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Research paper

Effects of Paclobutrazol on Growth and Antioxidant Content of Brassica Rapa Var. Oleifera

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Abstract

The experiment was conducted with the main objective was to study the effects of Paclobutrazol (PBZ) on growth and antioxidant content of *Brassica rapa* var. *oleifera*. The PBZ was applied at various concentrations (0, 5, 10, 15 and 20 mg /L) through seed soaking and seedling as soil drenching. The growth performance data collection was conducted on weekly basis starting from week 3 until week 6 of planting while antioxidant content analyses were conducted on day 38. It was found that PBZ affected the growth and antioxidant content of *Brassica rapa* var. *oleifera*. The soil drenching techniques was found significantly affect the plant height, number of leaves and internode length. Similarly, both ascorbic acid and phenolic content were significantly affected by the technique. It was found that application of 10mg/L and 5mg/L of PBZ has recorded highest amount of ascorbic acid and phenolic content, respectively. This study has proven that the application of PBZ using soil drenching technique on *Brassica rapa* var. *oleifera* has contributed to enhancement of its antioxidant content which is also one of the important component in healthy diet intake.

Keywords: *Paclobutrazol (PBZ); Brassica rapa var. oleifera; seed soaking; soil drenching; antioxidant*

1. Introduction

Fruits and vegetables are important components for healthy diet due to substantial amount of natural antioxidants contents. Majority of antioxidants can be found in food such as fruits and vegetables, nuts, grains, and some meats, poultry and fish but the most abundant types of antioxidants contained in fruits and vegetables include vitamin C, carotenoids, and phenolics [1]. It has been reported that sufficient amount of fruits and vegetables in daily diet intake can reduce risks and protect us from critical diseases including cancer and cardiovascular diseases. The fruits and vegetables intake guideline in our daily diet have been properly outlined by government agencies and health professionals worldwide. Antioxidants can be defined as substances that may protect or act as "free radical scavengers" and preventing damaging reaction within human cells by supplying positively charge atoms needed to neutralize unstable free radicals that are called "reactive oxygen species" (ROS) or "reactive nitrogen species" [2]. These free radicals are usually produced when our body breaks down food, or by environmental/air exposures like tobacco smoke and radiation. It can also cause serious health problems such as heart diseases, muscular degeneration, diabetes, cancer, inflammation, Parkinson's diseases, and asthma [1][2]. Structural-wise, antioxidants can exist in different chemical structures namely as beta-carotene, lutein, lycopene, lignan, selenium, vitamins C, vitamins E, and vitamins A and others [3].

Vegetables are categorized into seven different types namely bulb vegetables, fruit vegetables, inflorescence vegetables, leafy vegetables, root vegetables, stalk vegetables, and tuber vegetables. Leafy vegetables are also called potherbs, green vegetables, greens, or leafy greens where they are very important part in the human diet. Most leafy vegetables are rich in carotenoids (such as beta carotene), vitamin C, and are good sources of fiber and folate. They also provide varying amount of chlorophyll, iron, and calcium. *Brassica rapa* L. is one of the leafy vegetables which commonly known as mustard. It comes from the family Cruciferae family (Brassicacea) which is composed of 350 genera and 3500 species [4]. The name of crucifer originally comes from the shape of flowers, with four diagonally opposite petals in the form of a cross. There are several varieties in this species including *Brassica rapa* var. *rapa*, *Brassica rapa* var. *oleifera*, *Brassica rapa* var. *chinensis*, *Brassica rapa* var. *pekinensi*, *and Brassica rapa* var. *parachinensis*. *Brassica rapa* var. *oleifera* has green foliage [4]. The leaves are glabrous or slightly hispid when young, and the upper leaves are partially clasping the stem. The inflorescence is an elongated raceme. The flowers are pale yellow, concisely clustered at the top with open flowers borne at or above the level of terminal buds, and open upwards from the base of the raceme. They have height between 50-250cm and have 60-70% self-pollination but insect and wind also assist pollination. Brassica and many others Brassicacea genera contain glycosinolate compounds that are changed by the myrosinase enzyme to give bitter taste and goitro-genetic substances such as isothiocyanates, thiocyanates, nitrites and goitrin. Brassica is the vital genus which is important annual and biennial foliage and root vegetables [4].

Paclobutrazol (PBZ), with the chemical name of (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol), is a triazole type plant growth retardant which prevents gibberellins biosynthesis and involved in decreasing abscisic acid, ethylene and indole-3-acetic acid while increasing cytokinin levels. Trizoles have both plant growth regulator and fungicide effects

[5]. In addition, PBZ can also protect the plants from abiotic and biotic stress. It is also used to reduce the size of plant, improve compactness, and increase other abilities of plant to resist environmental stress [6][7]. The changes of plant morphological parameters observed in PBZ-treated plants include reduced plant growth and intermodal elongation, increase chlorophyll content, chloroplast enlargement, leaf tissue thickening, stimulation of root elongation by the inhibition of giberellin biosynthesis, increased cytokinin synthesis, and epicuticular wax formation [7][8][9][10]. PBZ is usually applied to the soil to be absorbed by the roots and transported via the xylem to the upper parts of the plant. Though the role of PBZ on growth performance of plants has been well reported, very limited informations available on the effect of PBZ application on antioxidant content in local vegetables. Hence, there is a need to investigate the efficacy of this compound in improving the antioxidant content in *Brassica rapa* var. *oleifera*. The main purposes of this study were to determine the optimum PBZ concentration for enhancing antioxidant content in *Brassica rapa* var. *oleifera* by using two different methods of PBZ application onto plant namely seed soaking (SS) and soil drenching (SD). The finding of this study will help in identification of better PBZ application method for effective increment of antioxidant content and adding beneficial properties of *Brassica rapa* var. *oleifera* thereby making it valuable crop plant. This will benefits human beings durable to infection.

2. Materials and Methods

2.1. Test Materials

The seeds and seedlings of 4 leaves of *B. rapa* var. *oleifera* purchased from a local nursery were used as test materials.

2.2. Methods

2.2.1. Seeds and Seedlings Treatments

The overall treatments on seed and seedlings were conducted under the rain shelter. The seeds were disinfected in 1% sodium hypochlorite solution for 10 min to eliminate possible seed-borne microorganisms. Then, the seeds were rinsed 3 times with distilled water. The seeds were randomly divided into 2 sets. First set of the seeds was germinated in germination trays. After 2 weeks, the seedlings were transferred to 15cm x 20cm polybags with potting mixture of 3:2:1 (peat moss: soil: sand). After 1 week of transfer, the plants with 6 leaves were subjected to PBZ application by soil drenching (SD). Second set of seeds was soaked with PBZ prior to germination followed by seedlings transfer into polybags with similar dimension mentioned earlier. This seed soaking (SS) method was applied at concentrations of 0 (control), 5, 10, 15 and 20 mg/L. Watering was carried out accordingly to maintain an adequate moisture level in the growth medium. The treatments were arranged according to a CRD with 6 replicates.

2.2.2. Growth Performance Measurement

The survival of the treated seeds and seedlings, and the growth performance of the plants were recorded weekly. The parameters recorded including plant height, crown diameter, the leaf size (length and width of leaves), number of leaves and chlorophyll content. The freshly harvested plants of each treatment were recorded for their fresh shoot weight, fresh root weight and the length of internode at 4th leaf. The chlorophyll content of those plants was measured using SPAD 502 Plus Chlorophyll Meter. The samples were also subjected to antioxidant analyses on 38th day of experiment. Two parameters were measured including Ascorbic Acid (AA) and Total Phenolic Content (TPC).

2.2.3. Determination of Ascorbic Acid (AA)

Ascorbic Acid (AA) content was quantitatively determined using the 2, 6-dichloroindophenol (DCIP) dye method as described by [11] and [12] with slight modification. In this procedure, 2 g of each fresh sample was mixed with 8 mL of cold extracting solution. This cold extracting solution was stored at 4 °C and consisted of 30 g metaphosphoric acid, 80 mL of acetic acid and distilled water added to 1000 ml. The mixture was homogenized in a blender for 1 min and then filtered through filter paper to eliminate particulates. The result was AA extract. To quantify AA content, 5 ml of AA extract was titrated with DCIP solution (i.e. 25 mg DCIP and 21 mg $NaHCO_3$ in 75 mL water) until pink colour. The DCIP solution was standardized with AA solution. All determinations were repeated three times, and the results were expressed as mg AA per 100 gram of fresh weight basis (unit written as mg100/g).

2.2.4. Determination of Total Phenolic Content (TPC)

The total phenolic acid of the *B. rapa* var. *oleifera* was determined by using Folin-Ciocalteu reagent following a slightly modified method [13] [14]. Gallic acid was used as a reference standard for plotting calibration curve. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The test material was dried at 60^{o}C in an oven and the dried sample was used to determine the phenolic content. Gallic acid stock solution was first prepared by dissolving 0.01 g dry gallic acid into 100 mL distilled water. The stock solution was used for the calibration curves of standard gallic acid. The standard gallic acid solutions of 0.0, 0.05, 0.1. 0.2, 0.3, 0.5 mg/L were then prepared accordingly. To determine the phenolic content in the sample, 2g dried leaf sample was placed in 40 mL distilled water and heated at 60^{o}C for 60 minutes. 100 µL sample solution was pipetted into small bottle. All the standard gallic acid solutions and sample solutions were each added with 1 mL of 50% Folin-Ciocaltue reagent and the mixture was left for 5 minutes. Then, 2 mL of 2% sodium carbonate solution and 7 mL distilled water were added into each mixture and agigated. The standard and the sample solutions were left in the dark for two hours and measured at 760nm by using UV-spectrophotometer. Quantitative results were then expressed with reference to gallic acid. Results on the total phenols were expressed as mg/g gallic acid equivalents (GAE) of dry extract.

2.2.5. Statistical Analysis

ANOVA was carried out at 5% level of significance. Treatment means were compared by using Tukey's Simultaneous Test.

3. Results and Discussion

3.1. Effects of PBZ on Growth Performance

As reported in Table 1, growth inhibition of *B. rapa* var. *oleifera* by PBZ in terms of height was noticeable with SD as compared to SS application. This result was in agreement with those reported by [6], [7], [15] and [16] that stated reduced plant height with higher PBZ concentration applied as soil drenching. According to [7], PBZ was found effective in reducing height of garden pansy plant (*Viola x wittrockiana* Gams.) from 8.17cm to 6.60cm when applied at 2.5pm and 5.0ppm, respectively. PBZ reduced plant height by blocking multiple steps in biosynthesis of gribberellins and steroids. The research conducted by [16] also reported that plant height of *Nerium oleandar* L. seedling was significantly inhibited by PBZ (37% reduction compared with the control). Research conducted by [17] also showed that the height of Okra significantly decreased with an increase in the concentration of PBZ applied to the soil.

Table 1: ANOVA and Mean Comparison for Plant Heights (in cm)

Source	df	*p*-value for period in days			
		21	28	32	36
Treatment	**1**	**0.619**	**0.022**	**0.000**	**0.000**
PBZ concentration	4	0.492	0.463	0.203	0.058
Interaction	4	0.741	0.602	0.360	0.584
Period (days)		21	28	32	36
Treatment					
SS		13.63 a	18.03 a	22.55 a	32.27 a
SD		15.11 a	15.37 b	18.29 b	21.30 b

Means with the same letter within the same column are not significantly different at 5% level of significance.

On the contrary, soil drenching of *B. rapa* var. *oleifera* seedling resulted in emergence of more leaves but the leaves were significantly smaller in terms of leaf width, as shown in Table 2 and 3, respectively. Application of PBZ to *B. rapa* var. *oleifera* by using SD technique resulted in the development of more leaves from 28 days after planting. Similarly, high application concentration of PBZ at 20 mg/L also resulted in increased number of leaves as compared to control treatment. This result was in agreement with those reported by [18] where PBZ was also found to increase the number leaves and roots in both wheat cultivars, i.e. Ghods and Karchia. Research conducted by [17] on Okra showed that number of leaves/plant increased with an increase in PBZ concentration with values ranged from 30.59 to 38.81 as compared to the control treatment.

Table 2: ANOVA and Mean Comparison for Number of Leaves

Source	df	*p*-value for period in days			
		21	28	32	36
Treatment	**1**	**0.471**	**0.002**	**0.000**	**0.000**
PBZ concentration	**4**	**0.157**	**0.000**	**0.000**	**0.000**
Interaction	4	0.344	0.234	0.505	0.867
Period (days)		21	28	32	36
Treatment					
SS		7.55 a	12.30 b	16.20 b	20.30 b
SD		7.30 a	14.55 a	20.75 a	27.10 a
Period (days)		21	28	32	36
PBZ concentration (mg/L)					
0		7.00 a	9.375 c	13.125 c	15.875 c
5		7.88 a	12.250 bc	17.00 bc	22.250 b
10		7.75 a	14.125 ab	19.250 b	23.875 b
15		6.75 a	14.875 ab	19.375 ab	25.625ab
20		7.75 a	16.50 a	23.625 a	30.875 a

Means with the same letter within the same column are not significantly different at 5% level of significance.

As shown in Table 4, neither SD nor SS was found significantly affect the leaf length of *B. rapa* var. *oleifera*. This finding was found in agreement with previous study conducted by [19] which reported that application of PBZ on white oak did not affect the leaf size (length and width) or twig growth at any time during the 7 years of his study. The effects of PBZ on plant growth inhibition were presumed to be dependent on genetic makeup of plants besides the application concentration and method as studied. However, [17] reported that the fifth leaf of okra plant showed significant decreased leaf area with all concentrations of PBZ studied as compared with the control treatment. Results from previous study conducted by [20] also showed that increasing the concentration of PBZ treatment resulted in decreased shoot length, internode length, leaf area, leaf index, weight, length, diameter of fruit and also fruit acidity. Overall, as shown in Table 5 and Fig. 1, the crown diameter of *B. rapa* var. *oleifera* under study was not significantly different irrespective of any concentration or the PBZ application technique.

Table 3: ANOVA and Mean Comparison for Leaf Width

Source	df	*p*-value for period in days			
		21	28	32	36
Treatment	**1**	**0.027**	**0.000**	**0.000**	**0.000**
PBZ concentration	4	0.193	0.265	0.337	0.491
Interaction	4	0.223	0.819	0.732	0.764
Period (days)		21	28	32	36
Treatment					
SS		7.88 a	9.50 a	11.00 a	12.4 a
SD		7.40 b	8.20 b	9.175 b	10.75 b

Means with the same letter within the same column are not significantly different at 5% level of significance.

Table 4: ANOVA for Leaf Length

Source	df	*p*-value for period in days			
		21	28	32	36
Treatment	1	0.453	0.092	0.028	0.131
PBZ concentration	4	0.170	0.652	0.754	0.654
Interaction	4	0.287	0.871	0.902	0.296

Table 5: ANOVA for Crown Diameter

Source	df	*p*-value for period in days			
		21	28	32	36
Treatment	1	0.390	0.288	0.787	0.626
PBZ concentration	4	0.494	0.889	0.270	0.171
Interaction	4	0.731	0.970	0.597	0.287

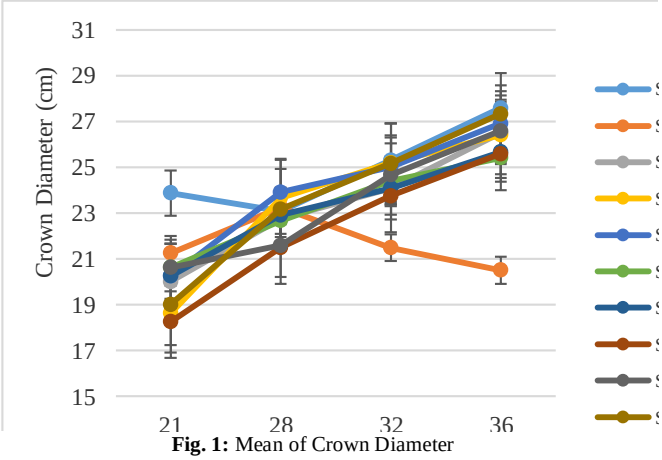

Fig. 1: Mean of Crown Diameter

Table 6 shows the statistical analysis for internode length measurement conducted on day 38 of experiment. The internode length was significantly affected by PBZ application using SD technique where reduction of internode length was noticed in *B. rapa* var. *oleifera.* The plants had significantly shorter internodes as compared to those treated by PBZ using SS. All PBZ treated plants also had significantly shorter internode length as compared to control treatment. Increasing of PBZ treatment concentration also significantly decreased the internode length of *B. rapa* var. *oleifera.* PBZ application at 20 mg/L was more effective in decreasing the internode length as compared to those treated with lower concentrations. This result was also similar to those reported by [21]. They reported PBZ treatment on *Lycopersicon*

esculentum Mill. has recorded 68% reduction and 22% increase of internode length and stem thickness, respectively.

Table 6: ANOVA and Mean Comparison for Internode Length on Day 38

Source	df	*p*-value for period on Day 38
Treatment	**1**	**0.000**
PBZ concentration	**4**	**0.000**
Interaction	4	0.079
Treatment		
SS		1.2150 a
SD		0.7250 b
PBZ concentration (mg/L)		
0		1.5625 a
5		1.0000 b
10		0.9250 b
15		0.7125 b
20		0.6500 b

Means with the same letter within the same column are not significantly different at 5% level of significance.

As shown in Table 7, application of PBZ to *B. rapa* var. *oleifera* by means of SS or SD did not significantly affect the relative chlorophyll content over the study period of 5 weeks. Fig. 2 also clearly show that PBZ application at concentrations up to 20 mg/L also did not significantly affect the chlorophyll content of *B. rapa* var. *oleifera*. This finding was found contrary to study reported by [22] that PBZ treatment increased the relative water and chlorophyll content of mango seedlings.

Table 7: ANOVA for Chlorophyll Content

Source	df	*p*-value for period in days			
		21	28	32	36
Treatment	1	0.110	0.941	0.079	0.110
PBZ concentration	4	0.321	0.951	0.284	0.321
Interaction	4	0.953	0.184	0.664	0.953

As can be seen in Table 8, fresh shoot weight was significantly affected by the PBZ treatment. In specific, seedlings drenched with PBZ generally had relatively lower fresh weight as compared to those treated with SS as shown in Table 9. This finding was found aligned to study reported by [8] where by using SD technique, the PBZ application was markedly as second most effective agent (after uniconazole) for suppressing shoot growth of *Euryops. Pectiantus* Cass.

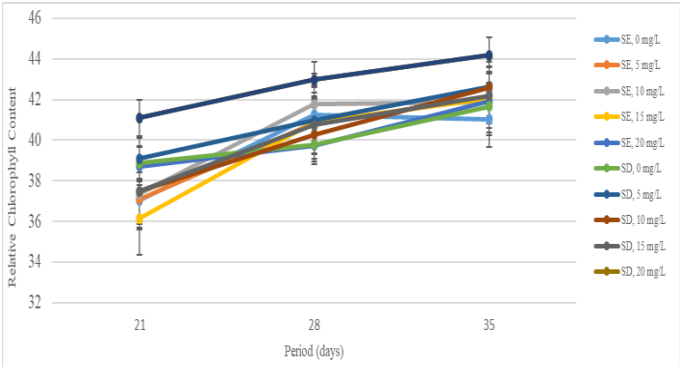

Fig. 2: Mean of Chlorophyll Content

On the other hand, PBZ did not significantly affect both root growth and root:shoot ratio as clearly shown by Fig. 3 and Fig. 4, respectively. In relation to the fresh root weight, [23] also reported that fresh weight, number and diameter of roots were not affected by PBZ. However, [24] stated that PBZ treated tomato plantlets recorded higher fresh weight of roots as compared to control in NaCl salinity media under in vitro condition.

Table 8: ANOVA of Shoot Weight, Root Weight and Root:Shoot Ratio on Day 38

Source	df	*p*-value for period in days		
		Shoot	Root	Root: Shoot
Treatment	**1**	**0.009**	0.217	0.629
PBZ concentration	4	0.764	0.285	0.091
Interaction	4	0.862	0.924	0.437

Table 9: Mean Comparison for Shoot Weight on Day 38

Source	Fresh weight of shoot (g)
Treatment	
SS	59.5 a
SD	46.04 b

Means with the same letter within the same column are not significantly different at 5% level of significance.

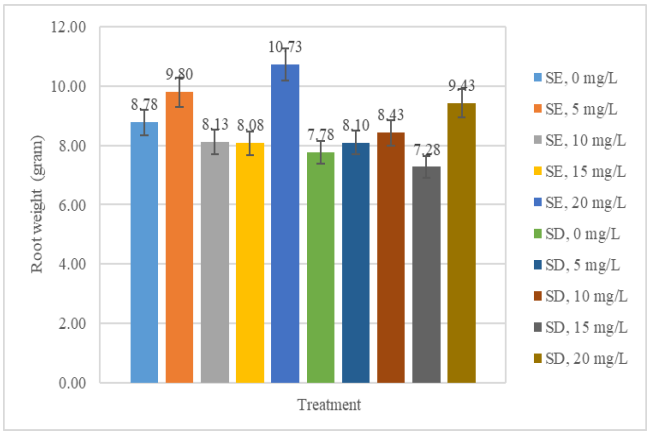

Fig. 3: Mean of Fresh Root Weight

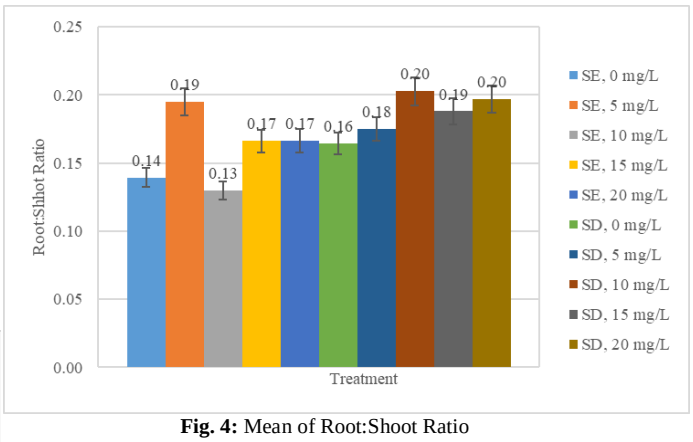

Fig. 4: Mean of Root:Shoot Ratio

3.1. Effects of PBZ on Antioxidant Content

Antioxidant content in terms of AA in *B. rapa* var. *oleifera* was significantly affected by PBZ application method and concentration and the result is shown in Table 10. Plants treated with SD method had significantly recorded higher AA content as compared to SS method. It was found that interaction between SD method and 10 mg/L PBZ resulted in the highest AA content. Several studies including [6], [25], [26], [27] and [28] also reported that application of PBZ induced biochemical adaptations including increasing the AA. According to study conducted by [25], PBZ application was found increased the AA content of leaves and stem tissues of *Ocimum sanctum* to 158.18% and 119.02% over the control on Diammonium Phosphate (DAP) fertilizer. According to [26], PBZ treated drought stressed plants maintained higher levels of antioxidant and scavenging enzymes. Previous study by

[27] also reported increased AA contents in the leaves and storage roots of triazole treated sweet potato plants. An increase in AA content was also reported by [28] in uniconazole treated tomato seedlings and paclobutrazol treated *Dioscorea rotundata.* As shown in Fig. 5, the phenolic content was generally higher in plants applied with PBZ as SD of seedling as compared to SS treatment. Application of PBZ by SD method at concentration of 5 mg/L gave highest amount of phenolic content.

Table 10: ANOVA and Mean Comparison of AA on Day 38

Source	df	*p*-value for period on Day 38
Treatment	**1**	**0.000**
PBZ concentration	**4**	**0.000**
Interaction	**4**	**0.000**
Treatment		
Seed		6.133 b
Seedling		11.199 a
PBZ concentration (mg/L)		
0		12.332 a
5		11.110 b
10		7.777 b
15		6.222 b
20		5.888 b
Interaction		
Treatment	PBZ Concentration	
SS	0	5.999 c
	5	5.333 c
	10	7.110 b c
	15	5.777 c
	20	6.444 b c
SD	0	5.777 c
	5	7.110 c
	10	17.554 a
	15	9.777 b
	20	15.776 a

Means with the same letter within the same column are not significantly different at 5% level of significance.

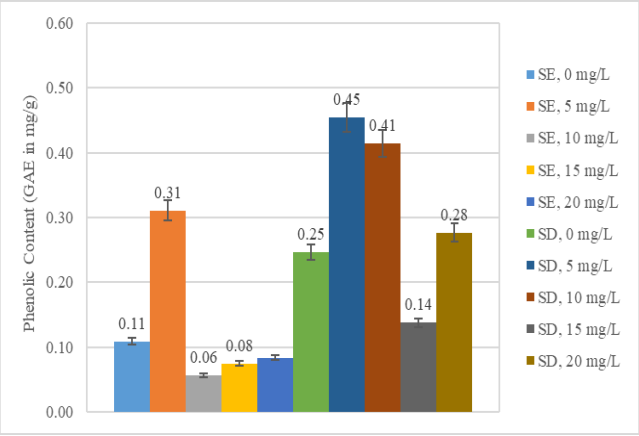

Fig. 5: Mean of Phenolic Content (GAE in mg/g)

4. Conclusion

From the results of this study, it can be concluded that the application of PBZ affected the growth performance and antioxidant content of *B. rapa* var. *oleifera*. It was found that application of 10mg/L and 5mg/L of PBZ using soil drenching technique recorded highest amount of ascorbic acid and phenolic content, respectively. Further experiments considering higher PBZ application concentrations and times with other major phytochemical antioxidants in *B. rapa* var. *oleifera* are required in order to identify effective concentration for optimum plant growth.

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