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EFFECTS OF PARA-DICHLOROBENZENE AND SODIUM AZIDE ON YIELD AND YIELD ATTRIBUTING TRAITS OF SESAME (*Sesamum indicum* L.)

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ABSTRACT

Sesame is an important source of income and edible oil particularly in sub-Saharan Africa. Its cultivation is constrains by many factors including lack of improved varieties. This research was carried out to evaluate the effect of chemical mutagens (Sodium Azide and Para-dichlorobenzene) on yield and yield attributing traits in three sesame varieties (Ex-sudan, E-8 and JAN-IRI). Pot experiments were conducted during 2018 and 2019 dry seasons using completely randomized design (CRD). Seeds were treated with different concentrations (1.0 - 4.0mM) of Sodium Azide or Para-dichlorobenzene to generate M_0 plant which were allowed to produce seeds. The effect of mutagens on yield and yield attributing traits in Sesame was evaluated using M_1 plant which were derived from the seeds of M_0 plants. In JAN-IRI, 1.0 - 2.0Mm of the mutagens significantly ($p \leq 0.05$) decreased the days to first flowering emergence (52.0 days) and increased the number of capsule (229), length of capsule (2.4cm) and number of seed per capsule (45.0). In EX-SUDAN, days to first flowering emergence (29.0days), number of capsule per plant (110), length of capsule (3.2cm) and number of seed per capsule (59.0) were significantly increased by 1.0 and 2.0mM Sodium Azide and para-dichlorobenzene repectively. Sodium Azide and para-dichlorobenzene at concentrations ranging from 1.0-2.0mM signicantly increased the seed weight and yield in both JAN-IRI, EX-SUDAN and E-8. Sodium Azide and para-dichlorobenzene was very effecient in generating mutants with increased yield and yield attributing traits. These results suggested that the concentrations used in this study are optimum for inducing mutants of different traits in Sesame and the technology could be apply to generate materials for feature breeding programme.

Keywords: Sesame, Chemical Mutagens, Germination, Vegetative Characters, Genetic variability

INTRODUCTION

Sesame (*Sesamum indicum* L.) belongs to the Pedaliaceae family. It is one of the most ancient oil seed known to mankind and plays a major role in human nutrition (komivi *et al.*, 2017). The crop has deep tap roots system and well adapted to dry condition and poor marginal soils. It is called Queen of oil seed due to its high quality polyunsaturated stable fatty acid which restrains oxidative rancidity (Reddy, 2006). Sesame seeds occur in many colours depending on the cultivar. The most traded variety of sesame is off-white and other common colours included buff, tan, gold, brown, reddish, gray and black (Majumdar, and Roy, 1992). Sesame is widely grown by small-holder farmers and is a major cash crop in Nigeria (NAERLS, 2010). The world harvested 4.8 million metric tons of sesame seeds in 2017 and Nigeria produces estimated 450,000 tons of sesame seed in 2018 (FAO STAT, 2018). The major challenges to Sesame production in Nigeria are poor performance of the existing varieties, shattering ability, pests, diseases, abiotic stresses. Althought, efforts are being made to improve Sesame by relevant institutions, progress is constrained by narrow genetic base of the crop which necessitate the need for creating genetic variability upon which improvement can be made. Chemical and physical mutagens are agents that change the genetic materials, usually DNA of an organism by increasing the frequency of mutations above the natural background level (Ashish *et al.,* 2011). Muatagens have played a pivotal role in creating crop varieties with induced mutations resulting in significant increase in food production. Sodium azide is a powerful mutagen in plant and its efficiency depend on concentration and treatment duration (Al-Qurainy and Khan, 2009). It has been suuccessfully used in the improvement of rice, barley and oats, (Awan *et al.*, 2000). Sodium azide decreases the cellular level of catmodulin, which is a calcium binding protein participating in signal transduction and cell division and physiologically it inhibits the activity of Catalase, Peroxidase and Cytochrome Oxidase (Maryam and Kasimu, 2016). Para-dichlorobenzene is a chlorinated aromatic hydrocarbon ($C_6H_4Cl_2$) used in various cytological studies owning to its polyploidizing property (Khan and Goyal, 2009). The objective of this study was to evaluate the effects of chemical mutagens on sesame growth and physiology

MATERIALS AND METHODS

Pot trials were conducted during 2018and 2019 dry seasons at the Screenhouse of the Department of Plant Biology, Bayero University, Kano, which lies on latitude 11^0 58N and longitude $8\,^0$ 30E with altitude of 440m above sea level. Seeds of the two improved varieties (EX-SUDAN and E-8) and one local variety (JAN-IRI) were collected from Kano State Agricultural and Rural Development Authority (KNARDA), Kano Nigeria. A total of one hundred (100) good seeds of each variety were selected and surface sterilized in 1.0% Sodium hypochlorite (NaOCl) for 1 minute and rinsed three (3) times in sterile distilled water. Ten (10) seeds were sorted in three replicates and were placed in Nylon net bags for easy handling. The seeds were soaked in different concentrations of Sodium azide (0.00mM, 1.0mM, 2.0mM, 3.0mM and 4.0mM) or Para-dichlorobenzene (0.00mM, 1.0mM, 2.0mM 3.0mM and 4.0mM) for three (3) hours. The seeds were then rinsed three times in sterile distilled water to remove the excess chemicals. The treated seeds were planted and resulting plants (M_0) were allowed to produce seeds. The seeds of the M_0 were collected and used to generate M_1 which were used to evaluate the effect of the mutagens. Seeds were sown in polypots (30 x 30 x 40cm) containing 7kg of the mixture of top soil and compost manure (ratio 3:1). All standard cultural practices were maintained and fertilizer was applied based on the procedure described by Chude *et al*, (2012). Data was collected at vegetative maturity on number of leaves, plant hight, number of braches, chlorophyll content. Data on the seed yield was collected at the end of the reproductive cycle when pods were fully meatured. Data was subjected to Analysis of Variance (ANOVA) using SPSS soft were version 23 (www.ibm.com) and significant means were separated using Turkey's Honestly significant diffrence (HSD).

RESULTS AND DISCUSSIONS

The result of the interaction between sesame varieties and different concentrations of Sodium azide and Para-dichlorobenzene is presented (Table 1). The results showed significant interaction ($p \leq 0.05$) between plant varieties and the different concentrations of the two mutagens. In this research, it was observed that, low concentrations of the mutagens produced significantly lower number of days to first flowering when compared to control and the rest of treatments. A decrease in days to first flowering was observed in JAN-IRI variety treated with 2.0Mm Sodium Azide (52.0 days) and 1.0Mm Para-dichlorobenzene (57.0days) when compared with control and the remaining treatments. In EX-SUDAN variety, lower number of days to first flowering was recorded in plant treated with lower doses of 1.0Mm Sodium Azide (30.0days) and 2.0Mm Para-dichlorobenzene (29.0days). Similarly decrease in days to first flowering was also recorded in E-8 variety treated with low dose of 2.0Mm Sodium Azide (34.0days) and 1.0Mm Para-dichlorobenzene (35.0days) when compared to control and other treatments. The reduction of days to flowering in mutagenic treatment could be best explained due to inhibition of biological processes necessary for flowering including mitotic processes. This work was in line with work of Dhanavel *et al*., (2008) who worked on cowpea and stated that, low doses of chemical mutagens interfered with synthesis of enzyme accelerates the degradation of existing enzyme involve in the formation of Auxin and thus, reduce the days to flowering and maturity. Reduced days to flowering due to mutagenic treatments might be the result of damage of cell constituents at molecular level or altered enzyme activity. These findings are in closed agreement with earlier report of Khan and Goyal, (2009).

The data analysis indicated significant increase ($p \leq 0.05$) with respect to the number of capsule per plant (Table 2). The highest number of capsules was recorded in JAN-IRI treated with 2.0Mm Sodium Azide (215) and 1.0Mm Para-dichlorobenzene (229) when compared to control and other treatments. Increase in number of capsule also recorded in EX-SUDAN treated with 2.0Mm Sodium Azide (110) and 1.0Mm Para-dichlorobenzene (105). Similarly, E-8 recorded the highest number of capsules when treated with 2.0Mm Sodium Azide (120) and 1.0Mm Para-dichlorobenzene (103). Since capsules are seeds bearing structures, their increase has positive correlation with the yield of seed. Sing and Kole (2005) reported increase in variability for number of capsule per plant following mutagenic treatments of Sodium Azide in *Vigna mungo* . Mensah *et al.,* (2005) reported that, number of capsules increased significantly as the concentration of mutagen decrease. Reduce in number of capsule could be best attributed to physiological disturbances due to increase in mutagen concentrations which might have probably affect some biological pathway in the treated seeds. This might have reduced the capsule number. It is also probable that the treated seeds soaked in lower concentration of Sodium Azide and Para-dichlorobenzene have readjusted response to treatment, thus reduced the number of capsules.

The effects of mutagen and variety interaction on length of capsule per plant is shown (Table 3). Length of capsule per plant significantly ($p \leq 0.05$) increases in all the varieties. The JAN-IRI variety treated with 2.0Mm Sodium Azide (2.4cm) and 2.0 Mm Para-dichlorobenzene (2.4cm) recorded the highest capsule length when compared to control and other treatments. In EX-SUDAN, highest length of capsules was recorded in 2.0Mm Sodium Azide (3.3cm) and 2.0Mm Para-dichlorobenzene (3.2cm). The highest capsule length in E-8 variety was observed in 3.0Mm Sodium Azide (3.11cm). the result was in conformity with finding of Diriba Shanko (2017) in Cowpea, who reported that ability of lower doses of chemical mutagens to enter the cell of leguminous plant may interact with DNA to induce physiological damages and chromosomal aberration which could be best detected and measured from early flowering emergences of seedling fertility improvement or increase in pods and seed formation. Increase in capsule number, length of capsules and number of seed per pod after Para-dichlorobenzene or Sodium Azide treatment has been linked to polyploidal sign and successful mutagen treatments.

Total number of treated and untreated seed per Capsule was estimated for all the different concentrations of Sodium Azide and Para-dichlorobenzene across the three varieties used in this study (Table 4). The number of seed per Capsule was significantly ($p \leq 0.05$) increase with decrease in dose concentration. In JAN-IRI variety, highest number of seed was counted in plant treated with 2.0Mm Sodium Azide (45.0) and 1.0Mm Para-dichlorobenzene (45.0) but in EX-SUDAN, maximum number of capsules were counted in plant treated with 2.0 Mm Sodium Azide (53.0) and 2.0Mm (59.0) Para-dichlorobenzene. Similarly, in E-8 variety highest number of capsules was recorded at low doses of 1.0Mm Sodium Azide (55.0) and 1.0Mm (51.0) Para-dichlorobenzene. The low concentration of Sodium Azide and Para-dichlorobenzene was shown to increase seed number when compared to other mutagen treatments. Artificial inducing of chemical mutagen of Sodium Azide and Para-dichlorobenzene at low concentration lead to the alteration of sesame plant genome integrated by environmental signals by increasing the rate of cellular division. It is probably observed that the mutagen might have influenced the activities of cytokinnins which is paramount importance in the fundamental processes of flower and fruit development including cell division and morphogenesis. The biological damages might consider as an indication of doses level and mutagenic effects. Diriba Shanko (2017) reported that the effect of chemical mutagen induced variation with varying mutagen doses by selecting the optimal doses for genotype in any plant species which is vital in breeding program.

Weight of hundred seed per plant was also estimated for the different concentration of the mutagens in all the sesame varieties (Table 5). Hundred seed weight was significantly ($p \leq 0.05$) increase in plant treated with low dose of chemical mutagens. In JAN-IRI variety, seed weight increased at 1.0Mm Sodium Azide (0.31g) and 2.0Mm Para-dichlorobenzene (0.28g). In EX-SUDAN, maximum weight was observed in seed treated with 2.0Mm Sodium Azide (0.45g) and 1.0Mm Para-dichlorobenzene (0.43g). Similarly, in E-8 variety seed weight was increased in seed treated with low dose of 1.0Mm Sodium Azide and 2.0Mm Para-dichlorobenzene (0.43g). This finding was in agreement with work of Kavera and Nadaf (2017) on groundnut, who reported that Sodium Azide and Para-dichlorobenzene at low concentrations induced markedly vegetative growth leading to the formation of higher number of branches, leaves, capsules, testa texture and seed vigour. This is possibly associated with doses. Kumar and Yadav (2010), reported that the seed treated with highest doses of Sodium Azide, Para-dichlorobenzene and Gamma rays showed highest reduction in germination percentages, higher days to flowering, reduced in the number of capsules and maturity periods. Ati and Adamu (2016) reported that, treatment with low dose of mutagen showed reduction in days to flowering and increase in fruit vigour and length.

Total seed yield was estimated for the different concentration of the mutagens in the all the Sesame varieties (Table 6). The maximum seed yield per plant was obtained in EX-SUDAN treated with low dose of 1.0Mm Sodium Azide (11.60g) and 1.0Mm Para-dichlorobenzene (10.60g). Seed yield in E-8 variety was also significantly ($P\leq0.05$) increased when subjected to 2.0mM Paradichlorobenzene (10.80g) or 4.0mM Sodium Azide (10.50g). In JAN-IRI variety, seed weight was significantly ($P\leq0.05$) higher in plant treated with low dose of 2.0Mm Sodium Azide (6.60g) when compared to control and other treatments. This is clear that seed yield in the M$_1$ plants was affected by the low dose concentrations of the mutagens and this can be seen from the interaction of differences varieties of sesame with the different concentrations of the mutagens. From these results, we inferred that treating sesame seeds with Sodium Azide or Paradichlorobenzene at concentration range around 1.0 – 2.0mM induced genetic changes which allow generation of mutants with considerable increase in seed yield. However, the genetic bases and inheritance of this trait in the subsquent generations is yet to established. Waghmare and Mehra, (2003) reported an increase in yield of bread wheat varieties and Soya bean when subjeted to different doses of chemical mutagens such as Ethylmethane Sulphonate, Sodium azide, Nitrosomethyl Urea and physical mutagens like Gamma rays. Preussa and Brilta, (2003) reported that, chemical mutagen at low concentration induces growth stimulation by altering the hormonal signaling network in the cells by increasing anti-oxidative capacity of the cells. Growth inhibition due to high doses of chemical mutagens and irradiation could be due to cell cycle arrest during somatic cell division and various damages to the entire genome.

CONCLUSION

Base on the results obtained from this study, it is clear that Sodium Azide and Paradichlorobenzene at concentrations of 1.0 – 2.0Mm induced changes in the days to first flowering emergence, number of capsule per plant, length of capsule, number of seed per capsule, hundred seed weight and the seed weight per plant in three different varieties of Sesame. These results suggested that the concentrations used in this study are optimum for inducing mutants of different traits in Sesame. Previous studies have examined the effect of low doses of Sodium Azide and Paradichlorobenzene which induced mutations in Soy bean, cowpea, Mungo bean and tomato. Thus, our results may proved useful in applying this technology to generate breeding materials in Sesame. Since yield increment being the main objectives in most of the breeding program, mutation breeding had played a key role in achieving the goals.

Table 1: Variety by Mutagen Interaction on Number of Days to Flowering

		Number of Days to Flowering		
Mutagen	**Concentration** (mM)	**JAN-IRI** **Mean±SE**	**Ex-sudan** **Mean±SE**	**E-8** **Mean±SE**
Control	0.0	$70.00^{a}\pm0.2$	$44.00^{b}\pm0.2$	$49.00^{b}\pm0.2$
S.A.	1.0	$65.00^{c}\pm0.3$	$30.00^{e}\pm0.2$	$45.00^{c}\pm0.2$
S.A.	2.0	$52.00^{e}\pm0.2$	$45.00^{b}\pm0.2$	$34.00^{e}\pm0.2$
S.A.	3.0	$68.00^{ab}\pm0.2$	$46.00^{ab}\pm0.4$	$32.00^{e}\pm0.2$
S.A.	4.0	$65.00^{c}\pm0.2$	$38.00^{c}\pm0.2$	$37.00^{cd}\pm0.1$
PDB	1.0	$57.00^{e}\pm0.2$	$34.00^{d}\pm0.2$	$35.00^{e}\pm0.2$
PDB	2.0	$68.00^{ab}\pm0.3$	$29.00^{e}\pm0.2$	$45.00^{c}\pm0.2$
PDB	3.0	$68.0^{ab}\pm0.3$	$49.00^{a}\pm0.2$	$52.00^{a}\pm0.1$
PDB	4.0	$67.00^{b}\pm0.2$	$50.00^{a}\pm0.2$	$36.00^{d}\pm0.2$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) $P\leq 0.05$. S.A. ═ Sodium azide, PDB ═ Para-dichlorobenzene, SE± ═ Standard Error.

Table 2: Variety by Mutagen Interaction on Number of Capsules per Plant

		Number of Capsules per Plant		
Mutagen	**Concentration** (mM)	**JAN-IRI** **Mean±SE**	**Ex-sudan** **Mean±SE**	**E-8** **Mean±SE**
Control	0.0	$142.00^{c}\pm3.6$	$87.00^{b}\pm1.7$	$72.00^{b}\pm3.2$
S.A.	1.0	$160.00^{bc}\pm2.8$	$73.00^{bc}\pm1.7$	$92.00^{ab}\pm1.5$
S.A.	2.0	$215.00^{a}\pm6.3$	$110.00^{a}\pm1.5$	$120.00^{a}\pm3.1$
S.A.	3.0	$185.00^{b}\pm4.3$	$97.00^{a}\pm2.2$	$74.00^{b}\pm1.5$
S.A.	4.0	$212.00^{ab}\pm1.7$	$85.00^{ab}\pm0.9$	$103.00^{a}\pm2.3$
PDB	1.0	$229.00^{a}\pm4.9$	$105.00^{a}\pm0.9$	$103.00^{a}\pm2.3$
PDB	2.0	$146.00^{c}\pm6.3$	$87.00^{b}\pm1.7$	$65.00^{b}\pm1.2$
PDB	3.0	$147.00^{c}\pm4.7$	$72.00^{c}\pm0.7$	$78.00^{ab}\pm3.0$
PDB	4.0	$150.00^{c}\pm10.6$	$56.00^{c}\pm0.7$	$68.00^{b}\pm2.3$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) $P\leq 0.05$: S.Azide ═ Sodium azide, PDB ═ Para-dichlorobenzene, SE ═ Standard Error.

Table 3: Variety by Mutagen Interaction on Length of Capsule per Plant

		Length of Capsule per plant (cm)		
Mutagen	**Concentration** (mM)	**JAN-IRI Mean ±SE**	**Ex-sudan Mean ±SE**	**E-8 Mean ±SE**
Control	0.0	$1.70^{b}\pm0.5$	$2.40^{b}\pm0.5$	$2.40^{b}\pm0.3$
S.A.	1.0	$2.20^{ab}\pm0.5$	$2.80^{ab}\pm0.6$	$2.90^{ab}\pm0.2$
S.A.	2.0	$2.40^{a}\pm0.3$	$3.30^{a}\pm0.7$	$2.90^{ab}\pm0.2$
S.A.	3.0	$2.20^{ab}\pm0.6$	$2.80^{ab}\pm0.7$	$3.11^{a}\pm0.8$
S.A.	4.0	$1.60^{b}\pm0.8$	$2.80^{ab}\pm0.9$	$2.83^{b}\pm0.7$
PDB	1.0	$2.20^{ab}\pm0.9$	$2.80^{ab}\pm0.7$	$2.40^{b}\pm0.20$
PDB	2.0	$2.40^{a}\pm0.8$	$3.20^{a}\pm0.7$	$2.90^{ab}\pm0.5$
PDB	3.0	$2.20^{ab}\pm0.3$	$2.60^{b}\pm0.6$	$2.81^{b}\pm 0.4$
PDB	4.0	$2.20^{ab}\pm0.3$	$2.80^{ab}\pm0.7$	$2.60^{b}\pm0.5$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) P≤ 0.05. S.Azide ═ Sodium azide, PDB ═ Para-dichlorobenzene, SE± ═ Standard Error.

Table 4: Variety by Mutagen Interaction on Number of Seeds per Capsule

		No. of Seeds / Capsule		
Mutagen	**Concentration** (mM)	**JAN-IRI Mean±SE**	**Ex-sudan Mean±SE**	**E-8 Mean±SE**
Control	0.0	$35.00^{b}\pm1.7$	$48.00^{ab}\pm1.8$	$41.00^{b}\pm1.7$
S.A.	1.0	$40.00^{b}\pm1.3$	$42.00^{b}\pm1.3$	$55.00^{a}\pm1.2$
S.A.	2.0	$45.00^{a}\pm1.8$	$53.00^{a}\pm1.2$	$43.00^{b}\pm1.2$
S.A.	3.0	$42.00^{b}\pm1.5$	$47.00^{b}\pm1.7$	$46.00^{b}\pm2.0$
S.A.	4.0	$43.00^{ab}\pm1.0$	$40.00^{b}\pm1.2$	$43.00^{b}\pm2.2$
PDB	1.0	$45.00^{a}\pm1.2$	$51.00^{b}\pm1.8$	$51.00^{a}\pm1.9$
PDB	2.0	$43.00^{ab}\pm1.5$	$59.00^{a}\pm1.3$	$42.00^{b}\pm1.9$
PDB	3.0	$39.00^{b}\pm1.3$	$51.00^{b}\pm1.4$	$47.00^{ab}\pm1.2$
PDB	4.0	$41.00^{b}\pm1.0$	$46\ .00^{b}\pm1.3$	$40.00^{b}\pm1.3$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) P≤ 0.05. S.Azide ═ Sodi um azide, PDB ═ Para-dichlorobenzene, SE ═ Standard Error.

Table 5: Variety by Mutagen Interaction on weight of Hundred Seeds Per Plant (in gram)

		Weight of Hundred Seeds Per Plant (in g)		
Mutagen	**Concentration** (mM)	**JAN-IRI Mean±SE**	**Ex-sudan Mean±SE**	**E-8 Mean±SE**
Control	0.0	$0.24^{b}\pm 0.10$	$0.41^{ab}\pm 0.03$	$0.39^{ab}\pm 0.07$
S.A.	1.0	$0.31^{a}\pm 0.30$	$0.38^{b}\pm0.06$	$0.42^{a}\pm 0.03$
S.A.	2.0	$0.22^{b}\pm 0.13$	$0.45^{a}\pm0.06$	$0.36^{b}\pm 0.09$
S.A.	3.0	$0.23^{b}\pm 0.19$	$0.38^{b}\pm0.07$	$0.41^{b}\pm 0.03$
S.A.	4.0	$0.26^{ab}\pm 0.10$	$0.37^{b}\pm 0.03$	$0.41^{b}\pm 0.02$
PDB	1.0	$0.26^{ab}\pm 0.49$	$0.43^{a}\pm 0.05$	$0.43^{a}\pm 0.03$
PDB	2.0	$0.28^{a}\pm 0.06$	$0.41^{ab}\pm 0.05$	$0.39^{ab}\pm 0.03$
PDB	3.0	$0.22^{b}\pm 0.60$	$0.38^{b}\pm 0.07$	$0.38^{b}\pm 0.04$
PDB	4.0	$0.26^{b}\pm 0.50$	$0.39^{b}\pm 0.06$	$0.38^{b}\pm 0.09$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) P≤ 0.05 probability level. Key: S. Azide ═ Sodium azi de, PDB ═ Para-dichlorobenzene, SE ═ Standard Error.

Table 6: Variety by Mutagen Interaction on Seed weight (gram/plant)

		Grains Yield Per Plant (g)		
Mutagen	**Concentration** (mM)	**JAN-IRI Mean±SE**	**Ex-suda n Mean±SE**	**E-8 Mean±SE**
Control	0.0	$2.49^{f}\pm 0.02$	$5.9^{g}\pm 0.26$	$4.30^{i}\pm 0.11$
S.A.	1.0	$4.14^{def}\pm 0.67$	$11.6^{a}\pm 0.2$	$7.90^{cd}\pm 0.27$
S.A.	2.0	$6.60^{a}\pm 0.19$	$6.4^{fg}\pm0.4\ 0$	$10.57^{a}\pm 0.11$
S.A.	3.0	$6.50^{ab}\pm 0.13$	$8.6^{bc}\pm 0.67$	$5.82^{gh}\pm 0.19$
S.A.	4.0	$4.12^{ef}\pm 0.10$	$8.4^{cd}\pm 0.80$	$10.50^{ab}\pm 0.5$
PDB	1.0	$6.45^{bc}\pm 0.49$	$10.6^{a}\pm 0.40$	$4.40^{i}\pm 0.20$
PDB	2.0	$5.64^{bcde}\pm 0.06$	$10.40^{ab}\pm0.34$	$10.80^{a}\pm 0.14$
PDB	3.0	$4.99^{bcde}\pm 0.60$	$7.6^{cde}\pm 0.90$	$4.40^{i}\pm 0.13$
PDB	4.0	$6.36^{bcd}\pm 0.50$	$8.8^{bc}\pm 0.67$	$7.30^{def}\pm 0.10$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) P≤ 0.05 probability level. Key: S.Azide ═ Sodium azide, PDB ═ Para-dichlorobenzene, SE ═ Standard Error.

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