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Research Article

Mutagenic effect of sodium azide (NaN_3) on M_2 generation of *Brassica napus* L. (variety Dunkled)

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Abstract

The present study was aimed to investigate the effect of different doses (0.2%, 0.4%, 0.6% and 0.8%) of sodium azide (SA) on M_2 generation of *Brassica napus* (variety Dunkled) on some qualitative, quantitative and biochemical parameters. The result showed that days to germination, days to flowering and days to siliqua maturation were delayed (9.4, 98 and 153 days respectively) in higher treatment as compared to control (5.4, 96 and 147 days respectively). Higher concentration of SA (0.8%) decreased germination percentage (88.56%), plant height (97.95 cm), stem diameter (3.49 cm), number of branches/plant (3.49), number of leaves/plant (10), number of siliqua/plant (64.68), number of seed/siliqua (20.09) and 1000 seeds weight (3.91g) as compared to control. An increase was noticed for siliqua length (5.42 cm) in higher concentration of SA (0.8%) as compared to control (5.24 cm). Proximate analysis showed a significant decrease in oil, oleic acid and glucosinolates contents percentage (42.6%, 54.9%, and 58.5 µmol/g respectively) in higher concentration of SA (0.8%) as compared to control (44.7%, 58.1% and 71.3 µmol/g respectively) while a significant increase was noticed in proteins, moisture, linoleic acid and erucic acid percentage (28.4%, 5.9%, 9% and 37.3% respectively) in higher concentration of SA (0.8%) as compared to control (26.8%, 5.5%, 8.4% and 35.2% respectively.

Keywords: Sodium Azide (SA); *Brassica napus*; morphological and biochemical parameters

Introduction

Rapeseed (*Brassica napus*) is also known as rape and Rapa. It is a bright yellow flowering annual herb of family Brassicaceae/ Cruciferae. The family comprised of over 338 genera and about 3,710 species distributed worldwide **[1]**. The family is cosmopolitan in nature but it is one of the cultivated medicinal food plants in middle Asia, North Africa and West

Europe [2]. Rapeseed cultivars show significantly different responses depending on weather conditions to different irrigation levels [3]. The increase of temperature has a negative effect on seed yield of spring oil rape seed, nitrogen show significant effect on seed yield and flowering, phosphorous concentration is not important while potassium concentration effect on the contrary [4]. Rapeseed and mustard is the important crop of Brassica group grown as oilseed crop in Pakistan. Oilseeds are rich in proteins (36-40 %) and in addition they contain a high level of fat contents (40-45%) [5, 6]. Rapeseed (*Brassica napus* L.) is now the third most important source of edible oil in the world after soybean and palm oil [7]. In Pakistan, after cotton it is the second most important source of oil. It contributes about 17% to the domestic production of edible oil [8]. In Pakistan during the year 2010-11 total cultivated area under rapeseed was 439 thousand acres producing 157 thousand tons of seed, which yielded 50 thousand tons of oil while in 2011-12¸ the total cultivated area under rapeseed cultivation was 575 thousand acres, producing 203 thousand tons of seed, which yielded 61 thousand tons of oil [9]. *Brassica* vegetables have been related to the prevention of cancer and degenerative diseases, owing to their glucosinolates and phenolic contents [10]. Combination of physical and chemical mutagen show enhancing effect on different parameter of *Brassica napus* [11]. One hundred and sixty three cultivars of annual oil seed crops developed using induced mutations. The maximum number of cultivars have been released in soybean (58), followed by groundnut (44), sesame (16), linseed (15), rapeseed (14), Indian mustard (8), Caster bean (4), white mustard (3) and sunflower (1) [12]. During the past seventy years, worldwide more than 2250 varieties have been released that have been derived either as direct mutants or from their progeny [13] and according to FAO/IAEA mutant varieties database there are 2541 mutant cultivars, 30 of which are of *Brassica*. Among the different breeding method induced mutation has been extensively and successfully used for genetic improvement of any yield attributes either qualitative or quantitative in nature [14]. Chemical mutagenesis is considered as an effective mean in improving the yield and quality trait of crop plants [15]. Sodium azide (NaN3) is one of the most powerful chemical mutagens used to induce mutation in crop plants [16]. The present study was carried out to investigate the effect of sodium azide induced mutation on the qualitative and quantitative characters of *Brassica napus* L.

Materials and methods

The seeds of M_2 generation of *Brassica napus* variety Dunkled were obtained from the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar. They were presoaked in distilled water for 4 to 6 hours at temperature 25^0C. Sodium azide solutions of four different concentrations were prepared i.e. 0.2%, 0.4%, 0.6%, and 0.8%. Seeds were soaked in all these different concentration of solution for 5 hours. The control seeds were soaked in distilled water for 5 hours.

Sowing

Seeds of M_2 generation of *Brassica napus (*variety Dunkled) were sown in the green house of botanical garden, Islamia College, Peshawar on 24th November 2014. The treated seeds of each dose were sown in 5 pots while seeds of control were sown in separate 5 pots. The seedling survival, growth, morphological changes and seeds setting were carefully observed.

Morphological parameter

Various agronomic and morphological parameters were measured during the plants life span. It includes the following:

Days to germination, germination percentage, days to flowering, plant height, number of branches /plant, number of siliqua/plant, number of seeds/siliqua, number of leaves/plant, siliqua length, days to siliqua maturation and 1000 seeds weight

Biochemical parameter

Oil contents, protein contents, moisture contents, glucosinolates, oleic acid, linoleic acid and erucic acid

Statistical analysis

Experiment was set up in randomize complete block design with five replica per treatment. Data were statistically analyzed to find out coefficient of variation for each parameter. Two tests were performed i.e. analysis of variation (ANOVA) and least significant difference (LSD) at $\alpha = 0.05$ using Statistics 10.0 software.

Proximate analysis

Proximate analysis of seeds was carried out at national institute of food and agriculture (NIFA), Peshawar, Pakistan.

Results and discussion

Mutation is a change in a genetic sequence at small level like substitution of a single DNA building block or nucleotide base with another nucleotide base. Meanwhile larger mutations can affect many genes on a chromosome along with substitutions; mutation can also be caused by insertions, deletion or duplications of DNA sequences. In the current study, results of the parameters studied are given below.

Germination

The result of table 1 and figure 1 show the effect of various concentration of sodium azide on days to germination in M_2 generation of *Brassica napus* L. The mean value showed that higher concentration of sodium azide may delay the germination. Days to germination increased slightly at 0.2%, 0.4%. 0.6% and 0.8% are (6.4, 7.8, 8 and 9.4 days respectively) as compared to control (5.4). The reduction in seed germination in mutagenic treatment had been explained due to delayed or inhibition of physiological and biological processes for seed germination including enzyme activity and inhibition of mitotic process **[17, 18]**. These results are also in the agreement with the finding of **[19]** on *Lycopersicun esculentum* and **[20]** on *Arachis hypogaea*.

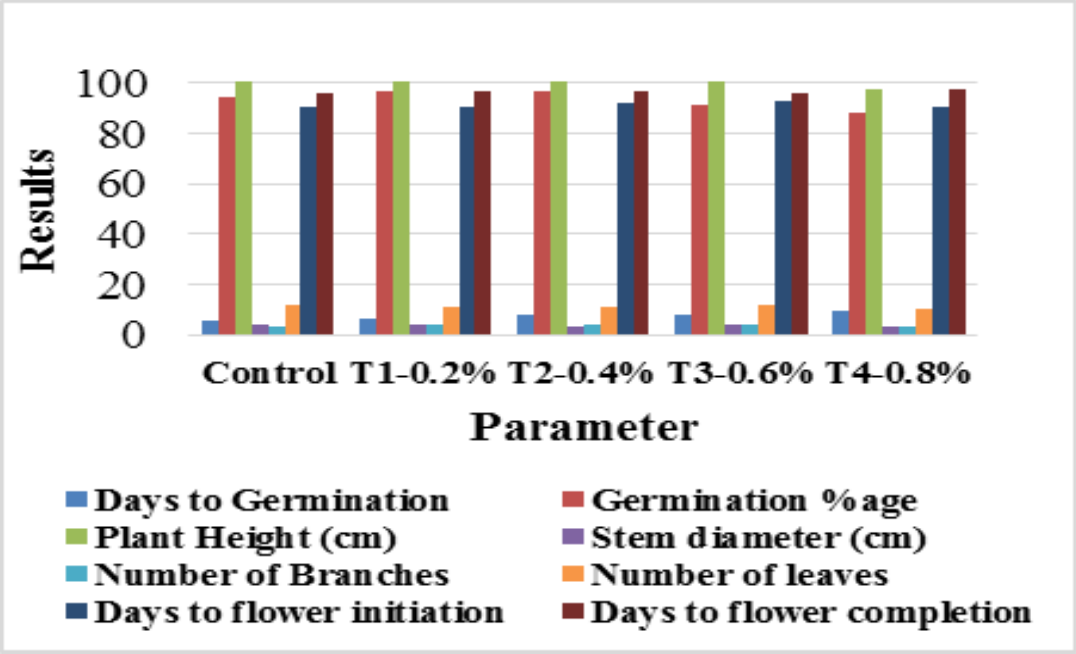

Figure 1. Showing the effect of various concentration of sodium azide on days to germination

Table 1. Effect of various concentration of sodium azide on some morphological characters in M_2 generation of *Brassica napus* L.

Treatments	A	B	C	D	E	F	G	H
Control	5.4^{d}	94.284^{a}	107.32^{ab}	3.84^{a}	3.62^{a}	12^{a}	91^{a}	96^{a}
T1-0.2%	6.4^{c}	97.142^{a}	107.5^{ab}	3.734^{ab}	3.79^{a}	11^{ab}	91^{a}	97^{a}
T2-0.4%	7.8^{b}	97.142^{a}	107.06^{ab}	3.598^{ab}	3.74^{a}	11^{ab}	92^{a}	97^{a}
T3-0.6%	8^{b}	91.426^{a}	110.13^{a}	3.818^{a}	3.81^{a}	12^{a}	93^{a}	96^{a}
T4-0.8%	9.4^{a}	88.568^{a}	97.95^{b}	3.494^{b}	3.49^{a}	10^{b}	91^{a}	98^{a}
LSD at α 0.05	0.9513	1.8657	10.181	0.2554	0.9356	1.8657	4.151	2.7357

A. Days to Germination, **B**. Germination %age, **C.** Plant height (cm), **D**. Stem diameter (cm), **E.** Number of branches, **F.** Number of leaves, **G.** Days to flower initiation, **H**. Days to flower completion

Germination percentage

The results showed that there is no significant effect of various concentration of sodium azide on germination percentage. The lowest mean value for germination percentage were recorded at 0.6%, 0.8% (91.42% and 88.56 respectively) while the highest mean value were recorded at 0.2% and 0.4% (97.142% each) as compared to control (94.28%). This study also confirms the finding of **[18]**, **[21-27]** who reported that germination percentage, shoot height, root depth, leaflet areas, chl a, chl b and the initial level of fluorescence (F_0) decreased with NaN3 treatment in pea. The present result is also in agreement with the finding of **[28]** who observed almost adverse effect of higher concentration of sodium azide on germination percentage (Table 1 and Figure 1).

Plant height

The data in table 1 and figure 1 show that mean value of plant height was increased (107.5 cm and 110.3 cm) at 0.2% and 0.6% respectively and decreased (107.06 cm and 97.95 cm) at 0.4% and 0.8% respectively as compared to control (107.32). Sodium azide i.e. 0.8% has adversely affected the plant height (97.95 cm). The work of **[17]** also showed almost the same results on stem height which was reduced by higher concentration of sodium azide. Similar results were also obtained by **[22]**, **[29-33]**.

Stem diameter

Table 1 and figure 1 represent the effect of various concentration of sodium azide on M_2 generation on stem diameter. Control showed the highest value for the mean of stem diameter (3.84 cm), and decreased (3.81 cm, 3.73 cm, 3.59 cm and 3.49 cm) at 0.6%, 0.2%, 0.4% and 0.8% respectively. Result showed that various concentrations of sodium azide adversely affected the stem diameter. These results are in agreement with the finding of **[34, 35]** on *Phyllanthus odontadenius* **[33]** on *Browallia speciosa.*

Number of branches

The data of table 1 represent that there was no significant effect of sodium azide on number of branches/plant on M_2 generation of *Brassica napus* L. The highest mean value for the number of branches were found at 0.2% (3.79) followed by 0.4% (3.74), 0.6% (3.81) and the lowest mean value for the number of branches was found at 0.8% (3.49) as compared to control i.e. 3.84. These results confirm the finding of **[35, 32]**. These results also confirm the finding of **[36]** who observed reduction in number of branches with increasing dose of sodium azide.

Number of leaves/ stem

Data presented in Table illustrate the effect of various concentration of sodium azide on number of leaves/stem in M_2 generation of *Brassica napus* L. Control and 0.6% showed the highest value for the mean number of leaves/stem (12 leaves/stem). While other treatments showed a decreasing tendency in the mean values of number of leaves/stem were recorded i.e. 0.2%, 0.4% and 0.8% are (11, 11 and 10 leaves/stem respectively). The stimulative effect of sodium azide might be attributed to cell division rates as well as activation of growth hormone e.g. auxin as reported by **[37]**. These results are in agreement with the findings of **[30]** on *Helianthus annuus* and **[20]** on *Arachis hypogaea* and *Browallia speciosa* **[33]**

Flowering initiation and completion

Table 1 represents the effect of various concentration of sodium azide on flowering initiation in M_2 generation of *Brassica napus* L. The result showed that there is no significant value on days to flowering initiation while flower completion was slightly affected. The lowest mean value was observed at 0.8%SA (98 days) while the highest meant value was recorded in control (96 days). Higher concentration of sodium azide delayed flowering. These results are parallel with the results of **[25, 38, 30]**.

Siliqua maturation

Data in Table 2 illustrate the effect of various concentration of sodium azide on siliqua maturation in M_2 generation of *Brassica napus* L. there is significant effect of various treatments on siliqua maturation. Higher concentration of sodium azide may delay the siliqua maturation as compared to control. The highest mean value were found in 0.8% (153 days), while the lowest mean value were found in 0.4% (145 days) as compared to control (147 days) and other treatments.

Table 2. Effect of various concentration of sodium azide on yield production in M_2 generation of *Brassica napus* L.

Treatments	Siliqua maturation(days)	Siliqua length(cm)	Number of Siliqua/plant	Number of seeds/siliqua	1000 seed weight(gram)
Control	147^{bc}	5.24^{a}	78.78^{a}	22.63^{a}	4.13^{a}
T1-0.2%	149^{b}	5.06^{a}	82.77^{a}	20.43^{ab}	3.96^{a}
T2-0.4%	145^{c}	5.38^{a}	66.4^{a}	21.6^{ab}	4.04^{a}
T3-0.6%	149^{b}	5.57^{a}	71.36^{a}	22.74^{a}	4.01^{a}
T4-0.8%	153^{a}	5.42^{a}	64.68^{a}	20.09^{b}	3.91^{a}
LSD at α 0.05	3.5400	0.641	28.748	2.3479	0.4174

Length of siliqua

Data presented in figure 2 illustrates the effect of various concentration of sodium azide on siliqua length in M_2 generation of *Brassica napus* L. The mean values show that there is no significant effect of Sodium azide on siliqua length. It shows that spike length was maximum (5.57 cm) at 0.6% and minimum (5.06 cm) at 0.2% as compared to control (5.24cm). Siliqua length at 0.4% and 0.8% are 5.38cm and 5.42cm respectively. Increased in mean value for siliqua length was also observed by **[39]**.

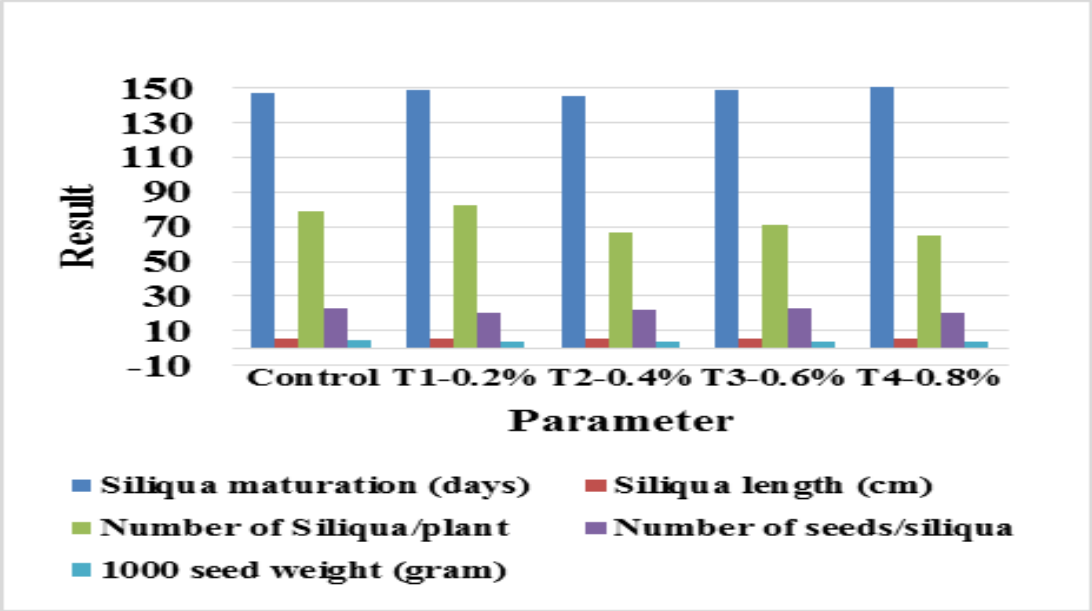

Figure 2. Illustrates the effect of various concentration of sodium azide on siliqua length

Number of siliqua/plant

Table 2 show average number of siliqua/plant at various concentration of sodium azide on M_2 generation of *Brassica napus* L. In control number of siliqua/plant was 78.78, and increase was noticed at 0.2% (82.77 siliqua/plant). Then decrease in the mean values for number of siliqua/plant in 0.6% (71.36%) and 0.8% (64.68 siliqua/plant) as compared to control. A decrease of siliqua was noticed in 0.4% (66.40 siliqua/plant) as compared to control and other doses. In 0.4% treatment a bifurcated siliqua was also obtained. [11] showed that siliqua/plant was enhanced by gamma rays and EMS (Ethyl methane sulphonate). This was also confirmed by [32]. Higher concentration of sodium azide reduced siliqua/plant. Similar finding were obtained by [26, 40]. However, present study disagrees with the finding of [41] who noticed increase in number of pods/plant with higher concentration of sodium azide.

Number of seed/siliqua

The data of table 2 and figure 2 represent the effect of various concentration of sodium azide on number of seed/siliqua in M_2 generation of *Brassica napus* L. Number of seed/siliqua was significantly affected by chemical treatment. The highest mean value for number of seed/siliqua was recorded at 0.6% (22.74 seed/siliqua) and the lowest mean value for number of seed/siliqua was recorded at 0.8% (20.09) as compared to control (22.63). Number of seed/siliqua at 0.2%, 0.4% is 20.43 seed/siliqua and 21.60 seed /siliqua respectively. The result revealed that the number of seed/siliqua was not improved by chemical treatment in M_2 generation of *Brassica napus* L. Similar results was obtained by [42] on *Sesame indicum* and [27] on *Arachis hypogaea* L. Increased number of seeds at 0.7% was also recorded by [32].

1000 seeds weight

Table 2 and figure 2 show the effect of various concentration of sodium azide on 1000 seed weight in M_2 generation of *Brassica napus* L. The mean value showed that there is no significant effect of sodium azide on 1000 seeds weight in gram. The weight was maximum at control (4.13g) and minimum at 0.8% (3.91g) as compared to other treatment i.e. 0.2% (3.96g), 0.4% (4.04g) and 0.6% (4.01g). Result showed that various concentration of sodium azide

adversely effected 1000 seed weight. Present study is dispute with the finding of [20] who observed an increase in 1000 grain weight at different concentrations. [32] also recorded an increase in 1000 grain weight at higher treatment. Present study also contradict finding of [43, 44].

Chemical analysis of seeds

The qualitative analysis of seeds was carried out using NIRS (Near Infra-Red Reflectance Spectroscopy) which works on the principle of measuring the absorption of light energy or wavelengths of each component at its characteristic frequency in the near IR region.

Oil percentage

Table 3 and figure 3 show the effect of various concentration of sodium azide on oil percentage in M_2 generation of *Brassica napus* L. The data obtained revealed that the oil content decreased significantly with high concentration of sodium azide. The lowest oil contents were recorded at 0.6%SA and 0.8% SA (43.9% and 42.6% respectively) as compared to control (44.7%) and other treatments. Our results also confirmed the findings of [11].

Table 3. Effect of various concentration of sodium azide on some bio chemical parameters in M_2 generation of *Brassica napus* L.

Treatments	Oil percentage	Protein percentage	Glucosinolates µmol/g	Moisture percentage	Oleic acid (18:1) percentage	Linoleic acid(18:03) percentage	Erucic acid(22:01) percentage
Control	44.7^{bc}	26.8^{a}	71.3^{a}	5.5^{a}	58.1^{a}	8.4^{a}	35.2^{b}
T1-0.2%	45^{ab}	27.8^{a}	61.9^{bc}	6.2^{a}	56.2^{b}	8.7^{a}	37.8^{ab}
T2-0.4%	47.2^{a}	26.1^{a}	64.9^{b}	5.6^{a}	56.4^{b}	7.8^{a}	40^{a}
T3-0.6%	43.9^{bc}	27^{a}	71.5^{a}	5^{a}	58.2^{a}	8.7^{a}	27.5^{c}
T4-0.8%	42.6^{c}	28.4^{a}	58.5^{c}	5.9^{a}	54.9^{c}	9^{a}	37.3^{ab}
LSD at α 0.05	2.2851	3.0659	3.6154	1.3259	1.4926	1.6583	2.9322

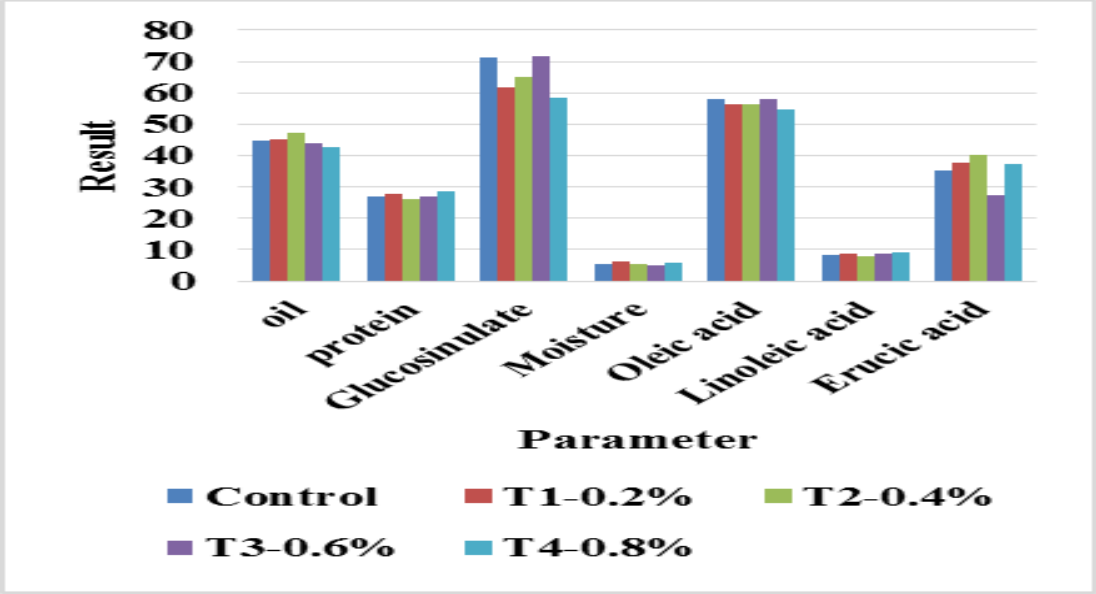

Figure 3. Showing the effect of various concentration of sodium azide on oil percentage

Proteins percentage

Table 3 show the effect of various concentration of sodium azide on Protein percentage in M_2 generation of *Brassica napus* L. The treated seed were analyzed for protein contents and the result showed that with high concentration of sodium azide increased the protein content i.e. 0.2% SA (27.8%), 0.6% SA (27.0.%), 0.8% SA (28.5%) except 0.4% SA which show a deviation and decrease protein content (26.1%) is noted as compared to control (26.8%). Similar results were reported by [45, 46].

Glucosinolates (GSL) content

The data of table 3 and figure 3 illustrate that when treated seed were analyzed for GSL content and the result revealed that increasing concentration of sodium azide significantly decreased the GSL content except at 0.6% SA which show a deviation and increase in GSL content is noted.

Moisture content (MC)

The result of table 3 and figure 3 revealed that high concentration of sodium azide increased the moisture content i.e. 0.2% SA (6.2%MC), 0.4% SA (5.6%MC), 0.8% SA (5.9%MC), except 0.6% SA (5.0%MC) as compared to control (5.5%MC).

Oleic acid (18:1) content

The data of table 3 and figure 3 represent the effect of various concentration of Sodium azide on Oleic acid content in M_2 generation of *Brassica napus* L. The analysis of seed revealed that high concentration of sodium azide decreased oil content i.e. 0.2% SA (56.2%), 0.4% SA (56.4%), 0.8% SA (54.9%) except at 0.6% SA (58.2%) as compared to control (58.1%).

Linolenic acid (18:3) content

Table 3 and figure 3 shows that linolenic acid content is increased with high concentration of sodium azide. 0.8% SA showed the highest mean value (9.0%) while 0.4% SA gave the lowest mean value (7.8%) as compared to Control (8.4%) and other doses i.e. 0.2% SA and 0.6% SA (8.7% and 8.7% respectively). Similar results were also observed by [47].

Erucic acid (22:1) content

The data of table 3 and figure 3 show the effect of various concentration of sodium azide on erucic acid in M_2 generation of *Brassica napus* L. The data revealed that result changed variously compared to control. 0.4% SA gave the highest mean value (40%) while 0.6% SA showed the lowest mean value (27.5%) as compared to control (35.2%) and other doses.

Recommendation

It is recommended that if 0.4% treatment of sodium azide is given to *Brassica napus* and worked on its different stages and environmental factor, so it is likely to obtain bifurcated siliqua which will increase the crop productivity and oil production.

Authors' contributions

Conceived and designed the experiment: WM Khan, Performed the experiment: S Ahmed, S Hussain & SS Shah, Analyzed the data: WM Khan, N Umar & S Ali, Contributed reagents/ materials/ analysis tools: MS Khan & N Akhtar, Wrote the paper: S Ahmed & WM Khan.

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