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In vitro Evaluation of the Effects of Sodium Azide on the Germination and Seedling Development of *Sesamum radiatum* and *Sesamum indicum*

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ABSTRACT: Mutation breeding is a widely recognized and effective method for enhancing crop production. Research has shown that induced mutations have the potential to significantly increase yield and improve various quantitative traits in plants. This method holds promise for the continued improvement of agricultural practices and the development of high-performing crop varieties. This study evaluated the mutagenic effects of sodium azide on seed germination and seedling development of two species of sesame. *Sesamum radiatum* and *Sesamum indicum* were subjected to varying concentrations of sodium azide ranging from 0.00% (control) - 0.250% for 24 hours after which they were grown in the laboratory. The mean weight of seed ranged between 1.97g in *Sesamum indicum* and 1.05g in *Sesamum radiatum*. The highest mean germination percentage was 98% and 100% for *Sesamum radiatum* and *Sesamum indicum*, respectively at day 7 with 0%, 0.0165%, 0.025%, 0.033% and 0.25% while the lowest average germination percentage was 86.67% and 98.33% produced by treatment levels: 0.033% and 0.063% for *Sesamum radiatum* and *Sesamum indicum* respectively. The analyses indicated that there was no interaction between species and sodium azide on germination percentage at P=0.54. The effect of treatments on species had no significant difference at P=0.97 on radicle length, number of days to foliage formation and length of hypocotyl. The analysis revealed that the main effect on species was found to be significant for germination percentage at a level of P≤ 0.05. Furthermore, radicle length, number of days to foliage formation, length of hypocotyl, and seedling height exhibited significant differences at P≤ 0.05, indicating that the species played a noteworthy role in these parameters. This suggests that species had a more pronounced influence on most of the parameters studied, underscoring the fact that different species reacted diversely to the mutagen.

Keywords: Mutagen, sodiun azide, *sesamum* sp, germination, seedling development

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INTRODUCTION

Mutagenesis is the process by which an organism's deoxyribonucleic acids (DNA) experience change, resulting in a gene mutation. A mutation is a permanent and heritable change in genetic material, which can result in altered protein function and phenotypic changes. Mutagens are substances that alter or change the genetic material of an organism; hence they are termed genotoxic (Durland and Ahmadian-Moghadam., 2022). Sodium azide (NaN_3) is widely used to induce mutagenesis within *in vitro* plant systems.

Plant breeders have generated new varieties using chemical mutagens for many decades (Dubey *et al*., 2017). Sodium azide (NaN_3) is widely regarded as a relatively safe to handle and very efficient chemical mutagen that is both inexpensive and non-carcinogenic (Gómez *et al*.,2019). *Sesamum radiatum* is a perennial herb found in the tropical areas of Africa and belongs to the Pedaliaceae Family. The Yoruba tribe in the South-Western part of Nigeria calls it "Ewe atura" which means 'leaves that bring relaxation and health to the body'

Plate 1: (A) is showing the *Sesamum radiatum* plants at flowering stage. (B) is showing the seeds of *Sesamum radiatum*. (C) is showing the *Sesamum indicum* plants at flowering stage. (D) is showing the seeds of *Sesamum indicum*

possibly because they relieve constipation and cure other ailments on ingestion. The leaves, seeds and oil serve as food especially in farming communities in Nigeria. The seeds have been reported to have a crude protein content of 22.9%. The leaves are also used for treating various stomach ailments. The decoction of the leaves is used for the treatment of catarrh, eye pains as well as bruises and erupted skins. The decoction of combined roots and leaves has been reported to have anti-viral and antifungal activity (Ogunlesi *et al.,* 2010). It is a leafy vegetable locally called Ekuku gogoro in Yoruba language, beni or gingelly (English), 'ridi' (Hausa) and belonging to the group of indigenous vegetables that grow in small quantity in the rural areas. The plant occurs throughout the tropics mainly as a weed, where it is gathered in the wild and used as a potherb. It is one of the many neglected leafy vegetables of the tropics despite its medicinal benefits (Edutan *et al*., 2014).

Yadav *et al*. (2022) reported that the low yields and production recorded by Sesamum globally is attributable to lack of arable lands, increasing biotic and abiotic stress. Meanwhile, Singh *et al*. (2022) in their study suggested that mutation breeding is known to have contributed immensely to the improvement of agricultural practices and the development of high-performing crop varieties. However, the present study aimed to evaluate the mutagenic effects of sodium azide on seed germination and seedling development of two species of Sesame (Plate 1). Sesame production in Nigeria probably began in the middle belt (North central) region of the country and later spread to other parts where it is commonly grown by small holder farmers (USAID, 2002; Iorlamen *et al*., 2014). The major producing areas in order of priority are Nasarawa, Benue and Jigawa States. Other states where it is cultivated in small scale include Cross River, Ebonyi, Niger, Gombe, Katsina, Yobe and Borno States. Others include Kwara, Adamawa, Plateau, Kogi, Bauchi, Kebbi and Taraba State (Oko, 2020).

Sesame (*Sesamum indicum* L.) is an important oilseed crop that is grown in various parts of the world,

particularly in Asia and tropical Africa. It is a drought-tolerant crop that can grow in different types of soil, including those that are low in fertility (Wacal *et al*., 2024). Sesame is widely grown in the Northern and Central parts of Nigeria. This study evaluated the mutagenic effects of sodium azide on seed germination and seedling development of two species of sesame.

MATERIALS AND METHODS

Source of material (sample)

The two different varieties of Sesame were collected directly from farmers: *Sesamum radiatum* (black benniseed) was collected from the farmers in Jos Plateau State while *Sesamum indicum* (white benniseed) was gotten from the farmers in Otukpa, Benue state Nigeria. The chemical mutagen used was obtained from African Center for Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos.

Experimental site

The research work was conducted at African Center of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos; Laboratory work included: viability test, mutagenic treatment of the selected seeds and germination. Seedling growth was monitored.

Germination

The seeds were treated using the concentration and method of Mensa *et al.* (2007). Randomly selected seeds were divided into six groups of 300 seeds each for both varieties. Each of the groups was subjected to the varying concentrations outlined below:

i. 0ml of NaN_3 + 100ml of distil water = 0% concentration 'control' (C_0)
ii. 0.016g of NaN_3 +100ml of water = 0.016% concentration (C_1)
iii. 0.033g of NaN_3 + 100ml of water = 0.033% concentration (C_2)
iv. 0.063g of NaN_3 + 100ml of water = 0.063% concentration (C_3)
v. 0.125g of NaN_3 + 100ml of water = 0.125% concentration (C_4)
vi. 0.250g of NaN_3 + 100ml of water = 0.250% concentration (C_5)

300 seeds of the five different groups and the control groups were put on a clean cloth and tied into a knot of each variety. The five different groups of seeds were soaked in the varying concentrations of NaN_3, and the sixth group representing the control was soaked in distilled water. These seeds were allowed in their various treatments for 24 hours after they were removed and washed thoroughly in running water to remove excess chemicals and exudates. Twenty seeds from each treatment were sown in petri dishes containing filter paper damped with 0.5 ml of distilled water to observe germination and growth. The percentage of germination was calculated after seven days using the following formular:

$$\% \text{ Germination} = \frac{\textit{Total number of germinated seeds}}{\textit{Total number of seeds plated}} \; x \; \frac{100}{1} \text{ (Mensah } \textit{et al}., 2007).$$

Data collection and analytics

The germination count and the growth parameters for the laboratory work included: length of radicle, length of hypocotyl, height of seedling, number of seeds that form foliage and number of days to foliage formation. The data were subjected to a two-way analysis using R Core Team (2023).

RESULTS

Germination percentage

Figure 1 shows the daily germination percentage of *Sesamum radiatum* observed *in vitro* when induced with different concentrations of sodium azide. The highest germination percentage was observed at 7 days after sowing (DAS) with a 98% success at a concentration dosage of 0.02% of sodium azide, while the lowest germination percentage was observed at 10% at 2DAS with a concentration dosage of 0.25%, and the difference was statistically significant (P≤0.05). Figures 1 show the daily germination percentage of *Sesamum radiatum* observed *in vitro* when induced with different concentrations of sodium azide. The highest germination percentage was observed at 7DAS with a 98% success at a concentration dosage of 0.02% of sodium azide, while the lowest germination percentage was observed at 10% in 2DAS with a concentration of 0.25%, and the difference was statistically significant (P≤0.05). However, over 80% germination was recorded after the seven (7) day period. Figure 2 shows the response of *Sesamum indicum* to the different concentrations of sodium azide and this was quite uniform across period of investigation with exception to Day 1, where the germination responses were significantly different (P≤0.05) from other periods upon investigation.

Effect of sodium azide treatments on the weight of seeds of *sesamum radiatum* and *sesamum indicum*

The mean weight of seed ranged between 1.97g in *Sesamum indicum* and 1.05g in *Sesamum radiatum* and

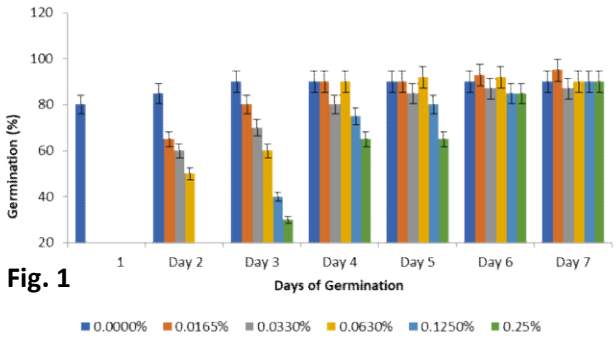

Fig. 1

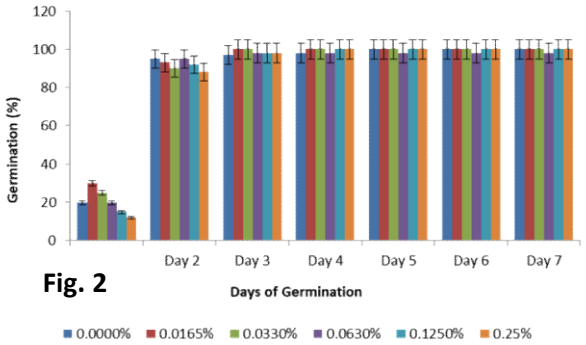

Fig. 2

Figure 1) Effect of sodium azide on germination percentage of *Sesamum radiatum.*
Figure 2) Effect of Sodium Azide on Germination Percentage of *Sesamum indicum.*

Table 1: Main effects of concentration of sodium azide (nan_3) on weight of seed, germination rate and length of radicle of *sesamum* species.

Treatment	WOS (g)	GR (%)	RL (cm)
Species			
Sesamum indicum	1.97±0.56a	0.99±0.011a	3.59±0.31a
Sesamum radiatum	1.05±0.48b	0.90±0.05b	1.48±0.64b
Concentration of NaN_3			
0%	1.47±0.92e	0.95±0.06a	3.36±0.75a
0.0165%	1.67±0.41c	0.97±0.04a	2.48±0.93b
0.033%	1.69±0.70b	0.93±0.07a	2.69±1.24c
0.063%	1.57±0.88d	0.95±0.04a	2.40±1.05d
0.125%	1.74±0.73a	0.94±0.07a	2.29±1.59e
0.250%	0.91±0.19f	0.95±0.08a	1.99±1.32f
LSD	0.01	0.05	0.07
Level of significance			
Species	≤0.001	≤0.001	≤0.001
Concentration	≤0.001	Ns	≤0.001
Species: Concentration	≤0.001	Ns	≤0.001
CV (%)	0.00	4.42	0.17

Means that are followed with the same letters within a column are not statistically significant at 5% level of probability. NB: WOS – Weight of seed; GR – Germination rate; RL – Length of radicle; CV – Coefficient of variability; NAN_3 – Sodium azide

the difference was statistically significant (P ≤0.001) as shown in (Table 1). The treatment: 0.125% of Sodium azide had the highest mean seed weight (1.74), which significantly differed at (P ≤0.001) from that of 0.033%, 0.0165%, 0.063%, 0% and 0.250%, with values of 1.69, 1.67, 1.57, 1.47 and 0.91 respectively (Table 1).

Effect of sodium azide treatments on the germination rate of *sesamum radiatum* and *sesamum indicum*

The mean germination rate ranged between 0.99 in *Sesamum indicum* and 0.90 in *Sesamum radiatum* and was statistically significant at P ≤0.001 (Table 1). The highest mean germination rate (0.97) was recorded in 0.0165% treatment which was 4.2% higher than the lowest mean germination rate (0.93) which was statistically significant at P ≤0.001. In *Sesamum radiatum,* germination percentage reduces with increase in concentration. The highest germination percentage was observed in the control (0.0%) having 80% germination 1 DAS and the lowest was recorded in all the treated seeds having 0% germination (0.0165% - 0.250%). This persisted until 7 DAS where all treated seeds reached the minimum 75% germination while in *Sesamum indicum* both the control (0.00%) and the treated seeds (0.0165% - 0.250%) recorded 12% - 20% germination 1 DAS and 98 -100% 7 DAS.

Effects of sodium azide treatments on the radicle length of *sesamum radiatum* and *sesamum indicum*

The mean radicle length of *Sesamum indicum* was

Table 2: Interaction Effects of *Sesamum* Species and Concentration of Sodium Azide on the Weight Change of Seed.

Species	Concentration of Sodium azide					
	0.00%	0.0165%	0.033%	0.063%	0.125%	0.250%
Sesamum indicum	2.31d	1.3f	2.33c	2.38b	2.41a	1.09g
Sesamum radiatum	0.63l	2.05e	1.05i	0.77j	1.07h	0.74k
LSD	0.01					

Mean followed by the same letters across rows are not statistically significant at 5% level of probability.

Table 3: Interaction Effects of *Sesamum* Species and Concentration of Sodium Azide on the Length of Radicle.

Species	Concentration of Sodium azide					
	0.00%	0.0165%	0.033%	0.063%	0.125%	0.250%
Sesamum indicum	4.05a	3.34e	3.82b	3.37d	3.75c	3.21f
Sesamum radiatum	2.68g	1.63h	1.55i	1.44j	0.84k	0.78l
LSD	0.01					

Means followed by the same letters across rows are not statistically significant at 5% level of probability.

3.59cm while *sesamum radiatum* was 1.48cm which differed statistically at P ≤0.001 (Table 1). The 0% treatment had the highest radicle length (3.36cm) and statistically differed at (P ≤0.001) from 0.0165%, 0.033%, 0.063%, 0.125% and 0.250%.There was a significant interaction effect between *Sesamum* species and the concentration of Sodium azide (Table 2). The mean weight size ranged from 2.41g in *Sesamum indicum* at a concentration dosage of 0.125% to 0.63g in *Sesamum radiatum* at 0.00% concentration of Sodium azide, and was statistically significant at (P ≤0.001). Prior to seed soaking in Sodium azide, the weight of the seed for *Sesamum indicum* and *Sesamum radiatum* were 0.80g and 0.65g, respectively.

This indicated that Sodium azide had a significant effect on the seed weight of *Sesamum indicum* at a dosage concentration of 0.0125% and *Sesamum radiatum* at a concentration of 0.0165%. The significant interaction effect between *Sesamum* species and the concentration of Sodium azide on radicle length is shown in (Table 3). The radicle length was highest in Sesamum indicum (4.05cm) for the control and lowest at a dosage concentration of 0.250% in *Sesamum radiatum* (0.78cm) and was statistically significant (P ≤0.001).

Effects of Sodium azide treatments on the length of hypocotyl of *Sesamum radiatum* and *Sesamum indicum*

The mean length of hypocotyl ranged between 4.05cm in *Sesamum indicum* and 2.89cm in *Sesamum radiatum* and differed statistically at P≤0.001 (Table 4). The treatment 0.033% produced plants with the highest mean length of hypocotyl (3.62cm) which was 13.57% higher than the lowest mean length of hypocotyl (3.16cm) observed in 0% (control) and was statistically significant at P≤0.001.

Effects of sodium azide treatments on the number of days to foliage formation of *sesamum radiatum* and *sesamum indicum*.

Table 4 also shows that the number of days to foliage formation varied from 19.50 observed in *Sesamum indicum* and 16.50 in *Sesamum radiatum*. Analysis indicated significant difference at P≤0.001. The highest mean number of days to foliage formation was observed in 0.0165%, 0.033% and 0.063% respectively while the lowest mean number of days to foliage formation was recorded in 0.125% and 0.250%, and it significantly differed at P≤0.001 (Table 4).

Effect of sodium azide treatments on the seedling height of *sesamum radiatum* and *Sesamum indicum*.

In *Sesamum indicum*, the seedling height attained a mean length of 4.25cm exceeding the average of 3.09cm observed in *Sesamum radiatum* and it was statistically significant at P ≤ 0.001. The concentration 0.033% of Sodium azide had the highest seedling height 3.83cm and differed statistically at (P≤0.001) from 0%, 0.0165%, 0.063%, 0.125% and 0.250% treatments (Table 4).

DISCUSSION

The differences observed in weight of seeds after the imbibition of the mutagen could be genetic. This could also explain why the two species of *Sesamum* responded differently to the induced concentrations of the mutagen in their germination rates. This is consistent with the findings of Zhang *et al*. (2012) and Kaaby *et al*. (2015).
The effect of NaN_3 on the percentage germination in *Sesamum indicum* may be as a result of the effect of the physiological and biological activities due to enzyme

Table 4: Main Effect of *sesamum* species and concentration of sodium azide (NaN_3) on length of hypocotyl, number of days to foliage formation and seedling height

Treatment	LOH (cm)	NODFF	SH
Species			
Sesamum indicum	4.05±0.51a	19.50±0.51a	4.25±0.52a
Concentration of NaN_3	2.89±0.20b	16.50±2.28b	3.09±0.20b
0%	3.16±0.06f	18.00±1.09b	3.36±0.06f
0.0165%	3.34±0.37e	19.00±0.00a	3.54±0.37e
0.033%	3.62±0.72a	19.00±1.09a	3.82±0.72a
0.063%	3.57±0.80c	19.00±1.09a	3.77±0.80c
0.125%	3.60±0.90b	16.50±2.73c	3.80±0.90b
0.250%	3.55±1.06d	16.50±3.83c	3.75±1.06d
LSD	0.01	0.09	0.01
Level of significance			
Species	≤0.001	≤0.001	≤0.001
Concentration	≤0.001	≤0.001	≤0.001
Species: Concentration	Ns	Ns	ns
CV (%)	0.35	0.43	0.39

Means that follow the same letters within a column are not statistically significant at 5% level of probability. NB: LOH – Length of Hypocotyl; NODFF – Number of days to foliage formation; SH – Seedling height; CV – Coefficient of variability; NAN_3 – Sodium azide

activities, hormonal imbalances, and inhibition of mitotic process. The inhibitory effect of NaN_3 on germination could be traced to the effect of azide anions which are strong inhibitors of cytochrome oxidase, and in turn inhibited oxidative phosphorylation (Ikhajiagbe and Oshomoh, 2013).

Sodium azide exerted no influence on the germination rate of *Sesamum indicum.* This might be as a result of the genetic makeup of the two species, concentration of sodium azide that was used or the duration of exposure as observed by Ikhajiagbe and Shittu, (2015).

Germination percentage is a critical indicator of seed viability and initial growth potential. In this study, it was found that the main effects of species on germination percentage was significant, indicating that there were inherent differences between the two Sesame species in terms of their germination capabilities.

The number of days to foliage formation increased with increase in concentration and the highest number of days was found in 0.033%, 0.0163% and 0.063% while the least number of days was found in 0.125% and 0.250%. This could mean a response to stress condition that warranted early foliage formation. It is not certain what it would look like if this were monitored beyond the period of study in question. This appears to be consistent with the findings of Singh *et al*. (2022) who reported that higher concentration of NaN_3 significantly reduced seed germination and seedling growth parameters in Kalanamak rice.

The expression of the Length of hypocotyl and Seedling height might be as a result of environmental effects (Singh *et al*., 2022). The decrease in radicle length of Sesame with increase in concentration of the mutagen in this research was similar with the reports of Mensah, (2007), Singh *et al*. (2022) and Udofia *et al*. 2022.

Conclusion

This study has revealed that the mutagen had a significant effect on the germination and seedling development of the two *sesamum* species. Generally, *Sesamum indicum* responded significantly to Sodium azides compared to *Sesamum radiatum*. The interaction effects between the two *Sesamum* species and the mutagen also explained that the responses of germination rate and seedling development to the different concentrations differed between the two species. Therefore, the use of the mutagen concentration: 0.033% as pre-treatment of *Sesamum* seeds is recommended.

Highlights

300 seeds of two *Sesamum* species were evaluated for germination and seedling development pre-treated with Sodium azides.

Five concentrations (0.0165%, 0.033%, 0.063%, 0.125% and 0.250%) of Sodium azides and a control group (0.00%) were used as treatments on the seed prior to *in vitro* evaluation.

Sesamum indicum responded significantly to the mutagen compared to *Sesamum radiatum*

0.033% concentration of the Sodium azides had a significant effect on the germination and seedling traits of the *Sesamum* species.

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