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Physiological and Morphological Effects of Paclobutrazol Doses on Early Development of Canola (*Brassica napus* L.) Seedlings

Paclobutrazol Dozlarının Kanola (*Brassica napus* L.) Fidelerinin Gelişimi Üzerindeki Fizyolojik ve Morfolojik Etkileri

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Abstract: In this study, the effects of different doses of paclobutrazol (PBZ) on early seedling development of rapeseed (*Brassica napus* L.) were investigated by measuring some physiological and morphological parameters. PBZ, a plant growth regulator known for its ability to inhibit gibberellin biosynthesis, was applied by spraying on the leaf and stem surfaces of plants at five different concentrations (0, 150, 300, 450, 900 ppm) to evaluate the effects on growth parameters, chlorophyll content, relative water content (RWC), leaf area, shoot and root properties, electrolyte leakage and leaf extract properties (pH and electrical conductivity) of rapeseed seedlings. The results showed that PBZ had different dose-dependent effects on rapeseed seedlings, causing significant reductions in leaf area and volume at high doses. Although PBZ had no significant effect on chlorophyll content, an improvement in water retention (RWC) was observed at 300 ppm. Furthermore, PBZ doses of 150–300 ppm enhanced membrane stability, as indicated by reduced electrolyte leakage. In contrast, higher doses caused morphological abnormalities such as leaf thickening and deformation. While doses of 300 ppm and below were found to be appropriate, PBZ application during the seedling stage had minimal impact on overall plant development.However, the effects of PBZ applications during later growth stages on drought and heat stress should be further investigated.
Keywords: Paclobutrazol, Rapeseed, Canola, *Brassica napus* L, Seedling development, Plant growth regulator

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Öz: Bu çalışmada, paclobutrazolün (PBZ) farklı dozlarının kolza tohumunun (*Brassica napus* L.) erken fide gelişimi üzerindeki etkilerini bazı fizyolojik ve morfolojik parametreler ölçülerek incelenmiştir. Gibberellin biyosentezini inhibe etme yeteneğiyle bilinen bir bitki büyüme düzenleyicisi olan PBZ'ün, kolza fidelerinin büyüme parametreleri, klorofil içeriği, bağıl su içeriği (RWC), yaprak alanı, sürgün ve kök özellikleri, elektrolit sızıntısı ve yaprak özütü özellikleri (pH ve elektriksel iletkenlik) üzerindeki etkisini değerlendirmek için beş farklı konsantrasyonda (0, 150, 300, 450, 900 ppm) bitkinin yaprak ve gövde yüzeylerine püskürtülerek uygulanmıştır. Sonuçlar, PBZ'nin kolza tohumu fideleri üzerinde doza bağlı farklı etkilerinin olduğunu, yüksek dozlarda yaprak alanı ve hacminde önemli azalmalara neden olduğunu göstermiştir. PBZ klorofil içeriğini önemli ölçüde değiştirmezken, RWC'de 300 ppm'de su tutulumunun iyileştiği görülmüştür. Ayrıca, 150–300 ppm PBZ dozlarının daha düşük elektrolit sızıntısı ile stabil bir membrana neden olduğu belirlendi. Daha yüksek dozlar ise yaprak kalınlaşması ve deformasyonu gibi morfolojik anormalliklere yol açtı. 300 ppm ve alt dozları uygun dozlar olsa da fide döneminde PBZ uygulamasının bitki gelişimi açısından önemli bir katkısı görülmemiştir. Ancak, sonraki büyüme dönemlerindeki uygulamaların kuraklık ve sıcaklık stresi üzerine etkileri araştırmalıdır.
Anahtar kelimeler: Paclobutrazol, Kolza, Kanola, *Brassica napus* L, Fide gelişimi, Bitki büyüme düzenleyicisi

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INTRODUCTION

Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops globally, providing a significant source of edible oil, animal feed, and biodiesel (Raboanatahiry et al., 2021). As global demand for sustainable agriculture and bioenergy increases, enhancing the yield and quality of rapeseed has become a critical focus of agricultural research. In the 2023/2024 season, global rapeseed production was 88.77 million metric tons. Production volume increased year by year in major producers such as Canada, the European Union, China and India (United States Department of Agriculture, 2024). This widespread cultivation underscores the crop's significance in meeting the nutritional and energy needs of a growing population.

The economic importance of rapeseed extends beyond its role as a major source of vegetable oil. The by-products of oil extraction, such as rapeseed meal, serve as high-protein animal feed, making rapeseed integral to the livestock industry. Additionally, rapeseed oil is increasingly used as a feedstock for biodiesel production, contributing to the global shift towards renewable energy sources (Kirkegaard et al., 2021). This versatility has solidified rapeseed's status as a vital crop in both agricultural and industrial sectors. However, the growth and development of rapeseed are susceptible to various environmental stress factors. To mitigate these challenges and optimize plant growth, the use of plant growth regulators (PGRs) has become increasingly common in agriculture. Paclobutrazol (PBZ), a triazole-based PGR, is notable for its role in modulating plant growth by inhibiting gibberellin biosynthesis. This inhibition regulates root and shoot development, enhances plant compactness, and improves stress tolerance (Sofy et al., 2020; Maheshwari et al., 2022).

Numerous studies have highlighted the impact of PBZ on the morphology, physiology, and biochemical properties of various plant species. For instance, in wheat (*Triticum aestivum* L.), PBZ reduces plant height while increasing chlorophyll content, thereby enhancing resistance to salinity stress by regulating ion homeostasis and stress-related gene expression (Pal et al., 2016). Similarly, PBZ application in soybean (*Glycine max* L.) reduces plant height and increases both plant and seed yield under varying plant densities (Maheshwari et al., 2022).

In rapeseed cultivation, PBZ has been reported to reduce plant height, thereby mitigating lodging-a common challenge that leads to seed loss and reduced yield. PBZ also positively affects pod shattering, contributing to improved seed yield and harvest quality (Kuai et al., 2015).

This study investigates the effects of different PBZ doses during the early seedling development stage of rapeseed. By evaluating the impact of various PBZ doses on the vegetative characteristics of rapeseed, this research aims to provide valuable insights into optimizing growth management and enhancing resistance to environmental stresses in rapeseed cultivation.

MATERIAL AND METHOD

The study was conducted at the Seed Science and Technology Laboratory of Abant İzzet Baysal University, Faculty of Agriculture, following a randomized complete block design with four replications. The experiment involved the application of five different PBZ doses (0, 150, 300, 450, 900 ppm) on the Laktriz cultivar, a spring-type rapeseed (*Brassica napus* L.).

Experimental Setup and Growth Conditions

Cocopeat-filled 2-liter pots were used, irrigated to saturation with Hoagland solution twice weekly after the first week. Seventy seeds were sown per pot, and thinning was performed post-emergence to leave 50 seedlings per pot. The seedlings were grown in a growth chamber under a 16-hour photoperiod with an irradiance of 35 $\mu mol\ m^{-1}\ s^{-1}$. The temperature was maintained at 24 ± 1°C with a relative humidity of 55–60%. PBZ solutions were applied twice at the seedling stage, on the 20th day after sowing, directly to the root zone on the cocopeat surface. On the 35th day, the seedlings were harvested for measurement.

Growth and Physiological Measurements

The growth and physiological parameters of the rapeseed seedlings were measured using a variety of methods. Chlorophyll content was determined with an Apogee Chlorophyll Concentration Meter (MC-100), which provided SPAD values indicative of chlorophyll concentration. Leaf area (LA) was measured using Easy Leaf Area software, with ten seedlings per replication analyzed for accuracy. Relative water content (RWC) of the leaves was calculated using the formula provided by Mullan and Pietragalla (2012). The formula used was:

$$RWC(\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} x100 \quad (1)$$

Where FW represents the fresh weight, TW the turgid weight, and DW the dry weight of the leaves.

Leaf volume was determined using the water displacement method. Electrolyte leakage (EL) was measured following the method described by Lutts et al., (1995), with some modifications. Leaves were excised, washed, and dried with filter paper. A 0.2 g fresh weight of leaves was cut into small pieces and immersed in 20 mL of deionized water, incubated at 25°C for 3 hours, and the initial electrical conductivity (EC1) was recorded. Subsequently, the samples were boiled at 100°C for 30 minutes to release all electrolytes, cooled to 25°C, and the final electrical conductivity (EC2) was measured. Electrolyte leakage was expressed as:

$$EL(\%) = \frac{\mathrm{EC1}}{\mathrm{EC2}} x100 \quad (2)$$

Shoot thickness was measured 3 cm above the soil surface using a digital calliper. Shoot fresh weight and dry weight were determined by weighing ten seedlings per replication using an analytical balance. For dry weight measurement, the shoots were dried in an oven at 65°C for 24 hours before weighing. Additionally, shoot and root lengths were measured from ten seedlings per replication. Leaf pH and electrical conductivity (EC) were also assessed. For this, five grams of fresh leaves were ground to a paste and extracted using 50 cm^3 of deionized water. The pH and EC of the leaf extract were then measured using a calibrated digital pH and EC meter (Multifunction Tester).

Results (except EC and pH values) are presented as mean values with standard deviations (±SD). Data were analyzed using a one-way ANOVA at $P < 0.05$ significance level with JMP 13.0 software. Differences between means were determined using the LSD test.

RESULTS AND DISCUSSION

This study revealed the effects of different doses of paclobutrazol (PBZ) application to rapeseed (*Brassica napus* L.) seedlings on chlorophyll content, relative water content (RWC), leaf area, shoot and root properties, electrolyte leakage and leaf extract properties (pH and electrical conductivity). PBZ doses caused statistically significant differences in all properties except Chlorophyll SPAD value and Shoot thickness (Table 1).

PBZ dose applications caused significant differences at the 5% level ($p<0.01$) in Relative water content (RWC) properties, while they caused significant differences at the 1% level ($p<0.01$) in Leaf volume, Electolyte leakage, Leaf area, Shoot fresh weight, Shoot dry weight, Shoot height and Root height properties.

Chlorophyll content (SPAD)

No statistically significant differences in chlorophyll SPAD values were observed among PBZ doses, though the highest SPAD value was at 900 ppm (Figure 1). This shows that PBZ has a limited effect on photosynthesis during the seedling period, but it may also be related to the fact that PBZ application time was on the 20th day and the uprooting and measurement time was on the 35th day. Earlier PBZ applications may cause different results in this and other traits. While many studies reported positive PBZ effects on chlorophyll content due to stress adaptation mechanisms, similar to this study, some studies did not report any significant changes (Berova and Zlatev, 2000; Kuai et al., 2015; Xia et al., 2018; Sharma et al., 2023; Banoo et al., 2022; Hajihashemi and Ehsanpour, 2013).

Relative Water Content (RWC)

Significant differences in RWC were observed, with the highest content at 300 ppm, suggesting PBZ may enhance water retention in rapeseed seedlings (Figure 1). This aligns with findings that moderate PBZ doses improve drought tolerance by increasing water use efficiency (Li et al., 2023; Jungklang et al., 2017).

Table 1. Effects of PBZ doses on chlorophyll content, relative water content (RWC), leaf volüme, electrolyte leakage, shoot thickness, leaf area, shoot fresh and dry weight, shoot and root height of canola seedlings.

Çizelge 1. PBZ dozlarının kanola fidelerinde klorofil içeriği, bağıl su içeriği (RWC), yaprak hacmi, elektrolit sızıntısı, sürgün kalınlığı, yaprak alanı, sürgün taze ve kuru ağırlığı, sürgün ve kök uzunluğu üzerine etkileri.

Doses	Chlorophyll SPAD value	Relative water content (RWC)	Leaf volume (cm^3 per plant)	Electolyte leakage	Shoot thickness (mm)
0	47.46 ± 2.14	77.03 ± 3.0 b	5.17 ± 0.31 a	0.042 ± 0.00105 c	1.26 ± 0.051
150	46.07 ± 1.33	81.63 ± 2.6 b	2.6 ± 0.46 bc	0.033 ± 0.00047 e	1.27 ± 0.164
300	46.43 ± 4.56	86.75 ± 2.9 a	3.4 ± 0.62 b	0.036 ± 0.00067 d	1.38 ± 0.017
450	42.00 ± 1.73	81.62 ± 2.9 b	1.8 ± 0.35 c	0.075 ± 0.00211 a	1.38 ± 0.068
900	54.43 ± 10.91	80.45 ± 1.93 b	2.4 ± 1.23 bc	0.059 ± 0.00102 b	1.03 ± 0.235
	NS	*	**	**	NS
Doses	**Leaf area (cm^2 per plant)**	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Shoot height (cm)**	**Root height (cm)**
0	34.25 ± 14.74 a	0.84 ± 0.074 b	0.158 ± 0.023 a	22.03 ± 1.80 c	18.57 ± 0.91 a
150	22.14 ± 3.59 ab	1.38 ± 0.159 a	0.127 ± 0.023 ab	28.174 ± 1.44 a	15.6 ± 1.91 b
300	13.51 ± 4.67 bc	1.01 ± 0.165 b	0.167 ± 0.015 bc	25.93 ± 0.72 b	14.53 ± 0.85 bc
450	9.13 ± 3.42 bc	1.00 ± 0.044 b	0.087 ± 0.012 cd	24.6 ± 0.8 b	12.76 ± 1.42 c
900	7.23 ± 4.32 c	1.01 ± 0.072 b	0.083 ± 0.015 d	26.5 ± 0.7 ab	14.6 ± 1.11 bc
	**	**	**	**	**

* Significant differences at the 0.05 probability level; ** Significant differences at the 0.01 probability level; NS : Nonsignificant at the 0.05 probability level.

Leaf Volume and Area

Both leaf volume and area decreased with increasing PBZ doses, consistent with PBZ's growth-retarding effects by inhibiting gibberellin biosynthesis (Banoo et al., 2022; Matsoukis et al., 2014; Jyothsna et al., 2022) (Figure 1). The decrease in leaf area and leaf volume per plant observed with increasing doses of PBZ showed abnormalities at the 900 ppm dose, with leaf wrinkling and thickening causing deformation. The control group had the highest leaf volume and area, reinforcing PBZ's inhibitory effect on vegetative growth.

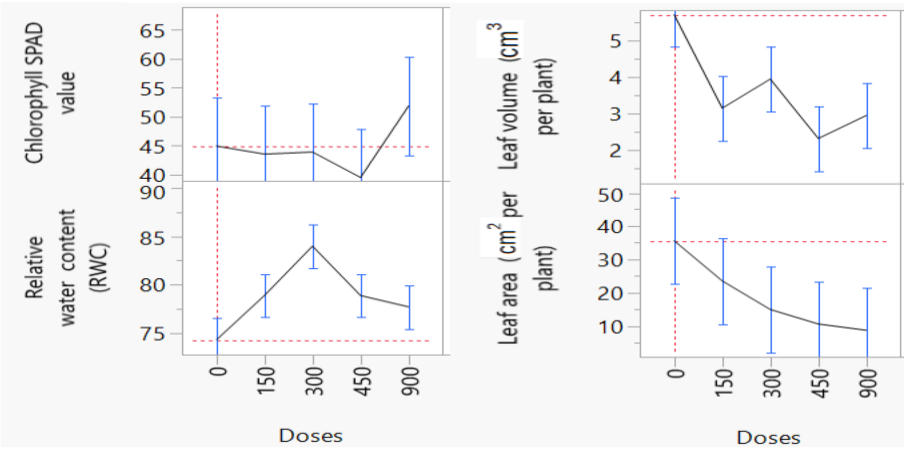

Figure 1. Changes in chlorophyll content (SPAD), relative water content (RWC), leaf area and leaf volume according to PBZ doses.

Şekil 1. PBZ dozlarına göre klorofil içeriği (SPAD), bağıl su içeriği (RWC), yaprak alanı ve yaprak hacmindeki değişimler.

Electrolyte Leakage, EC, and pH of Leaf Extract

Electrolyte leakage was highest at 450 ppm, with 150 ppm PBZ resulting in the most stable membrane level (Figure 2). The electrical conductivity value of the leaf extract was lowest in control plants and highest at the 450 ppm PBZ dose. Previous studies have reported that PBZ maintains cell membrane stability in the presence of extra stress conditions, but in the absence of stress factors, as in our study, it has been determined that PBZ doses of 150–300 ppm should not be exceeded for rapeseed seedlings (Jungklang et al., 2017, Somasundaram (2022). No significant differences in leaf pH were observed among doses.

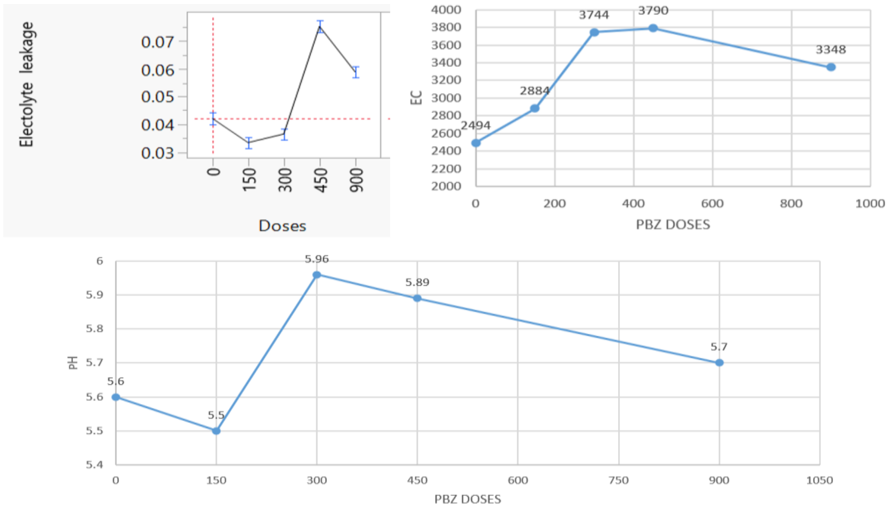

Figure 2. Changes in electrolyte leakage, and leaf extract properties (electrical conductivity and pH) depending on the PBZ doses.

Şekil 2. PBZ dozlarına bağlı olarak elektrolit sızıntısında ve yaprak ekstrakt özelliklerinde (elektriksel iletkenlik ve pH) meydana gelen değişimler.

Stem and Root Characteristics

PBZ shortens the shoot length of rapeseed and makes it resistant to lodging (Tesfahun and Menzir, 2018, Wu et al., 2022, Ali et al., 2024). In this respect, the effects of PBZ on roots and stems are important. In our study, the observation that stem fresh weight was the highest at 150 ppm and stem dry weight was the highest in the control group indicates that low to medium PBZ doses may initially promote biomass accumulation due to reduced water loss (Figure 3).

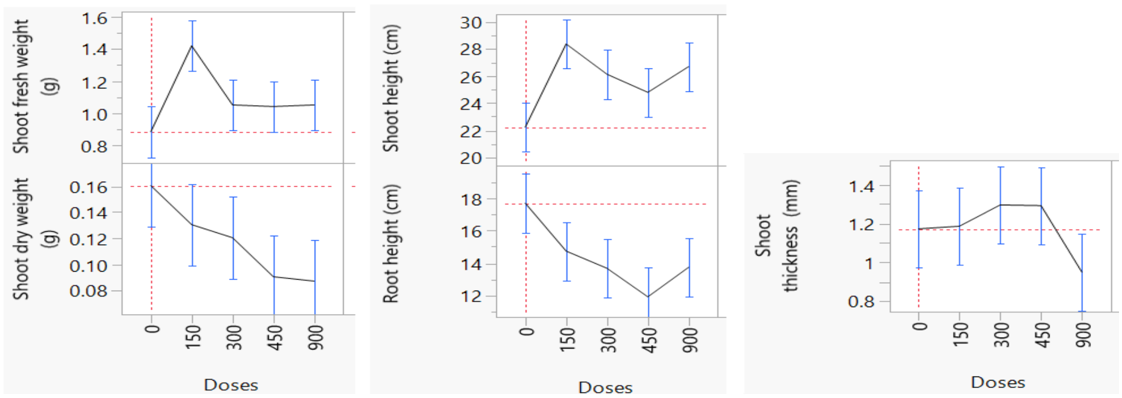

Figure 3. Changes in shoot and root characteristics (shoot dry and fresh weight, shoot and root height, shoot thicknees) according to PBZ doses.

Şekil 3. PBZ dozlarına göre sürgün ve kök özelliklerindeki değişimler (sürgün kuru ve taze ağırlığı, sürgün ve kök yüksekliği, sürgün kalınlığı).

However, as PBZ doses increase, its inhibitory effects become more pronounced and lead to a decrease in dry weight as seen at higher doses. Similarly, the longest stem length at 150 ppm and the highest root length in the control group indicate that the effect of PBZ on roots and stems affects leaf growth more than root and stem growth between the 20th and 35th days of application. Studies should be conducted to determine whether PBZ application at an earlier time may have a greater effect on roots and stems. The lack of significant difference in stem thickness between doses indicates that this parameter may also be less sensitive to PBZ during this period.

CONCLUSION

The effects of PBZ (Paclobutrazol) on rapeseed seedlings showed dose-dependent changes. While high doses caused a decrease in leaf area and volume, chlorophyll content did not change significantly. At 300 ppm dose, RWC (relative water content) improved and lower electrolyte leakage was observed. However, high doses caused morphological problems such as leaf thickening and deformation. Although doses of 300 ppm and below seemed appropriate, it was stated that applications during the seedling period had limited contribution to plant development. For a more comprehensive evaluation, future studies are recommended to investigate the effects of PBZ doses on drought and heat stress during the later stages of plant development.

CONFLICT OF INTEREST

The author has no conflicts of interest to declare.

DECLARATION OF AUTHOR CONTRIBUTION

All processes of the study were carried out by the author.

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