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Response of growth Retardants Paclobutrazol and Cycocel on Morphological Characteristics in Indian Mustard (*Brassica juncea* L.) Genotypes under Rainfed Condition

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Abstract
The present investigation was conducted during the 2020-22 in field at Advanced Centre for Rainfed Agriculture (ACRA), Dhiansar, SKUAST-Jammu. Laboratory work was carried out in the Division of Plant Physiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha. The main aim of this study was to evaluate the effects of Cycocel (CCC) and Paclobutrazol (PBZ) as the growth regulators, on mustard crop under rainfed condition. *Brassica juncea* L. genotypes (RH-30, Pusa bold and RSPR-01) were taken for the study. PBZ in different concentration (100, 150, 200 and 250 ppm) and CCC (500, 800, 1100 and 1400 ppm) were applied at vegetative stage (40 days after sowing). A significant reduction in plant height was recorded in plants treated with PBZ @ 200 ppm and CCC @ 1100 ppm in all genotypes of *Brassica juncea* in comparison with their control. Paclobutrazol impedes gibberellin biosynthesis through blocking ent-kaurene synthesis in the metabolic pathway of gibberellin production, resulting in reduced amounts of active gibberellins and consequent reduction in stem elongation. Maximum number of leaves and branches were observed in PBZ @ 200 ppm and CCC @ 1100 ppm and minimum was recorded in control. Leaf area were significantly decreased in plants treated with PBZ @ 200 ppm and CCC @ 1100 ppm in all genotypes of *Brassica juncea* in comparison with their control. Maximum dry weight of plants was recorded in plants treated with PBZ @ 200 ppm and CCC @ 1100 ppm in comparison to control. Chlorophyll SPAD were observed highest in plants treated with PBZ @ 200 ppm and CCC @ 1100 ppm in all genotypes of *Brassica juncea* in comparison with their control. Application of PBZ @ 200 ppm and CCC @ 1100 ppm exerted a profound effect on the growth characters in mustard crop.

Keywords: Mustard, *Brassica juncea* L., paclobutrazol, cycocel, ent-kaurene, leaf area

Introduction
Mustard (*Brassica juncea* L.) is one of the most ancient oilseed crop belongs to the family cruciferous and genus brassica. It is a member of the cruciferous family. For its high-quality oil, the crop is grown practically everywhere in the nation, and it has a huge export potential. Rapeseed-mustard ranks first among all oilseed crops in terms of oil yield despite being the second most significant edible oilseed crop in India after peanuts due to its higher oil content (35–45 percent) (Kumar *et al.*, 2018) [4]. In oilseed brassicas, triazole fungicide paclobutrazol (PBZ) is a plant growth inhibitor, has been shown to decrease plant height, improve oil yield contributing features, increase branching, and resistance to lodging. As a result, the seed quality is improved. It is a synthetic plant growth regulator which influences plant growth and development and triazole-type inhibitor of gibberellin (GA) biosynthesis. Ent-kaurene oxidase, an enzyme in the GA biosynthetic pathway that catalyses the oxidation of ent-kaurene to ent-kaurenoic acid, is inhibited by this substance. Gibberellin is recognised to be its opponent in plants. It affects plants like tomato, mustard, apple, and numerous cereal crops by decreasing the content of gibberellin, lowering internodal development to create sturdier stems, increasing root growth, producing early fruit set, and increasing seed set (Banoo *et al.*, 2019) [9]. Cell division still takes place when gibberellin production is blocked, but the new cells do not elongate, leading to a thicker stem and short internodes with the same number of leaves (Jungklang *et al.*, 2017) [2]. Additionally, paclobutrazol can be applied chemically to lower the risk of lodging in cereal crops (Kamran *et al.*, 2018; Desta and Amare, 2021) [3, 10]. It is possible that the PBZ-induced change in leaf area during deficit irrigation relates to more

effective water utilisation. Reduced shoot growth caused by the PBZ-induced suppression of GA production has been linked to variations in the root/shoot dry weight ratio (Bayat and Sepehri, 2012) [1]. Cycocel (CCC or Chlormequat chloride) and PBZ both block the GA cycle in stages I and II, respectively. Instead of eliminating it or competing with it for the active site, cycocel prevents the production of gibberellins. This is based on the observation that *Fusarium moniliforme*, a fungus, does not make gibberellic acid when cycocel is present. Cell wall thickness and the quantity of vascular bundles were shown to increase after cycocel treatment. CCC is an anti-gibberellin growth retardant, that also play a major role in drought tolerance (Pirasteh-Anosheh *et al.*, 2016) [16]. Paclobutrazol modifies the sink-source relationship by reallocating carbohydrates to other organs, which leads to increased flowering. Plant growth regulators are recognised to boost physiological efficiency including photosynthetic ability of plant and play key role in generating better crop yields (Raza *et al.*, 2017) [17].

Materials and Methods

The study was conducted during the 2020-22 in field at Advanced Centre for Rainfed Agriculture (ACRA), Dhiansar, SKUAST-Jammu. Laboratory work was carried out in the Division of Plant Physiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha. The experiment was laid out in randomized block design (RBD) with nine treatments and three replications. Seeds of *Brassica juncea* genotypes RH-30, Pusa bold and RSPR-01 were procured from the Division of Plant Breeding and Genetics, FoA, SKUAST-J, Chatha. The foliar spray of PBZ and CCC were given at vegetative stage (40 Days After Sowing (DAS). Plant height of the tagged plants was measured in centimetres as the distance from ground level to the tip of the plant at harvest. Number of leaves was recorded from selected plants from each treatment. Number of branches was recorded from selected plants from each treatment. Total biomass or dry weight of plant in grams was recorded from selected plants from each treatments. The leaf surface area one side was calculated manually and recorded throughout the period of crop development (Blanco and Folegatti, 2003) [6]. SPAD value were taken after exposure to cold stress. SPAD (Soil Plant Analysis Development) measurements were made with SPAD-502 instrument. SPAD readings taken around the midpoint of each leaf, upper, middle and lower leaf on one side of the midrib and values were averaged.

Results and Discussion

Plant height (cm)

A significant reduction in plant height was recorded in CCC treated plants @ 1100 ppm (72.4 cm, 71.6 and 66.4 cm) followed by PBZ @ 200 ppm (80.4, 75.7 and 70.4 cm respectively) in all three varieties RH-30, RSPR-01 and Pusa bold in comparison to control (134.6 cm, 125.4 cm and 112.8 cm respectively). As evident from table 1, CCC when applied @ 500 ppm, 800 ppm and 1400 ppm, also significantly reduced plant height (88.6, 79.6 and 74.7 cm), (83.3, 74.2 and 70.5 cm) and (74.9, 73.5 and 67.6 cm) respectively in all three varieties. Similar pattern was noticed when all the varieties were treated with different level of PBZ @ 100, 150 ppm, 200 ppm and 250 ppm in comparison to control, whereas under rainfed environment, maximum plant height (134.6 cm) were obtained by variety RH-30 (drought resistant) in comparison to other drought susceptible varieties RSPR-01 (125.4 cm) and Pusa bold (112.8 cm). Similar findings were also reported by Soumya *et al.* (2017) [7]. According to Tesfahun and Menzir (2018) [11], plant height reduction strongly associated with reduced elongation of the internodes, rather than lowering the number of internodes and they found uppermost internodes to be shortened under paclobutrazol application.

Table 1: Effect of PBZ and CCC on plant height (cm) in *Brassica juncea* genotypes at physiological maturity

Plant height (cm)				
Treatments	**Variety**			
	RH-30	**RSPR-01**	**Pusa Bold**	**Mean**
T1 - Control	134.6	125.4	112.8	124.2
T2- PBZ @ 100 ppm	91.3	84.4	79.6	85.1
T3- PBZ @ 150 ppm	85.8	79.4	73.7	79.6
T4- PBZ @ 200 ppm	80.4	75.7	70.4	75.5
T5- PBZ @ 250 ppm	82.3	76.2	72.5	77.0
T6- CCC @ 500 ppm	88.6	79.6	74.7	80.9
T7- CCC @ 800 ppm	83.3	74.2	70.5	76.0
T8-CCC @ 1100 ppm	72.4	71.6	66.4	70.1
T9- CCC @ 1400 ppm	74.9	73.5	67.6	72.0
Mean	88.1	82.2	76.4	
SE (m)±	Variety (V) = 0.65 Treatments (T) =1.14 Variety x Treatments (VxT) =1.97			
CD at 5%	Variety = 1.81 Treatments = 3.22 Variety x Treatments =5.61			

Number of leaves and number of branches

A marked increased in number of leaves was observed in CCC treated plants @ 1100 ppm (40.0, 37.6 and 34.0) followed by PBZ @ 200 ppm (38.3, 35.3 and 31.6) in all three varieties RH-30, RSPR-01 and Pusa bold in comparison to control (27.3, 24.3 and 18.0 respectively). As evident from table 2, CCC when applied @ 500 ppm, 800 ppm and 1400 ppm, also significantly increased number of leaves (34.6, 30.6 and 27.6), (36.0, 33.6 and 30.6) and (39.3, 36.6 and 33.0 respectively) in all three varieties. Similar pattern was noticed when all the varieties were treated with different level of PBZ @ 100, 150 ppm, 200 ppm and 250 ppm in comparison to control, whereas under rainfed environment, maximum number of leaves (27.3) were obtained by variety RH-30 (drought resistant) in comparison to other drought susceptible varieties RSPR-01 (24.3) and Pusa bold (18.0). Number of branches was significantly increased in CCC treated plants @ 1100 ppm (41.3. 35.6 and 33.3) followed by PBZ @ 200 ppm (39.3, 33.6 and 30.6) in all three varieties RH-30, RSPR-01 and Pusa bold in comparison to control (24.3, 18.0 and 13.0 respectively). As evident from table 3, CCC when applied @ 500 ppm, 800 ppm and 1400 ppm, also significantly increased number of branches (32.0, 26.3 and 23.3), (37.3, 31.3 and 27.3) and (39.0, 33.6 and 31.0 respectively) in all three varieties. Similar pattern was noticed when all the varieties were treated with different level of PBZ @ 100, 150 ppm, 200 ppm and 250 ppm in comparison to control, whereas under rainfed condition, maximum number of branches (24.3) were obtained by variety RH-30 (drought resistant) in comparison to other drought susceptible varieties RSPR-01 (18.0) and Pusa bold (13.0). The same trend also reported by Pal *et al.,* (2016) [5], Xia *et al.,* (2018) [8]. PBZ application has reduced plant height, improved stem diameter and leaf number, altered root architecture (Pal *et al.,* 2016) [5] directly contributed to yield increase, and indirectly reduced the event of lodging. Higher number of branches per plant, shoot dry weight was recorded in sufficiently watered rape plant as compared to mild and severe drought affected rape plants (Mehanna *et al.,* 2013) [15]. Prakash *et al.,* (2015) [12] recorded that the application of cycocel at 120 ppm reduced plant height and increased number of branches and leaves.

Table 2: Effect of PBZ and CCC on number of leaves in *Brassica juncea* genotypes at 90 DAS

Number of leaves				
Treatments	**Variety**			
	RH-30	**RSPR-01**	**Pusa Bold**	**Mean**
T1 - Control	27.3	24.3	18.0	23.2
T2- PBZ @ 100 ppm	32.6	28.6	25.3	28.8
T3- PBZ @ 150 ppm	35.6	32.3	28.3	32.0
T4- PBZ @ 200 ppm	38.3	35.3	31.6	35.0
T5- PBZ @ 250 ppm	37.3	34.3	30.6	34.0
T6- CCC @ 500 ppm	34.6	30.6	27.6	30.9
T7- CCC @ 800 ppm	36.0	33.6	30.6	33.4
T8-CCC @ 1100 ppm	40.0	37.6	34.0	37.2
T9- CCC @ 1400 ppm	39.3	36.6	33.0	36.3
Mean	35.6	32.5	28.7	
SE (m)±	V = 0.37 T = 0.64 VxT = 1.16			
CD at 5%	V = 1.05 T= 1.82 V x T = 3.16			

Table 3: Effect of PBZ and CCC on number of branches in *Brassica juncea* genotypes at 90 DAS

Number of branches				
Treatments	**Variety**			
	RH-30	**RSPR-01**	**Pusa Bold**	**Mean**
T1 - Control	24.3	18.0	13.0	18.4
T2- PBZ @ 100ppm	30.6	24.3	21.3	25.4
T3- PBZ @ 150ppm	35.6	29.3	26.3	30.4
T4- PBZ @ 200ppm	39.3	33.6	30.6	34.5
T5- PBZ @ 250ppm	36.0	31.6	29.6	32.4
T6- CCC @ 500ppm	32.0	26.3	23.3	27.2
T7- CCC @ 800ppm	37.3	31.3	27.3	31.9
T8-CCC @ 1100ppm	41.3	35.6	33.3	36.7
T9- CCC @ 1400ppm	39.0	33.6	31.0	34.5
Mean	35.0	29.2	26.1	
SE (m)±	V = 0.37 T =0.64 VxT = 1.11			
CD at 5%	V = 1.05 T= 1.82 V x T =3.15			

Leaf area (cm^2)

Data presented in Table 4 showed a significant reduction in leaf area in CCC treated plants @ 1400 ppm (62.17, 57.12 and 55.29 cm^2) followed by PBZ @ 250 ppm (65.02, 60.12 and 56.37 cm^2) in all three varieties RH-30, RSPR-01 and Pusa bold in comparison to control (81.21, 76.12 and 70.22 cm^2 respectively). Chlormequat chloride when applied @ 500 ppm, 800 ppm and 1100 ppm, also significantly decrease leaf area (72.03, 65.29 and 62.44), (68.12, 61.33 and 59.12) and (63.19, 58.20 and 56.23 respectively) in all three varieties. Similar pattern was noticed when all the varieties were treated with different level of PBZ @ 100, 150 ppm and 200 ppm in comparison to control, whereas under rainfed condition, maximum leaf area (81.21) were obtained in variety RH-30 (drought resistant) in comparison to other drought susceptible varieties RSPR-01 (76.12) and Pusa bold (70.22). The result are in agreement with other researchers (Pal *et al.*, 2016) [5]. Leaf area of *Brassica juncea* decreases under drought stress and with increase in intensity of drought more reduction in leaf area occurs (Raza *et al.*, 2017) [17]. Reduction in leaf area under water deficit conditions is mainly due to less leaf expansion and leaf area adjustment process. The PBZ induced reduction in leaf area may be linked to inhibition of GA biosynthesis whereas small leaves helps in minimum loss of water by the leaf surface and it also helps in improvement of water use efficiency

Table 4: Effect of PBZ and CCC on leaf area (cm^2) in *Brassica juncea* genotypes at 90 DAS

Leaf area (cm²)				
Treatments	**Variety**			
	RH-30	**RSPR-01**	**Pusa Bold**	**Mean**
T1 - Control	81.21	76.12	70.22	75.85
T2- PBZ @ 100 ppm	75.13	69.15	64.12	69.46
T3- PBZ @ 150 ppm	69.09	64.22	60.33	64.54
T4- PBZ @ 200 ppm	66.15	61.25	57.29	61.56
T5- PBZ @ 250 ppm	65.02	60.12	56.37	60.50
T6- CCC @ 500 ppm	72.03	65.29	62.44	66.58
T7- CCC @ 800 ppm	68.12	61.33	59.12	62.85
T8-CCC @ 1100 ppm	63.19	58.20	56.23	59.20
T9- CCC @ 1400 ppm	62.17	57.12	55.29	58.19
Mean	69.12	63.64	60.15	
SE (m)±	V = 0.46 T =0.83 VxT =1.38			
CD at 5%	V = 1.31 T= 2.26 V x T =3.93			

Chlorophyll (SPAD value)

A marked increased in chlorophyll (SPAD value) was observed in CCC treated plants @ 1400 ppm (77.00, 68.00 and 61.33) followed by PBZ @ 250 ppm (75.33, 61.66 and 55.00) in all three varieties RH-30, RSPR-01 and Pusa bold in comparison to control (55.33, 46.00 and 39.33 respectively). As evident from table 5, CCC when applied @ 500 ppm, 800 ppm and 1100 ppm, also significantly increased chlorophyll (SPAD) (65.66, 55.66 and 49.33), (69.66, 59.33 and 53.66) and (74.66, 65.33 and 58.66 respectively) in all three varieties. Similar pattern was noticed when all the varieties were treated with different level of PBZ @ 100, 150 ppm and 200 ppm in comparison to control, whereas under rainfed environment, maximum chlorophyll (SPAD) (55.33) were obtained by variety RH-30 and minimum in RSPR-01 (46.00) and Pusa bold (39.33). The results are in conformity with early reports (Soumya *et al.*, 2017; Kamran *et al.*, 2018) [7, 3]. Exposure to various abiotic stresses including water stress reduces the level of total chlorophylls due to disintegration of thylakoid membranes and destruction of chloroplasts is caused by different reactive oxygen species. CCC had stimulated the vegetative growth and hence has caused an increased accumulation of metabolic components especially carbohydrates such as chlorophyll and carotene. Similarly chlorophyll content increased with the application of chlormequat chloride in faba bean (Reddy *et al.*, 2013) [14]. The effect of cycocel in increasing chlorophyll contents may be due to the reduction in cell size resulting in dense cytoplasm (Prat *et al.*, 2008) [13].

Table 5: Effect of PBZ and CCC on chlorophyll (SPAD value) in *Brassica juncea* genotypes at 90 DAS

Chlorophyll (SPAD value)				
Treatments	**Variety**			
	RH-30	**RSPR-01**	**Pusa Bold**	**Mean**
T1 - Control	55.33	46.00	39.33	46.88
T2- PBZ @ 100 ppm	63.00	52.33	46.66	53.99
T3- PBZ @ 150 ppm	69.00	56.33	51.66	58.99
T4- PBZ @ 200 ppm	73.33	61.66	55.00	63.33
T5- PBZ @ 250 ppm	75.33	64.00	58.33	65.88
T6- CCC @ 500 ppm	65.66	55.66	49.33	56.88
T7- CCC @ 800 ppm	69.66	59.33	53.66	60.88
T8-CCC @ 1100 ppm	74.66	65.33	58.66	66.21
T9- CCC @ 1400 ppm	77.00	68.00	61.33	68.77
Mean	69.21	58.73	52.66	
SE (m)±	V = 0.52 T =0.90 VxT =1.57			
CD at 5%	V = 1.48 T=2.57 V x T =4.46			

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