NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 54 Number 2, December 2023 Pg. 50-55
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Evaluation of the Effect of Sodium Azides on Growth and Yield of Pepper (*Capsicum* spp. L).

[1]Udogu, O. F., [1]Nkaa, F. A. and [2]Njoku, D. N.

[1]Michael Okpara University of Agriculture, Umudike
[2]National Root Crop Research Institute, Umudike
Corresponding author's email: okwudiri.favour@mouau.edu.ng

Abstract
The study was conducted to investigate the effect of sodium azide on the growth and yield parameters of Pepper (*Capsicum* spp. L). Pepper Seeds were purchased from the National Seed Council Umudike, Abia State. The experiment was set up at Michael Okpara University of Agriculture, Umudike Screen House. The experiment was set up in Complete Randomized Design (CRD) with three replications. The seeds were put inside six different beakers, each containing 20 seeds. The seeds were pre-soaked in distilled water for 3 hours and the floating seeds were eliminated during this period. Various concentrations of sodium azide (0.0%, 0.01%, 0.02%, 0.04%, 0.05%, 0.06% and 0.07%) were prepared. The pre-soaked seeds were later soaked in different concentrations for 3 hours and then placed on the Petri dish for germination percentage and we planted the seeds in our experimental site. The data for germination percentage, plant height, number of leaves, leaf area, stem girth and the number of fruits were obtained and were subjected to the ANOVA test and the mean was separated using Least Significant Difference (LSD) at the statistical significance of 95 % confidence interval. From the result obtained, sodium azide significantly reduced the germination percentage, plant height, stem girth, leaf area, number of leaves and number of fruits of pepper with increasing concentration. However, there are possibilities of having better results in the second mutant generation (M_2) since the second generation was not assessed in this work.
Keywords: Sodium azide, pepper, growth, yield, Pepper, and effect

Introduction

Mutations are essential tools used by the geneticist to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops. Mutations are known to enhance the genetic variability of crop plants and result in a large number of high-yielding varieties of several crops (Pavadai *et al*., 2010; Mehta and Nair, 2011). According to Kleinhofs *et al.* (1998), the ability of these mutagens to enter the cells of living organisms and interact with nucleotide bases in DNA to produce the general toxic effect associated with their mutagenic properties; thus, their effects are mainly due to the direct interaction with the nucleotides

Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. Van Harten (1998) has reported the role of chemical mutagens in enhancing genetic variability in higher plants. Genetic variability is fundamental to successful breeding programs in vegetatively and sexually propagated plants. Mutation produces genetic variability that enhances evaluation and selection for improved crop traits such as; improved yield, shelf-life, and stress tolerance. It is known that various chemicals have positive or negative effects on living organisms. Chemical mutagens generally produce induced mutations which lead to base pair substitutions, especially GC - AT (guanine: cytosine adenine: thymine) resulting in amino acid changes, which change the function of proteins but do not abolish their functions as deletions or frameshift mutations mostly do (Jander *et al*., 2003). These chemo mutagens induce a broad variation of morphological and yield parameters in comparison to normal plants. Sodium azide (NaN_3), has been demonstrated to have mutagenic effects and it has proved to be one of the most powerful mutagens in crop plants (Menda *et al*., 2004). It is a common bactericide, pesticide and industrial nitrogen gas known to be highly mutagenic in several organisms, including plants and animals (Menda *et al*., 2004; Mackenzie *et al*., 2005). Mensah and Obadoni (2007) reported the utilization of NaN_3 in generating genetic variability in plant breeding in barley, groundnut and some other crops. The effect of NaN_3 on seed germination and plant survival has also been reported in *Stevia rebaudiana* seeds by Pande and Khetmalas (2012) and in *Nigella, Plantago* and *Trigonella* by

Prabha *et al.* (2011). Pearson *et al.* (1995) found that various concentrations of NaN_3 delayed the initiation of plant growth in barley. On the other hand, Fay (1995) observed some positive effects of NaN_3 in wild oat seed. In wheat, NaN_3 reduced the root depth and shoot height (Srivastava *et al*., 2011) and in tomato, it decreased each germination percentage, seedling height, seedling survival, number of leaves per seedling, height at maturity, number of leaves per plant and yield per plant, where these effects are increased with increasing NaN3 concentration (Adebola, 2013). This research work investigated the impact of sodium azide on the germination percentage and vegetative growth parameters of pepper.

Materials and Method

Source of planting materials and study area

Seeds of *Capsicum frutescens* were obtained from the National Seed Council of Nigeria within the premises of the National Root Crop Research Institute (NRCRI), Umudike, Abia State. Sodium azide (crystalline). Buffer solution (pH=4.0), distilled water, petri dishes, beakers of different measurements and weighing balance were present in the undergraduate laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umuahia, Abia State. The experiment was conducted between the 24^{th} of September to October 2019 within the premises of the Michael Okpara University of Agriculture, Umudike. Umudike is in the rainforest belt of Nigeria and lies on latitude 05° 28°N and longitude 07° 32°E 245mm (N.R.C.R.I., 1993). It has an average rainfall of 2,200 mm and is 123m above sea level. Minimum and maximum temperatures are 22.41°C and 30°C respectively, with a total annual mean rainfall of 1,245mm (N.R.C.R.I., 1993).

Experimental design/ statistical analysis

The experiment was set up in a CRD (Complete Randomized design). It has six treatments and one control and was replicated three (3) times. The data obtained were subjected to Analysis of variance (ANOVA). Their treatment means were separated using the Duncan Multiple Range test (DMRT) and the Least Significant Difference (LSD) at a statistical significance of 95% confidence interval.

Preparation of mutagens and treatment

One hundred and fifty seeds of the pepper variety were selected into seven groups and put into a 100ml beaker. The first group served as the control whereas the other groups represented various sodium azide concentrations. Presoaking of the seeds in water was done for two hours, and then the seeds were removed and air-dried for 30 minutes. Six different concentrations of sodium azide solutions were prepared namely 0.00%, 0.02%, 0.05%, 0.1%, 0.2%, 0.5% and 1.0%. All were measured as w/v i.e. 0.1g, 0.25g, 0.50g, 0.55g, 0.70g and 0.95g of mutagen (NaN_3) which was weighed into 100ml of distilled water to arrive at the various concentrations above. Two tablets of phosphate buffer were added to each of the concentrations to maintain the pH of the solutions at 4.0 as well as to avoid the dissociation of the mutagen. After the preparations, the presoaked seeds were soaked into freshly prepared concentrations for four hours with intermittent shaking to provide uniform treatment to the soaked seeds. After the treatment time, the seeds were removed from the concentrations and washed thoroughly in running tap water three times to remove the residual chemicals on the seeds. The seeds were dried at room temperature for 30 minutes before planting took place at the screen house.

Measurement of growth parameters

The following growth parameters were measured at 21-day intervals after planting was done.

Germination percentage: this was checked 14 days after planting. Germination percentage (%) was calculated as follows

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds} \times 100}{\text{Number of seeds planted}}$$

Plant height (cm): this was measured from the soil level to the meristematic tip using a meter rule.

The number of leaves: this was done by visual counting of the leaves as the plant grows.

Number of branches: this was done by visual counting of the branches as the plant grows.

Plant canopy- this was by visual counting of the canopy as the plant grows.

Leaf area- this was done by calculating the leaf length and width of the plant at every two-week interval using the formula of Padron *et al.* (2016).

Results and Discussion

Effect of sodium azide on the germination percentage of C. frutescens

Table i shows the effect of sodium azide on the germination percentage of *C. frutescens*. The result obtained showed that seedling germination varied with the different concentrations of sodium azide. Germination was maximum at the control (0.00%), followed by T4 0.2% which had 75% germination. T2 0.05%, T3 0.1%, T5 0.5% and T6 1.0% ranged from 60% to 65% germination while the lowest germination percentage was recorded at T1 0.02% with 55%.

Effect of sodium azide on the plant height of C. frutescens 2 to 8 weeks after planting

The result in Figure I revealed that the mean height of the plants ranged from 2.7 to 7.03 cm in week 2, 2.9 to 11.07 cm in week 4, 3.5 to 12.73 cm in week 6 and 3.8 to 15.73 cm in week 8. It was observed that the control had the highest plant height compared to the other treatments in all the weeks recorded. Treatment one (0.02%) recorded the highest plant height among the treatments after the control followed by Treatment three (0.1%) and Treatment four (0.2%). From the results Treatment two (0.05%), Treatment five (0.5%) and Treatment six (1.0%) were affected negatively by the sodium azide. It is therefore noted that sodium at high concentrations can inhibit the growth of *C. frutescens.* At week two after planting, there was a significant decrease in plant height concerning sodium azide treatment. The same report was observed in week four (4), six (6) and eight (8) after planting. There were significant decreases in plant height as compared to the control.

Effect of sodium azide on the number of leaves of C. frutescens 2 to 8 weeks after planting

The result in Figure ii shows the mean number of leaves of the plants. The mean values of the number of leaves ranged from 4.6 to 9 leaves in week 2, 5 to 10 leaves in week 4, 5.3 to 11.3 leaves in week 6 and 6 to 12.3 leaves in week 8. It was also observed that the control had the highest number of leaves compared to the other treatments in all the weeks recorded. The T1 (0.02%) recorded the highest number of leaves among the treatments after the control followed by T3 (0.1%) and T4 (0.05%). Similar to the plant heights, number of leaves were also inhibited in T2 (0.05%), T5 (0.5%) and T6 (1.0%). These treatments showed poor results in several leaves. The data analysis revealed that the number of leaves in control for all the weeks analysed was significantly different ($p \geq 0.05$) from the treatments.

Effect of sodium azide on the number of branches of C. frutescens 2 to 8 weeks after planting

The mean values for several branches are displayed in Figure iii below. From the results shown, it was observed that the different treatments had varying numbers of branches in all the weeks observed (2, 4, 6 and 8). The control had the highest number of branches compared to the other treatments in all the weeks recorded. This was followed by T1 0.02%, T3 0.1%, T4 0.05%, T6 1.0% and T5 0.5%. T2 0.05% recorded the lowest value for several branches. The result of the analysis of variance showed that the control was significantly different $p \geq 0.05$ from the values obtained in other treatments.

Effect of sodium azide on the plant canopy of C. frutescens 2 to 8 weeks after planting

The mean values for the plant canopy are revealed in Figure iv below. From the results shown it was observed that the different concentrations of the mutagen had varying mean values for plant canopy in all the weeks observed (2, 4, 6 and 8). The control had the highest mean values for plant canopy compared to the other treatments in all the weeks recorded. This was followed by T1 0.02%, T3 0.1%, T4 0.05%, T6 1.0% and T5 0.5%. T2 0.05% recorded the lowest mean values for plant canopy. The result of the analysis of variance showed that the control treatment in all weeks was significantly different $p \geq 0.05$ from the values obtained in other treatments.

Effect of sodium azide on the plant canopy of C. frutescens 2 to 8 weeks after planting

The mean values for leaf area are shown in Table ii below. From the results it is shown that sodium azide improved the leaf area of *C. frutescens* at T3 0.1 % which performed better than other treatments in all weeks except weeks 2, 6 and 8 which the control treatment recorded the highest leaf area. The T2 0.05%, T5 0.5% and T6 1.0% were inhibited by the sodium azide as they didn't record high values for leaf area in all weeks studied. T3 0.1% and T4 0.05% recorded higher values than the T2 0.05%, T5 0.5% and T6 1.0% respectively. The result indicates that the leaf area of the pepper was inhibited by the sodium azide treatments at high concentrations.

The data obtained in this study showed significant effects of sodium azide on the growth parameters investigated in *C. frutescens.* Germination was maximum in the control but decreased in the different concentrations of sodium azide. Reduction in germination induced by sodium azide has been reported by many researchers. Khan *et al*., (2004) observed reductions in seedling germination of *Vigna radiata* treated with sodium azide. The reduction in seedling germination observed in this study agrees with the works of Asad *et al.* (2014) who reported a reduction germination percentage of *P. vulgaris* as a result of sodium azide treatments. The effect of NaN_3 on seed germination and plant survival was reported in *Stevia rebaudiana* seeds by Pande and Khetmalas (2012) and in *Nigella, Plantago* and *Trigonella* by Prabha *et al.* (2011). The latter three crops showed minimum germination at 4.5 % concentration of NaN_3 for eight hours of duration of exposure. Similarly, Pearson *et al.* (1995) found that various concentrations of NaN_3 delayed the initiation of plant growth in barley. On the other hand, Fay (1995) observed some positive effects of NaN_3 in wild oat seed.

In the present study, the control performed better in all growth parameters recorded which include plant height, number of leaves, number of branches and plant canopy. The lowest concentration of sodium azide treatment showed better results compared to treatments with high concentrations of sodium azide. This result is also in agreement with the report of Adamu and Aliyu (2007) who reported increased growth in tomatoes due to sodium azide treatments at low concentrations. The increase in the number of leaves and number of branches of *C. frutescens* in the lowest concentration of sodium azide treatment signifies the ability of sodium azide to initiate more foliar buds. This is in agreement with the report of Maluszyiski *et al.* (2001) who reported an increase in leaf area among maize mutants.

From the study, high concentrations of sodium azide treatment on *C. frutescens* influenced the ability of the plants to grow well. It was observed that the data on the mean values of plant height, number of leaves, number of branches and plant canopy of the plants with high concentrations of sodium azide were poor when compared to the control treatment. This observation agrees with the works of Adamu and Aliyu (2007) who reported a decrease in the shoot and root lengths of tomato (*Lycopersicon* esculentum) soaked in sodium azide. In wheat and tomato, NaN_3 reduced the shoot height, also decreased each germination percentage, seedling height, seedling survival, number of leaves per seedling, height at maturity, number of leaves per plant and yield per plant, where these effects are increased with increasing NaN_3 concentration (Srivastava *et al*., 2011; Adebola, 2013).

The result for the leaf area indicated that the leaf area was inhabited by the sodium azide treatments at high concentrations. This is supported by Salim *et al.* (2009) who reported that sodium azide is a strong mutagen, and the growth of plant parts is strongly inhibited by increasing its concentration and treatment duration. The mutational effects of this mutagen have been observed on tomatoes and it was very effective in inducing mutations concerning germination percentage, root length, seedling height, seedling survival, number of branches per plant, leaf area and yield per plant respectively (Adamu and Aliyu, 2007). Adamu and Aliyu (2007) also stated that the effect of different concentrations of NaN_3 treatment on root length was observed in different crops used for the research.

Conclusion

Sodium azide had significant effects on the vegetative growth of the *C. frutescens*. The effects were observed to be concentration-dependent. From the findings, it's clear that a small quantity of sodium azide can improve the vegetative growth of pepper. Further studies should be conducted to evaluate the effect of sodium azide on the yield parameters of *C. frutescens.* The mutagenic effect of sodium azide on the anatomy of plants should be investigated.

References

Adamu, A. K. and Aliyu, H. (2007). "Morphological effects of Sodium azide on tomato (*Lycopersicon esculentum* Mill.)," *Sci. World J*., **2** (4): 9-12.

Adebola, M. O. (2013). "Mutagenic effects of sodium azide (NaN_3) on morphological characteristics of tomato (*Lycopersicum esculentum*)," *Research Journal of Science and IT Management*, **2** (4): 1-5.

Asad, V. A., Kumari, R. U., Amutha, R., Kumar, T. S., Hepziba, S. J. and Kumar, C. R. A. (2014). "Effect of chemical mutagen on the expression of characters in arid common beans (*P. vulgaris* L.Walp.)," *Research Journal of Agriculture and Biological Sciences*, **5** (6): 1115 – 1120.

Fay, P. K. (1995). "The effect of germination stimulators on wild oat (*Avena fatua* L.) emergence in the field," The North Central Weed Control Conference 30.110111. pp. 56.

Jander, G., Baerson, S. R., Hudak, J. A., Gonzalez, K. A., Gruys, K. J. and Last, R. L. (2003). "Ethyl methane sulfonate saturation mutagenesis in Arabidopsis to determine the frequency of herbicide resistance," *Plant Physiol*., **131**: 139-146.

Khan, S., Wani, M. R. and Parveen, K. (2004). "Induced genetic variability for quantitative traits in *Vigna radiata* (L.) Wilczek," *Pak. J. Bot*., **36**: 845-850.

Kleinhofs, A., Owais, W. and Nilan, R. (1998). "Sodium Azide; A chemical mutagen," *Mutation Research*, **55**: 165–195.

Mackenzie, J. L., Scade, F. E., Le, Q. H., Bureau, T. E. and Schoen, D. J. (2005). "Genomic mutation in lines of Arabidopsis thaliana exposed to ultraviolet-B radiation," *Genetics*., **171**: 715-723, 2005.

Maluszyiski, D., Munir, N., Safdar, I. and Naz, S. (2001). "Effect of induced mutation for varietal improvement in some local maize cultivars," *The Journal of Animal and Plant Sciences*, **25** (1): 234-242.

Mehta, A.K. and Nair, R. (2011). "Use of gamma irradiation for prolonging the shelf life of garden pea (*Pisum sativum* L.)", *Plant Mutation Report,* **2**: 38-45.

Menda, N., Semel, Y., Peled, D., Eshed, Y. and Zamir, D. (2004). "In silico screening of saturated mutation library of tomato," *Plant J*., **38**: 861-872.

Mensah, J. K. and Obadoni, B. (2007). "Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.)", *African Journal of Biotechnology,* **6** (6): 668-671.

Pande, S. and Khetmalas, M. (2012). "Biological Effect of Sodium Azide and Colchicine on Seed Germination and Callus Induction in Stevia Rebaudiana", *Asian Journal of Experimental Biological Sciences*, **3** (1): 93-98.

Pavadai, P., Girija, M. and Dhanavel, D. (2010). "Effect of gamma rays on some yield parameters and protein content of soybean in M2, M3 and M4 generation," *Journal of Experimental Science*, **1**: 8-11.

Pearson, O. W., Nillan, R. A. and Sander, C. (1995). "The effect of sodium azide on cell processes in the embryonic barley shoot", *Radiation Botany,* **15**: 315-322.

Prabha, R., Dixit, V. and Chaudhary, B. R. (2011). "Comparative spectrum of sodium azide responsiveness in plants", *World Journal of Agriculture Sciences*, **7**(1): 104-108.

Srivastava, P., Marker, S., Pandey, P. and Tiwari, D.K. (2011). Mutagenic Effects of Sodium Azide on the Growth and Yield Characteristics in Wheat (*Triticum aestivum* L em. Thell.). *Asian Journal of Plant Sciences,* **10** (3): 190-201.

Van Harten, A. M. (1998). Mutation Breeding: Theory and Practical Applications. Cambridge University Press, Cambridge, UK.

Table i: Effect of sodium azide on the germination percentage of *C. frutescens*

Treatment (%)	Germination percentage%
Control 0.00	100
T1 0.02	55
T2 0.05	65
T3 0.1	60
T4 0.2	75
T5 0.5	65
T6 1.0	60

Table ii: Effect of sodium azide on the leaf area (cm^2) of *C. frutescens* 2 to 8 weeks after planting

Treatment %	2WAP	4WAP	6WAP	8WAP
Control 0.00	1.72 ± 0.47^{b}	4.68 ± 1.46^{c}	8.62 ± 3.89^{e}	15.14 ± 6.34^{d}
T1 0.02	1.67 ± 0.74^{b}	3.80 ± 1.68^{c}	6.11 ± 2.14^{c}	9.90 ± 3.20^{b}
T2 0.05	0.27 ± 0.00^{a}	0.72 ± 0.00^{a}	1.57 ± 0.00^{a}	2.77 ± 0.00^{a}
T3 0.1	0.80 ± 0.10^{a}	5.38 ± 6.74^{d}	7.77 ± 8.99^{d}	11.16 ± 11.12^{c}
T4 0.2	1.16 ± 1.17^{b}	3.43 ± 2.32^{c}	4.53 ± 2.43^{b}	6.33 ± 2.96^{b}
T5 0.5	0.17 ± 0.11^{a}	1.56 ± 0.42^{b}	1.47 ± 0.32^{a}	2.77 ± 0.70^{a}
T6 1.0	0.53 ± 0.19^{a}	1.64 ± 0.08^{b}	2.40 ± 0.17^{a}	2.71 ± 0.01^{a}

Values are mean ± SD, and values with different subscripts on the same column are significantly different i.e. $P \leq 0.05$

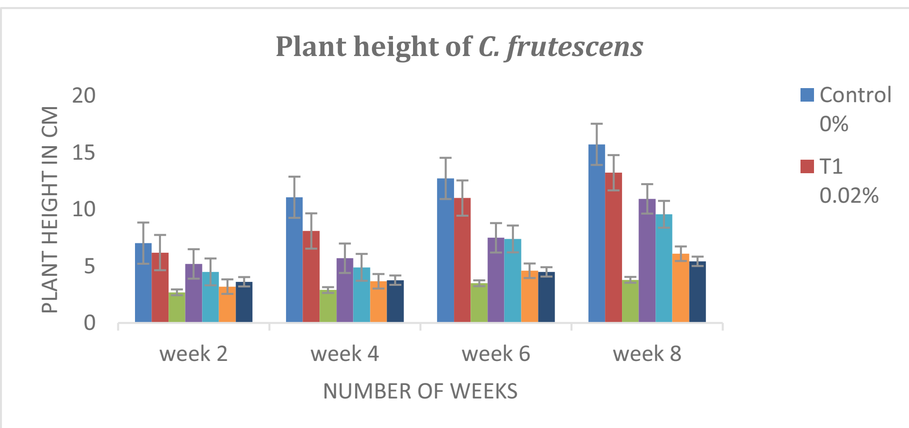

Fig. i Effect of sodium azide on the plant height of C. frutescens

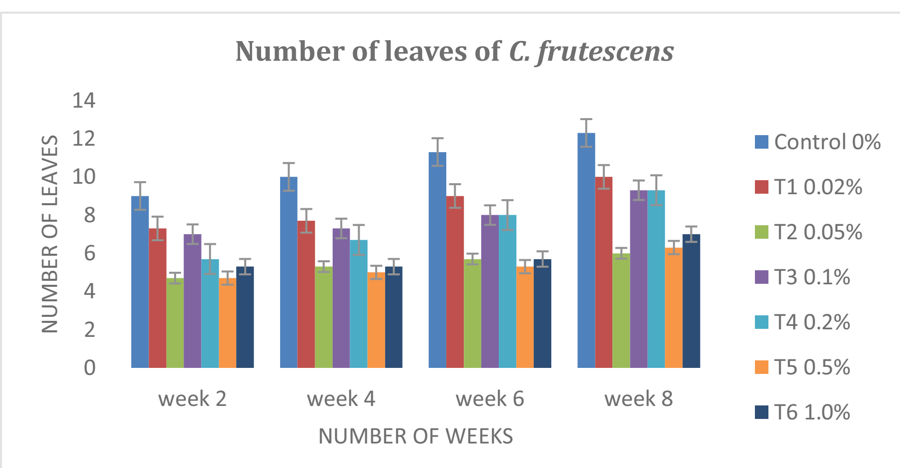

Fig. ii Effect of sodium azide on the number of leaves of C. frutescens

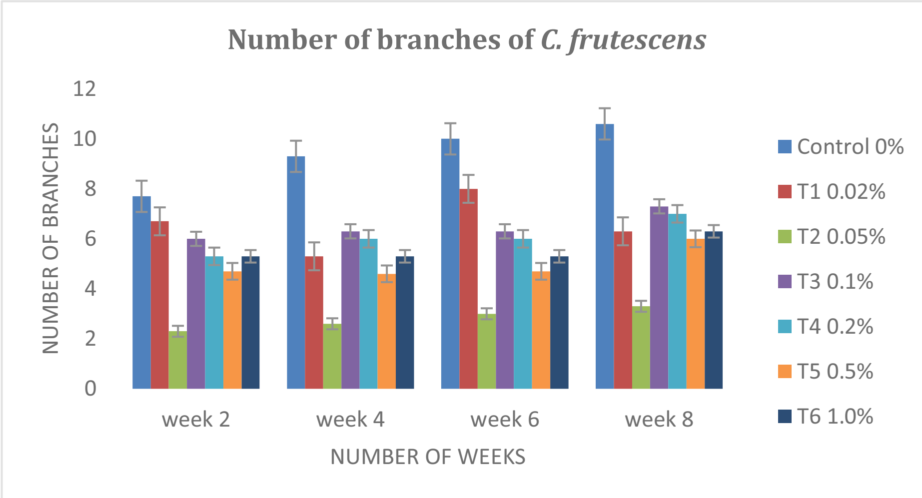

Fig. iii Effect of sodium azide on the number of branches of C. frutescens

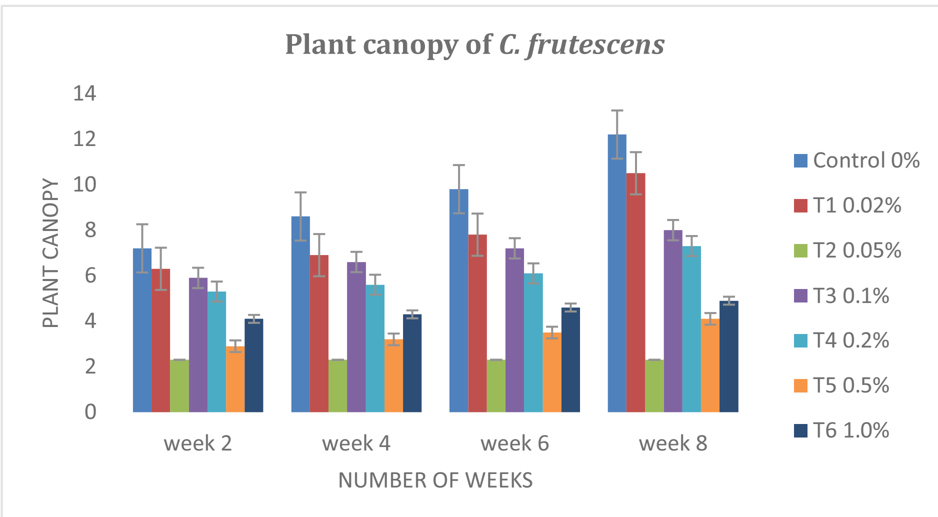

Fig. iv Effect of sodium azide on the plant canopy of C. frutescens