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Paclobutrazol enhances yield and secondary compound accumulation in *Curcuma longa* L.

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Abstract The results indicated that the application of different concentrations of PBZ at 60 days preharvest had an impact on yield after harvest at 9 months while the color of turmeric remained relatively consistent. In all treatments, the rhizome color was yellow to orange. The fresh weight of the rhizome showed no significant differences within the range of 0-800 mg/L PBZ treatments, with the lowest yield observed in 1,200 mg/L PBZ treatment. The dry weight percentage was highest when treated with 800 mg/L PBZ for the mother rhizome and 600 mg/L PBZ for the finger rhizome. Regarding the accumulation of secondary compounds, highly significant differences among the treatments were found for the content of total flavonoids and total phenolics while curcumin showed significant differences. The treatment with 1,200 mg/L PBZ had the highest total phenolics content at 78.18 mg GAE/gDW and total flavonoids at 295.54 mg QUE/gDW. PBZ-treated plants also showed higher curcumin content and percentage than the non-treated plants. These findings indicated that the application of PBZ has the potential to improve both the yield and quality of turmeric, which is advantageous for cultivating this valuable medicinal plant.

Keywords: Turmeric, Plant growth regulator, Curcumin, Phenolics, Flavonoids

Introduction

Turmeric (*Curcuma longa* L.) is a perennial herbaceous plant belonging to the family Zingiberaceae. The plants are extensively cultivated in warm climates across various regions of the world (Setzer *et al*., 2021). Turmeric has traditionally been employed as a spice and food additive due to its distinct yellow color, taste enhancement qualities (Prasad *et al*., 2014), and its ability to improve the palatability and storage stability of food products. Additionally, turmeric possesses numerous beneficial properties, including antioxidant effects (Tanvir *et al.*, 2017), anti-inflammatory properties (Lee *et al*., 2020), and potential anti-cancer activities (Mbese *et al*., 2019).

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The mother rhizome and finger rhizome of turmeric are rich in bioactive compounds, which exist in the form of non-volatile curcuminoids and volatile oils (Itokawa *et al*., 2008). Among these compounds, curcuminoids play a crucial role as the primary bioactive components of turmeric (de Oliveira Filho *et al.,* 2021). Particularly curcumin a type of curcuminoid, showed significant biological activities, including antibacterial effects (Mody *et al.*, 2019) antioxidant properties, antitumor and anti-inflammatory actions, anti-cancer potential (Baldi *et al*., 2020) as well as anti-acidogenic, radioprotective, and neuroprotective properties (Urošević *et al*., 2022; Oglah *et al*., 2022).

Plant growth regulators can affect the primary and secondary metabolites of various plant species (El-Sayed *et al*., 2022). Paclobutrazol (PBZ) is a triazole-type plant growth retardant known chemically as, (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentane-3-ol). It functions by inhibiting gibberellins biosynthesis, leading to a decrease in abscisic acid, ethylene, and indole-3-acetic acid levels, while simultaneously increasing cytokinin levels (Desta and Amare, 2021). In addition to its plant growth regulator and fungicide effects (Nazarudin *et al*., 2007). PBZ can also protect the plants from abiotic and biotic stress. It is also used to reduce the size of plants, improve compactness, and increase other abilities of plants to resist environmental stress (Xia *et al*., 2018; Biswas *et al*., 2018), i.e., drought stress (Hajihashemi and Ehsanpour, 2013) and salt stress (Waqas *et al*., 2017).

PBZ is typically applied as a soil drench, where it is taken up by the plant root and transported through the xylem to the upper part of the plant, the apical meristem (Desta and Amare, 2021). Not only the influence of PBZ on plant growth performance has been extensively investigated, but exogenous PBZ has been applied as an elicitor to enhance the secondary compound synthesis and accumulation in some medicinal plants. PBZ was found to stimulate the terpenoid pathway, leading to the accumulation of terpenes (Desta and Amare, 2021). In *Ocimum sanctum*, Gopi *et al.* (2009) reported a significant increase in the total phenolics content due to PBZ treatments, with the increase being particularly pronounced at the higher PBZ dosages. In *Arachis hypogaea,* Sankar *et al*. (2007) found that PBZ increased the levels of α-tocopherol.

Limited information is available regarding the PBZ impact on the production of secondary compounds in turmeric. Therefore, it is necessary to investigate the effectiveness of PBZ in enhancing the content of secondary compounds in turmeric. The objectives of this study were to determine the optimal concentration of PBZ for improving both yield and secondary compound content in turmeric.

Materials and methods

Material preparation and experiment

For land preparation and planting, the following procedures were carried out. Tillage was performed before the beginning of the rainy season, involving the reduction of soil size through shoveling. Subsequently, plowing was conducted, and planting grooves were done. The spacing between rows was set at 75 centimeters while the distance between individual mats or plants space was maintained at 30 centimeters. Turmeric was planted using stems weighing between 20 - 30 grams. To provide essential nutrients, a 13-13-21 fertilizer was incorporated into the soil at a rate of 50 kilograms per rai, with rice straw being utilized for moisture retention purposes.

During cultivation, specific actions were undertaken. Weeding activities were initiated once the turmeric plants germinated and reached a shoot length of approximately 5-10 centimeters. Additionally, ammonium sulfate fertilizer (21-0-0) was applied at the rate of 50 kg per rai. Following the initial weeding, a second weeding was conducted after 4 weeks of planting. Regularly watering was carried out every 3 days, and additional weeding procedures were performed 3-4 more times until turmeric growth and development stabilized.

PBZ treatments were administered to the soil at 60 days preharvest using the soil drench method. PBZ used in the study were 0, 600, 800, and 1,200 mg/L. After 9 months of growth, the rhizomes were harvested.

Yield analysis

After harvesting, mother and finger rhizomes were carefully washed, and their fresh weight was recorded. Following this, the mother rhizomes and finger rhizomes were sliced and dried in a hot air oven at 50 ºC. The resulting dry weight was then recorded, and the percentage of dry weight was calculated by (dry weight/fresh weight) x 100.

The quality and secondary compound analysis

Preparation of extract from turmeric finger rhizomes involved the following steps, the finger rhizomes were thoroughly washed to remove any impurities. They were cut into small pieces and dried in a hot air oven at 50 ºC for 3 days. Once completely dried, the turmeric piece was finely powdered. The powder was used to measure color values using a color measuring instrument, the Minolta CR-40.

A quantity of 0.5 g of turmeric powder was mixed with 20 mL of 95 % ethanol and allowed to macerate for 3 days (x3). The ethanolic extract was filtrated using Whatman filter paper No.1. The filtrated extract was collected in glass bottles and tightly sealed, stored at a temperature of 14 ºC for quantitative analysis of the secondary compounds. The turmeric extract was analyzed for the quantification of important constituents using a UV-Vis spectrophotometer (TG90 UV-Vis P&G Instrument®). The curcumin content was quantified by measuring its absorbance at 520 nm. The method was modified from Jasim and Ali (1988) and Arifin *et al*. (2022), using curcumin as a standard solution. The total phenolic content was determined using the Follin-Ciocalteu Phenol Test (Singleton and Rossi, 1965), with modifications based on the method by Ranatunge *et al.* (2017). The absorbance was measured at a wavelength of 760 nm, and gallic acid was employed as the standard solution. The total flavonoid content was measured using the Aluminum chloride colorimetric method, with modifications based on the method by Chang *et al*. (2002), with adapted from Baskar *et al*. (2011). The absorbance was measured at a wavelength of 415 nm, and quercetin was used as a standard solution.

Statistical analysis

The experiments were conducted using a completely randomized design with 4 treatments, each having 4 replications, and 10 plants in each replication. The data was evaluated through an analysis of variance (ANOVA). After the ANOVA, the mean differences among treatments were compared using Duncan's Multiple-Range Test (DMRT).

Results

Yield

Significant variations in the fresh weight of the mother rhizome were found among PBZ treatments. Notably, the higher concentration of PBZ at 1,200 mg/L reduced fresh weight when compared to 600-800 mg/L and the control group. Moreover, significant differences were observed in both the dry weight and dry weight percentage of the mother rhizome among the various PBZ treatments. Application of 800 mg/L PBZ resulted in the highest recorded mother rhizome dry weight and dry weight percentage, at 13.24 g and 24.90% respectively (Table 1).

While the quantity and dry weight percentage of finger rhizomes remained consistent across PBZ treatments, significant differences were found in both the

fresh weight and dry weight of these rhizomes among the treatments. Particularly noteworthy was the enhancement in fresh weight observed in rhizomes treated with 600 mg/L PBZ compared to those treated with 1,200 mg/L PBZ and the control, with no statistically significant difference detected compared to the 800 mg/L PBZ treatment. Furthermore, the 600 mg/L PBZ treatment had the highest recorded dry weight of finger rhizomes at 47.62 g (Table 2).

Quality improvement

The color of finger rhizome powder plays a crucial role in determining the quality and market value of turmeric products. Therefore, investigating the impact of PBZ treatment on the color attributes of finger rhizome powder yielded interesting results. After drying the finger rhizomes at 50 ºC in a hot air oven, color measurements were taken using a colorimeter to assess the L* (lightness), a* (redness), and b* (yellowness) values of the powder. In our analysis of the finger rhizome fresh powder color, no significant differences were observed in the L*, a*, and b* values among the PBZ-treated plants (Table 3, Table 4, and Figure 1). In all treatments, the powder exhibited a yellow-orange color. However, it is noteworthy that the color appeared slightly darker in the higher concentration of PBZ (800-1,200 mg/L) compared to the lower concentrations of PBZ (0-600 mg/L).

Table 1. Fresh weight, dry weight, and dry weight percentage of mother rhizomes per plant of turmeric after various concentrations of paclobutrazol were applied at 60 days preharvest, and rhizomes were harvested at 9 months old

Paclobutrazol (mg/L)	**Fresh weight** (g)	**Dry weight** (g)	**Dry weight Percentage** (%)
0	57.30a	9.75b	17.02b
600	57.67a	9.61b	16.66b
800	53.17a	13.24a	24.90a
1,200	38.83b	6.39c	16.45b
F-test	*	*	*
C.V.%	42.56	30.43	24.58

Means within the same column followed by the same letter are not significantly different.
*= significantly different at the $p < 0.05$ level

Table 2. Number, fresh weight, dry weight, and dry weight percentage of finger rhizomes per plant of turmeric after various concentrations of paclobutrazol were applied at 60 days preharvest, and finger rhizomes were harvested at 9 months old

Paclobutrazol (mg/L)	**Numbers**	**Fresh weight** (g)	**Dry weight** (g)	**Dry weight Percentage** (%)
0	13.42	201.76b	41.17b	20.41
600	15.93	243.36a	47.62a	19.57
800	14.75	221.33ab	40.57b	18.33
1,200	13.93	195.33b	36.09b	18.48
F-test	ns	*	*	ns
C.V.%	18.72	29.15	26.41	10.33

Means within the same column followed by the same letter are not significantly different.
ns and * = not significant and significantly different at the $p < 0.05$ level

Table 3. L* a* and b *values of fresh finger rhizomes after various concentrations of paclobutrazol were applied at 60 days preharvest and finger rhizomes were harvested at 9 months old

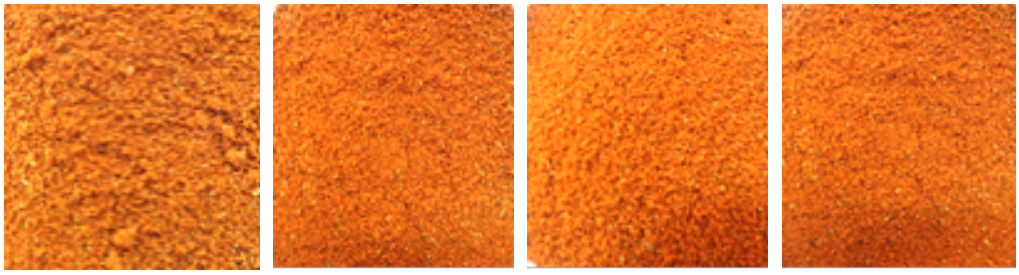
Paclobutrazol (mg/L)	**Color values**		
	L*	a*	b*
0	28.24	15.22	42.57
600	30.64	16.47	43.64
800	31.18	16.22	44.09
1,200	31.05	15.68	39.87
F-test	ns	ns	ns
C.V.%	5.16	5.64	4.12

ns= not significant

Table 4. L* a* and b *values of finger rhizome powder after various concentrations of paclobutrazol were applied at 60 days preharvest and finger rhizomes were harvested at 9 months old

Paclobutrazol (mg/L)	**Color values**		
	L*	a*	b*
0	30.01	14.95	41.09
600	29.87	13.76	41.32
800	31.03	13.82	41.80
1,200	31.31	13.84	41.93
F-test	ns	ns	ns
C.V.%	5.16	5.64	4.12

ns= not significant



A **B** **C** **D**

Figure 1. Finger rhizome powder after various concentrations of paclobutrazol; 0 (a), 600 (b), 800 (c), and 1,200 (d) mg/l were applied at 60 days preharvest, and finger rhizome were harvested at 9 months old

The analysis of secondary compound contents, including curcumin, total phenolics, and total flavonoids, revealed significant differences among the PBZ treatments in the finger rhizome powder of turmeric plants. This observation was made after applying various concentrations of paclobutrazol 60 days before harvest, with the finger rhizomes being harvested at 9 months old. Our result found that the application of PBZ resulted in an improvement in the content and percentage of curcumin in the finger rhizomes when compared to the non-treated plants. Specifically, the treatment with 600 and 1,200 mg/L PBZ showed higher curcumin content at 72.23 and 76.74 mg/gDW or 7.22 and 7.67 % of curcumin percentage in the finger rhizomes compared to the non-treated plants but not significant from 800 mg/L PBZ (Table 5).

The application of PBZ resulted in an improvement in the content and percentage of total phenolics in the finger rhizomes compared to the non-treated plants. Specifically, the treatment with 1,200 mg/L PBZ exhibited the highest content and percentage of total phenolics in the finger rhizomes, measuring 78.18 mg GAE/gDW or 7.82% (Table 6). Additionally, the 600 and 800 mg/L PBZ treatments also showed higher total phenolic content and percentage compared to the non-treated plants, although the differences were not statistically significant. Similarly, the application of PBZ, particularly at high concentrations, enhanced the content and percentage of total flavonoids in the finger rhizomes compared to the non-treated plants. The treatment with 1,200 mg/L PBZ exhibited the highest content and percentage of total flavonoids in the finger rhizomes, measuring 295.54 mg QE/gDW or 29.55% (Table 7). However, the 600 and 800 mg/L PBZ treatments did not show significant differences in total flavonoid content and percentage compared to the non-treated plants.

Table 5. Curcumin content and percentage in finger rhizome of turmeric plant after various concentrations of paclobutrazol were applied at 60 days preharvest and finger rhizome were harvested at 9 months old

Paclobutrazol	**Curcumin**	
(mg/L)	(mg/gDW)	(%)
0	51.50 b	5.15 b
600	72.23 a	7.22 a
800	63.30 ab	6.33 ab
1,200	76.74 a	7.67 a
F-test	*	*
C.V.%	9.12	8.47

Means within the same column followed by the same letter are not significantly different.
*= significantly different at the $p < 0.05$ level

Table 6. Total phenolic content and percentage in finger rhizome of turmeric plant after various concentrations of paclobutrazol were applied at 60 days preharvest and finger rhizome were harvested at 9 months old

Paclobutrazol	**Total phenolics**	
(mg/L)	(mg GAE/gDW)	(%)
0	43.53 c	4.35 c
600	56.51 b	5.65 b
800	64.57 b	6.46 b
1,200	78.18 a	7.82 a
F-test	**	**
C.V.%	23.58	18.49

Means within the same column followed by the same letter are not significantly different.
*= significantly different at the $p < 0.05$ level

Table 7. Total flavonoid content and percentage in finger rhizome of turmeric plant after various concentrations of paclobutrazol were applied at 60 days preharvest and finger rhizome were harvested at 9 months old

Paclobutrazol	**Total flavonoids**	
(mg/L)	(mg QE/gDW)	(%)
0	189.11 b	18.91 b
600	189.71 b	18.97 b
800	205.90 b	20.59 b
1,200	295.54 a	29.55 a
F-test	**	**
C.V.%	8.45	6.87

Means within the same column followed by the same letter are not significantly different.
**= significantly different at the $p < 0.01$ level

Discussion

Yield

The application of PBZ has demonstrated its potential in enhancing biomass accumulation in turmeric plants, affecting fresh weight, dry weight, and the percentage of dry weight of mother rhizome, particularly at a concentration of 800 mg/L. This enhancement in biomass accumulation is associated with increased dry matter content, which is likely influenced by reduced GA activity in the rhizome tissue, leading to an augmented sink strength that attracts more assimilates and subsequently enhances dry weight, as suggested by Desta and Amare (2021). These results align with previous research on various plant species, such as cassava, potatoes, and sesame, which also reported the impact of PBZ on fresh and dry weight.

Notably, our findings indicate that for PBZ-treated finger rhizome dry weight percentage tends to decrease with higher concentrations of PBZ at 1,200 mg/L, suggesting a concentration-dependent effect on this aspect of rhizome development. These findings underscore the importance of optimizing PBZ concentration for maximizing turmeric rhizome yield, as the effects appear to be different in dose. It's worth noting that PBZ's impact on different plant species varies, as it was found to decrease dry matter in some plants like *Jatropha curcas*, impacting seed weight and size. On the other hand, in the case of potatoes, PBZ application, especially at lower concentrations, increased tuber fresh and dry weight, with diminishing returns at higher concentrations, consistent with findings by Mabvongwe *et al*. (2016).

In conclusion, our study demonstrates that PBZ application can significantly influence turmeric rhizome yield, with the 800 mg/L concentration resulting in the highest mother rhizome dry weight. and the dry weight percentage of finger rhizomes improved with 600 mg/L PBZ treatment. However, overall rhizome yields were found in 800 mg/L treatment.

Further research is imperative to unravel the underlying mechanisms driving these outcomes and to provide specific recommendations for optimizing turmeric cultivation practices.

Quality improvement

In our analysis of the color of finger rhizome powder color, the PBZ-treated plants showed no significant differences in the L*, a*, and b* values when compared with the non-treated plant. These results contrast with the previous studies, which have shown that the application of paclobutrazol (PBZ) can

enhance the pigments in plants. For example, Takane *et al*. (2019) found that the highest content of anthocyanin was inversely proportional to the increase in PBZ doses in *Adenium obesum*. This increase is attributed to the elevation of abscisic acid (ABA) hormone levels, as ABA plays a role in anthocyanin biosynthesis (Soumya *et al*., 2017). Abd El-Aal and Mohamed (2017) also found that increasing the concentration of paclobutrazol resulted in chlorophyll content values of *Pelargonium zonal* comparable to those obtained with non-treated plants. According to Yusop *et al*. (2018), PBZ-treated plants showed an increase in chlorophyll content due to the inhibition of gibberellin biosynthesis and the promotion of cytokinin synthesis.

These findings suggest that while our study did not reveal significant color differences in PBZ-treated plants, the effect of PBZ on pigment levels may vary depending on plant species, PBZ dosage, and the specific pigments being assessed. Additional research is necessary to clarify the interactions between PBZ and pigment biosynthesis in different plant species.

Secondary compound contents

Our findings indicate that the application of PBZ significantly boosted both the content and percentage of total phenolics within finger rhizomes when compared to untreated plants. Notably, the most remarkable results were observed at 1,200 mg/L. Moreover, PBZ, especially at higher concentrations, elevated the content and percentage of total flavonoids in finger rhizomes in comparison to untreated plants. The treatment with 1,200 mg/L PBZ exhibited the highest values. The increase in secondary compounds can be attributed to the action of PBZ, which functions as a plant growth regulator by retarding cell elongation without affecting the rate of cell division (Desta and Amare, 2021). This effect is achieved by temporarily inhibiting gibberellins (GAs) biosynthesis through the prevention of the oxidation of ent-kaurene to ent-kaurenoic acid, accomplished by inactivating cytochrome P-450-dependent oxygenases (Cavalcante *et al*., 2020). When GA biosynthesis is restricted, more precursors in the terpenoid pathway accumulate and are diverted to stimulate abscisic acid (ABA) biosynthesis (Opio *et al*., 2020). It is important to note that the response to PBZ treatment can vary depending on factors such as plant species, the specific secondary compounds involved, and the dosage of PBZ applied. In soybeans, Dinler *et al*., (2022) reported that increasing the concentration of PBZ led to an increase in the proline content, and a high concentration of BPZ (20 mg/L) was found to be effective in promoting total flavonoid accumulation in soybeans. In *Brassica rapa* var. oleifera (Yusop *et al*., 2018), the application of 5-10 mg/L of PBZ resulted in the highest phenolic content. In *Ocimum sanctum*, PBZ

treatments increased the ascorbic acid content in root tissue by 142.18% and in leave tissue by 158.18% compared to the control (Nair *et al*., 2009). Additionally, PBZ application during various growth stages leads to a significant increase in total phenolic content in *O. sanctum*. Gopi *et al*. (2009) also reported a significant increase in the total phenolics content due to PBZ treatments, with the increase being particularly pronounced at the higher PBZ dosages. PBZ was found to stimulate the terpenoid pathway, leading to the accumulation of several terpenes (Desta and Amare, 2021). Furthermore, PBZ increased the levels of α-tocopherol in *Arachis hypogaea* (Sankar *et al*., 2007) and *O. sanctum* (Nair *et al*., 2009).

Our results suggest that PBZ application could be a promising approach for increasing the beneficial secondary compounds in turmeric, such as curcumin, total phenolics, and total flavonoids in the finger rhizome, which are associated with its various medicinal properties. Further studies may focus on exploring the underlying mechanisms of PBZ-induced changes in secondary compound content.

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