



ASSESSMENT OF MUTAGENIC EFFICIENCY AND DETERMINATION OF OPTIMAL DOSE OF SODIUM AZIDE ON VEGETATIVE AND FRUIT PARAMETERS OF EGGPLANT (*SOLANUM MELONGENA*)

Animasaun, D.A.,¹ Hanafi, T. M.,¹ Azeez, M. A.² and Adedibu P.A.¹

¹Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, P. M. B. 1515, Ilorin, Kwara State, Nigeria.

²Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Oyo State, Nigeria;

*Corresponding author: animasaun.ad@unilorin.edu.ng, biostanleydayor@yahoo.com.au

Abstract

This study was conducted to determine the effect and optimal concentration of sodium azide on the vegetative and fruit parameters of eggplant (*Solanum melongena*). Seeds of eggplant (NG/SA/JAN/09/157) collected from National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria were soaked in varying concentrations of 0 mM, 10 mM, 20 mM, 30 mM and 40 mM sodium azide for 6 hr. The seeds were grown in the screenhouse using complete randomized design in three replicate. Data were collected on growth, qualitative and quantitative flower as well as fruit related traits for each of the treatments, the data were subjected to analysis of variance (ANOVA). The results showed that 10 mM treatment plants had best performance for vegetative parameters evaluated. Although higher concentrations (30-40 mM) reduced germination and seedling vigour, however, 40 mM significantly promoted early maturity, higher fruit size and weight. Sodium azide concentrations evaluated did not affect leaf pigmentation, fruit colour and shape. The study concluded that characters desired will determine the effective dose of sodium azide to apply, as low doses favoured vegetative characters while higher doses favoured fruit quality. Since higher fruit yield in *S. melongena* is of interest to the breeders, the 40 mM dosage is therefore appropriate and the mutants created over the years could further be screened for the crop improvement.

Keywords: chemosensitivity; mutagenesis; mutation breeding; *Solanum melongena*

INTRODUCTION

Solanum melongena L. popularly known as eggplant is a member of the family *Solanaceae*. Eggplant is an agronomical important crop closely related to *S. aethiopicum* (scarlet eggplant) and *S. macrocarpon* which are native to Africa (Sekara *et al.*, 2007). Although eggplant is generally grown as an annual crop, it is actually an herbaceous perennial (Rowell *et al.*, 2002). Cultivation of eggplant is highly concentrated, with 90% of output coming from five countries; China (58%), India (25%), Iran, Egypt and Turkey contributes significantly, in addition, Queensland and New South Wales produces eggplant commercially (Ullio, 2012). In tropic Africa, Nigeria in particular, eggplant is widely cultivated by local rural farmers mainly in the norther part of the country.

Eggplant leaves and fruit are nutritious, containing low calories, fats, sodium and non-starchy (Lindgren *et al.*, 2008). The fruit can be cooked as vegetable, some African varieties could be eaten raw as vegetable fruit. The fruit is rich in fibre, potassium, magnesium, folic acid, vitamin A and B₆ (Praca *et al.*, 2004). Eggplant is a warm season crop and does not tolerate frost, very cool or high temperature and humidity affects its yield (Valencia *et al.*, 2000). Furthermore, extended periods of soil saturation may result into buildup of pathogens that could cause root decay in the crop (Bliss *et al.*, 2004). The plant is susceptible to insect, pest, nematode, fungi and bacterial attacks (Praca *et al.*, 2004), which cause reduction in yield with great economy lost. Consequently, development of resistant and high yield varieties has been put forward as a solution to this problem, and to achieve this mutagenesis could be a veritable tool.

Induced mutation breeding is an efficient tool for generating variability and creating diversity for genetic improvement of economic crops (Adamu *et al.*, 2004). It has great potentials and could be used as a complimentary approach in genetic improvement of crops. Various mutagenic agents are used to induce favorable mutations for the improvement of various traits in crops, ornamental and

medicinal plants (Ahloowalia *et al.*, 2001; Mehandijev *et al.*, 2001; Mensah *et al.*, 2007; Rajib *et al.*, 2012). Sodium azide is one of the commonly used mutagens in crop plants. It has been reportedly affected plant physiology and decrease cyanide resistant respiration in tobacco callus (Wen and Liang, 1995). Also, sodium azide mutagenic effects have been studied in crop plants, and its mutagenic potential reported in several screening assays (Rines, 1985; Raicu and Mixich, 1992; Sarma *et al.*, 2001; Prina and Favres, 2004; Mensa *et al.*, 2007; Mostafa, 2011; Animasaun *et al.*, 2014).

Despite the reports of mutagenic effects of sodium azide in crop plants, information on its effect on the growth and productivity of eggplant (*Solanum melongena*) is not well studied. Therefore, the present study is aimed at evaluating the effects of sodium azide on the germination, growth and yield of eggplant to determine the optimum concentration for improved performance of the crop which can further be screened and utilise in the crop improvement.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the University of Ilorin Botanical Garden, located in the Southern Guinea Savanna zone of Nigeria (N 08° 28' 53.3", E 04° 40' 28.9"). The annual rainfall is 1200 mm and temperature varies between 33°C and 34°C.

Seeds collection and viability test

Eggplant (*Solanum melongena* NG[SA]JAN[09]157) seeds were collected from National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. The seeds were subjected to viability test using floatation method by soaking the seeds in sterile water, well stirred and allow to settle for about 20 min. Seeds that sank to the bottom were considered viable, the floating seeds (non-viable) were discarded. The viable seeds were air-dried in preparation for mutagenic treatments.

Chemical Treatment

Dry viable seeds were exposed into freshly prepared different concentrations (0 mM, 10 mM, 20 mM, 30 mM and 40 mM) of sodium azide for 6 hr. the 0 mM seeds which serves as control were soaked in distilled water for the same period. After 6 hrs. the seeds were washed off residual chemical and air-dried before sowing.

Pot experiment

The treated seeds of *S. melongena* were sown into planting bags (25 cm x 35 cm) containing garden soil. The experiment was laid out in a completely randomized design (CRD) with three replications for each of the treatments. Watering and other cultural practices were carried out as required. Data were collected on quantitative and qualitative characters at two weeks interval. Parameters evaluated include number of leaves, leaf length, leaf breadth, plant height, number of branches fruit size, number of flower per plant, number of fruits per plant, fruit dimension, fruit weight and fruit colour.

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS statistical package. The means were separated with Duncan Multiple Range Test at 0.05 α -level. Traits correlations among the treatment concentrations were also analyzed.

RESULTS

The response of the treated plants to different concentrations of sodium azide varied in quantitative characters studied. Highest percentage germination (80%) was recorded in seeds treated with 10 mM, followed by 0 mM, 20 mM (75%), 30 mM (65%) while the lowest (60%) was observed in seeds treated with 40 mM for 6 hr (Figure 1). The performance of the treated eggplants in term of growth in response to different sodium azide concentrations showed highest plant height occurred in 10 mM across the weeks of study (Figure 2). In contrast, higher concentrations of 30 and 40 mM adversely affected plant height. Likewise, 10 mM of sodium azide for 6 hr treatment favoured leaf formation in *S. melongena* (Figure 3). The average number of leaf per plant across the studied weeks was in order of 10 mM > 0 mM > 20 mM > 30 mM > 40 mM; the least number of leaf per plant was found in 40 mM plants. Leaf length and width of the treated eggplant varied with concentrations of

sodium azide used. Longer and broader leaves were obtained with 10 mM treatment followed by 0 mM meanwhile, leaf dimensional traits were negatively affected by higher concentrations of 20-40 mM (Figures 4 & 5). It was noted that as concentrations increases above 10 mM, performances of the eggplant decreases in term of leaf length and width. The trend was also similar for number of branches per plant. Although branches formation commenced at 6 WAS, 10 mM treated plants have highest number of branches, but at 10 WAS, there was improvement in branch formation by 40 mM plants.

At maturity (12 WAS), plant height was significantly highest (53.33 cm) in 10 mM, similar for 0 and 20 mM and the least were in 30 and 40 mM, where plant height were not significantly difference (Table 1). This trend was similar for number of leaf per plant for which 10 mM plant had significantly highest number of leaves and the least occurred in 40 mM treated plants. Considering leaf length, there was no significant difference in the performance of 10 and 20 mM plants, 0 and 30 mM concentrations also produced similar effects while the least were found in 40 mM treated plants. Surprisingly, all the concentrations produced similar leaf blade width with the exception of 40 mM which leaves were significantly narrow. At 12 WAS, 10 mM plant were the most branched (5.41) followed by 40 mM (4.66), branches per plant were not significantly different for 0, 20 and 30 mM treated plants.

Commencement of flowering and fruit set in *S. melogena* - NG[SA]JAN[09]157 is shown in Plate 1. The flower and fruit related parameters performance in response to different concentrations of sodium azide varied significantly (Table 2). Earlier maturity and higher number of flowers were achieved with higher concentrations (30 and 40 mM). Nevertheless, fruit formation was similar for 0 and 10 mM plants. The number of fruits per plant was also significantly higher for 30 and 40 mM, but statistically similar for 0, 10 and 20 mM plants. Other fruit parameters such as fruit length, width and weight were significantly favoured by higher concentrations treatment of sodium azide. The qualitative traits observed for the treatments were basically similar, no remarkable difference was observed in characters such as growth habit, flower and fruit colour as well as the fruit shape. With respect to the 0 mM plants, the aforementioned qualitative traits were similar for all the treated plants.

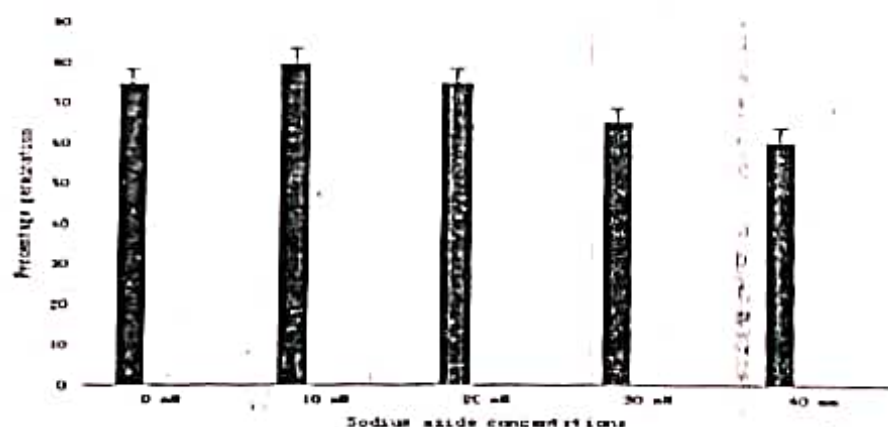


Fig. 1. Percentage germination of eggplants (*Solanum melogena* - NG[SA]JAN[09]157) treated with different concentrations of sodium azide. The bars are mean values $\pm$ standard error.

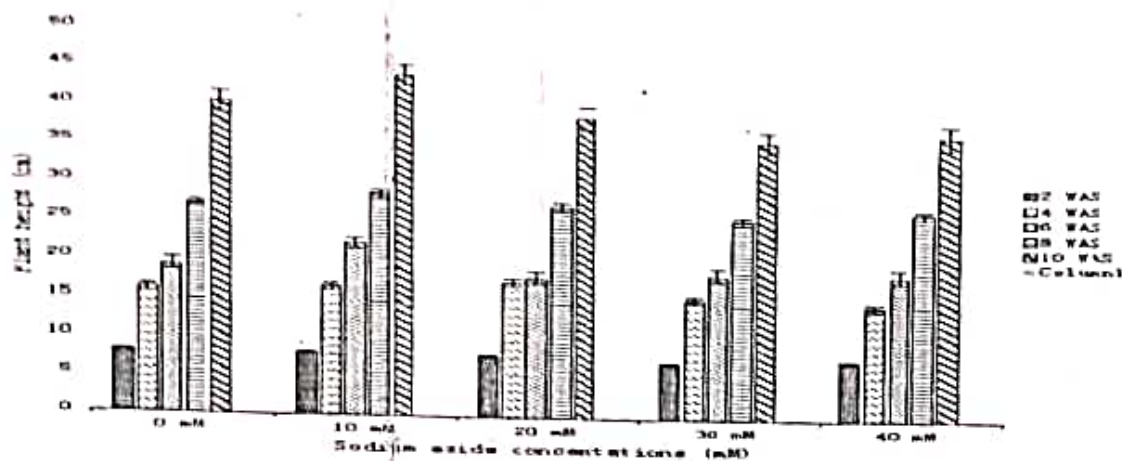


Fig. 2. Plant height (cm) of eggplant (*Solanum melogena* - NG|SA|JAN|09|157) in response to different concentrations of sodium azide
The bars are mean values $\pm$ standard error. WAS=Week after sowing.

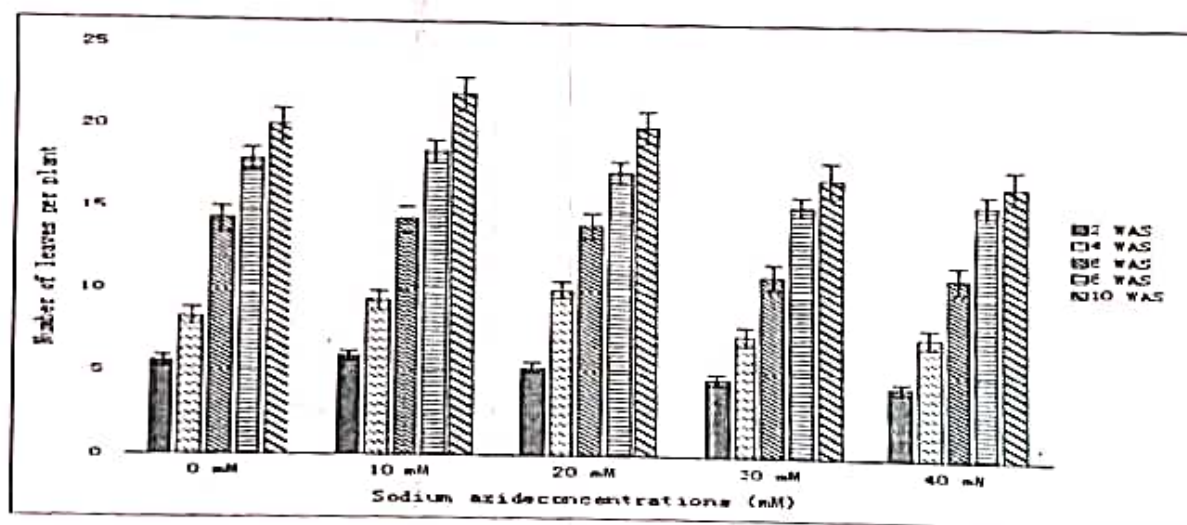


Fig. 3. Number of leaves (cm) of eggplant (*Solanum melogena* - NG|SA|JAN|09|157) in response to different concentrations of sodium azide
The bars are mean values $\pm$ standard error. WAS=Week after sowing.

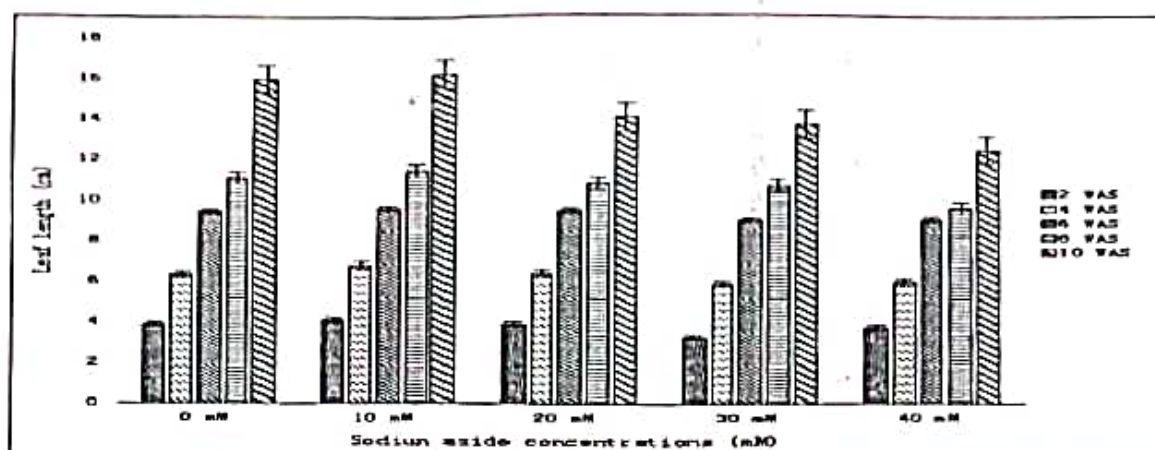


Fig. 4. Leaf length (cm) of eggplant (*Solanum melogena* - NG|SA|JAN|09|157) in response to different concentrations of sodium azide

The bars are mean values $\pm$ standard error. WAS=Week after sowing.

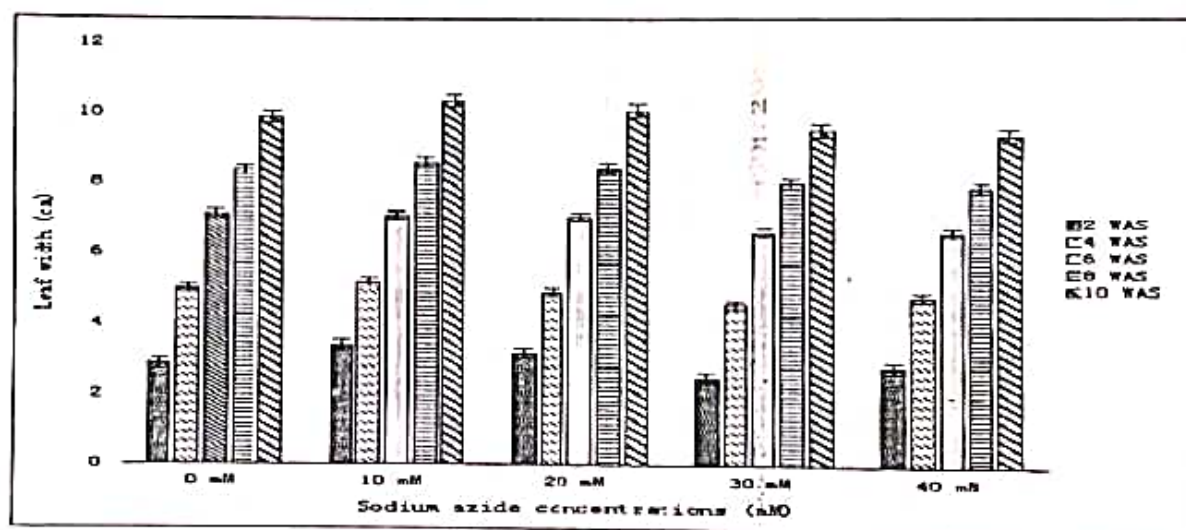


Fig. 5. Leaf width (cm) of eggplant (*Solanum melogena* - NG|SA|JAN|09|157) in response to different concentrations of sodium azide

The bars are mean values $\pm$ standard error. WAS=Week after sowing.

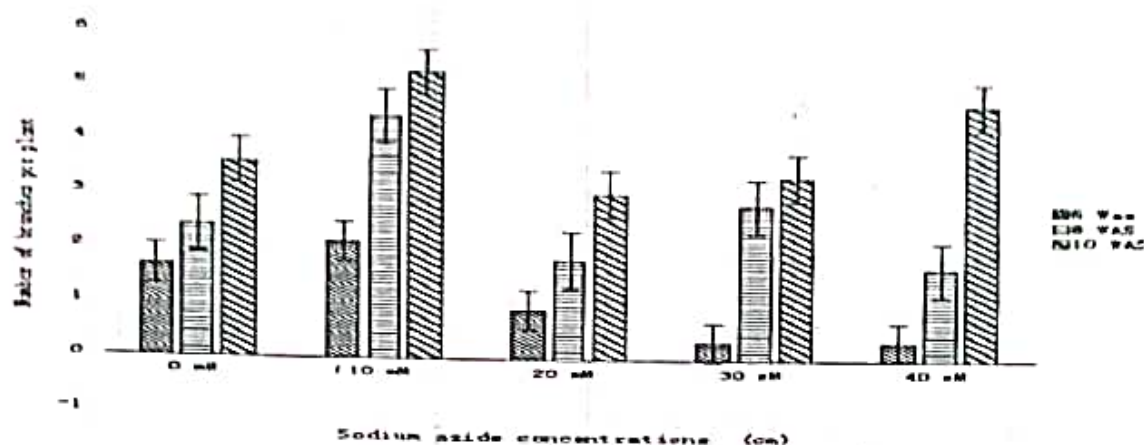


Fig. 6. Number of branches of eggplant (*Solanum melogena* - NG|SA|JAN|09|157) in response to different concentrations of sodium azide

The bars are mean values $\pm$ standard error. WAS=Week after sowing.

Table 1. The effect of different concentrations of sodium azide on fruit and flower characters of *Solanum melogena* (NG|SA|JAN|09|157) at 12 weeks after sowing (12 WAS)

SA Conc.	PLH (cm)	NLP	LBL (cm)	LBW (cm)	NBP
0 mM	47.05 ^b	24.33 ^b	11.78 ^{ab}	10.62 ^a	3.56 ^c
10 mM	53.33 ^a	29.10 ^a	12.10 ^a	10.32 ^a	5.41 ^a
20 mM	41.00 ^b	24.13 ^b	12.41 ^a	9.97 ^{ab}	3.12 ^c
30 mM	39.67 ^{bc}	21.25 ^{bc}	10.68 ^b	10.35 ^a	3.45 ^c
40 mM	38.89 ^c	18.67 ^c	9.67 ^c	8.39 ^b	4.66 ^b

SA Conc: Sodium azide concentrations; PLH: Plant height; NLP: Number of leaves per plant; LBL: Leaf blade length; LBW: Leaf blade width; NBP: Number of branches per plant.

Means in carrying the same superscript along a column are not significantly different at $p \leq 0.05$

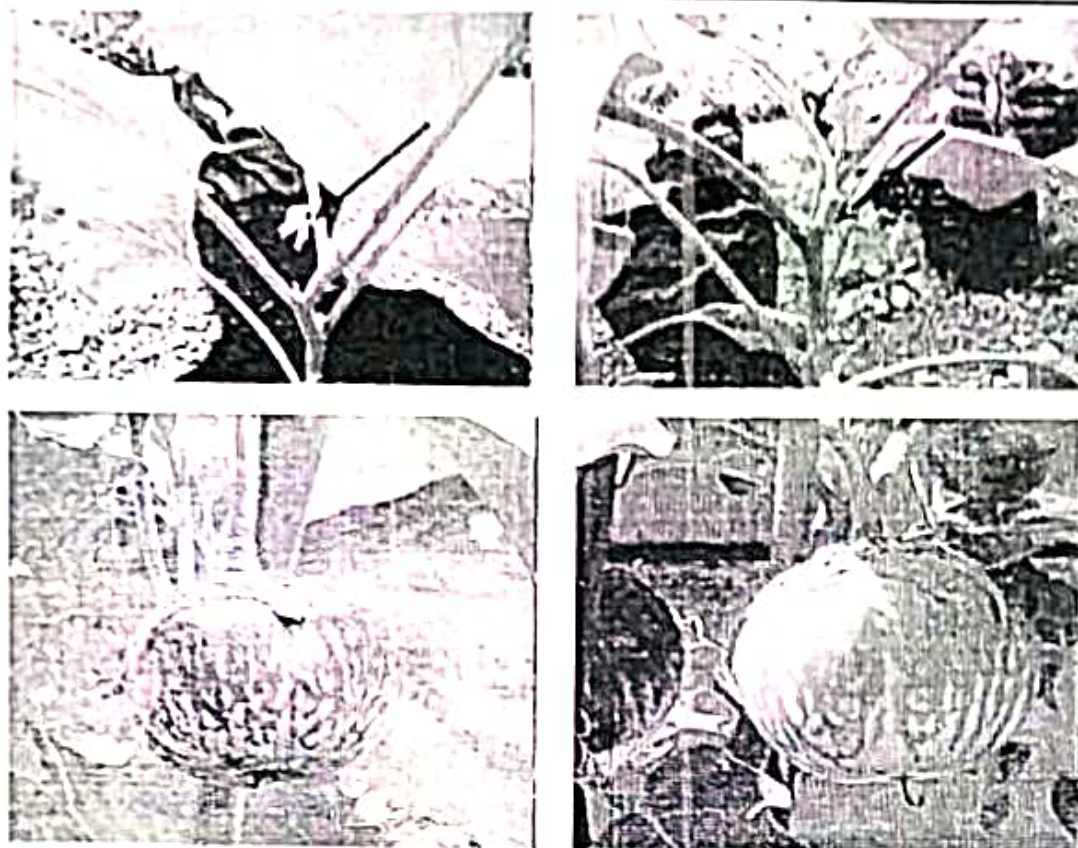
Table 2. The effect of different concentrations of sodium azide on flower and fruit related characters of *Solanum melogena* (NG|SA|JAN|09|157)

SA Conc.	NFL	NFP	FRL	FRW	FSL	FFW
0 mM	11.05 ^{bc}	8.00 ^b	5.28 ^{ab}	6.62 ^c	2.11 ^{bc}	32.00 ^c
10 mM	10.33 ^c	8.78 ^{ab}	4.91 ^b	7.32 ^b	2.18 ^{ab}	29.07 ^d
20 mM	12.00 ^b	8.13 ^{ab}	4.78 ^b	10.27 ^a	1.79 ^b	34.70 ^{bc}
30 mM	12.67 ^{ab}	10.25 ^a	5.26 ^{ab}	10.35 ^a	2.63 ^{ab}	43.80 ^a
40 mM	14.89 ^a	9.67 ^a	6.11 ^a	11.39 ^a	2.74 ^a	42.23 ^a

SA Conc: Sodium azide concentrations; NFL: Number of flower per plant; NFP: Number of fruit per plant; FRL: Fruit length; FRW: Fruit width; FSL: Fruit stock length; FFW: Fruit weight.

Means in the same superscript along the column are not significantly different at $p \leq 0.05$

Table 3. Qualitative traits of *Solanum melogena* - NG|SA|JAN|09|157 in response to different concentrations of sodium azide treatment



SA Conc	PGH	LHR	FCL	FSP	FPS	FRC
0 mM	Upright	Few	Bluish violet	Oval/round	Pendant	Lite green
10 mM	Uprigh	Few	Bluish violet	Oval/round	Pendant	Lite green
20 mM	Uprigh	Few	Bluish violet	Oval/round	Pendant	Lite green
30 mM	Uprigh	Few	Bluish violet	Oval/round	Pendant	Pale green
40 mM	Uprigh	Medium	Bluish violet	Oval/round	Pendant	Pale green

PGH: Plant growth habit, LHR: Leaf hairiness; FCL: Flower colour; FSP: Fruit shape; FPS: Fruit position; FRC: Fruit colour.

Plate 1. Commencement of flowering and fruit set for sodium azide treated eggplants *Solanum melogena* (NG/SA/JAN/09/157)

DISCUSSION

Application of sodium azide on crop is easy and inexpensive for improvement of agronomic traits. It has been used in various crops to improve their yield and quality (Rines, 1985; Raicu and Mixich, 1992; Sarma et al., 2001; Prina and Favres, 2004; Animasaun et al., 2016). The qualitative and quantitative characters evaluated in this study showed variation in most characters, though there are characters with no significant differences in plant responses to the applied treatments. Whereas,



low concentration of sodium azide (10 mM) promoted germination rate and percentage, higher concentrations (30 and 40 mM) caused delay in germination and decreased in germination percentage of the treated seeds. The present result agreed with earlier reports (Mensah and Obadoni, 2006; Mensah *et al.* 2007, Animasaun *et al.*, 2016). This signified that high doses of the mutagen could lower the germination rate. Thus, germination percentage of the seeds decreased with higher concentrations. The delay in germination and reduced germination frequency could be due to negative mutagenic activities such as embryo damage, deactivation, mutation of the enzymes or disruption of the pathways that are required for seed germination.

The observed reduction in the vegetative characters of higher sodium azide dose treatment plant in this study concurred with the findings of Mensah *et al.* (2007) where increased concentrations of sodium azide and colchicines reduced vegetative characters in sesame. It further reinforced the report of Sarma *et al.* (2001) who observed that increased concentration of sodium azide led to significant reduction in vegetative growth of rice. Likewise, Adamu and Aliyu (2007) elucidated that sodium azide mutagenic efficiency increased with increasing mutagenic dose, while mutagenic effectiveness decreased with mutagenic dose in tomato. Makeen and Babu (2010) noted that that mutagenicity and effectiveness of sodium azide on soybean is highest at the lower doses. However, these reports are at variance with the findings of Poomananda and Hosakatee (2009), who reported a decrease in plant height of *Guizotia abyssinica* treated with low dosages of sodium azide and gamma rays. In the present study, it was evident that the optimal concentration of sodium azide to induce vegetative growth in eggplant was 10 mM, concentrations above this threshold, in particular concentrations of 30 and 40 mM negatively affected leaf formation and other foliar parameters. This could result into reduced photosynthate and ultimately affected plant growth negatively.

Interestingly, although vegetative growth and vigour were favoured by low sodium azide concentrations in *S. melongena*, flower and fruit related traits were significantly enhanced by higher concentrations (30 and 40 mM) with the optimal performance achieved with 40 mM. Mutagenesis using higher dosage of both physical and chemical mutagens have been reportedly used to improve fruit related characters in crop plants (Daudu and Falusi, 2011). According to Bolbhat and Dhumal (2012), combined activities of gamma irradiation and ethyl-methanesulphurnate (EMS) significantly increased yield and seed weight of *Macrotyloma uniflorum* (horsegram) consistently at M₁ and M₂ generations. Early maturity, higher fruit number and heavier fruit were induced by higher concentrations of 30 and 40 mM in this study. This was possible due to ability of such higher concentrations to cause sufficient mutation and facilitate earlier gene switch to repress vegetative growth and cause reproductive and flowering genes to upstream for flowering and fruit set. Some earlier studies have also reported that higher concentration of sodium azide promotes early maturity and high fruit or seed production in crop plants (Mensah *et al.*, 2007; Adamu and Aliyu, 2007; Animasaun *et al.*, 2014). The observed similarity in qualitative traits of the treatment plants showed that the evaluated sodium azide concentrations did not produce visible effects on the plant and the fruit quality of the *S. melongena*. This suggests that the concentrations are within the range of dosage for qualitative stability of the plant.

CONCLUSION

The present study showed that sodium azide could be used for mutagenic improvement of *S. melongena*. It was noted that germination, growth and fruit production were affected by different sodium azide concentrations, higher concentrations (30-40 mM) adversely affected germination and seedling vigor. 10 mM had optimal and significant positive effects on vegetative growth of the crop. However, higher concentrations, in particular, the 40 mM, produced early maturing mutants with higher fruit yield which are desire characters for commercial cultivation of eggplant. Therefore, the study concludes that quality of the character desired will determine the effective dose of sodium azide to apply, as low doses favoured vegetative characters while higher doses favoured flowering and fruit related traits. Since, higher fruit yield in *S. melongena* is of interest to the growers, application of the 40 mM sodium azide for 6 hrs could therefore be utilised to enhance fruit yield.



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