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ORIGINAL RESEARCH ARTICLE

Evaluating the Lethal Dose (LD_{50}) and Mutagenic Effects of Sodium Azide on Germination and Quality Traits in Two Varieties of Tomato (*Solanum lycopersicum*)

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ABSTRACT
Sodium azide is a chemical mutagen that has been used to produce resistance in various susceptible crops to improve their yield and quality traits against harmful pathogens. The lethal dose and mutagenic effects of sodium azide on the germination percentage of two varieties of tomatoes (Beefsteak and San Marzano) were investigated with the aim of optimizing a suitable mutagen concentration with variability that could be exploited in the improvement of quality traits in Tomato plants. This study was carried out in the Green House at the Department of Biotechnology, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State (AE-FUNAI). The two varieties of tomato seeds were collected locally as fresh seeds from the taxonomic department and were treated with sodium azide at different concentrations of 0.0%, 0.2%, 0.4%, and 0.6% w/v. The seed was sown and monitored for 30 days with everyday documentation of germination percentage, survival percentage, and lethal dose. Statistical analysis using significant difference (LSD) at 95% probability level was employed to analyze the effects. The results obtained from this study show that there is a steady decrease in germination and survival percentage with increased concentration of sodium azide in both varieties of tomatoes when compared with the control. The lethal dose (LD_{50}) was determined upon germination and survival of the tomato varieties. The highest LD_{50} was calculated by linear regression to be 0.950 for the beefsteak variety, which was significantly higher than 0.794 for the san marzano variety at 50% germination was recorded under treatment 0.6% NaN3. These concentrations are therefore considered as the LD_{50} values. Sodium azide via mutation at low concentrations improves some important quality traits of tomatoes.

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INTRODUCTION

The tomato (*Solanum lycopersicum*) is regarded as one of the most valuable horticultural crops due to its high nutritional content and sensory appeal, in addition to its economic significance. Usually, it takes the form of consumable fresh and processed goods. The tomato is a tropical annual fruit and vegetable that has its origins in South America (Abdullah *et al.*, 2020). Today, thousands of cultivars with a variety of fruit kinds and ideal development circumstances under various growing environments are selected for their edible fruits, which are grown all over the world (Cherie, 2010; Hartz, 2020). Named for its worst shape with wrinkled skin (beefsteaks) and huge steaks, pointy shape, tomato plants, including the San Marzano and Beefsteak, are often meaty texture vegetable fruits with sweet flavor usually for hamburger toppings, pizza toppings, sandwich toppings, canning, and marinara sauce. In the Campania region of Italy, the little hamlet of San Marzano sul Sarno is the birthplace of San Marzano, although beefsteaks are native to South America and have since spread around the world, particularly in West Africa (Ghana and Nigeria). It is anticipated that this will enable the crop to be cultivated in more places. On the other hand, there is a great deal of unrealized potential for African tomato production to increase both in terms of quantity and quality (Chen *et al.*, 2015). As climate patterns become increasingly unpredictable, it is necessary to regularly produce resistant and adaptable types in order to ensure sustained output.

In order to create genetic variety in breeding programs, an induced mutation is essential. Spontaneous mutations occur seldom, and the resulting changes are insufficient to be useful in breeding. Thus, chemical or physical mutagens are used to create artificial mutations

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(Chaudhary *et al.*, 2019). Induced mutations are a significant factor in improving global food security, as emphasized by Kharkwal and Shu (2010). Various mutagens are used on seeds to improve their agronomic characteristics. One such mutagen is sodium azide (NaN_3), a strong chemical that is known to cause point mutations, transversion, and the A-T > G.C base pair transition (Gruszka *et al.*, 2012; Sadiq and Owais, 2000). When the ideal dose of a chemical mutagen for a particular crop or target tissue has not previously been determined, a dose-response curve must be created. Therefore, the purpose of this work is to determine the sodium azide concentrations required for inducing mutations in two tomato accessions found in Eastern Nigeria, using varying sodium azide concentrations.

MATERIALS AND METHOD

Study Area

In Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, the current study was conducted (AE-FUNAI). Situated in the southeast region of Nigeria, Ebonyi state is roughly located between latitudes 5/40' and 6/45'N and longitudes 7/30' and 8/28'E. The climate in Ebonyi is tropical, either savanna-like or wet and dry. Averaging 29.34°C (84.81°F), the city's annual temperature is -0.12% colder than that of Nigeria. On average, Ebonyi experiences 210.46 wet days (57.66% of the time) and 206.97 millimeters (8.15 inches) of precipitation annually.

Collection of Plant Materials

Fresh tomato seeds from the taxonomic department in Abakaliki, Ebonyi State, were collected locally, and the seeds—beefsteak and san marzano—were identified at the taxonomic unit. At the Department of Biotechnology and Microbiology's shared laboratory at Alex Ekwueme Federal University in Ndufu, Ikwo, the fruits and vegetable seeds were dried at room temperature for optimal induction.

Seed Treatment

A pH 3 buffer was used to dilute the sodium azide solution in distilled water. For each of the two tomato kinds, a total of 400 sound seeds were chosen. Four groups of one hundred seeds each were added to a 250 ml beaker. For the two types, solutions were made in six levels (0.0, 0.2, 0.4, and 0.6% w/v). In this investigation, the control was limited to water adjustments. The method utilized to determine these concentrations was taken from the report by Eze and Dambo (2015). It involved measuring out 0.2%, 0.4%, and 0.6% of the salt and dissolving it in 1000 ml of distilled water to create the solution, while the 0.0% solution consisted of 1000 ml of distilled water that had been pre-soaked in distilled water for three hours. After the seeds were pre-soaked, they were soaked for five hours with sporadic shaking to guarantee homogeneity in freshly made buffer 3 sodium azide concentrations of 0.2%, 0.4%, and 0.6% w/v and 0.0% as control. Following the prescribed treatment period, the seeds were extracted from the solutions and properly cleaned under running tap water five times to eliminate any remaining chemicals. Following this, the mutated seeds were promptly planted in a polybag at the greenhouse to serve as nursery seeds.

Data collection

Germination percentage: Over the course of 30 days, germination was seen in all treatments. For 30 days, the number of plants that germinated in each treatment was counted every 30 days to determine the germination percentage. The computation involved dividing the total number of sowed seeds by the number of seeds that germinated. The formula below was utilized to determine the germination percentage:

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

Survival %: At 30 days following sowing, the number of plants that survived in each treatment was counted to determine the survival percentage. It was determined by multiplying the total number of sowed seeds by the total number of plants that survived.

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

Lethal Dose (LD_{50}): To determine the LD_{50}, the number of plants that survived in each treatment was tallied 30 days following seeding. It was determined by dividing the total number of seeds that germinated by the total number of seeds that survived.

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

Statistical analysis

The data collected was analysed using Statistical Analysis Software (SAS version 9.1) while means were separated using least significant difference (LSD) at 95% probability level.

RESULTS

Effects of Different Concentrations of Sodium Azide on Germination Percentage of Tomato

Table 1 shows the outcome of sodium azide's effects on the two tomato types' germination percentages (Beefsteak and San marzano). The results of the study demonstrate that the two tomato varieties' germination percentages were impacted by the mutagen concentrations, resulting in a progressive decline in germination with every increase in mutagen concentration. According to data, the germination rate of the San Marzano variety ranged from 83.5% to 67.5%. In the control group, the highest germination percentage of 96.0% was recorded. The lowest germination percentage was recorded in the greatest concentration of 0.6% counted 67.5% germination, and treatment 0.2% sodium azide counted 83%, followed by 0.4% (78.5%). According to the results, the Beefsteak variety's germination percentage ranged from 71% to 55%. In the control group, the highest germination percentage of 98.0% was recorded. The lowest germination percentage was recorded in the greatest concentration of 0.6%, counted 55% germination, and treatment 0.2% sodium azide, which was 71%, followed by 0.4% (65%). Table 1 illustrates the results of an analysis of variance that indicated the two tomato varieties' germination percentages were very significant ($p>0.05$), to the interaction was not substantially different ($p>0.05$).

Table 1: Effects of different concentrations of sodium azide on the germination percentage of tomato plants.

Sodium Azide Treatment (%)	Germination percentage (%)	
	San Marzano	Beefsteak
0.0	96	98
0.2	83	71
0.4	78.5	65
0.6	67.5	55
Total	**1.05**	**3.28**
LSD(0.05) Acc	***	***
LSD(0.05) Conc.	***	***
LSD(0.05) inter.	NS	NS

NS = not significant, *** = highly significant., Acc = Accession

Effects of Different Concentrations of Sodium Azide on Survival Rate of Tomato

The result of the effects of different concentrations of sodium azide on the survival rate of the two varieties is presented in Figure 1 and Figure 2. A steady decrease in survival percentage was observed with increased concentrations of sodium azide in both varieties of tomatoes. The untreated San Marzano variety had the highest percentage of seedling survival at 81%, followed closely by 0.2% NaN3 concentration (77%), 0.4% NaN3 concentration (72.5%), and least 0.6% NaN3 concentration (45%). A similar effect was seen in the beefsteak variety. The untreated beefsteak variety had the highest percentage seedling survival of 84%, followed closely by 0.2% NaN3 concentration (68%), 0.4% NaN3 concentration (62%), and least 0.6% NaN3 concentration (55%) survival percentage.

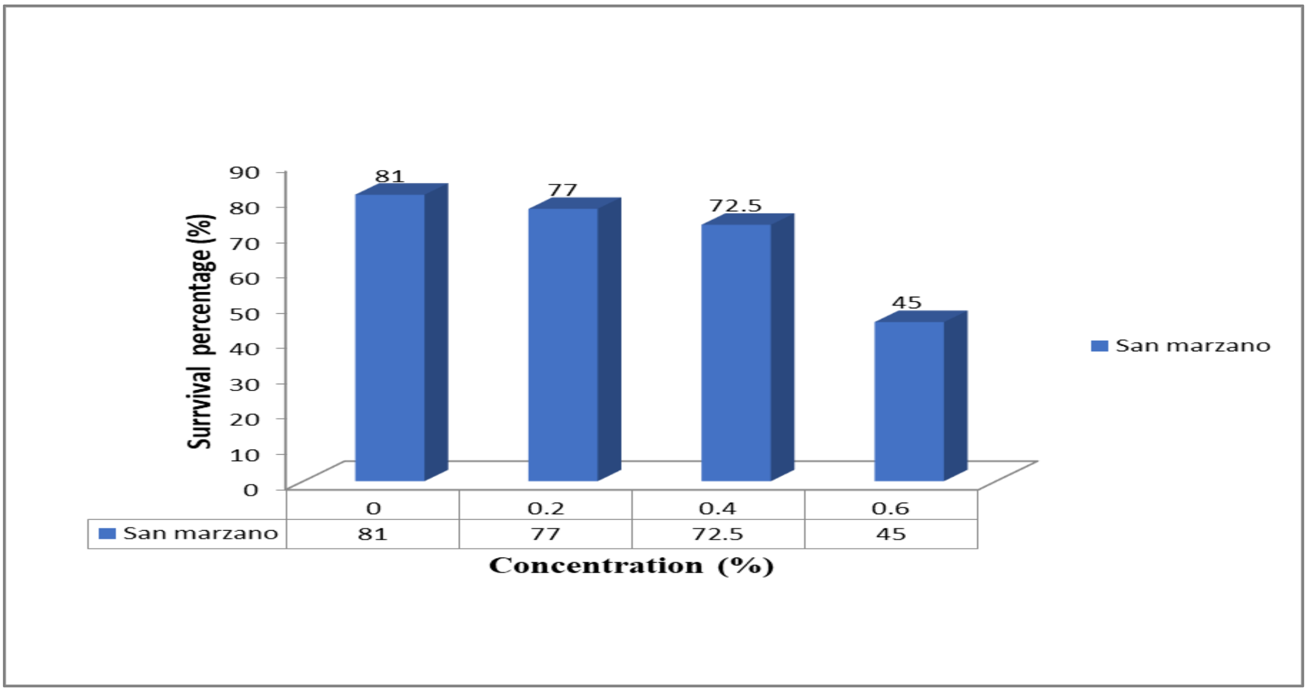

Figure 1: Effects of different concentrations of sodium azide on the survival percentage in San Marzano.

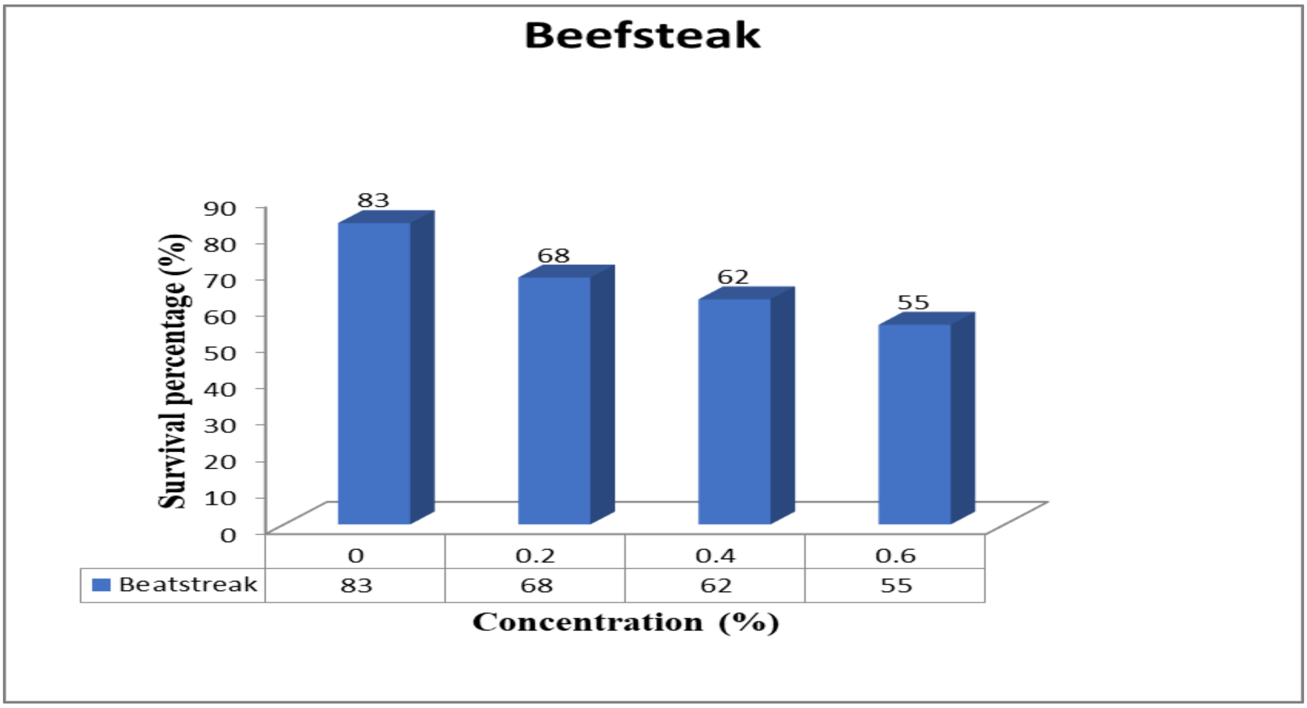

Figure 2: Effects of different concentrations of sodium azide on the survival percentage of Beefsteak.

Lethal dosage of sodium azide in the two varieties of tomatoes.

The LD_{50} was obtained under sodium azide dosage and survival percentage. There was a general trend of decrease in survival percentage with increase in dosage. However, the result of survival percentages to dosage was specific for each species, resulting in significant difference in LD_{50}. The highest LD_{50} was calculated by linear regression to be 0.950 for the beefsteak variety, which was significantly higher than 0.794 for the San Marzano variety. Therefore, 50% lethality was recorded at 0.6% concentration for the San Marzano variety and 0.7% sodium azide concentration for the beefsteak variety.

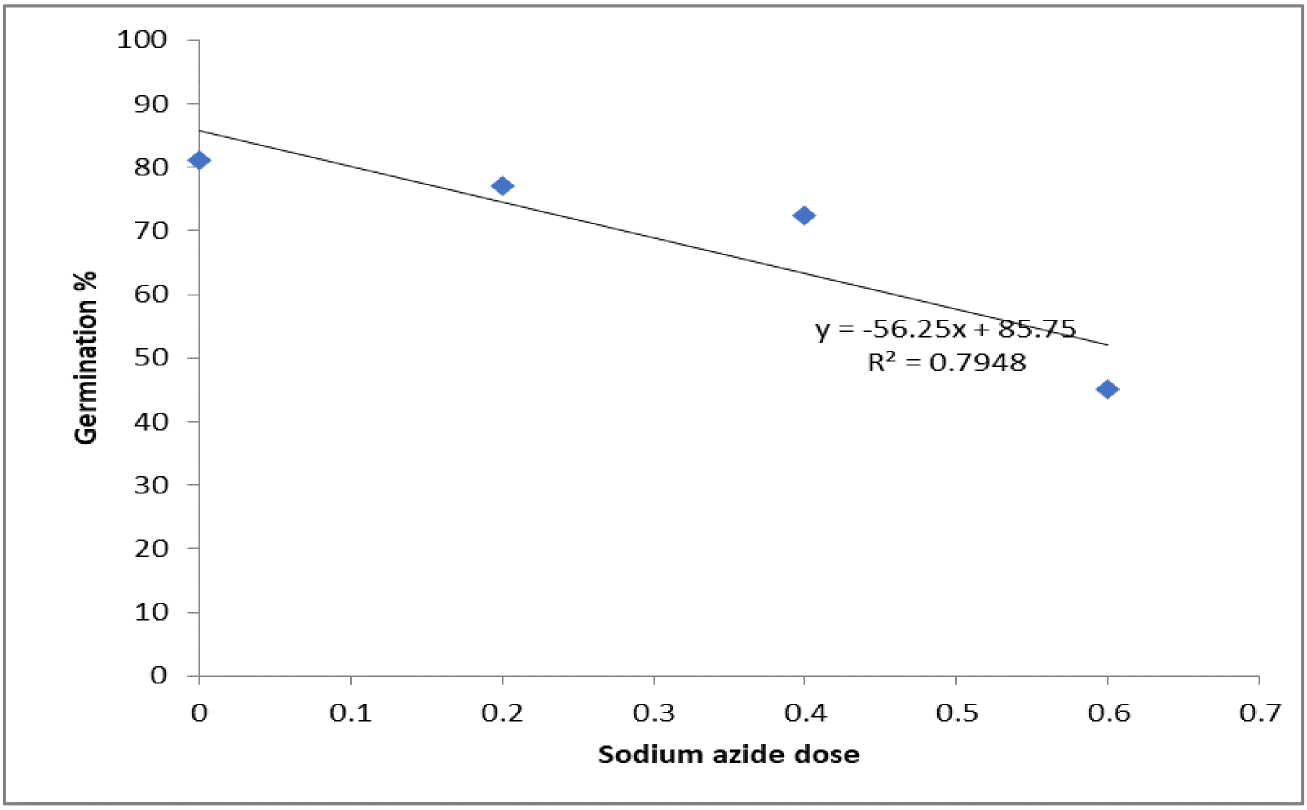

Figure 3: Lethal dose (a dose-response curve) of sodium azide on San Marzano

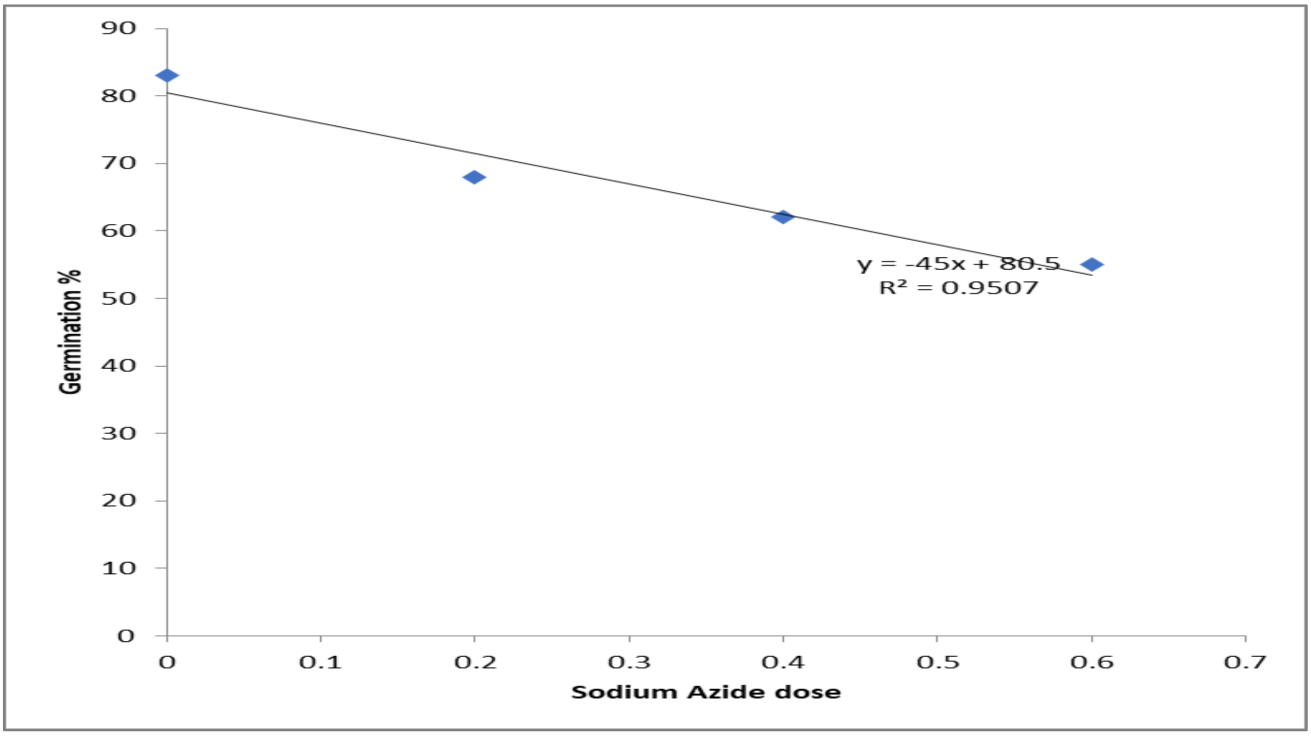

Figure 4: Lethal dose (a dose-response curve) of sodium azide on beefsteak

DISCUSSIONS

Mutagenesis research has not extensively examined the optimization of mutagen concentrations on crop plants, particularly tomatoes, for a desirable plant cultivar despite the well-documented mutagenic effect of some chemical mutagens on seed germination and survival rates. As every species, variety, and genotype has a completely different radio sensitivity, the first step in mutation breeding is to determine the dose, according to Owais and Kleinhofs (1988). Because doses below LD_{50} favor a plant's ability to recover from treatments, higher doses increase the risk of inducing either positive or negative mutations, making LD_{50} the ideal dose that maximizes favorable mutation frequency while causing the least amount of damage to the plant. The LD_{50} test is therefore carried out before any mutation breeding procedure in order to prevent excessive loss of genuine experimental materials. This work has contributed to the body of knowledge regarding the impact of mutagens on crop plant survival rates and seed germination. Based on the study's findings, sodium azide treatment had an impact on two of the tomato types that were investigated. The observation was made that sodium azide concentrations showed morphological modifications of varying kinds. According to the results, the mutagen concentrations inhibited seed germination, especially at higher levels.

Previous research by Omosun *et al.* (2021) on two pigeon pea accessions indicated similar inhibitory effects on seed germination. They also found that the two accessions treated with varying dosages of EMS had lower germination and survival rates. Hasegawa and Inome (2012) in rice, Adeosun *et al.* (2020) in tomato varieties, Raina *et al.* (2018) in cowpea, Adamu *et al.* (2004) in tomatoes, Gichner and Veleminsky (1977) in Arabidopsis, and Ilbas *et al.* (2005) in barley have all previously found similar inhibitory effects on seed germination. The present investigation's outcome is consistent with the findings of Adamu and Aliyu's (2007) paper, which documented decreases in tomato variety seed germination after treatment with varying concentrations of sodium azide. According to Liamngee *et al.* (2017), a rise in sodium azide concentration allowed the mutagen to hinder the availability and consumption of energy, interfere with enzyme activity, and stop vital processes that could cause the embryo to die. The findings of Gunasekaran and Pavada (2015) on groundnuts and Bala *et al.* (2019) on two tomato cultivars (*Solanum lycopersicum mill*) further corroborate the decline in seed germination and survival percentages seen in this study.

According to the study's death curves, the LD_{50} varies with plant species, type, and state, as well as the point at which lethality is noted. This study's lethal dosage was determined by looking at the survival and germination rates at increasing chemical mutagen concentrations and the percentage of survivorship at those concentrations. As the quantities of the mutagens increased, the study's observations showed that mortality increased as well. This supported the study's mutagen mortality curves and illustrated how sodium azide affected LD_{50} as it varied between the two tomato varieties. The results of Omosun *et al.* (2021) were supported by this report, which said that the LD_{50} values vary depending on the crop species, varieties, other plant materials' seeds, types of treatments, raising techniques, and other aspects. Raina *et al.* (2018) found that cowpeas treated with varying doses of sodium azide and gamma-ray had LD_{50} values of 0.64%. These results are in strong accord with the determination and variation of the lethality of tomato types on the mutagen concentrations observed in this study.

CONCLUSION

A mutagen's impact is contingent upon the concentration used as well as the species of plants that are impacted. Consequently, the process must be optimized to ensure a high mutation frequency without jeopardizing the viability of the seed. With sodium azide, this adjustment is crucial since a high concentration significantly inhibits seed germination in a variety of species. The study's findings also demonstrated the effectiveness of sodium azide in causing mutations in both sorts of seedlings based on their germination and survival rates. Lower concentrations of mutagens stimulate the growth hormone and enzymes responsible for tomato quality, yield, and growth, whereas high concentrations of mutagens have the opposite effect. This explains why sodium azide has a stimulatory impact at lower doses. As a result, sodium azide-induced mutagenesis at lower concentrations can greatly increase tomato seedling output and quality agronomic attributes.

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