RESEARCH ARTICLE

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Optimization of lethal dose (LD_{50}) and effect of chemical mutagen on yield characteristics and quality traits on three varieties of *Capsicum chinense* Jacq.

ABSTRACT
The popularity of Habanero pepper (*Capsicum chinense* Jacq.) among consumers is increasing, and the purpose of the study is well-founded, as the nutritional value and medicinal uses of these pepper varieties are essential and necessary to increase the production through induced mutagenesis. Thus, the development of ideotypes of peppers with high production potential is the main goal of the habanero variety improvement initiatives. The present study was conducted to determine the LD_{50} dose and yield parameters induced by EMS in three *Capsicum chinense* cultivars datil pepper, Aji dulce (Ose Ibeku) and Habanero chile (Ose Nsukka) under laboratory conditions. The study was conducted by exposing the seeds to different concentrations of EMS (0.1%, 0.3%, 0.5%, 0.7% and 0.9% v/v) for 5 hours of exposure, with water as the control. The effect of the EMS mutagen on germination and survival rates for the three varieties was reported, and the collected data was subjected to statistical analysis using linear regression, analysis of variance, and least significant difference analysis. The results obtained from the germination percentage show an inversely proportional relationship, expressed in a decrease in this indicator with increasing concentrations of the mutagen among the treated plants. The lethal dose (LD_{50}) was determined at EMS concentrations of 0.8%, 0.5% and 0.5% (v/v) and the optimal dose was determined for the three pepper varieties. The results of the analysis of variance in pepper varieties show that with the induced mutations with 0.1% EMS and 0.3% EMS, the yield characteristics in the M_1 generation of all three varieties increase.

Key words: Habanero pepper, ethyl methane sulphonate, lethal dose (LD_{50}), yield characters, Buffer 7, new cultivar

Introduction

Habanero pepper (*Capsicum chinense* Jacq.) is a vegetable crop native to the Caribbean and Yucatan peninsula of Mexico that is cultivated every year in tropical and subtropical climates. Habanero pepper belongs to the family of Solanaceae and the genus of *Capsicum*. Chinense peppers, which include the datil pepper, Aji dulce (Ose Ibeku), and Habanero chile (Ose Nsukka), are characterized by their intense and potent floral flavors. They are also infamous for their ugly form, which includes wrinkled skin and long, stinger-like bottoms. Its moisture content falls within the ranges documented for other commercial pepper types, and it undergoes processing to extract its capsaicin and oleoresins for use in a variety of goods (Ibeh & Egbucha, 2021). Pepper's popularity among consumers is growing, and its industrial applications are expanding globally to keep up with the world's population expansion, because of its nutritional values and medicinal needs around the world therefore, there is a need to increase pepper production.

An estimated 3.9, 4.0, and 34.5 million tons of dried red, yellow, and green peppers were produced globally on average in 2016, accounting for almost 60% of the total Habanero pepper production worldwide (Jankowicz-Cieslak & Till, 2016). Due to its intense flavor, pungency and widespread use in various cuisines across the globe, pepper is the most expensive spice and condiment globally. It has a high concentration of vitamins, minerals, and the antioxidant carotene (lycopene) (Ibeh & Egbucha, 2023; Fattori et al., 2016). Cultivations of this plant have been reported in many countries like Indian, Portuguese, etc. down to West Africa like Nigeria especially in Northern Nigeria where Datil pepper are grown and sold to other regions of the country, as well as Eastern Nigeria, particularly in Umuahia North, Abia

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State, where the Ibeku people cultivate Aji dulce, also known as Ose Ibeku, and Habanero chile, also known as Ose Nsukka, as an economic crop in Enugu State. Notwithstanding pepper's worldwide significance, habanero pepper yield and production are impacted by biotic (such as disease and pests) and abiotic (such as low soil fertility and drought) challenges. Thus, developing pepper ideotypes with high production potential and lethal dose sensitivity to the ideal dosage required for pepper plant cultivation is the primary objective of habanero pepper improvement initiatives.

According to Bado et al. (2015), chemical mutagens, such ethyl methane sulphonate (EMS), have been effectively applied to a variety of crops and are more effective than physical mutagens, like gamma radiation, at causing a higher frequency of crop trait mutations. A large-scale mutagenesis program must be started after mutagenesis and dosage fixation are optimized. In order to compare the effects of the mutagen on seeds treated under various conditions, the term "lethal dosage" refers to the fixation of dosage in mutagen that causes a 50% reduction in seed germination after the seeds are exposed to the mutagen for a specific period of time and under specific conditions (Manzila et al., 2020; Usharani et al., 2017). Variations in crop species, genotype, mutagen, and environmental factors during mutagenesis also affect LD_{50} values (Arjun et al., 2020; Lalitha et al., 2019). By enhancing mutant germplasm and using it to create superior varieties of various crops, induced mutations have been essential in addressing issues related to global food and nutritional security (Asif & Khalil, 2019). In this study, the optimal concentration of EMS suitable for mutation of pepper was examined to determine the fix dose suitable for three pepper accessions and to determine the optimal dose for improvement of some yield traits.

Materials and Methods

Experimental site and plant materials

The current investigation was conducted at Michael Okpara University of Agriculture Umudike's screen house. According to NRCRI Meteorological Reports, Umudike is located in latitude 05º 291 N and longitude 07º 331 E in Nigeria's South-East agricultural zone, in the rainforest. The region is roughly 100 000 square meters in size and is located 8-10 kilometers east of the capital of Abia State, Umuahia. It features distinct wet and dry seasons and a humid tropical climate. The dry season begins in November and lasts until February, whereas the rainy season lasts for eight months, from March to October. Over a ten-year period, Umudike's average yearly rainfall falls between 1568.4 mm and 2601.3 mm. At the Origba market in Umuahia North LGA, Abia State, three types of pepper - Datil pepper, Ajidulce, locally known as Ose Ibeku, and Habanero chile, locally known as Ose Nsukka - were gathered locally as fresh seeds (Figure 1). The taxonomic unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, examined the habanero pepper in order to properly identify the fruits.

Seed treatment

Seed treatment was done using three varieties of healthy and high-quality Habanero pepper seeds (100 seeds carefully labeled in each glass beaker of 500 ml) were freshly removed from the fruits and pre-soaked in distilled water for three hours in order to prepare the EMS in accordance with the methodology of Mba (2013). To guarantee consistency, the pre-soaked seeds were immersed in freshly made buffer 7 EMS concentrations at 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, and 0.0% as a control, for five hours while being shaken intermittently. Following the treatment period, the seeds were

a b c

Figure 1. *Freshly extracted and air dried a) Datil pepper seed b) Ose Ibeku (Ajidulce) and c) Ose nsukka (Habanero chile) seed from the fruits.*

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taken out of the solutions and given five thorough washes under running water to get rid of any remaining chemicals. For twenty-five minutes, the seeds were allowed to air dry. To produce the M_1 generation, seeds were sown right away. Three replications of a randomized complete block design (RCBD) were used to set up the experiment. The plot had a total of 54 pot bags and was 13 m by 43 m. The planting area was 0.3 m × 0.7 m, and each bag contained one plant weeks after transplant from the nursery plants (for the yield parameters). To prevent plant growth inhibition, the soil was a combination of sieved, analyzed loamy and sandy soil from the university botanical garden. The nursery plants were planted in May 2024 and transplanted in June 2024. In September 2024, the fully grown plants were harvested as M_1 generation seeds and stored for future research. ANOVA was performed for the randomized complete block design (RCBD) using Genstat Twelfth edition software 8 on data derived from the yield parameters (number of days to first flowering, number of fruits per plant, number of seeds per plant, and weight of fresh fruit). Differences that were significant were found at the 5% probability level.

Data Collection

Germination percentage

The sprouting percentage was calculated by counting the number of plants that germinated in each treatment 16 days after seeding. The calculation was based on the number of seeds that germinated divided by the total number of seeds that sowed. After that, it was computed using the formula below:

$$\text{Sprouting percentage (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

Survival percentage

To calculate the survival percentage, the number of plants that survived in each treatment was tallied 30 days following seeding. The calculation was based on the total number of survival plants divided by the total number of sowed seeds:

$$\text{Survival percentage (\%)} = \frac{\text{Total number of survival plants}}{\text{Total number of seeds sown}} \times 100$$

Lethal Dose LD_{50}

For LD_{50}, the number of plants that survived in each treatment was counted 20 and 30 days after seeding. In order to compute it, the total number of survived plants was divided by the total number of germinated seeds.

$$LD_{50}\ (\%) = \frac{\text{Total number of survival plants}}{\text{Total number of germinated seeds}} \times 100$$

Yield Parameter

Number of days to first flowering (NDFF)

This was determined by selecting three plants randomly from each treatment with a trace from the day of transplant to days to first flowering.

Number of fruits per plant (NFPP)

This was determined by counting and recording the fruits produced by three randomly selected plants (transplanted plants) in each plot.

Number of seeds per plant (NSPP)

This was determined by counting and recording the seeds produced by three randomly selected plants in each plot.

Weight of Fresh fruit (g) (WFF)

This was determined by selecting three plants per variety randomly from each treatment and using electrical weighing balance to weigh according to treatment and variety.

Results

Effects of different concentrations of EMS on the germination percentage

The effects of varying EMS concentrations on the germination rates of three different *Capsicum chinense* genotypes are displayed in Table 1. According to the study's findings, EMS had a detrimental effect on germination; the control seeds of the three varieties had the highest germination percentage (0.0%) at (datil pepper) 100%, 98% (Ose Ibeku) and 96.5% (Ose Nsukka) germination respectively. The 0.1% datil pepper treated seeds germination % with 93.2% success, followed by the least germinated % 0.9% (46.3). The treated seeds of 0.1% EMS in Ose Ibeku (Aji dulce) exhibited 72.7% germination and 0.9% EMS with least germinated % of 34.3%. The following germination rates were obtained in Ose Nsukka (Habanero, Chile): 0.1% EMS dose (74.3%) and 0.9% EMS dose (30%). The outcome showed an effect that was depending on concentration. According to the results, under equal conditions, the germination percentage gradually decreased as EMS concentrations increased. Mutagen concentrations had extremely significant ($p>0.05$) effects on the germination % of *Capsicum chinense* accession, according to analysis of variance, but the interaction was not statistically different ($p>0.05$), as indicated in Table 1.

Effect of different concentrations of EMS on the survival percentage

The survival percentage of Ose Ibeku, Ose Nsukka, and Datil pepper under varying doses (EMS concentrations) is displayed in Table 2. This percentage was computed by dividing the total number of seeds dispersed by the number of survival plants, and comparing the results to the control (non-

Table 1. *Effects of different concentrations of EMS on the germination (%) in three C. chinense varieties.*

EMS. conc. (%)	**Datil Pepper (Germination %)**	**Ose Ibeku (Aji dulce) (Germination %)**	**Ose Nsukka (Habanero chile) (Germination %)**
0.0	100	98	96.5
0.1	93.2	72.7	74.3
0.3	80.8	66.6	63.6
0.5	72.7	50	58.3
0.7	69.7	48.8	47.2
0.9	46.3	34.3	30
Total	80.92	68.07	54.15
$LSD_{(0.05)}$varieties	***	**	**
$LSD_{(0.05)}$Conc.	***	***	***
$LSD_{(0.05)}$Inter.	NS	NS	NS

Legend: NS=not significant,**=very significant,***=highly significant.

Table 2. *Effects of different concentrations of EMS on survival (%) of studied C. chinense varieties.*

EMS conc. (%)	**Datil Pepper (Survival, %)**	**Ose Ibeku (Aji dulce) (Survival, %)**	**Ose Nsukka (Habanero chile) (Survival, %)**
0.0	98.9	96	92.5
0.1	83.4	79.3	70.3
0.3	77	64.4	60.2
0.5	52.3	48.4	44.3
0.7	40.4	30.4	37.2
0.9	36.2	23.2	22.4
Total	48.9	46.07	44.15
$LSD_{(0.05)}$ varieties	***	***	***
$LSD_{(0.05)}$ Conc.	***	***	***
$LSD_{(0.05)}$ Inter.	***	***	**

Legend: NS=not significant, **=very significant, ***=highly significant.

treated) group. After 30 days of monitoring each variety's survival, the average percentage was noted. According to the study, a higher mutagen concentration led to a lower survival rate; thus, the three *capsicum chinense* varieties' seedling survival was reliant on the mutagen concentration. It was found that the seeds that were not treated (control 0.0%) had the highest survival rate among all of the treatment levels. The lowest mutagen concentration (0.1%) in datil pepper recorded 83.4% survival (%), compared to the control (non-treated) at 98.9% with the highest survival %. The results in Ose Ibeku (Aji dulce) showed a similar pattern, with 96% in control (0.0%) and 0.1%, 0.3%, 0.5%, 0.7%, and 0.9% as the other treatment levels. The maximum survival rate in Ose Nsukka (Habanero Chile) was 92.5% for control seeds (0.0%), whereas the survival percentages for treated seeds of 0.1% and 0.9% were 70.3% and 22.4%, respectively. According to the study, the minimum survival percentage for all varieties was 0.9% EMS concentration, and the survival percentage sharply decreased as the survival percentage reduced drastically with increase in mutagen concentration in all varieties. According to analysis of variance, the survival % varied significantly ($p>0.05$) depending on the concentrations, and there was a very significant interaction between the concentrations and varieties ($p>0.05$).

Effect of different concentrations of EMS on the lethal dose (LD_{50})

The effects of different concentrations of EMS on LD_{50} generally varies with plant species, the type and status of the plant material and the stages at which lethality was measured. LD_{50} was calculated in this study by dividing the total number of survival seeds by the total number of germinated seeds. Table 2 shows a general trend of a declining survival rate as dosage increased. Significant variations in LD_{50} were caused by the germination to dose outcomes, which varied depending on the varieties. The Ose Nsukka (Habanero chile) had the highest LD_{50} (0.965) determined by linear regression, which was significantly higher than the 0.945 and 0.939 determined for Datil pepper and Ose Ibeku (Aji dulce pepper), respectively (Figures 2, 3, 4). Similarly, EMS has different LD_{50} values dosage of each pepper variety for instance, Datil pepper's LD_{50} is attained at a higher concentration of 0.8% EMS treatment; therefore, an increase in EMS dose to 0.8% is required to reach LD_{50} with this varieties (Figure 2). The minimal lethal dose was seen in Ose

Ibeku (Aji dulce pepper) when the LD_{50} was recorded at 0.5% EMS treatment. This means that 50% of 100 Ose Ibeku seeds were recorded as survivors, while the remaining 50% resulted in mortality (Figure 3). The greatest lethal dose in the trial for 0.5% EMS therapy in Ose Nsukka (Habanero, Chile) was 58.3% (Figure 4).

Effects of different concentrations of EMS on the yield characters of three varieties of capsicum chinense during M1 generation.

The result of Table 3, illustrates the effect of different concentrations of EMS on yield parameters (Number of days to first flowering-NDFF, number of fruits per plant-NFPP, number of seeds per plant-NSPP and weight of fresh fruit-WFF) of *Capsicum chinense* accessions during M_1 generation. When comparing the three pepper varieties to the control, the results showed that the mutagen treatment accelerated early flowering. The lower concentrations of the mutagen (0.1% and 0.3% EMS treatment) caused early flowering in all three varieties, while the higher concentrations (0.5%, 0.7%, and 0.9% EMS treatment) caused a gradual delay in the flowering time of the plants across all varieties when compared to the control (0.0%).The number of days before first flowering varied significantly (P<0.05) between treatments and types, according to the analysis of variance results. Every plant, both treated and untreated, had its number of fruits tallied. It was discovered that the treated plant's fruit production varied more than that of the control plants. All cultivars showed minimum variances of 3.33, 9.67, and 0.00 at the EMS dose of 0.9%, indicating no viable fruit, while plants treated with 0.1% and 0.3% EMS dose showed the highest variance. A significant difference (p<0.01) was observed between the average number of fruits per plant and the EMS treatment at each variety when compared to the control. Each plant's number of seeds was counted for both treated and untreated plants. It was discovered that there was a greater variance in the treated plant's seed count when compared to control plants. The plants treated with 0.1% EMS dose showed the greatest variation across all kinds (99.33, 109.33, and 104.67), whereas the plants treated with 0.9% EMS dose showed the least variation across all varieties (42.33, 67.33, 0.00). Additionally, as compared to the control, there was a significant difference across the treatment, varieties, and interactions (p<0.01). Using an electronic weighing balance, the fresh fruit weights of all treated and untreated plants were determined. The weight of the fresh fruits from the treated plant varied more than that of the control plants, it was discovered. Variability was lowest in plants treated with 0.9% EMS dose (25.00, 22.33, 0.00) and highest in plants treated with 0.1% EMS dose (68.33, 46.67, 85.33) among the varieties. When compared to the control, there was also a highly significant difference (p<0.01) across the treatment, variety, and interaction.

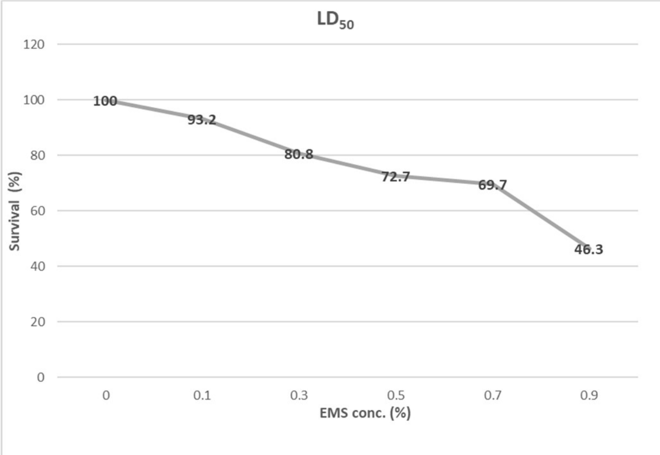

Figure 2. *Effect of different concentrations of EMS on the LD_{50} of Datil pepper.*

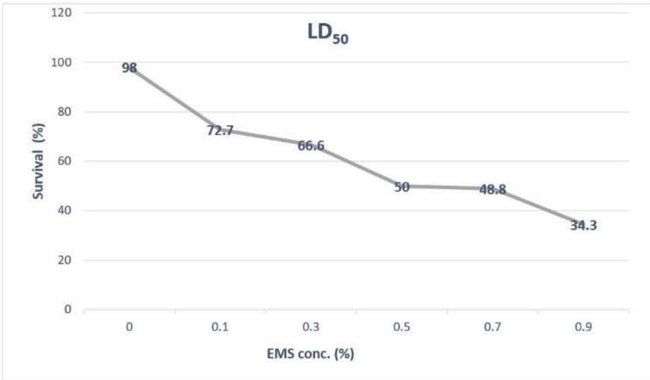

Figure 3. *Effect of different concentrations of EMS on the LD_{50} of Ose Ibeku (Aji dulce pepper).*

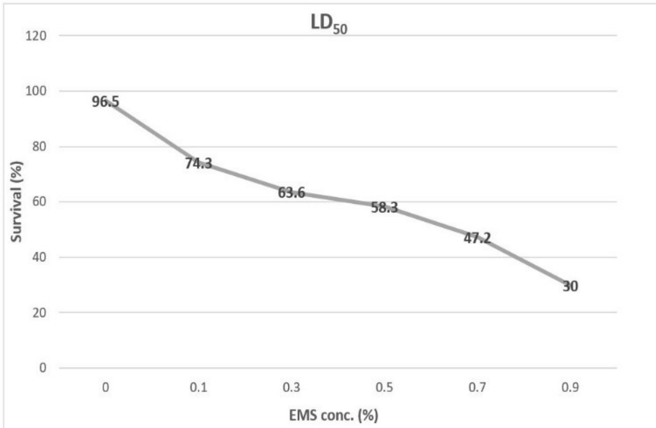

Figure 4. *Effect of different concentrations of EMS on the LD_{50} of Ose Nsukka (Habanero chile).*

Table 3. *Effects of different concentrations of EMS on the yield characters of three varieties Capsicum chinense during M_1 generation.*

Accession	EMSconc. (%)	NDFF	NFPP	NSPP	WFF
Datil Pepper	0.0	64.1	6.00	80.00	43.33
	0.1	40.0	15.00	99.33	68.33
	0.3	49.0	10.67	90.67	55.00
	0.5	50.0	6.67	55.67	44.67
	0.7	56.0	4.67	45.33	33.33
	0.9	56.1	3.33	42.33	25.00
	Total	35.2	7.72	68.9	44.9
Ose Ibeku (Aji dulce)	0.0	48.8	12.00	102.00	25.00
	0.1	39.2	34.33	109.33	46.67
	0.3	48.4	23.33	94.33	37.67
	0.5	48.4	15.00	87.33	29.00
	0.7	56.6	11.67	83.67	25.67
	0.9	58.0	9.67	67.33	22.33
	Total	33.5	17.7	90.5	31.06
Ose Nsukka (Habanero chile)	0.0	48.0	15.00	95.67	55.00
	0.1	35.0	25.67	104.67	85.33
	0.3	39.0	15.67	97.67	55.67
	0.5	56.3	11.67	83.33	42.00
	0.7	64.1	6.33	59.00	31.00
	0.9	0.00	0.00	0.00	0.00
	Total	26.9	9.30	72.7	50.2
LSD(0.05)varieties.		**	***	***	***
LSD(0.05)Conc.		**	***	***	***
LSD(0.05)Inter.		*	***	***	***

Legend: NS=not significant,*=significant,**=very significant,***=highly significant, NDFF=Number of days to first flowering, NFPP=Number of fruit per plant, NSPP=number of seeds per plant, WFF=weight of fresh fruit.

Discussion

EMS mutagenesis is a successful method for introducing genetic variation into plant populations. In this investigation, we treated three different varieties of *Capsicum chinense* with 0.1%, 0.3%, 0.5%, 0.7%, and 0.9% EMS. We then measured the yield of M_1 generation, the fatal dosage, the germination rate, and the survival rate. There have been reports of a concentration-dependent effect of chemical mutagens on seed germination. The findings of this investigation showed that when the amounts of mutagens increased, the amount of EMS treatment that was applied to the seeds of the three pepper varieties (Datil pepper, Ose Ibeku, and Ose Nsukka) decreased. The findings indicated that the quantities of mutagens had an inhibitory effect on seed germination, especially at higher dosages. In two pigeon pea accessions treated with varying dosages of EMS, Omosun et al. (2021) showed similar inhibitory effects on seed germination, resulting in a drop in germination and survival %. Similar findings of Kim et al. (2006) caused EMS in Arabidopsis, Shah et al. (2015) in barley, and Banjare et al. (2017) in garlic. The findings of this study are consistent with those of Jabeen & Mirza (2004), who used EMS to increase genetic variety in *Capsicum annum* L. This study's findings were consistent with those of Bind & Dwivedi (2014), who found that cowpea treated with gamma and ethyl methane sulphonate mutagens had a lower survival rate.

The LD_{50} of this study varies generally with plant species, the type, status of the plant material, and the level at which lethality is recorded on the mortality curves, as shown in this study. Lethal dosage in this study was obtained based on the germination percentage and survival percentage observed at the increase in mortality rate with an increase in chemical mutagen concentrations. From the observation of this study, the mortality increased with increase in mutagen concentrations and its support the curves mortality of the mutagens in this study and shows the effects of EMS on LD_{50} as it varies within the three pepper varieties. This study supported the findings of Yadav et al. (2016), who found that LD_{50} values vary depending on crop species, varieties, other plant components, seeds, treatment types, growing techniques, cultural customs, and other aspects. The results of Talebi et al. (2012), who documented the LD_{25} and LD_{50} values based on the growth reduction of seedlings following EMS treatment with 0.25% and 0.50% on the rice variety, are also noteworthy. According to Sharamo et al. (2021), who reported LD_{50} values of 0.64% in barley treated with varying concentrations of EMS, the lethality of these pepper cultivars and the differences in mutagen concentrations revealed in our investigation are in excellent accord.

Plant breeders greatly benefit from the long-standing practice of improving the yield parameter in mutagenesis. The findings of this investigation demonstrated that EMS can be applied to enhance the yield characteristics of the pepper cultivars under investigation. Each variety has a different rate of fruit growth. The mutagen may improve the effects of growth promoters on seeds, especially in the kinds with the lowest amounts, which could have a good influence on yield. Among the cultivars in this study, EMS treatment increases cytological effects, as seen by the earlier flowering period at 0.1% range to 0.3% EMS. The results of the quantity of seeds per plant vary by variety and are different for the lowest concentrations (0.1% and 0.3%). The stimulatory effects of the mutagen at the lowest dose on the productive system of the variety may be the cause of the increased effects. This study supports the findings of Patil et al. (1997), who examined chili pepper seeds treated with 0.1%, 0.2%, and 0.5% EMS and dimethyl sulfate for 12 and 18 hours. According to the fresh fruit weight results, there is a 0.1% and 0.3% EMS variation amongst the cultivars. The results of this investigation on the weight of fresh fruits were consistent with the findings of Badawi et al. (2015) regarding the successful application of EMS to enhance potato yield, growth characteristics, and genetic variability. The results of this study, which examined the effects of EMS on morphological features in *Cyperus esculentus L.* at higher concentrations (0.5%, 0.7%, and 0.9%), were in conflict with those of Zhang et al. (2020), who found that 0.9% EMS treatment with 6 hours exposure time positively improved traits in agronomic characters and yield. The findings of Zhang et al.'s (2020) research revealed that 0.9% EMS improves and amplifies *Cyperus esculentus*'s morphological and yield characteristics. The current study recommended that in order to increase the variety of *Capsicum chinense* in subsequent research, breeders should utilize EMS treatment at doses ranging from 0.1% to 0.5%.

Conclusion

One of the most potent and efficient chemical mutagens used in plant biotechnology to create genetic diversity and new cultivars is ethyl methane sulphonate (EMS). Mutagenesis aims to provide a specific and effective dose of mutagen concentration that can be used to mutate various plant material accessions for agronomic and yield enhancement. In order to ensure a high mutation frequency without compromising seed viability, the process must be optimized. Because a high concentration significantly lowers seed germination in several species, this adjustment is crucial for EMS. The study's findings also showed how effective EMS is at causing pepper to mutate. The findings demonstrated that the germination rate of the plants was considerably impacted by mutagen treatments. The study's observations of morphological characteristics and yield attributes demonstrate that the pepper plants treated with EMS produced the greatest diversity across all metrics. Because mutagens at lower concentrations activate the enzymes and growth hormones that are crucial for yield and fruit quality, EMS has a stimulatory effect at lower doses, whereas greater concentrations of mutagens have an inhibitory effect on fruit improvement. Thus, ethyl methane sulphonate-induced mutagenesis at lower doses can greatly enhance fruit output and quality agronomic parameters in *Capsicum chinense*.

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