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Effects of sodium azide on seed germination of common beans (*Phaseolus vulgaris)*

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Abstract

The use of sodium azide (NaN_3) to determine the effect of induced mutations on seed germination and seedling growth of *Phaseolus vugaris* L. was carried out. The seed were exposed to NaN_3 solution (0.25, 0.5, 1.0, 2.0, 3.0 and 4.0mM at pH 3) for 4 hours and were thereafter rinsed in tap water to remove excess mutagens. Compared to the control, the percentage germination of seeds treated with 0.5mM at days 1 and 2 were 96.7% and 100% respectively. Percentage germination of seeds treated with 1.0mM was 10% and 53% on the first and second days respectively. Seedling growth was enhanced by low doses (0.25, 0.5 and 1.0mM) of the mutagen, while higher doses (2.0, 3.0 and 4.0mM) inhibited growth of the seeds. At day 7, radicle lengths (mm) were higher with seeds treated with 0.25mM NaN_3 compared with those treated with 0.5mM and 1.0mM NaN_3. The response of the plumule length at day 7 was also higher with 0.25mM NaN_3 compared with 0.5mM and 1.0mM NaN_3. The results indicate that NaN_3 is suitable for creating variability in cowpea at low concentrations.

Keywords: Induced Mutation, Mutagens, Plumule length, *Phaseolus vulgaris*, Sodium Azide, Seed germination

Introduction

Mutation experiments on the seed quality of cereals induced by various mutagens may shed some light on the issue. Induced mutations occur more or less randomly in the genome; even their target cannot be directed. Only one of the (two or more) alleles of a locus is affected, inheritance is almost ever recessive, therefore homozygosity is normally required for proper expression. Accordingly, results were more often useful in self-pollinating plant species. On the other hand, mutant heterosis has been repeatedly reported **(**Micke, 1968, 1969, 1976; Romer *et al.,* 1974**)** and specific mutations for example concerning male sterility **(**Daskalov *et al.*, 1988**)** or grain quality traits **(**Robbelen, 1990**)** proved useful in cross-pollinating species.

Amano (1985) studied carefully the changes induced in the *waxy* loci of rice and maize using EMS, UV, thermal neutrons, and gamma-rays. Amano **(**1981, 1985**)** and Yatou *et al.,* **(**1991**)** looked at the expression of the *waxy* gene in pollen and in the endosperm**.** The authors confirmed a destructive effect of all the mutagens used, but

the kind and degree of destruction differed between the mutagens applied, from impairment of proper code transcription (mainly by EMS) to total inactivation or even completes deletion of the locus.

Another interesting insight can be derived from studies on the effect of chemical mutagens on a particular locus in barley (*Mla12*), responsible for a specific resistance to *Erysiphe graminis* **(**Jorgensen, 1996**)**.
Chemical mutagens are the one cause of mutations in living organism. It is known that various chemicals have positive or negative effects on living organisms. Many of these chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals **(**Yuan *et al.,* 1993**)**. These effects can occur both spontaneously and artificially following induction by mutagens. These chemo- mutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants. Dhanayanth *et al.,* **(**2000**)** and Bhat *et al.,* **(**2005**)** found chemical mutagens are to be more effective than physical ones, and many others researchers found the reverse case (Zeerak *et al.,* 1991**)**.

Sodium azide (NaN_3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. It has been reported that sodium azide affects plant physiology and decrease cyanide resistant respiration in tobacco callus (Wen *et al.,* 1995**)**. It is known to be highly mutagenic in several organisms, including plants and animals **(**Rines, 1985; Raicu *et al.,* 1992; Grant *et al.,* 1994**)** and its mutagenic potential has been reported in several screening assays. Sodium azide is marginally mutagenic in different organisms **(**Arenaz *et al.,* 1989**)** and it is not mutagenic in several others organisms such as *Drosophila* **(**Kamraand *et al.,* 1979**)** and *Arabidopsis* **(**Gichnerand *et al.,* 1977**)**. The mutagenicity is mediated through the production of an organic metabolite of azide compound **(**Owais *et al.,* 1988**)**. This metabolite enters into the nucleus, interacts to DNA, and creates point mutation in the genome. In order to understand its mutagenic mechanism, many studies in barley and bacteria have been performed in recent years **(**Gichnerand *et al.,* 1977**)**. Being a strong mutagen in plant, it affects the different parts of the plants and their growth developmental phenomena by disturbing the metabolic activities. The effect of chemical mutagens depends on the permeability of seed coat and nature of the mutagens. To enhance the mutagenic effectiveness and efficiency of NaN_3 and especially the metabolite, more knowledge about the effect of time, temperature of seed soaking and various concentration of NaN_3 are required. The pH value affects the rate of mutation with NaN_3.

Azide ion plays an important role in causing of mutation by interacting with enzymes and DNA in the cell. These azide anions are strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation process. In addition, it is a potent inhibitor of the proton pump **(**Kleinhofs *et al.,* 1974**)** and alters the mitochondrial membrane potential **(**Kleinhofs *et al.,* 1974; Zhang, 2000**)**. These effects caused by NaN_3 together may hamper ATP biosynthesis resulting in decreased availability of ATP molecule which may slow the germination rate and reduce the germination percentage. The another reason behind this is that seeds have probably developed tolerance to the inhibitory effect of NaN_3 on germination and had improved their physiological conditions on additional days with respect to seed germination. All living cells require energy in the form of ATP molecules to carry all biological reactions. At low energy level, the rate of biological reactions inside the cell decreases. Cheng *et al.,* (1988**)** treated barley seeds with sodium azide and found a significant decrease in the percentage germination. It is important to stress the fact that treatments with sodium azide, under the same conditions, produce a delay in the initiation of plant growth, as can be observed and mentioned by **(**Pearson *et al.,* 1975**)**. The various concentration of sodium azide showed different effects of mutagenesis as mentioned by many authors in literature. Kleinhofs *et al.,* **(**1974**)** suggested that 0.003 M NaN_3 dose increases

mutations in pea. The higher dose (2mM, 3mM and 4mM) of sodium azide also cause disturbance in genetical and physiological activities leading to the death of the cells of *Phaseolus vulgaris* seeds when treated with them. The greater sensitivity at higher mutagenic level has been attributed to various factors such as changes in the metabolic activity of the cells, inhibitory effects of mutagens and to disturbance of balance between promoter and inhibitors of growth regulators **(**Krishna *et al.,* 1984**)**. Sodium azide is a strong mutagen, and growth of plant parts are strongly inhibited with increasing its concentration and treatment duration. The mutational effects of this mutagen has been observed on tomato and it was very effective in inducing mutations with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant, and yield per plant respectively **(**Adamu *et al.,* 2007**)**.

The mechanism of action and the nature of mutations created by sodium azide are becoming understood and it has been accelerated by the discovery of a mutagenic metabolite formed by sodium azide. The chromosomes are damaged by sodium azide in mitosis, as seen in barley **(**Nillan *et al.,* 1975; Sander *et al.,* 1978**)**, in bean and in human leucocytes **(**Sander *et al.,* 1978**)** respectively. Thus, sodium azide induced chromosome aberration frequencies, which were similar or slightly superior to those of the untreated controls. The most predominant aberrations induced by sodium azide are translocations, lagging chromosome, bridges and sticky chromosomes. The numerous mutants produced under the treatment of sodium azide and great influence was found on seed shape and seed size of tested common bean mutants **(**Jeng *et al.,* 2010**)**.

Materials and Methods

Samples Collection

Viable seeds of common beans (*Phaseolus vulgaris*) were purchased from Makurdi, Benue State, Nigeria. The seeds were stored before processed for use (air-dried dormant seeds).

Preparation of Mutagenic Solution

Sodium azide solution was prepared by dilution in distilled water adjusted to pH 3 by using a pH 3 buffer. Solution was prepared in six levels (0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 mM). The control used in this study was water adjusted to a pH 3. The seeds were exposed to NaN_3 solution for 4 hours. The seeds were then thoroughly rinsed with tap-water to remove excess mutagens.

Planting of Seeds

The treated seeds were planted in sterile petri dishes stuffed with sterile cotton wool used as the absorbent. The cotton wool was damped in water and thereafter 30 seeds were placed on each of them. The seeds in the petri dishes were left for 7 days.

Parameters Measured

Data were collected on period to first germination (hours and mintues), percentage germination (%), radical length, plumule length, water imbibitions at 10 hours after planting (%), fresh weight of sprouted seeds at 5days after planting and dry weight of sprouted seeds at 5days after planting. The radical length and plumule length were measured in millimetres (mm), while fresh weight and dry weight of sprouted seeds were measured in grams. The values presented in tables 5, 6 and 7 are mean values ± standard deviations.

Results and Discussion

Table 1 explains the time taken for seeds treated in the various solutions to germinate after planting. Germination occurred in the control treatment at 5hours, 25mintues with 16 seedlings germinating initially. In treatment of seeds with 0.25 mMNaN_3 solution, germination started at 6hours 30mintues after planting. In treatment of seeds with 0.5mM NaN_3 solution, it took 8 hours for the first seed to germinate, while with the treatment of seeds in 1mM sodium azide solution, germination began at 11 hours 44mintues. No germination occurred in 2.0mM - 4.0mM NaN_3 treated seeds.

Mutations are the tools used by the geneticists to study the nature and function of the genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops **)**Adamu *et al.,* 2004**)**. Various mutagenic agents are used to induce favourable mutations at high frequency that include ionizing radiation and other chemical mutagens. Ahloowalia *et al.,*)2001**)** induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. Sodium azide can be used to improve the germination behaviour of *Phaseolus vulgaris* by applying it in low concentrations (1mM and lower concentrations).

The result showed that all the traits studied were affected by sodium azide treatment except the control. Table 1 explained the effects of NaN_3 treatments on period to first germination of treated cowpea seeds. The time after planting with corresponding time of seed germination was faster in the control (5hours, 25mintues) than those in 0.25mM (6 hours, 30 mintues), 0.5mM (8 hours) and 1mM (11 hours, 44mintues). Germination was delayed in seeds treated with 1mM NaN_3 solution. While 2mM, 3mM and 4mM NaN_3 concentrations did not support germination in *Phaseolus vulgaris* in a period of seven days. The implication of this is that mutagenic treatments significantly hindered germination in the seed of *Phaseolus vulgaris*.

The control solution and 0.25mM NaN_3 solution showed the same effect on percentage germination, in which there was 100% germination from day 1 to day 7, compared to when the concentration of NaN_3 was increased to 0.5mM and 1mM NaN_3.Highest germination percentage was observed with 0.25mM NaN_3 solution as against the least germination percentage observed with 1mMNaN3 solution from day 1 to day 3. No germination occurred in seeds treated in 2, 3 and 4mM NaN_3 solutions. The effect of the mutagen on radicle length showed that there is continuous increase in length of the radicle from day 1 to day 7 of seeds treated with control, 0.25, and 0.5 NaN_3 solutions respectively. Continuous increase in length of radicle was observed in seeds treated with 1mM NaN_3 from day 2 to day 7.The effect of the mutagen on plumule length showed that there is continuous increase in length of the plumule from day 1 to day 7 of seeds treated with control, 0.25, and 0.5 NaN_3 solutions respectively. Continuous increase in length of plumule was observed in seeds treated with 1mM NaN_3 from day 3 to day 7.Highest radicle and plumule lengths were observed with 0.25mM NaN_3 solution as against that observed with 1mM NaN_3 solution at day 7.

Sodium azide is highly soluble in water, but fewer number of hydrozoic ions are produced in water, and at low pH the quantities of NaN_3 dissociated to hydrozoic acid which is theoretically many times greater (at pH 3 there is approximately 19 times more hydrozoic acid than at pH 6, for the same concentration of NaN_3), and that would be the condition for better penetration through the cell membrane and create mutations in the genome of a cell **)**Nilan *et al.,* 1973; Kleinhofs *et al.,* 1974**)**. The result of the study showed that the high dose of NaN_3 (2 - 4mM NaN_3) treatments on *Phaseolus vulgaris* seeds resulted in them not germinating. Mutagen causes changes

to the DNA that can affect the transcription and replication of the DNA, which in severe cases can lead to cell death. The mutagen produces mutations in the DNA, and deleterious mutation can result in aberrant, impaired or loss of function for a particular gene, and accumulation of mutations may lead to diseases.

Different mutagens act on the DNA differently. Powerful mutagens may result in chromosomal instability **)**Huang *et al.,* 2003**)** causing chromosomal breakages and rearrangement of the chromosomes such as translocation, deletion, and inversion. Such mutagens are called clastogens.
The relevance of this research to crop improvement is to increase yield, product quality, cause winter hardiness (to survive winter stress) and bringing about resistance to insect pests, plant pathogens, heat and drought, soil stress, lodging (bending or breaking over of plants before harvest) and shattering (fall out of seeds before harvest). It is also used to create genetic variability of the plant for desirable traits and provide the tools to overcoming the limitations of variability in the plant. The role of mutation breeding in increasing the genetic variability for desired traits in various crop plants have been proved beyond doubt by a number of scientists **)**Tah, 2006; Khan *et al.,* 2009; Mostafa, 2011**)**.

Conclusion

Induced mutations used successfully for variety development affected essential structural genes common to almost all forms of living creatures and preserved over millions of years. It seems also unlikely, that the mutations concern any other gene that has assumed during the course of evolution an essential position in the gene network of the particular species, because such mutations would have resulted in loss of vigour and probably death (e.g. chlorophyll mutations).

Considering the use of *in-vitro* culture techniques in mutation breeding, the following might be concluded: All genetic alterations caused eventually by physical or chemical mutagens can be expected also from somaclonal variation. In addition, one may expect activation of transposons, but this also suspected for some chemical mutagens. Somaclonal variation will be compounded with epigenetic effects. This makes the use for crop improvement more difficult, particularly in asexually propagated plants, since crossing and reselection would be required to sort out desired mutations from other alterations. *In-vitro* selection is handicapped by a-typical gene expression. Use of haploids derived from another culture has found its best application in the doubled-haploids-technique, which leads faster to homozygosity for more effective selection.

Experimental mutagenesis has now a more restricted, but better defined place among the methods of applied genetics. In addition to the use of induced mutations in crop improvement, there is now a most valuable use in fundamental genetics and plant physiology. This will benefit breeders through more effective strategies for crop improvement. However, if a breeder wants new genes with new gene products, he would definitely have to resort to gene engineering and use mutagen treatments as a supplementary tool. In the treatment of seeds with 2, 3 and 4mM sodium azide solution, no germination occurred. This shows that these concentrations do not support germination of the beans seeds. It's positive to say that with all the parameters measured, increase in concentrations of NaN_3 above 1mM will not support germination in cowpea (*phaseolus vulgaris*) at pH 3, but will support germination and growth at pH 3 with concentrations ranging from 1mM NaN_3 and lower concentrations.NaN_3 causes negative effect of inhibiting germination and growth at high concentrations, as seen in this study.

Therefore, NaN_3 is a strong mutagen, and affected the parameters measured in the study carried out on *phaseolus vulgaris* and thus it should be used further on this species to improve its agronomic traits and also produce resistance to them against biotic and abiotic stress by creating mutation.

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Conflict of Interests

The authors have declared that no conflicting interest exists.

Tables, Figures and Charts

Table 1: Effects of NaN_3 treatments on period to first germination of treated cowpea seeds

Treatments ($mM\ NaN_3$ solution)	Time after Planting
0 (Control)	5hrs, 25mins
0.25	6hrs, 30mins
0.5	8hrs
1.0	11hrs, 44mins
2.0	No germination occurred
3.0	No germination occurred
4.0	No germination occurred

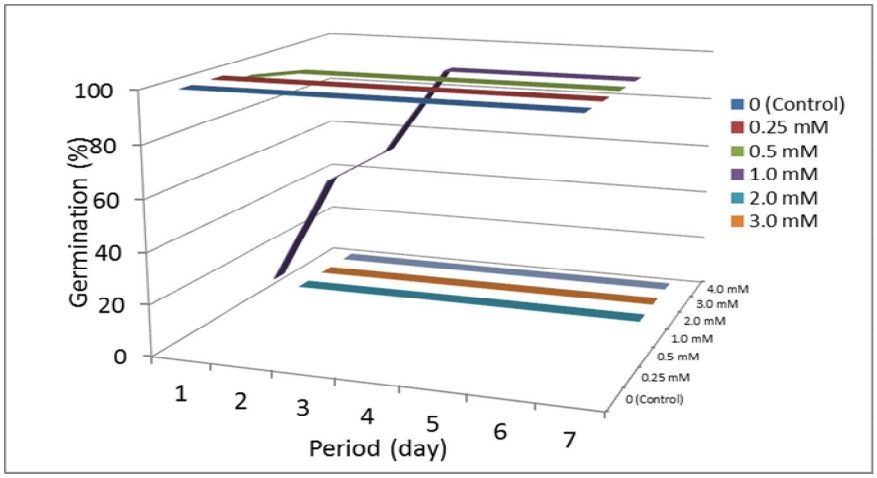

Figure 1: Effects of NaN_3 treatments on percentage germination of cowpea seeds.

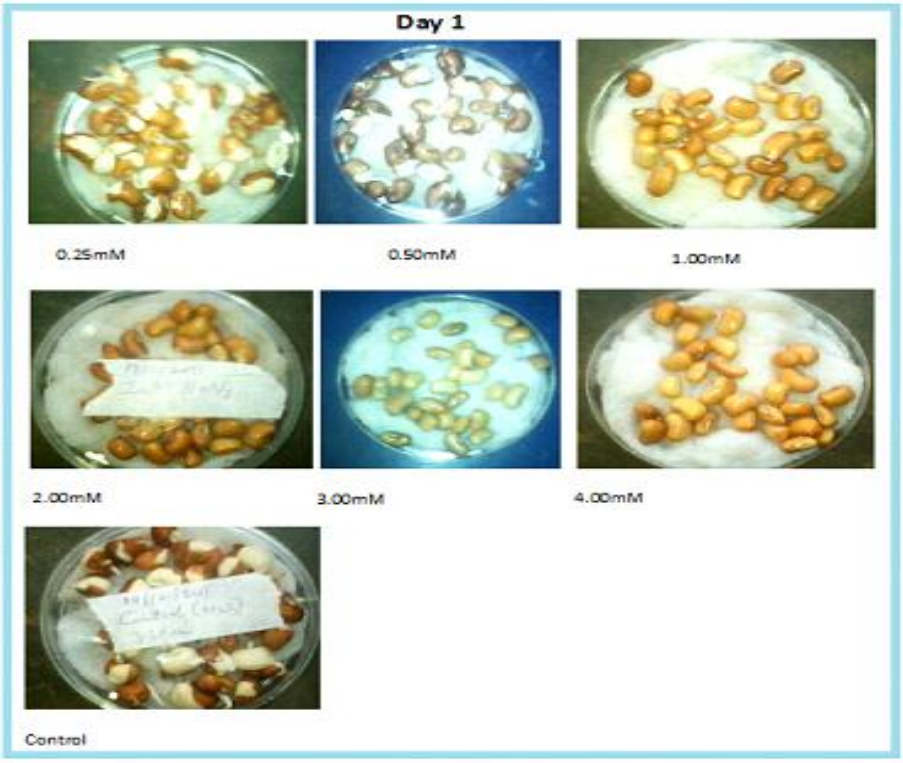

Plate 1: Treated cowpea seeds on the first day of sowing in petri dishes.

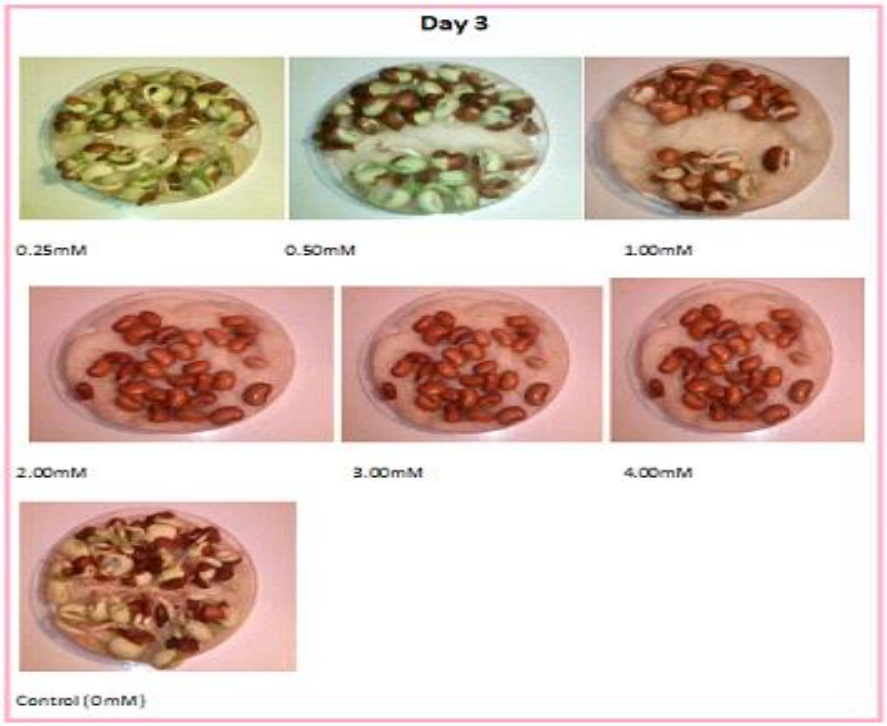

Plate 2: Germinated cowpea seeds treated with NaN_3 solution on the 3rd day of germination.

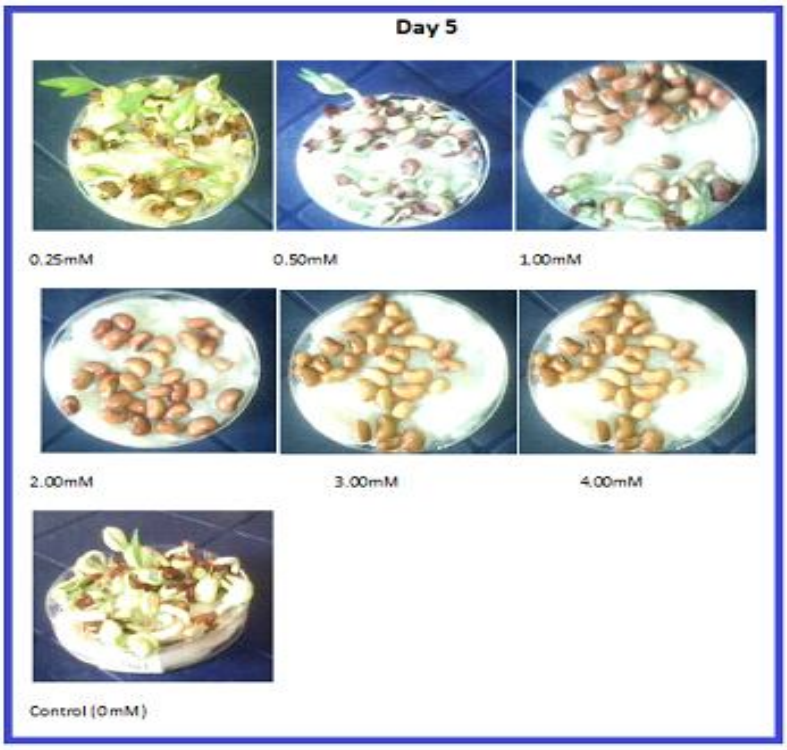

Plate 3: Germinated cowpea seeds treated with NaN_3 solution on the 5th day of germination.

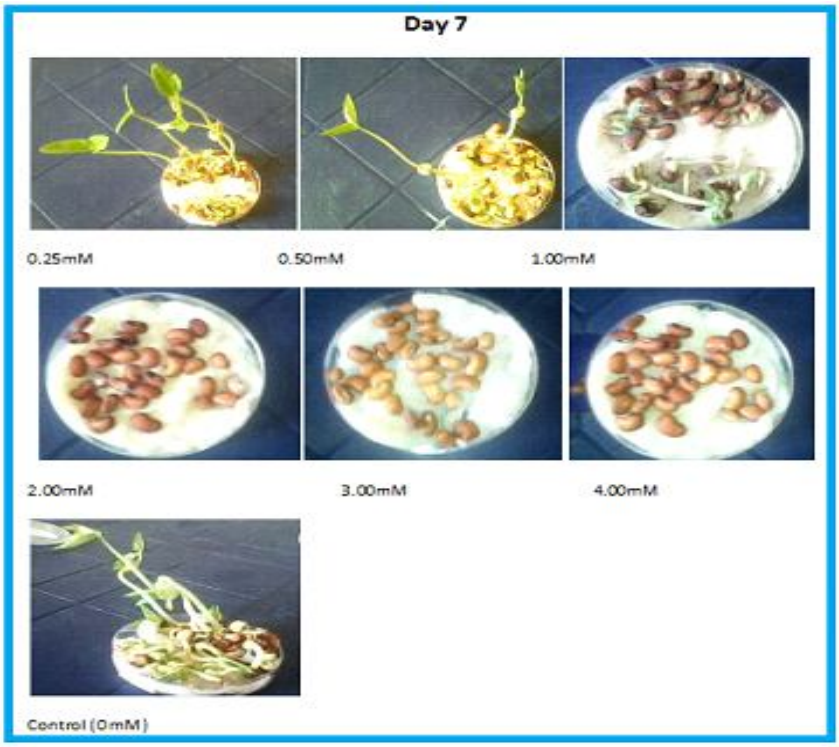

Plate 4: Germinated cowpea seeds treated with NaN_3 solution on the 7th day of germination.

Table 3: Effects of NaN_3 treatments on radicle length (mm) of germinated seeds of cowpea.

Treatments (mM NaN_3 solution)	Day1	Day2	Day3	Day4	Day5	Day6	Day7
0mM (Control)	14.30	19.90	22.40	23.50	24.50	25.70	26.30
0.25mM	12.10	17.90	18.60	20.20	22.50	22.90	23.00
0.5mM	13.80	19.90	20.10	21.20	21.40	21.60	21.90
1.0Mm	0	12.60	18.10	20.80	21.00	21.10	21.90
2.0mM	0	0	0	0	0	0	0
3.0Mm	0	0	0	0	0	0	0
4.0Mm	0	0	0	0	0	0	0

Table 4: Effects of NaN_3 treatments on plumule length (mm) of germinated seeds of cowpea.

Treatments (mM NaN_3 solution)	Day1	Day2	Day3	Day4	Day5	Day6	Day7
0 (Control)	7.30	12.90	18.60	21.00	22.80	23.40	24.30
0.25	7.20	11.20	15.10	16.20	16.70	19.70	20.60
0.5	4.90	12.20	16.50	19.50	20.70	21.40	21.90
1.0	0	0	10.00	12.50	14.75	15.60	18.75
2.0	0	0	0	0	0	0	0
3.0	0	0	0	0	0	0	0
4.0	0	0	0	0	0	0	0

Table 5: Effects of NaN_3 treatments on water imbibition of germinated seeds of cowpea at 10 hours after planting

Treatments (mM NaN_3 solution)	Water imbibition (%)
0 (Control)	45.2 ± 6.98
0.25	58.6 ± 6.92
0.5	55.2 ± 5.24
1.0	63.9 ± 8.63
2.0	71.8 ± 2.13
3.0	70.9 ± 4.38
4.0	71.3 ± 5.93

Values presented in mean ± standard deviations.

Table 6: Effects of NaN_3 treatments on fresh weight (g) of sprouted seeds of cowpea at 5 days after planting.

Treatments (mM NaN_3 solution)	Weight (grams)
0 (Control)	2.596 ± 0.071
0.25	2.668 ± 0.098
0.5	2.719 ± 0.109
1.0	3.109 ± 0.104
2.0	No germination
3.0	No germination
4.0	No germination

Values presented in mean ± standard deviations.

Table 7: Effects of NaN_3 treatments on dry weight (g) of sprouted seeds of cowpea at 5 days after planting

Treatments (mM NaN_3 solution)	Dry weight (grams)
0 (Control)	0.531 ± 0.032
0.25	0.687 ± 0.099
0.5	0.863 ± 0.123
1.0	0.901 ± 0.123
2.0	No germination
3.0	No germination
4.0	No germination

Values presented in mean ± standard deviations.

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