plant : The results of analysis of variance of the effects of EMS concentration on the number of branches were shown in Table 3. The result showed that in at 3WAT and 6WAT there was no significant difference at $P > 0.05$. At 9WAT there was significantly difference at $P < 0.05$. Following the mean separations. Among the treatments, data obtained in datil pepper showed that 0.1% EMS treatment recorded maximum number of branches both secondary and primary branches in all the weeks after transplant while 0.9% EMS treatment showed minimum number of branches in all the weeks after transplant. The results in Oselbeku detected that 0.1% EMS treatment recorded maximum number of branches both secondary and primary branches in all the weeks after transplant while 0.9% EMS treatment showed minimum number of branches in all the weeks after transplant. In OseNsukka, Among the treatments 0.3% EMS treatment recorded the maximum number of branches at 9WAT (10.67) followed by the 0.1% EMS treatment with mean value of 10.00 while 0.9% EMS treatment showed minimum number of branches in all the weeks after transplant.

Table 3: Effects of different concentrations EMS on the number of Branches per plant.

Varieties	EMS conc. (%)	3 WAT	6 WAT	9WAT
DATIL PEPPER	0	1.00 ± 0.00	2.00 ± 0.58	8.33 ± 1.17
	0.1	1.33 ± 0.19	3.67 ± 0.84	11.00 ± 2.52
	0.3	1.33 ± 0.19	3.00 ± 0.58	8.33 ± 1.84
	0.5	1.33 ± 0.19	3.33 ± 0.19	10.33 ± 2.36
	0.7	1.00 ± 0.00	1.67 ± 0.19	7.33 ± 1.64
	0.9	1.00 ± 0.00	1.33 ± 0.19	6.33 ± 1.84
	Total	1.167	2.50	8.61
OSE IBEKU	0	1.00 ± 0.00	1.33 ± 0.19	8.00 ± 1.15
	0.1	1.33 ± 0.19	4.67 ± 1.17	13.33 ± 1.50
	0.3	1.00 ± 0.00	2.67 ± 0.51	11.33 ± 1.68
	0.5	1.00 ± 0.00	1.33 ± 0.19	8.33 ± 0.19
	0.7	1.00 ± 0.00	2.00 ± 0.58	6.67 ± 2.22
	0.9	1.33 ± 0.00	1.67 ± 0.19	4.67 ± 1.54
	Total	1.111	2.28	8.72
OSE NSUKKA	0	1.00 ± 0.00	3.33 ± 0.38	9.00 ± 0.67
	0.1	2.00 ± 0.58	4.67 ± 0.38	10.00 ± 1.20
	0.3	1.00 ± 0.00	3.00 ± 0.58	10.67 ± 0.38
	0.5	1.00 ± 0.00	2.00 ± 0.58	6.67 ± 0.77
	0.7	1.00 ± 0.00	2.00 ± 0.58	5.33 ± 1.39
	0.9	1.00 ± 0.00	1.00 ± 0.00	1.67 ± 0.19
	Total	1.17	2.67	7.22
	LSD _(0.05)	NS	NS	*

NS= not significant, *=significantly different.

Effects of different concentrations of EMS on stem girth (mm): The results of the analysis of variance on the stem girth derived from different concentrations of EMS on the three pepper varieties at 3WAT, 6WAT and 9WAT are shown in the table 4. The result obtained shows that at 3WAT there was no significantly different at $P > 0.05$. There was significantly difference at 6WAT and 9WAT ($P < 0.05$). The separations of means using least significant difference showed that at 6WAT and 9 WAT across the treatments differs significantly. In Datil pepper treatment 0.1% EMS measured maximum stem girth with a mean values of 0.27mm at 3WAT, 0.50mm at 6WAT and 0.87mm at 9WAT whereas treatment 0.9% EMS measured minimum stem girth with mean values of 0.13mm at 3WAT, 0.33mm at 6WAT and 0.60mm at 9WAT when compared with other treatment levels .The control (0.0%) however, measured 0.27mm at 3WAT, 0.40mm at 6WAT and 0.73 at 9WAT. In Oselbeku also, the separation of the means showed that the highest millimeter measured on the stem girth were observed on the lowest EMS dose (0.1%) with mean values (0.27mm, 0.50mm and 0.83) (table 4) when compared with other treatment levels and the least millimeter was measured on the highest EMS treatment (0.9%) with the mean values 0.13mm,0.33mm and 0.57mm respectively. In OseNsukka similar result was obtained at 0.1% EMS treatment which measured 0.30mm at 3WAT, 0.57mm at 6WAT and 0.90mm at 9WAT as maximum stem girth whereas treatment 0.9% EMS measured minimum stem girth with mean values of 0.17mm at 3WAT, 0.33mm at 6WAT and 0.57mm at 9WAT when compared with other treatment levels and the control(0.0%) however, measured 0.30mm at 3WAT, 0.47mm at 6WAT and 0.80 at 9WAT (table 4).

Table 4: Effects of different concentrations EMS on stem girth (mm).

Varieties	EMS conc. (%)	3 WAT	6 WAT	9WAT
DATIL PEPPER	0	0.27 ± 0.02	0.40 ± 0.03	0.73 ± 0.05
	0.1	0.27 ± 0.02	0.50 ± 0.03	0.87 ± 0.05
	0.3	0.27 ± 0.05	0.43 ± 0.05	0.77 ± 0.04
	0.5	0.23 ± 0.07	0.42 ± 0.05	0.73 ± 0.04
	0.7	0.17 ± 0.04	0.40 ± 0.04	0.70 ± 0.03
	0.9	0.13 ± 0.02	0.33 ± 0.04	0.60 ± 0.06
	Total	0.222	0.428	0.783
OSE IBEKU	0	0.27 ± 0.05	0.43 ± 0.02	0.70 ± 0.03
	0.1	0.27 ± 0.05	0.50 ± 0.06	0.83 ± 0.05
	0.3	0.20 ± 0.07	0.40 ± 0.07	0.80 ± 0.03
	0.5	0.13 ± 0.04	0.27 ± 0.02	0.70 ± 0.06
	0.7	0.13 ± 0.05	0.43 ± 0.05	0.70 ± 0.07
	0.9	0.13 ± 0.02	0.33 ± 0.02	0.57 ± 0.04
	Total	0.189	0.394	0.717
OSE NSUKKA	0	0.30 ± 0.07	0.47 ± 0.03	0.80 ± 0.03
	0.1	0.30 ± 0.00	0.57 ± 0.04	0.90 ± 0.03
	0.3	0.30 ± 0.03	0.50 ± 0.02	0.87 ± 0.02
	0.5	0.27 ± 0.02	0.47 ± 0.05	0.73 ± 0.04
	0.7	0.27 ± 0.02	0.47 ± 0.05	0.67 ± 0.04
	0.9	0.17 ± 0.02	0.33 ± 0.02	0.57 ± 0.02
	Total	0.267	0.467	0.756
LSD _(0.05)	NS	*	**	

NS= not significant, *=significantly different, **=very significant.

Effects of different concentrations of EMS on number of days to first flowering (days) : The result of the effects of different concentration of EMS on days to first flowering in the three pepper varieties. The results obtained on the pepper varieties revealed that the mutagen treatment enhanced early flowering when compared with the control plants. The present study shows that the lower concentration of the mutagen induce early flowering in the three pepper varieties. Observations across the three varieties of pepper plants during the stages of flowering showed that increase in the concentrations of the mutagen caused a gradual delay in flowering time of the plants. In Datil pepper the result of the analysis of variance on the number of days to first flowering showed that there was significant difference ($P < 0.05$) across treatment for number of days to first flowering in datil pepper (Table 5). Data recorded in datil pepper revealed that early flowering was induced by treatment 0.1% EMS (40.0days) and followed by 0.3% EMS (49.0days) when compared with the control plant (0.0%) which flowered under 64.1 days. In OseIbeku , there was also significantly difference at $p < 0.05$. Ose Ibeku, showed that the plants treated with 0.1% EMS flowered first (39.2days) followed by 0.3% and 0.5% EMS treatment (48.4days and 48.4days) when compared with the control (48.8days). In OseNsukka, the effect of the EMS dose on the number of days to first flowering were also significantly different at $p < 0.05$. OseNsukka followed the same trend. The results showed that the plants treated with 0.1% EMS flowered earlier (35.0days) followed by 0.3% EMS (39.5days). 0.9% EMS treatment showed no visible flower during this study .The control recorded 48.1days (table 5).

Table 5: Effects of different concentrations of EMS on number of days to first flowering (days).

EMS conc. (%)	DATIL PEPPER	OSE IBEKU	OSE NSUKKA
0	64.1	48.8	48.1
0.1	40.0	39.2	35.0
0.3	49.0	48.4	39.5
0.5	50.0	48.4	56.3
0.7	56.0	56.6	64.1
0.9	56.1	58.0	0
Total	35.2	33.5	26.9
LSD _(0.05)	*	*	*

*= significant

Effects of different concentration of EMS on the yield parameters of three habanero pepper: The results obtained from the yield characters of three habanero peppers showed variations on the parameters measured (table 6). Number of fruits of all the treated and untreated plants were counted. The results showed in number of fruits per plant observed that in habanero peppers there were highly significantly difference ($P \leq 0.01$). It was found that, variation was more in the number of fruits of the treated plant as compared with control plants. Maximum variance was observed in plants treated with 0.1% EMS dose (15.00) in Datil pepper and minimum

variation was observed in plant treated with 0.9% EMS dose (3.33). In OseIbeku, the maximum variance was observed in plant treated with 0.1% EMS dose (34.33) and minimum variation of 9.67 on EMS dose of 0.9%. In OseNsukka, similar results on the dose LSD was observed with the maximum variance of 25.67 on EMS dose 0.1% and minimum variance of 0.00 at the EMS dose of 0.9% which indicates no viable fruit was observed. The results observed on weight of fresh fruits (g) on the mutagen effects showed highly significantly difference ($P \leq 0.01$) across the varieties. Maximum variation was observed in plants treated with 0.1% EMS dose (68.33g) in Datil pepper and minimum variation was observed in plant treated with 0.9% EMS dose (25.00g) when been compared with the control. In OseIbeku, the maximum variation was observed in plant treated with 0.1% EMS dose (46.67g) and minimum variation of 22.33g on EMS dose of 0.9%. In OseNsukka, similar results on the dose LSD was observed with the maximum variance of 85.33g on EMS dose 0.1% and minimum variance of 0.00g at the EMS dose of 0.9% which indicates no fruit (Table 6). The results observed in number of seeds per plant showed highly significantly difference ($P \leq 0.01$). Treatment effects also were highly significantly ($P \leq 0.01$). Maximum variance was observed in plants treated with 0.1% EMS dose (99.33) in Datil pepper and minimum variation was observed in plant treated with 0.9% EMS dose (42.33) when been compared with the control. In OseIbeku, the maximum variance was observed in plant treated with 0.1% EMS dose (109.33) and minimum variation of 67.33 on EMS dose of 0.9%. In OseNsukka, similar results on this study was observed with the maximum variance of 104.67 on EMS dose 0.1% and minimum variance of 0.00 at the EMS dose of 0.9% which indicates no fruit.

Table 6: Effects of different of concentration EMS on the yield of Habanero pepper varieties.

Varieties	EMS conc. (%)	Number of fruit/plant	Weight of fresh fruits (g)	Number of seeds/plant
Datil pepper	0	6	0	0
	0.1	15	0	0
	0.3	10	6	0
	0.5	6	7	5
	0.9	3	3	0
OseIbeku	T o t a l	7	7	2
	0	12	0	0
	0.1	34	3	3
	0.3	23	3	3
	0.5	15	0	0
OseNsukka	0	7	1	6
	0	9	9	6
	T o t a l	17	7	7
	0	15	0	0
	0.1	25	6	7
	0.3	15	6	7
	0.5	11	6	7
	0	7	3	0
	0	9	0	0
	T o t a l	19	3	0
L S D (0 . 0 5)	*	*	*	*

*** = highly significant.

IV. Discussion

Effect of different concentrations of EMS on the growth and yield parameters of Habanero Pepper

Seeds improvements done by several authors using chemical mutagens especially EMS has yield great result over the years. Providing important traits for breeders to use in ever-increasing population. The result of this study showed that EMS treatments enhanced the growth parameters studied and its shows that the effects of EMS concentrations on the plant height are concentration –dependent in all the weeks observed. However, the concentration of 0.1% and 0.5% EMS treatment in Datil pepper increase the plant height when compared to other treatment levels and the control (0.0%). In OseIbeku and OseNsukka the lowest concentration of EMS (0.1%) increase the plant height when compared with the control and other treatments levels. This observation is in agreement with the report of Dhakshanamoorthy *et al.*⁶ in *Jatropha curcas* L. The result also agrees with the

findings of Alcantara *et al.*² on *Capsicum annum* L. seeds treated with EMS concentration. The decrease in the plant height with increase in EMS concentration observed in this study might be attributed to the inhibition or disturbances at the cellular as well as the physiological and cytological levels which probably resulted to reductions on the plant height. Mutagenesis improvement on plant height poses a positive effects for this particular pepper plant on this study, also resulted in the improvement of some growth and yield characters as a prove in this study. Plants with increase number of leaves were induced ranging from 0.1% to 0.5% EMS treatment respectively. Average increase in number of branches and stem girth was observed in the range of 0.1%, 0.3% and 0.5% EMS treatment because of its stimulatory effect of mutagens at lower concentrations. This observation is in line with that of the findings of Arisha *et al.*³ who reported on the physiological improvement of *Capsicum annum* L. induced in M₁ and M₂ generations. The morphological improvement across the treatment of this study showed that the lowest concentration of the mutagen activate the growth hormones responsible to enhance the morphological traits. Therefore the result of this study are in line with Jabeen and Mirza,¹⁰ Observations from this study shows significant different in most of the traits which indicated the existence of variation in the Habanero pepper varieties. This observation is in accordance with the reports of Sharamoet *et al.*¹⁶ on the treatment of barley seeds with same EMS concentration of this study. Improvement of the yield parameter is one of the long aged practice in mutagenesis and very beneficial to plant breeders. From the result of the present study its showed that EMS can be utilized to improve the yield characters of the pepper varieties studied. Increase in the number of fruits varies among the varieties. The positive effects on the yield could be that the mutagen enhances growth promoter's effects on the seeds optimum to the varieties at lowest concentrations. Observation of the earlier flowering time at 0.1% ranging to 0.3% EMS showed that EMS treatment enhances cytological effects among the varieties on this study. The result on the weight of fresh fruits shows variance at 0.1% and 0.3% EMS among the varieties. Findings on the weight of fresh fruits on this study was in agreement with the report of Badawi *et al.*⁴ on the effective use of EMS to improve genetic variability, growth characters and yield of potato. The findings of Zhang *et al.*²⁰ contradicted with this present study who reported the effects of EMS on morphological traits in *Cyperus esculentus* L. at higher concentrations (0.5%, 0.7% and 0.9%) and reported that 0.9% EMS treatment with 6hrs exposure time positively improved traits in agronomic characters and yield parameters. The findings of Zhang *et al.*²⁰ showed that 0.9% EMS increases and enhances the morphological and yield traits in *Cyperus esculentus*. The results of the number of seeds per plant differs increasingly among the lowest concentration (0.1% and 0.3%) and varies differently in varieties. The increase of the effects could be as a result of the stimulatory effects on the productive system of the varieties at the lowest dose of the mutagen. This report is in support with the work of Patilet *et al.*¹⁵ who reported on the seeds of chili pepper treated with 0.1%, 0.2% and 0.5% EMS and Dimethyl sulfate for 12 and 18hrs. The present study suggested that the use of EMS treatment ranging from 0.1 % to 0.5% EMS dose should be employed by the breeders to improve variability of *Capsicum chinense* in further studies.

V. Conclusion

The effect of a mutagen depends both on the concentration applied and on the varieties treated. Therefore, it is necessary to optimize the procedure to assure a high mutation frequency without compromising seed viability. This optimization is essential for EMS because a high concentration drastically reduces seed germination in multiple species. The results obtained from this study have moreover proved the potency of EMS in inducing mutation in pepper. Observation from growth and yield characters of this study shows that EMS treated Habanero pepper generated maximum variation in all the parameters. This could be that EMS stimulates growth hormones and activate the plant promoter genes at the cytological levels. Observations of the present study further, observed beneficial agronomic improvement and yield characters at 0.1% and 0.3% EMS treatments respectively. The stimulatory effect of EMS at a lower dose is due to the fact that mutagens at lower concentration stimulate enzymes and growth hormone responsible for growth, yield and fruit quality while higher concentration of mutagens had inhibitory effect on fruits quality. Hence, fruit yield and quality agronomic traits in *Capsicum chinense* can be improved significantly through induced mutagenesis by ethyl methane sulphonate at lower concentrations.

References

- [1]. Ahloowalia, B., Maluszynski, M. and Nichterlein, K. Global impact mutation derived varieties. *Euphytica*.2004,135(2): 187–204
- [2]. Alcantara, T. P., Bosland, P. W. and Smith, D. W. Ethyl Methane Sulfonate induced mutagenesis of *Capsicum annum*. *Journal of Hereditary*.1996,7:239–41
- [3]. Arisha, M. H., Shah, S. N., Gong, Z. H., Jing, H., Li, C. and Zhang, H. X. Ethyl methane sulphonate induced mutations in M₂ generations and physiological variations in M₁ generation of peppers (*Capsicum annum* L.). *Frontiers Plant Sciences*.2015,6:1-11.
- [4]. Badawi, M. A., Sahar, S.T., Al-Hamada, R. I. and Abdelaziz, M. E. Effects of Ethyl methane sulfonate (EMS) mutagen on genetic variability, growth characters and yield of potato. *Middle East Journal of Agricultural Research*.2015,4(4):1076-1087.
- [5]. Bhat, T. A., Khan, A. H. and Parveen, S. Comparative analysis of meiotic abnormalities induced by gamma rays, EMS and MMS in *Vicia faba* L. *Indian Journal of Botanical Society*.2009,84:45–48

- [6]. Dhakshnamoorthy, D., Sevaraj, R. and Chidambaram, A. Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. *Rom. J. Biol.-Plant Biol.* 2010, 55(2):261-266.
- [7]. Fattori, V., Hohmann, M. S. and Rossaneis, A.C. Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses. *Molecules*. 2016, 21(7): 844.
- [8]. Genstat. Genstat for Windows Discovery, 25th Edition, Lawes Agricultural Trust, Rothamsted Experimental Station 2009, VSN International Ltd.
- [9]. Irfaq, M. and Nawab, K. (2003). Effect of gamma irradiation on some morphological characteristics of three Wheat (*Triticum aestivum* L.) cultivars. *Asian Journal of Plant Sciences*. 2003, 2(3):999-1003.
- [10]. Jabeen, N. and Mirza, B. Ethyl methane sulfonate enhances genetic variability in *Capsicum annuum*. *International Journal of Agriculture and Biology*. 2004, 4: 425-8.
- [11]. Materska M, and Perucka I. Antioxidant Activity of the Main Phenolic Compound Isolated from Hot Pepper Fruit (*Capsicum annuum* L.). *Journal of Agriculture and Food Chemistry*, 2005, 53(5):1750-1756.
- [12]. Mba, C., Afza, R., Bado, S. and Jain, S. M. Induced mutagenesis in plants using physical and chemical agents. In: Davey, M.R., Anthony, P. (eds) *Plant cell culture: essential methods*. Chichester: John Wiley & Sons, Ltd. 2010, 111-130.
- [13]. National Root Crop Research Institute, (NRCRI, 1999). Annual Report on Meteorological Data. Umudike, Nigeria
- [14]. Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., Miah, G., Pawar S.S, Bharude N.V. and Sonone S.S. Chilies as food, spice and medicine: A perspective. *International Journal of Pharmacy and Biological Sciences*. 2011, 1(3):311-318.
- [15]. Patil, J.L., Meshran, L. D. and Nandanwar, R. S. Induced quantitative variation in economic characters by chemical mutagens in chili. *Journal of Soils and Crops*. 1997, 7:15-18.
- [16]. Sharamo, F. F., Shimelis, H., Olaolorun, M. B., Korir, H., Indetie, A. H. and Mashilo, J. Determining ethyl methane sulfonate mediated (EMS) mutagenesis protocol for inducing high biomass yield in fodder barley (*Hordeum vulgare* L.). *Australian Journal of Crop Science*. 2021, 15(7):983-989.
- [17]. The Royal Society. Reaping the benefits; science and substantial intensification of global agriculture. Accessed October 1, 2009. Research Councils, UK. 207043.
- [18]. Usman, M. Principle and application of plant mutagenesis in crop improvement: review. *Biotechnology & Biotechnological Equipment*. 2016, 30(1): 1-16.
- [19]. Wani, M. R., Khan, S. and Kozgar, M. Induced chlorophyll mutagenic attempts to create genetic variability in Basmati rice. *Journal of Plant Breeding and Crop Sciences*. 2012, 4: 101-105
- [20]. Zhang, J., Han, X., Yang, S., Qian, H., Li, X. and Ye, Y. Effects of different EMS solution concentration and time treatment on morphological traits of *Cyperus esculentus* L. *Creative Commons Attribution License*. 2020, 203:200-6.