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Effect of Plant Growth Regulators on Biochemical Studies of Mustard [*Brassica juncea* (L.) Czern & Coss.] under Sodic Soil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field study was conducted to evaluate the "Effect of plant growth regulators on biochemical parameters of mustard [*Brassica juncea* (L.) Czern & Coss.] under sodic soil." An experiment was laid out in randomized block design with seven treatments and three replications. The field experiment treatments consist of foliar spray with GA_3 (50ppm, 100ppm, 150ppm) and salicylic acid (100ppm, 200ppm, 300ppm) were prepared on weight by volume basis as per desired concentration. Data confined to physiological changes like chlorophyll content, catalase activity were recorded at 40, 60 and 80 DAS. Oil content (%) in mature seeds was recorded at maturity. Significantly higher chlorophyll contents and catalase activity in leaves were estimated with the foliar spray of 100 ppm GA_3 (2.51, 5.05, 2.24) chlorophyll content mg g^{-1} fresh weight and (69.17, 173.80, 171.30) catalase activity EU mg^{-1} protein min^{-1} and Significantly higher oil content (%) in mature seeds of mustard were estimated with the foliar application of 100 ppm GA_3 (40.67 %) as compared to control. These biochemical parameters superior with foliar application of GA_3 100ppm followed by Salicylic acid 200ppm.

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Keywords: Plant growth regulators; mustard; oilseed crop; foliar application.

1. INTRODUCTION

The Indian mustard is an important oilseed crop, it's botanical name is *Brassica juncea* L. (2n=18) and it belongs to family Cruciferae. Rapeseed-mustard is the major rabi oilseed crop, which is sown during October-December and the harvesting begins from March onwards. Oil content of different species specific crop ranges from 30-48%. Indian mustard [*Brassica juncea* (L) Czern & Coss.] is predominantly cultivated in Rajsthan, Uttar Pradesh, Haryana, Madhya Pradesh and Gujarat. Mustard is cultivated in mostly under temperate climate. In India, it is the second most important edible oilseed crop after groundnut sharing 25-30% in Indian oilseed export generated money. The share of oilseeds is 14.1% out of the total cropped area, in India, rapeseed mustard accounts for 3% of it. Mustard seed is the second largest produced oilseed in the world. Rapeseed-mustard oil is used primarily for edible purpose and is the principle cooking oil in the mustard growing areas in the country. When compared to other edible oils, the rapeseed-mustard oil has the lowest amount of harmful saturated fatty acid. It also contains adequate amount of the two essential fatty acids (i.e., linoleic and linolenic acid) which are not present in many of other edible oils. The meal cake left after oil extraction forms important cattle feed and may be used as organic manure. The cake has minerals like S, P, Co, K, Mg, Zn and vitamins like Niacin, Thiamine, Riboflavin and Vit.E. A rich source of vitamins and minerals, Brassica contains many medicinal properties. There is an increasing interest in crop as a source of erucic acid for chemical industry. "Rapeseed-mustard seed was primarily used for human consumption because of low erucic acid and thus, becoming desirable edible oil. Plant growth regulators are organic compound other than nutrients that modify plant physiological process. They refer to artificially synthetic agents used for promoting or inhibiting the germination, root differentiation, and other plant physiological processes. Use of growth regulators increases the rate of photosynthesis by increasing the chlorophyll content per unit area and size of mesophyll cells of leaves" [1]. The application of PGR is known to play an important role in plant response to stress [2]. "Gibberellic acid is a naturally occurring growth hormone which regulates growth and development of plant. Gibberellic acid is associated with various plant growth and development processes such as seed germination, hypocotyls elongation, leaf expansion, floral initiation, uniform flowering, floral organ and development, reduced time to flowering, increased flower number and size and induction of some hydraulic enzymes in aleurone layer of cereal grain. Gibberellic acid (GA_3) is a phytohormone that is needed in small amounts at low concentration to accelerate plant growth and development. So, favourable conditions may be induced by applying growth regulators like GA_3 exogenously in proper concentration at a proper time in a specific crop. Several studies on different plant species have shown that the exogenous application of GA_3 can enhance the productivity of crops affecting the vital physiological process" [3]. "Salicylic acid has recently been recognized as a plant growth hormone & generates a wide range of metabolic & physiological responses thereby affecting their growth & development" (Hayat et al., 2010). "Salicylic acid has been found to play a key role in the regulation of plant growth, development, interaction with other organisms and in the responses to environmental stresses" [4,5,6]. The use of salicylic acid to induce resistance of plants to abiotic stress has received considerable attention. Exogenous application of Salicylic acid has been reported to influence several developmental and physiological processes, i.e., seed germination [7], transpiration rate [8], stomatal closure, membrane permeability, growth and photosynthesis [9]. Therefore, the present research was undertaken to determine whether or not applying foliar application of plant growth regulators to mustard plants will reduce any damaging effects and determined for their growth behaviour, biochemical characteristics and yield attributes. Salicylic acid is one of the numerous phenolic compounds found in plants. "The exogenous application of salicylic acid to plants generates diverse physiological effects, such as the inhibition of dry mass accumulation, the promotion of stomatal closure [8] and the inhibition of ethylene synthesis".

2. MATERIALS AND METHODS

The experiment was conducted on Indian mustard at Aachraya Narendra Deva University of Agriculture & Technology, Ayodhya during 2018. The experiment was laid out in RBD with three replications and 7 treatments of 3 concentrations of gibberellic acid and three concentrations of salicylic acid with control. The concentrations of gibberellic acid are 50, 100 & 150 ppm & salicylic acid 100, 200, 300 ppm.

2.1 Total Chlorophyll Content (mg g^{-1} fresh weight) (Arnon, 1949)

Total chlorophyll content was estimated according to method of Arnon(1949) and expressed as mg g^{-1} fresh weight of leaves. In this method taken, 200 mg of fresh leaves were homogenized in 5 ml of 80 % aqueousacetone centrifuged at 4000 rpm for 20 minutes. The supernatant was collected and residues was re-extracted with 5 ml of 80% acetone and centrifuged it. The supernatant was combined and volume was made at 20 ml with 80 % acetone. Optical Density (O.D.) was measured at 645 and 663 nm on spectrophotometer using 80% acetone as blank. The amount of total chlorophyll was calculated as follows:

$$\text{Total chlorophyll} = \frac{20.2XO.D(645)+8.02XO.D.(663)XVolume}{1000XWeight}$$

Where, W = weight of sample (mg fresh weight), V = final volume (ml), O.D. = Optical Density.

2.2 Catalase Activity (EU mg^{-1} protein min^{-1})

Catalase activity can be assayed calorimetrically according to method given in analytical biochemistry (Sinha,1972).Calculate facilitates the dismutation of H_2O_2 to water and oxygen according to the reaction.The enzyme plays an important role in association with SOD as well as in photorespiration and glycolate pathway. The reagents arePhosphate buffer (0.1 M), pH 7.0, Potassium dichromate acetic acid (5% potassium dichromate andglacialacetic acid in acid in 1:3 ratio) andH_2O_2(0.2 M). In this method 100 mg of fresh leaf material was homogenized with 10 ml of phosphate buffer 0.1 M (pH 7.0) and centrifuged at 10,000 rpm at 40^0Cfor 20 minutes. Collect supernatant and stored at low temperature. Used supernatant for enzyme assay as estimate the enzyme activity as given Chart 1.

Take 1 ml supernatant, 1ml H_2O_2, 3 ml phosphate buffer. Keepit at 37^0C for 3 minutes in water bath, take 1ml + 3ml ($K_2Cr_2O_7.CH_3COOH$). Keep in water bath for 10 monutes, take O.D. at 570 nm. Express result as enzyme unit $gram^{-1}$ freshweight or $gram^{-1}$ protein basis.

2.3 Oil Content (%)

Oil content was estimated by the conventional Soxhlet method. It was used for estimation of oil (AOAC, 1970). In this method seed samples were kept in the oven at 70^0C for removal of moisture. After removal of moisture the seeds were crushed in a pestle-mortar for extraction of oil.

$$\text{Oil content (\%)} = \frac{\text{Weight of oil flask+Ether extract}-\text{Weight of flask oil}}{\text{subsatance taken}} \text{ X } 100$$

3. RESULT AND DISCUSSION

Biochemical parameters of any crop played a major role for improving quality of any produce/products along with better yield which causes the maximum benefits as monetary value for the growers. The biochemical parameter like chlorophyll content, catalase activity and oil content have been significantly affected by foliar application of salicylic acid at various growth stages of the crop.

3.1 Total Chlorophyll Content (mg g^{-1} fresh weight)

The data present in Table 1 and Fig. 1 clearly showed that higher chlorophyll content (mg g-1 fresh weight) was noticed at 60 DAS. Among the treatments maximum chlorophyll content observed with GA_3 100 ppm (2.51, 5.05 and 2.24) at 40, 60 and 80 DAS and followed by salicylic acid 200 ppm (2.34, 4.86 and 2.06) respectively over control. Foliar application of plant growth regulators significantly improved the chlorophyll content in leaf upto 60 DAS and after that 60 DAS the declined trend was recorded in chlorophyll content in leaf. Similar results were reported by, Travaglia *et al.,* [10] in soybean, Sharma *et al.,* [11] in mustard seeds. Use of growth regulators increased the rate of photosynthesis by increasing the chlorophyll content per unit area and size of mesophyll cells of leaves [1].

Chart 1. Used supernatant for enzyme assay as estimate the enzyme activity

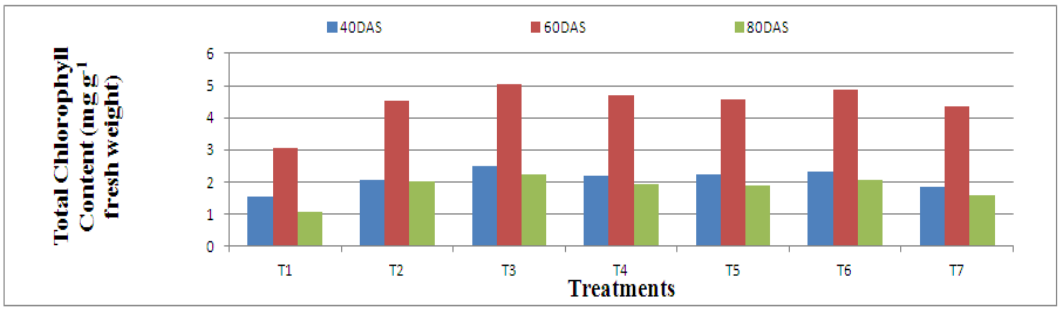
Test	Blank	Reagents
1.25 ml	-	H_2O_2
0.50 ml	0.50 ml	Enzyme extract
3.25 ml	4.50 ml	Phosphate buffer
5.00 ml	5.00 ml	Total Volume

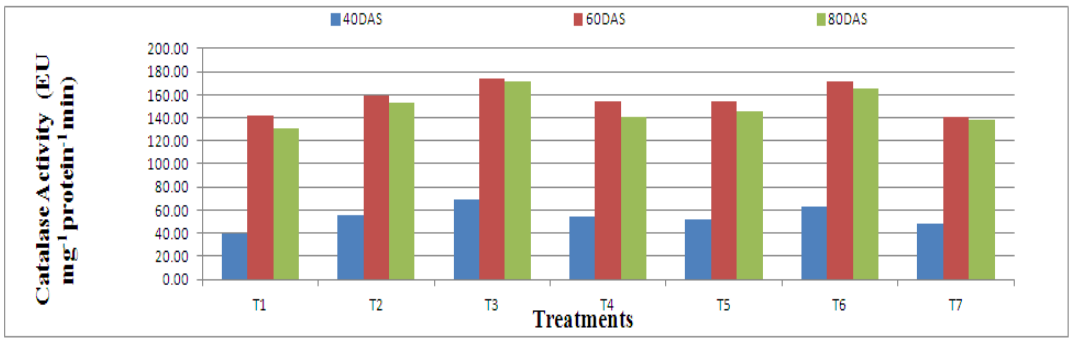
Tables 1,2 & 3. Variation in chlorophyll content, catalase activity and oil content of Indian Mustard having seven treatments including control are depicted respectively in the tables

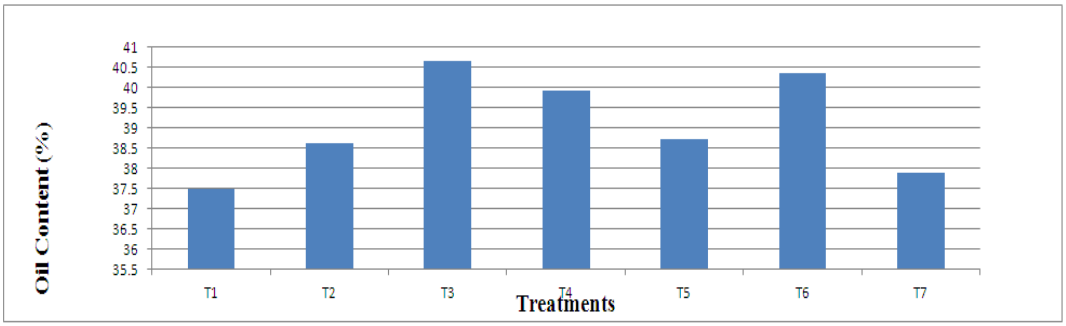
Treatments	Chlorophyll content (mg g^{-1} fresh weight)		
	40DAS	60DAS	80DAS
T_1 : Control	1.53	3.04	1.05
T_2 : GA_3 (50 ppm)	2.05	4.54	2.03
T_3 : GA_3 (100 ppm)	2.51	5.05	2.24
T_4 : GA_3 (150 ppm)	2.18	4.71	1.92
T_5 : SA(100 ppm)	2.25	4.56	1.87
T_6 : SA(200 ppm)	2.34	4.86	2.06
T_7 : SA(300 ppm)	1.84	4.36	1.60
SE m±	0.07	0.22	0.08
CD at 5%	0.22	0.68	0.26

Treatments	Catalase activity (EU mg^{-1}protein min^{-1})		
	40DAS	60DAS	80DAS
T_1 : Control	39.8	141.3	131
T_2 : GA_3 (50 ppm)	55.9	159.1	152.7
T_3 : GA_3 (100 ppm)	69.17	173.8	171.3
T_4 : GA_3 (150 ppm)	54.3	154.1	140.8
T_5 : SA(100 ppm)	52.17	154.3	145.3
T_6 : SA(200 ppm)	63.2	171	165.7
T_7 : SA(300 ppm)	47.6	142.2	138.1
SE m±	2.22	0.62	0.44
CD at 5%	6.9	1.92	1.36

Treatments	Oil content (%)
T_1 : Control	37.50
T_2 : GA_3 (50 ppm)	38.64
T_3 : GA_3 (100 ppm)	40.67
T_4 : GA_3 (150 ppm)	39.91
T_5 : SA(100 ppm)	38.74
T_6 : SA(200 ppm)	40.36
T_7 : SA(300 ppm)	37.90
SE m±	0.01
CD at 5%	0.04







Figs. 1,2 & 3. Total chlorophyll content, catalase activity and oil content are respectively shown in three different graphical bar plots

3.2 Catalase Activity (EU mg^{-1} protein min^{-1})

The data present in Table 2 and Fig. 2 indicate that catalase activity (EU mg-1 protein min-1) increased with 40, 60 DAS after that it was found declined at 80 DAS of crop growth. The maximum increased in catalase activity was recorded with foliar spray of GA_3 100ppm (69.17, 173.80 and 171.30) at 40, 60 and 80 DAS followed by foliar spray of salicylic acid 200 ppm (63.20, 171.00 and 165.70) respectively, over other treatments. Similar results were also reported by Eisvand *et al*., [12] in Agropyron alongatum.

3.3 Oil Content (%)

The data present in Table 3 and Fig. 3 indicate all the treatments showed increase in oil content percent in mustard seeds. Statistically significant oil content (%) was seen with foliar sprayed of GA_3 100 ppm (40.67%) followed foliar sprayed by salicylic acid 200 ppm (40.36%) over rest of the treatments. The oil content (%) increased significantly in all the treatments over control. This showed that the GA_3 has much influence on oil content. Similar findings were reported by Baydar [13] in safflower. Dawood *et al*., [14] reported that different concentrations of salicylic acid (0, 25, 50, 75 and 100 mg liter-1) before sowing significantly increased oil percentage.

4. CONCLUSION

Salinity stress during flowering lowers the oil quality of mustard by raising the levels of glucosinolates and erucic acid. Catalase is a type of anti-oxidative which directly regulates gene

expression against salinity stress in mustard plants resulting into trigger mechanism of protective functions of the enzymes. Chlorophyll content of plants were found maximum at 60 DAS and then decline, GA_3 100ppm is most effective in cholorophyll content. The maximum increased in catalase activity was recorded with foliar spray of $GA_3$100ppm [15,16].The application of GA_3 and Salicylic acid has increased the catalase activity in the mustard plants. Salicylic acid, a growth regulator, was very effective in reducing the negative impacts of salinity stress on mustard oil quality indices. On the other hand, it has also increased the seed oil content under higher salt-stricken conditions.The effect of GA_3 appears to be mediated via improving photosynthetic pigments, so increasing photosynthesis, significant growth as compared to check in sodic soil water, and increasing osmoregulant proline biosynthesis, thereby decreasing protein degradation. Use of plant growth regulators to see changes in oil quality of mustard. Statistically significant oil content (%) was seen with foliar sprayed of GA_3 100 ppm (40.67%) followed foliar sprayed by salicylic acid 200 ppm (40.36%). The oil content (%) increased significantly in all the treatments over control. This showed that the GA_3 has much influence on oil content. It is assumed that GA_3 is both economically and biochemically stable, whereas Salicylic acid has also procured a significantly beneficial administration for mustard under saline conditions. Mustard seed oil content and quality can be improved by both GA_3 and Salicylic acid under current climate changing scenario.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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