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Effects of Paclobutrazol Seed Priming on Seedlings Quality, Physiological and Bakanae Disease Index Characteristics of Rice (*Oryza sativa* L.)

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. Bakanae disease is a significant rice disease widely distributed in rice-growing regions worldwide. Therefore, the present investigation aimed to assess the optimal concentrations of paclobutrazol (PBZ) as a treatment for rice grains (cv. Sakha 108) to control bakanae disease, also evaluating its impact on grain germination, seedling growth parameters as well as disease index. Paclobutrazol concentrations had no significant impact on seed germination, regardless of whether the seeds were incubated with *Fusarium fujikuroi* or not. Application of PBZ, either alone or in combination with fungal pathogens, negatively impacted the rice seedlings' height. Paclobutrazol at 25, 50 and 100 mg/L, combined with the fungal pathogen positively impacted root length. Paclobutrazol at 3 and 6 mg/L mitigated the adverse impact on chlorophyll pigments content in infected seedlings. The highest proline contents were achieved by 100 mg/L PBZ alone or in combination with fungal pathogens. It has been observed that the application of PBZ, either alone or in combination with a fungal pathogen, leads to the enhancement of catalase, peroxidase, and polyphenol oxidase activities. The median lethal concentration of PBZ was 0.874 mg/L; applying low concentrations of paclobutrazol effectively increased the percentage of fungal growth suppression. Application of PBZ, at higher concentrations (50 and 100 mg/L), decreased infection percentage and disease severity index (DSI) significantly. These findings suggest that PBZ can be an effective treatment for controlling bakanae disease and enhancing resistance in rice plants.



KEYWORDS

Oryza sativa; *Fusarium fujikuroi*; chlorophyll; oxidative enzymes; proline; photosynthesis rate; stomata conductance

Nomenclature

<i>O. sativa</i>	<i>Oryza sativa</i>
<i>F. fujikuroi</i>	<i>Fusarium fujikuroi</i>
PBZ	Paclobutrazol
GA	Gibberellin
°C	Degree celsius
ml	Milliliter
min	Minute
vol	Volume
h	Hour
CFU	Colony forming units
mg/L	Milligram/litter
C	Colony diameter (mm) of control
T	Colony diameter of treated plate
PDA	Potato dextrose agar
Cm	Centimeter
LC ₅₀	Lethal concentration 50
FGP	Final germination percentages
CO ₂	Carbon dioxide
H ₂ O ₂	Hydrogen peroxide
PAR	Photo-synthetically active radiation
LTR	Leaf transpiration rate
μmol g ⁻¹	Micromole/gram
chl. a	Chlorophyll a
chl. b	Chlorophyll b
CAT	Catalase
POD	Peroxidase activity
PPO	Polyphenol oxidase
DSI	Disease severity index
IAA	Indole acetic acid
GAs	Gibberellins
ABA	Abscisic acid

1 Introduction

Rice is a major staple food crop that feeds more than half of the world's population. It provides the majority of calories for billions of people, particularly in Asia, Africa, and Latin America [1–3]. *Fusarium fujikuroi* Nirenberg, a teleomorph known as *Gibberella fujikuroi* (Sawada) Wollenw., is the

cause of bakanae, or foolish seedling disease of rice. There have been reports of rice production losses worldwide, ranging from 20% to 50% [4]. According to reports, seeds are the main source of Bakanae disease [5]. As well as the fungus that causes the disease is soil- and seed-borne. According to Chen et al. [6], the disease is primarily transmitted from one field to another through infected seeds. An essential component in understanding the spread of rice diseases is the recovery percentage of pathogens in different parts of the rice grain. According to Manandhar [7], the lemma had the highest pathogen recovery percentage, followed by palea, basal glumes, embryo, or endosperm. This implies that the lemma is the most likely part where the fungus will flourish and proliferate.

Bakanae disease symptoms can vary according to a number of circumstances, including age, cultivar resistance, and stress levels. Since younger plants are more vulnerable to infection and the effects of the fungus's gibberellin synthesis, symptoms are typically more noticeable in these plants. In general, severe bakanae symptoms are more likely to arise in stressed or weak plants [8]. Poor seedling emergence, elongated rice seedlings, fewer and thinner tillers, yellowish green leaves, crown rot, stunting, empty panicles, discolored grains, and possible wilting that resulted in plant mortality were among symptoms of the disease [4,9]. According to Wulff et al. [10], *F. fujikori* causes a lower percentage of seeds to germinate than other *Fusarium* species. Furthermore, numerous metabolites are produced by this pathogen, such as pigments like Bikaverin, mycotoxins, fusarins, fusaric acid, fusarubins or phytohormones, i.e., gibberellic acid [11,12]. Although all *Fusarium* species can impair seed germination and produce grain discoloration, *Fusarium fujikori* is thought to be more harmful than other species [10].

Synthetic growth inhibitor paclobutrazol (PBZ) is a member of the triazole chemical family. One of the most popular plant growth regulators in crop production is PBZ, a type of plant gibberellin (GA) biosynthesis inhibitor that may be applied as foliar spray or as soil drench that is absorbed by the roots. Depending on the desired outcome and type of plant, this flexibility enables the use of various application tactics [13]. According to Sponzel [13], PBZ prevents the oxidation of ent-kaurene, which inhibits the formation of GA (Anti-gibberellin). Additionally, it acts as a highly effective systemic fungicide and is utilized to treat a variety of economically significant fungal diseases [14]. Normally, seeds are treated with fungicides to control fungus disease. Seed priming is a pre-sowing treatment that is regarded as a cost-effective or convenient approach for improving crop production [15].

The infection mechanism of Bakanae disease depends on the enhanced production of gibberellin, leading to the accelerated growth of rice seedlings that are affected. Meanwhile, paclobutrazol inhibits gibberellin synthesis in the host plant, suggesting that it may also suppress gibberellin production in the pathogen. Utilizing specific concentrations of paclobutrazol could be key in managing the disease or at least mitigating its severity. Therefore, the current study was conducted to assess and determine the most effective PBZ concentrations for grain treatment against bakanae disease, as well as grain germination and seedling growth parameters.

2 Materials and Methods

2.1 Source of Pathogen

Fusarium fujikuroi strain was isolated, purified and identified by Rice Pathology Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Kafr El-Sheikh, Egypt.

2.2 Preparation of *F. fujikuroi* Inoculum for Artificial Inoculation

200 g corn meal: sand (1:5, vol/vol) medium in Glass bottles of 500 mL capacity was autoclaved for 30 min. at 1.5 or 2 atm, then inoculated with 3 mycelial plugs (5 mm in diameter) from edges of 7-day old *F. fujikuroi* culture and incubated at 25°C for 10 days/12 h illumination to induce sporulation [16].

2.3 Preparation of Conidial Suspension

F. fujikuroi isolate was cultured on sandy media for micro conidia spore production and incubated at $28 \pm 2^\circ\text{C}$ for approximately 15–20 days. A flask was filled with sterilized water and a sterile brush was used to remove the conidia that was growing. Muslin cloth was used to filter the conidial suspension, which was then adjusted to 5×10^6 conidial/mL using hemocytometer [17].

2.4 Screening Paclobutrazol Concentration

A wide range of paclobutrazol concentrations was used to determine the appropriate levels that may affect *F. fujikuroi* while maintaining the viability of the rice plant. As a first stage, effect of paclobutrazol on *F. fujikuroi* on medium was assessed at the following concentrations (0, 1.5, 3, 6, 12, 25, 50, 100, 150, 200, 250 and 500 mg/L). Relative to the outcomes, further experiments employed concentrations of no more than 100 mg/L. The activity of PBZ was investigated in terms of mycelial growth, colony forming units (CFU), and *F. fujikuroi* sporulation. The effect of PBZ on hyphal growth was investigated by adding previously indicated PBZ doses to potato dextrose agar (PDA). *F. fujikuroi* was inoculated onto PBZ-improved agar plates by inserting a 5 mm diameter disc from the margins of 5-day-old colonies into the center of each plate. Five replicate plates were utilized for each treatment. Following a period of seven days, colony diameter measurements were taken in order to determine the percentage of inhibition. This was done utilizing the following formula according to Aurangzeb et al. [18].

$$\text{percentage of inhibition} = \frac{C - T}{C} \times 100$$

where C = colony diameter (mm) of control, T = colony diameter of the treated plate.

Relative to the outcomes, further experiments employed concentrations of no more than 100 ppm. An estimation of the LC_{50} value was performed using probit analysis, according to Lei and Sun [19].

2.5 Preparation of Infected Seeds

Rice seeds (Sakha, 108) underwent multiple washes in sterile distilled water for approximately 5 min each. This washing process was repeated 3–4 times. Afterward, the cleaned seeds were left to dry in a clean area for about 1 h. Next, they were immersed in spore suspension of *F. fujikuroi* at varying concentrations and kept at a temperature of 25°C overnight to facilitate the attachment of conidia to the seed surface. Following this, the infected seeds were air-dried for an additional hour.

2.6 Hydroponic Germination System and Pots Experiments

Hydroponic germination system and pots experiments were carried out at Research Field of Rice, Research Department, Sakha Research Station Agricultural Research Center (ARC), Giza, and laboratories of Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, Egypt, on rice variety namely (Sakha 108) under greenhouse conditions. The experiments were conducted in order to study the effect of paclobutrazol (PBZ) at 1.5, 3, 6, 12, 25, 50, 100 mg/L and its interaction with bakanae disease (*Fusarium fujikuroi*) on pathological, morpho-physiological, biochemical or histological characters of rice at the seedling growth stage.

2.6.1 Hydroponic Germination System Experiment

A new hydroponic germination system of rice grains has been developed in order to observe the response of grain germination, seedlings growth and bakanae disease and its symptoms to PBZ concentrations. Two plastic containers (dishes) were used, one large (9 cm \times 9.5 cm and 5 cm in deep) and the other small (7 cm in diameter and 4.5 cm in deep), as shown in the following diagram (Fig. 1). The base of the small container was removed, and a hole (3 cm \times 3 cm) was made in the lid. One end of a strip of medical gauze was attached to the area of the hole, with the other end hanging down. The small

container was placed inside the large one, which was filled with water up to about halfway. The inoculated and uninoculated rice grains (20 grains) were placed in the area of the hole in the lid of the small container. Water is drawn up through capillary action to the rice grains. After germination, the roots grow downward, permeating the medical gauze.

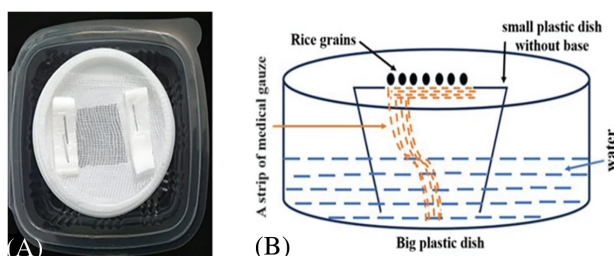


Figure 1: A hydroponic germination system was designed for rice germination and early seedling growth parameters measurements (A) and illustrating diagram (B)

2.6.2 Germination Parameters

The number of germinating seeds was counted every 2 days up to 15 days and considering grains germinated when the plumule emerged. At the end of the germination period, the final germination percentages (FGP), according to Kader [20].

2.7 Pots Experiment

Pots experiments were performed to study the effects of PBZ at 0, 1.5, 3, 6, 12, 25, 50, and 100 mg/L and its interaction with *Fusarium fujikuroi* inoculation on seedling growth parameters including seedling height, root length, dry weight, chlorophyll pigments, proline content, antioxidant enzymes, leaf CO_2 , Leaf H_2O_2 , net photosynthesis, transpiration rate as well as stomata conductance. Rice grains were planted in 20 cm (in diameter) plastic pots filled with clay soil. Rice grains were inoculated with *F. fujikuroi* in different PBZ concentrations. PBZ-treated rice grains with and without *F. fujikuroi* inoculation were sown, 20 grains/pots of equally distant-spaced plants per pot. Pots were immersed in water up to 4 cm above the soil surface. The germinated seeds were counted from the second day to the eighth day.

2.7.1 Seedling Growth Parameters

For seedlings growth parameters, samples were taken after 30 days from sowing to estimate the root length, seedling height (cm), and dry weights of seedlings (dried in an electric oven at 70°C for 72 h) g/plant. Ten randomly selected plants were marked from each replicate (3 replicates/treatment), and evaluations were conducted on them.

2.7.2 Transpiration Rate, Stomata Conductance, Leaf CO_2 and Net Photosynthesis

Assessing a plant's carbon (C) and water content involves evaluating stomata, diffusive conductance, and exchange rates of CO_2 or water vapor. To accomplish this, a portable porometer called the "steady-state porometer, LICOR, LI-1600, Lincoln, NE, USA" is employed. This porometer utilizes open gas exchange system that measures levels of incoming and outgoing CO_2 and H_2O_2 concentrations in a cuvette placed on or around leaves [21]. Stomatal conductance ($\text{cm}^{-2} \text{S}^{-1}$) is measured in fully expanded leaves, while the net photosynthetic rate (NPR, A) (g m^{-2}) utilized for gs models is estimated as follows: $A = A_{\text{max}} \times f(\text{PAR})$, where PAR (photo-synthetically active radiation) is obtained from on-site measurements taken in the morning, around noon, and in the afternoon. CO_2 concentration is calculated, and leaf transpiration rate (LTR) ($\mu\text{g cm}^{-2} \text{s}^{-1}$) is directly measured on the same leaf, representing the combined rates of adaxial and abaxial surfaces. Additionally, this device can be used to determine the

response of CO₂ curves in field. Adaxial and abaxial diffusive resistances are evaluated on the upper fully expanded leaf using the steady-state porometer during the measurement process.

2.7.3 Leaf H₂O₂ Content

The second leaf from the tips of 10 rice seedlings/replicate (3 replicates/treatment) was collected to measure the endogenous hydrogen peroxide (H₂O₂) content (μmol g⁻⁹ fresh weight), following the method described by Velikova et al. [22].

2.7.4 Chlorophyll Pigments

The concentration of chlorophyll pigments (chl. a, b or total chlorophyll) (μg/cm²) were estimated as a fresh area from the second leaf tip from 10 seedlings/replicate (3 replicates/treatment) using a spectrophotometer according to Moran and Porath [21].

2.7.5 Proline Content

The proline content (μmol g⁻¹) as a fresh weight in rice leaves (the second leaf from the seedling tip) was measured according to Bates et al. [23].

2.7.6 Antioxidant Enzymes

To conduct antioxidant enzyme assays, rice leaves that had been frozen were finely ground into powder using liquid nitrogen. The ground powder was then extracted using ice-cold 0.1M Tris-HCl buffer (pH 7.5), which also contained 5% (w/v) sucrose and 0.1% 2-mercaptoethanol, (3:1 buffer volume/FW). After homogenization, the mixture was subjected to centrifugation at 10,000 g for 20 min at a temperature of 4°C. Resulting supernatant was utilized for determining enzyme activity. Both the preparations for enzyme extraction and the enzyme assay itself were carried out at a temperature of 4°C.

The activity of Catalase, CAT (EC 1.11.1.6) was estimated by monitoring the disappearance of H₂O₂ at 240 nm according to Aebi's method [24]. Peroxidase activity, POD (EC 1.11.1.7) was measured according to Polle et al. [25]. Polyphenol oxidase, PPO (EC 1.14.18.1) activity was detected using the technique of Oktay et al. [26].

2.7.7 Evaluation of Paclobutrazol on Appearance of Bakanae Disease

The Bakanae disease severity index (DSI) was assessed in ten seedlings per replicate (3 replicates/treatment) at 30 days post-treatment using the disease severity scale (DSS), as described by Mohd Zainudin et al. [27].

2.8 Statistical Analysis

Data were calculated utilizing analysis of variance (ANOVA). Means were considered significantly different when the significance level (*p*) was equal to or less than 0.05. All calculations were determined as described by the statistical package of the Costate Program (1986). Duncan's multiple range tests were utilized to compare treatment means [28].

3 Results

3.1 Influence of PBZ Concentrations on *F. fujikuroi* Growth in Vitro

Figs. 2 and 3 indicate that paclobutrazol exhibited inhibitory effects on mycelial growth of *F. fujikuroi* in a dose-dependent manner. At concentrations of 1.5, 3, 6, 12 and 25 mg/L, paclobutrazol likely had some inhibitory effect on radial mycelial growth of *F. fujikuroi*, while the reduction may have been significant compared to the control. However, at concentrations of 12, 25, 50, and 100 mg/L, paclobutrazol had a more pronounced effect on mycelial growth. Specifically, at concentrations of 50 and 100 mg/L, paclobutrazol significantly reduced the linear growth of *F. fujikuroi*. This indicates that these concentrations were the critical doses at which paclobutrazol had a significant impact on the linear expansion of the fungal mycelium.

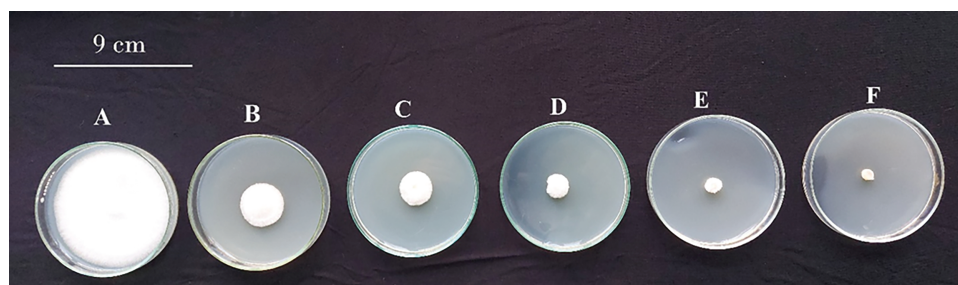


Figure 2: Effect of PBZ at various concentrations on linear growth of *Fusarium fujikuroi* at 7 days from incubation under lab conditions using potato dextrose agar media A: 0 mg/L, B: 1.5 mg/L, C: 3 mg/L, D: 6 mg/L, E: 12 mg/L and F: 25 mg/L

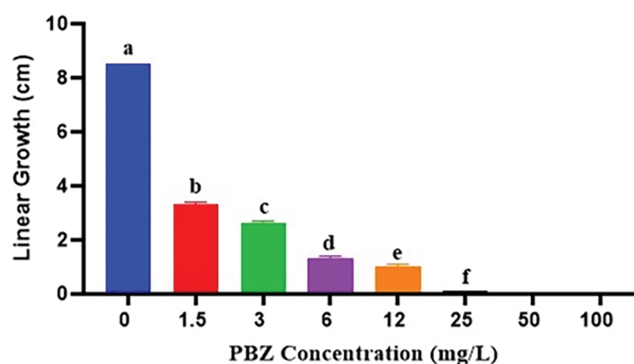


Figure 3: Effect of PBZ at different concentrations at 0, 1.5, 3, 6, 12, and 25 mg/L on linear growth of *Fusarium fujikuroi* at 7 days from incubation. Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)

3.2 Toxicological Studies

In lethal concentration 50 (LD_{50}), the doses of toxic substances that result in the death of a certain proportion of organisms are often used as indicators of acute toxicity. The Probit analysis findings indicate that the estimated median lethal concentration (LD_{50}) of PBZ was determined to be 0.847 mg/L. This value suggests that at low dosages of PBZ, it has ability to suppress mycelial development of *F. fujikuroi* (Table 1, Fig. 4).

Table 1: Effect of Paclobutrazol on the inhibition of mycelial growth of *Fusarium fujikuroi* *in vitro* sensitivity tests

Fungicide	Linear growth		
	Toxicity regression equations	R ²	LC ₅₀ (mg/L)
Paclobutrazol 25% EC	Y = 1.073x + 5.077	0.950	0.847

3.3 Seed Germination

Rice seeds priming with paclobutrazol (PBZ) at 100, 50, 25, 12, 6, 3, 1.5 and 0 mg/L with and without *Fusarium fujikuroi* incubation insignificantly impacted seed germination percentage (Fig. 5).

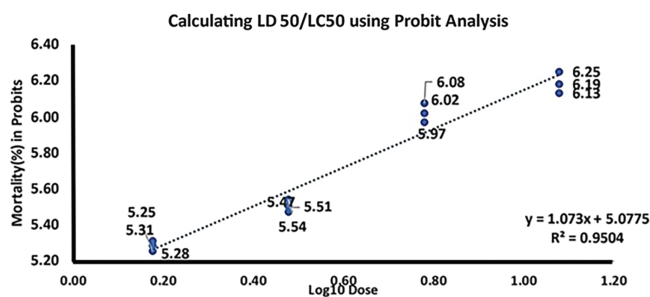


Figure 4: Effect of paclobutrazol fungicide on the inhibition of mycelial growth of *F. fujikuroi* *in vitro* sensitivity test with probit analysis

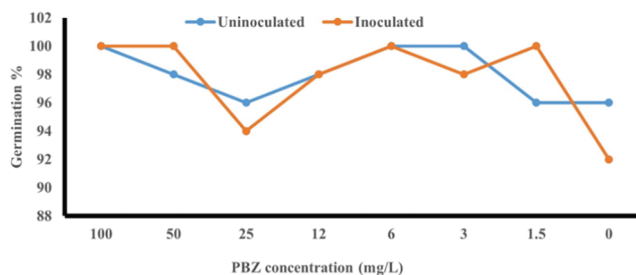


Figure 5: Rice seed germination percentage as affected by priming with PBZ at 100, 50, 25, 12, 6, 3, 1.5 and 0 mg/L concentrations with and without *F. fujikuroi* incubation after 4 weeks from seed sowing

3.4 Seedling Height

The data presented in Figs. 6 and 7 demonstrated that seed priming with PBZ at concentrations of 1.5, 3, 6, 12, 25, 50, and 100 mg/L significantly reduced rice seedling height compared to the control. There was an inverse relationship between increasing PBZ concentrations and seedling height reduction. Additionally, a significant increase in seedling height was observed in rice seeds inoculated with *F. fujikuroi* compared to uninoculated seedlings when combined with PBZ application.

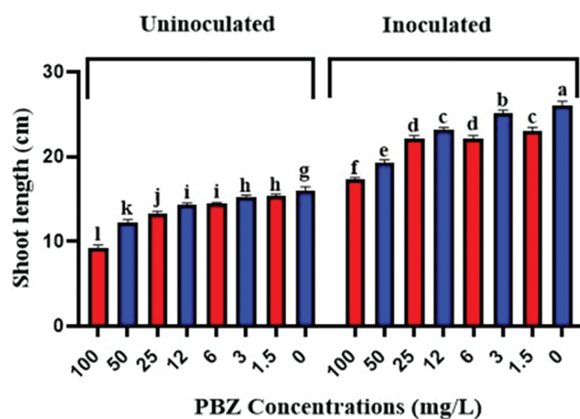


Figure 6: Rice seedling height as affected by priming with PBZ at 100, 50, 25, 12, 6, 3, 1.5 and 0 mg/L concentrations with (Infected) and without (Healthy) *F. fujikuroi* incubation after 4 weeks from sowing. Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)



Figure 7: Effect of seed priming with various concentrations of paclobutrazol (PBZ) at 0, 1.5, 3, 6, 12, 25, 50 and 100 mg/L with (A) and without (B) *F. fujikuroi* inoculation on seedling height at 4 weeks after sowing

3.5 Root Length and Lateral Roots Initiation

There is a negative relationship between increasing paclobutrazol (PBZ) concentrations and a reduction in root length. On the other side, a positive relationship was noted between the application of PBZ concentrations in combination with *F. fujikuroi* inoculation on root length (Fig. 8). It was noted that PBZ at 12 mg/L increased the initiation of lateral roots (Fig. 9).

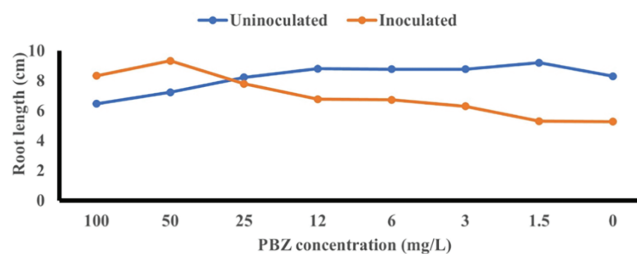


Figure 8: Effect of seed priming with different concentrations of paclobutrazol (PBZ) at 0, 1.5, 3, 6, 12, 25, 50 and 100 mg/L *F. fujikuroi* inoculation on root length at 4 weeks after sowing in the hydroponic system

3.6 Shoot and Root Dry Weight/Seedling

Data illustrated in Fig. 10 showed that treating plants with PBZ resulted in a significant reduction in both shoot and root dry weight values. The trend of decreasing shoot and root dry weight with increasing PBZ concentration was observed not only in the uninoculated plants but also under the inoculated condition. This suggests that the inhibitory effect of PBZ on shoot and root growth was consistent regardless of whether the plants were inoculated with *F. fujikuroi*. It is noted that, the interaction between PBZ and the inoculation with *F. fujikuroi*, resulting in lower root dry weight values compared to PBZ treatment alone.

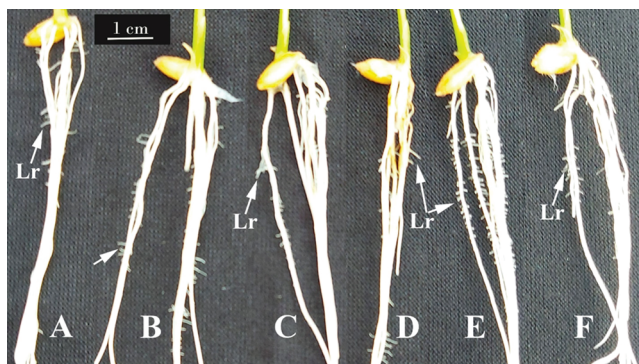


Figure 9: Effect of paclobutrazol (PBZ) at different concentrations on initiation of lateral roots at 2 weeks after seed sowing in hydroponic germination system, A: 0 mg/L, B: 1.5 mg/L, C: 3 mg/L, D: 6 mg/L, E: 12 mg/L and F: 25 mg/L at 2 weeks after seed sowing, Lr: lateral roots

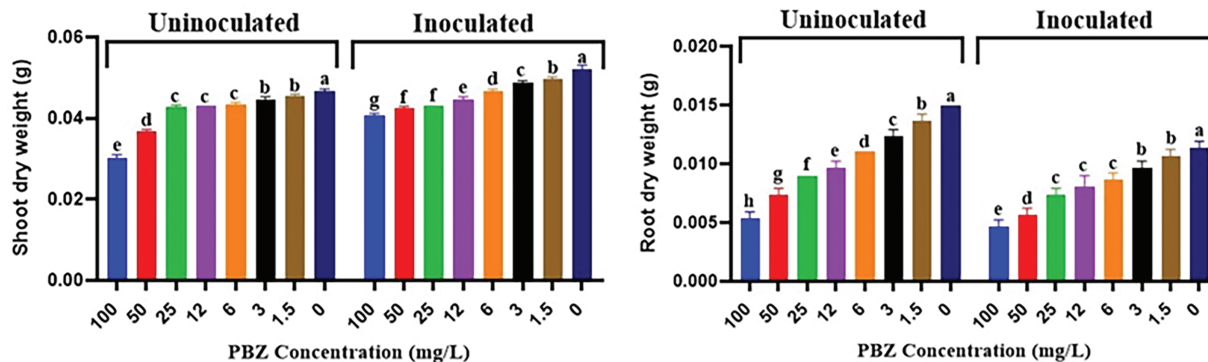


Figure 10: Effect of paclobutrazol (PBZ) at (0, 1.5, 3, 6, 12, 25, 50 and 100 mg/L) on shoot and root dry weight/plant at 4 weeks after seed sowing. Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)

3.7 Chlorophyll Content

Besides morphological effects, paclobutrazol also directly contributes to different physiological changes that are generally associated with optimized yield in different crops. Data illustrated in Fig. 11 revealed that PBZ at all used concentrations significantly increased chlorophyll content (chl. a, b or total) in comparison with the control. The highest chlorophyll content values were recorded at 6 and 3 mg/L PBZ treatments. It is noted that the interaction between PBZ and the inoculation with *F. fujikuroi*, resulting in lower chlorophyll a, chlorophyll b, or total chlorophyll values compared to the application of PBZ alone. When combined with *F. fujikuroi* inoculation, PBZ concentrations still significantly increased the content of chlorophyll pigments. However, the highest chlorophyll content was observed at a 12 mg/L PBZ concentration.

3.8 Proline Content

Rice seedlings treated with both PBZ and its interaction with *F. fujikuroi* inoculation exhibited higher proline concentrations than untreated seedlings. There is a link between increasing concentrations of PBZ and an increase in proline content in plants. This means that as PBZ concentration increased, the proline content in the plant also increased. The highest proline contents were detected by PBZ at 100 mg/L with and without fungal pathogen inoculation (Fig. 12). It is noted that the application of PBZ in combination with *F. fujikuroi* inoculation resulted in higher proline contents compared to uninoculated treatments.

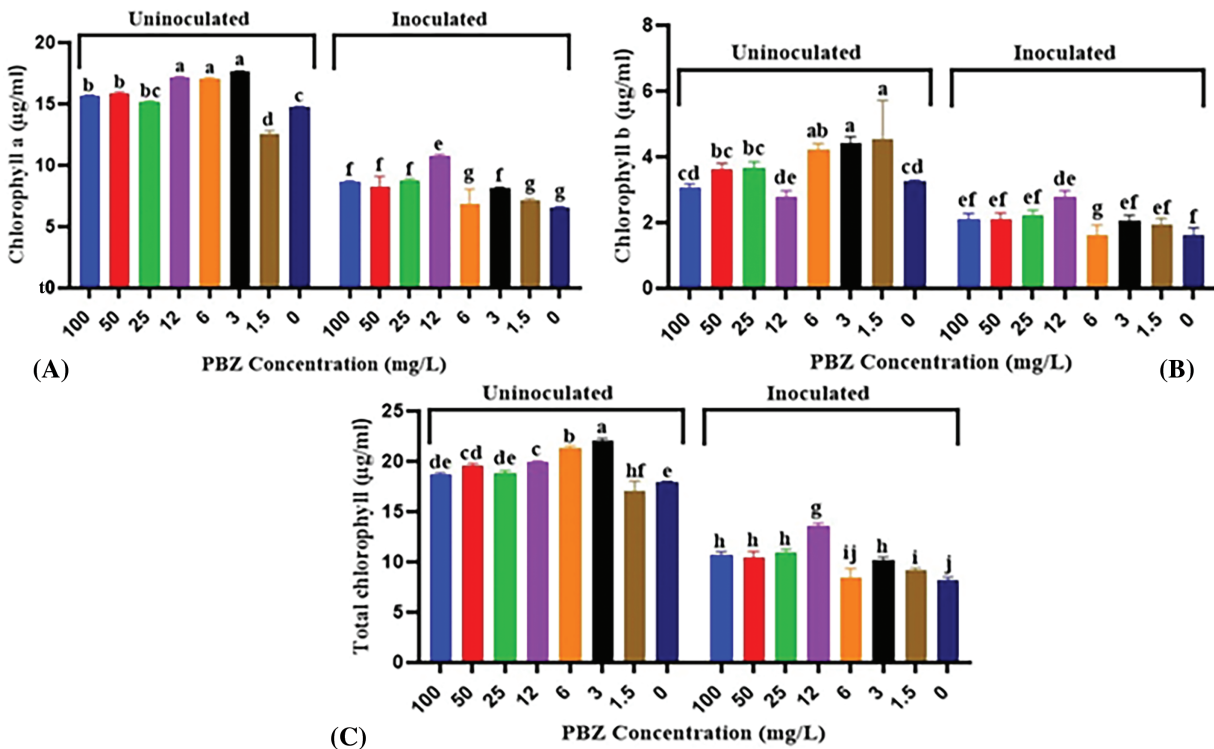


Figure 11: Effect of paclobutrazol (PBZ) at different concentrations and its combination with *F. fujikuroi* inoculation on chlorophyll pigments content of rice seedling at 4 weeks after sowing, (A) Chlorophyll a, (B) Chlorophyll b and (C) Total chlorophyll. Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)

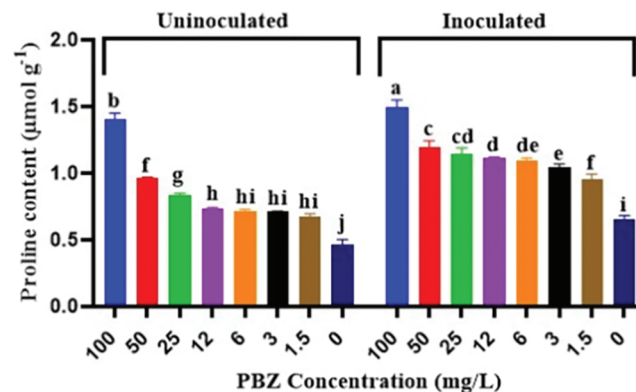


Figure 12: Effect of paclobutrazol (PBZ) at different concentrations (mg/L) and its combination with *F. fujikuroi* inoculation on proline concentration of rice seedling at 4 weeks after sowing. Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)

3.9 Antioxidant Enzymes

Data in Fig. 13 indicated that it appears that there is a relationship between the concentration of PBZ and activity of antioxidant enzymes, including catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO). As the concentration of PBZ increases, the activity of these enzymes also increases. Significant differences

were detected on CAT, POD and PPO activity between inoculated and uninoculated treatments without PBZ application (0 mg/L). More enzymes activities were noted in inoculated seedlings than uninoculated ones. Furthermore, when PBZ was applied, significant variances in enzyme activity were observed between inoculated and uninoculated treatments. Inoculated seedlings showed higher enzyme activity compared to uninoculated seedlings, even under PBZ application.

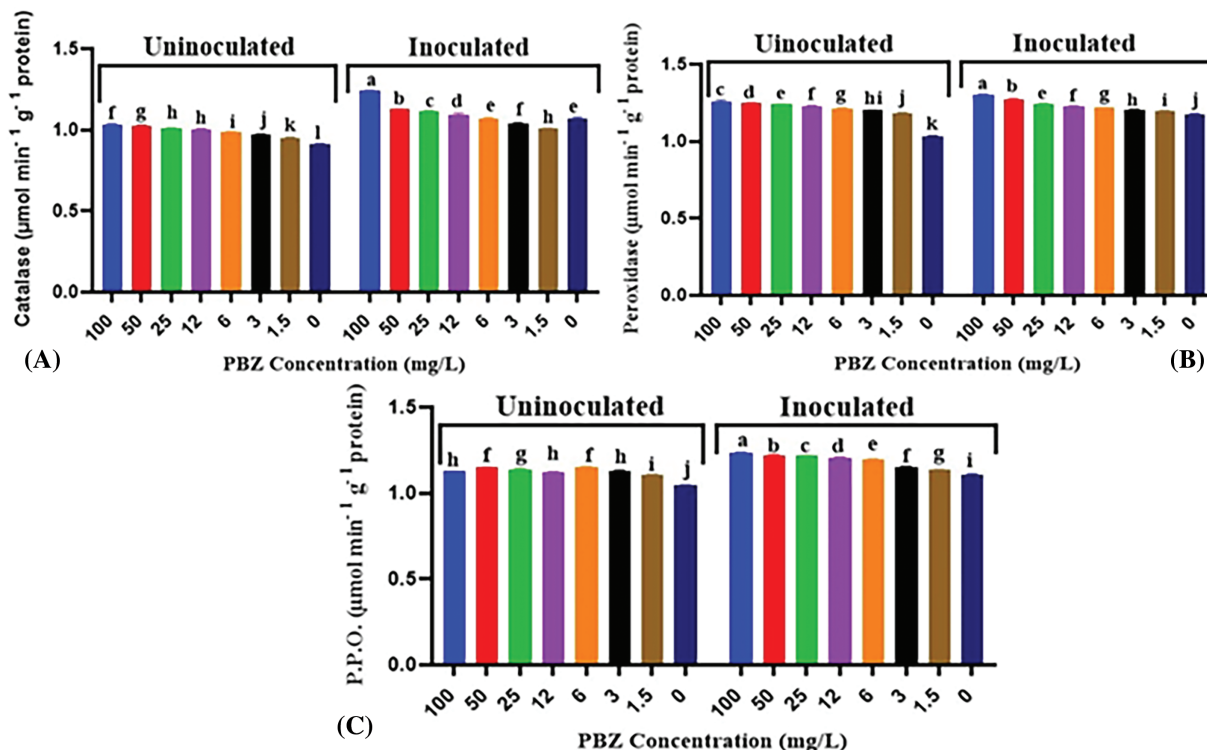


Figure 13: Effect of paclobutrazol (PBZ) at different concentrations (mg/L) and its combination with *F. fujikuroi* inoculation on antioxidant enzymes activity in rice seedling at 4 weeks after sowing, (A) Catalase, (B) Peroxidase and (C) Polyphenol oxidase (P.P.O). Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)

3.10 CO_2 Around the Leaf

During photosynthesis, plants absorb carbon dioxide (CO_2) from the atmosphere through the stomata, which is necessary for photosynthesis process. Data in Fig. 14A indicated that insignificant differences were detected in CO_2 around the leaf between inoculated and uninoculated rice seedlings under 0 mg/L PBZ. While significant increases in leaf CO_2 exchange rates were recorded by 12, 50 and 100 mg/L PBZ without fungus inoculation. The data from Fig. 13 suggests that there were no significant differences in CO_2 around the leaf levels between inoculated and uninoculated rice seedlings under the treatment of 0 mg/L PBZ.

3.11 Stomata Conductance

Stomatal conductance plays a crucial role in both photosynthesis and transpiration. Higher conductance allows more CO_2 for photosynthesis but also leads to more water loss through transpiration. It is influenced by various factors, including environmental conditions, such as light intensity, temperature, humidity, and concentration of CO_2 in the atmosphere. Data in Fig. 14B revealed that PBZ treatments without *F. fujikuroi* inoculation (Healthy) gave the highest stomatal conductance values compared with inoculated

rice seedlings except PBZ at 12 mg/L. Inoculated rice seedlings recorded the lowest stomatal conductance value without PBZ application.

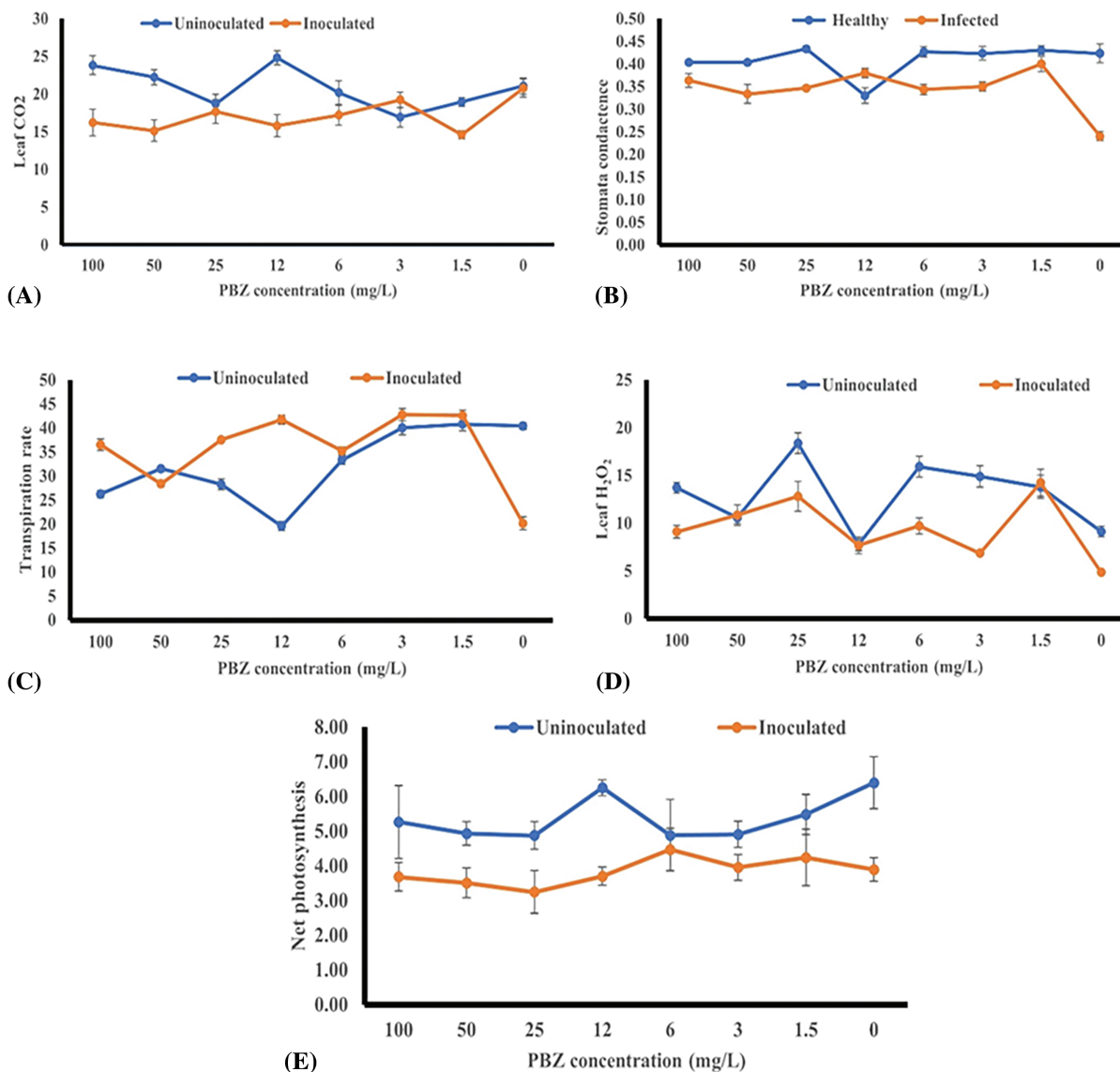


Figure 14: Effect of paclobotrazol (PBZ) at different concentrations (0, 1.5, 3, 6, 12, 25, 50 and 100 mg/L) and its combination with *F. fujikuroi* inoculation on CO₂ around leaf (μmol m⁻² s⁻¹) (A), stomatal conductance (cm⁻² s⁻¹) (B), transpiration rate (μg cm⁻² s⁻¹) (C), leaf H₂O₂ (μmol m⁻² s⁻¹) (D) and net photosynthesis (g m⁻²) (E) of rice seedling at 4 weeks after sowing

3.12 Transpiration Rate

Transpiration is an essential process for plants, as it facilitates the movement of water and nutrients from the roots to the leaves. However, excessive transpiration without adequate water uptake can lead to water stress and negatively impact plant growth. Data in Fig. 14C revealed that inoculated seedlings gave the lowest transpiration rate value. Under most PBZ concentrations, significant increases in transpiration rates were noted by inoculated seedlings compared to uninoculated rice seedlings.

3.13 Leaf H_2O_2

Data in Fig. 14D indicated that the highest leaf hydrogen peroxide (H_2O_2) content values were noted by most PBZ treatments without inoculation.

3.14 Net Photosynthesis

Data in Fig. 14E revealed that net photosynthesis values were found by all PBZ concentrations without *F. fujikuroi* inoculation.

3.15 Disease Index

Data in Fig. 15 illustrated disease index, including infection %, efficiency and disease severity index (DSI) in both hydroponic and pots experiments. An inverse relationship was observed between PBZ concentrations and infection % and disease severity index (DSI). PBZ at 50 and 100 mg/L gave the lowest values. At the same time, a positive relationship was found between PBZ concentrations and disease efficiency. This means that increasing the concentration of PBZ increases efficiency. Decreasing in infection % and DSI due to paclobutrazol acts as fungi growth inhibitor. The positive impact on infection percentage suggests that PBZ treatment, particularly at higher concentrations, increased the tolerance and/or resistance ability of the rice plants to infection by the fungal pathogen.

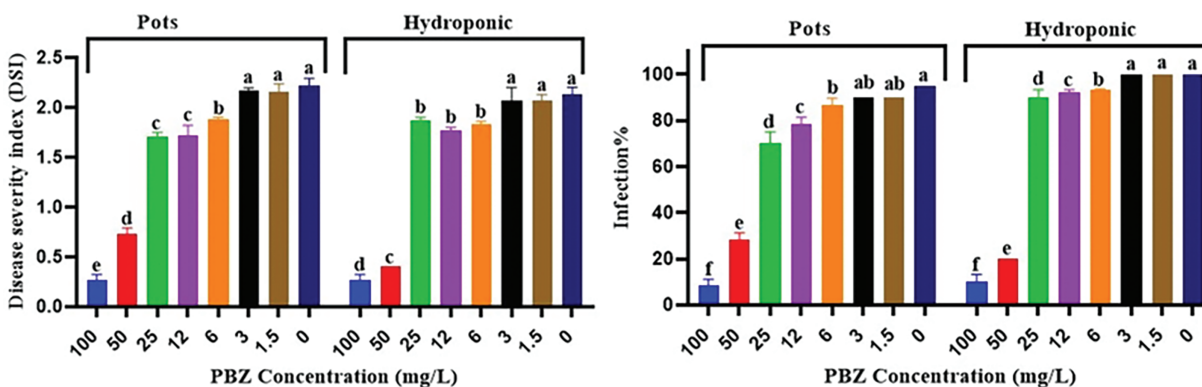


Figure 15: Effect of PBZ at 0, 1.5, 3, 6, 12, 25, 50 and 100 mg/L in combination with *F. fujikuroi* inoculation on infection % and disease severity index (DSI) in hydroponic and pots experiments. Letters that differ on each bar indicate significant contrast among treatments ($p < 0.05$)

4 Discussion

The median lethal dose (LD_{50}) is a commonly used measure, and it represents the dose at which 50% of the population being studied is expected to die. LD_{50} values are useful in assessing the relative toxicity of different substances, as substances with lower LD_{50} values are considered more toxic than those with higher LD_{50} values. LD_{50} values provide valuable information for evaluating the potential risks associated with exposure to toxicants in various organisms, i.e., fungal organisms [29]. Paclobutrazol can have inhibitory effects on mycelial growth in some fungi. Martínez et al. [30] indicated that Paclobutrazol at 0.05, 0.25, 1.25, and 6.25 mg/L reduced mycelial growth of *Botrytis cinerea* isolates. This may be due to its ability to interfere with the biosynthesis of gibberellins, a group of plant hormones that are involved in regulating plant growth or development. By suppressing gibberellin production, paclobutrazol can inhibit the elongation of fungal hyphae, thereby affecting mycelial growth. Additionally, the concentration of paclobutrazol used and the duration of exposure can also influence its effects on mycelial growth. However, the efficacy of paclobutrazol as a fungicide may vary depending on the specific fungus, its life stage, and other environmental factors. Phenamacril and ipconazole (triazole compounds) are both

antifungal compounds that have been tested for their activity against *F. fujikuroi* mycelial growth. When tested individually, both phenamacril and ipconazole demonstrated high antifungal activity against *F. fujikuroi* mycelial growth. Ipconazole is a triazole fungicide that acts by inhibiting the biosynthesis of ergosterol, a key component of fungal cell membranes. Without ergosterol, the integrity of the fungal cell membrane is compromised, leading to cell death. Ipconazole has demonstrated potent antifungal activity against a wide range of fungal pathogens, including *F. fujikuroi* [31].

Our results showed minimal changes in seed germination for both incubated and non-incubated rice seeds, which were not statistically significant (Fig. 5). Applying growth regulators like paclobutrazol to seeds can indeed lead to several challenges, including reduction or absence of seed germination and/or delay in seedling [15]. On the other hand, Dewi et al. [32] found that paclobutrazol at 12.5, 25, 50 and 100 mg/L concentrations did not affect black rice seed germination %. The effect of paclobutrazol on seed germination percentage may depend on plant species and the concentration used as well as incubation period. Soaking tomato seeds in paclobutrazol (PBZ) solutions, even at relatively low concentrations like 500 and 1000 mg L⁻¹, can indeed decrease germination compared to water-soaked control seeds [33]. The fact that the germination process is not affected by treatment with the different concentrations of paclobutrazol is a key point for its use in combating the bakanae disease in rice.

The reduction in rice seedling height may indeed be explained by significant and irreversible enlargement of small daughter cells resulting from meristematic cell divisions. Furthermore, reduction rates of new cell production can further contribute to suppressing seedling growth. As a result, both cell enlargement or division are inhibited, ultimately decreasing overall seedling growth parameters. There is a strong correlation between PBZ application and a reduction in plant growth [34,35]. Moreover, this effect may be attributed to decreased net photosynthetic plant rates due to the reduction in photosynthetic leaf area. Paclobutrazol (PBZ) is synthetic growth retardant and is known as anti-gibberellins [36,15]. It works by inhibiting the biosynthesis of gibberellins, which are plant hormones that promote stem elongation. The increase in seedling height observed in inoculation with *F. fujikuroi* could be attributed to the strain potentially producing gibberellin-like substances, which promote stem elongation [37]. Paclobutrazol at 1.5, 3, 6, 12, 25, 50 and 100 mg/L combined with *F. fujikuroi* inoculation decreased rice shoot height compared to infected and untreated plants. The highest reduction in seedling height was recorded at 50 and 100 mg/L PBZ. Paclobutrazol (PBZ) is known as a plant growth regulator. Its main function is to inhibit the production of gibberellins (GAs), which are plant hormones that promote growth and development. By reducing GA levels, PBZ can stunt plant growth [15]. Under PBZ application, the inoculation of *F. fujikuroi* resulted in an increase in shoot length compared to uninoculated seedlings. This indicates that the combination of PBZ application and *F. fujikuroi* inoculation had a positive impact on shoot growth. It is possible that *F. fujikuroi* infection may induce physiological changes or produce compounds that interfere with the normal growth processes regulated by PBZ.

An inverse relationship was observed between higher PBZ concentrations and a decrease in root length. Conversely, a positive correlation was found when PBZ were applied in combination with *F. fujikuroi* inoculation, resulting in increased root length. PBZ was observed to increase the diameter and length of fibrous roots and enhance lateral root formation in *Catharanthus roseus* plants [38]. Li et al. [39] indicated that PBZ at 6 mg/L significantly increased the lateral root number of *Phoebe bournei*. There is a correlation between an increase in number of lateral roots and an enhancement indole acetic acid (IAA) level. Auxin is essential for regulating the development and growth of roots. Indole acetic acid (IAA) plays a role in controlling cell division and elongation, thereby regulating the growth of primary and lateral roots through polar auxin transport (PAT). Auxins have been extensively studied and consistently found to have the most pronounced effect on rooting processes. They play a crucial role in stimulating cell division and elongation, promoting the formation and development of roots [40]. This process involves the formation of asymmetric distribution and concentration gradients of IAA in the roots.

At the same time, PBZ's ability to regulate root growth is achieved by modulating balance of crucial endogenous plant hormones, such as gibberellic acid (GA) and abscisic acid (ABA) [41,42]. As the concentration of paclobutrazol (PBZ) increased abscisic acid (ABA) levels in *P. bournei* roots initially decreased significantly and then increased. This dynamic fluctuation was closely associated with ABA biosynthesis and metabolism processes [43]. ABA exhibits different effects on root regulation depending on its concentration. At low concentrations, ABA promotes root growth. However, at high concentrations, it inhibits root development [44].

The reduction in seedling dry weight observed in the presence of PBZ can be attributed to inhibition of gibberellin (GA) biosynthesis. As mentioned earlier, PBZ acts by reducing the production of GAs, which are plant hormones that promote growth [15]. By inhibiting GA production, PBZ can potentially reduce the leaf size and overall photosynthetic area of the plant. Reduced photosynthetic area can hinder the plant's ability to capture sunlight and perform photosynthesis efficiently, which can further contribute to decreased biomass accumulation. The interaction between PBZ and the inoculation with *F. fujikuroi*, resulting in lower root dry weight. This indicates that the presence of *F. fujikuroi* had an additional impact on the reduction of root dry weight when combined with PBZ treatment. This suggests that, the reduction in root dry weight due to increasing ABA content [40]. At high concentrations, ABA can have a more pronounced inhibitory effect on root growth. The reduction in root dry weight suggests that the accumulation of ABA inhibits root biomass accumulation, potentially by interfering with cell division, elongation, or nutrient uptake processes [41]. The relationship between plant length (length of shoot and root) and dry weight is typically positive (Figs. 6, 8 and 10), indicating that as a plant becomes taller (with increased length), its overall biomass, including dry weight, generally increases as well.

Increasing chlorophyll content can be explained by more densely packed chloroplasts per unit leaf area due to a reduction in leaf area [42]. This could be due to an increase in the activity of oxidative enzymes (CAT, POX and PPO) that prevented cell maturation and cell death. PBZ-treated plants synthesized more cytokinin, which enhanced chloroplast development and chlorophyll biosynthesis or prevented chlorophyll catabolism [45,46]. The presence of *F. fujikuroi* may have contributed to the observed reduction in chlorophyll levels, potentially through mechanisms such as interference with chlorophyll synthesis or increased stress on the plants. On the other hand, PBZ synthesized more cytokinin, a class of plant hormones known for their role in promoting cell division and chloroplast development. This increased cytokinin synthesis, in turn, enhanced chloroplast development and chlorophyll biosynthesis in the treated plants [47,48]. Bakanae disease indeed causes various symptoms in rice plants, and etiolation is just one of them. This is the most characteristic symptom, causing abnormally thin leaves [9]. The fungus disrupts the pathways responsible for chlorophyll production, leading to less chlorophyll pigment being formed. Etiolation cases can involve accelerated breakdown of existing chlorophyll molecules. The fungus produces gibberellin-like compounds that mimic the plant hormone gibberellin. However, these fungal gibberellins disrupt the normal signaling pathways involved in chlorophyll production, leading to reduced synthesis [47]. Building on the known ability of paclobutrazol (PBZ) to inhibit gibberellin (GA) biosynthesis in plants, this study demonstrates that PBZ also reduces the growth of the rice pathogen *Fusarium fujikuroi*. This observed reduction in fungal growth suggests a potential link between PBZ's mode of action and the inhibition of gibberellin-like compound biosynthesis within the fungus. Further investigation is needed to confirm this hypothesis and explore the underlying mechanisms in detail.

The interaction between PBZ and *F. fujikuroi* inoculation had synergistic effect on proline accumulation in plants. The higher proline contents observed under the combined PBZ and *F. fujikuroi* treatment suggest that the fungal inoculation induced a greater stress response in the plants, potentially leading to increased proline synthesis. Proline acts as an osmolyte, meaning it accumulates in response to PBZ treatment. This accumulation helps maintain cell turgor pressure by drawing water into the cell, preventing dehydration and collapse of the cell membrane [49]. PBZ can alter gibberellin (GA) metabolism, and a decrease in

GA can indirectly lead to increased ABA biosynthesis [40]. Abscisic acid (ABA) plays a significant role in increasing proline content in plants [50]. In general, proline content values were higher in inoculated than uninoculated seedlings. The increase in proline could be due to the stress caused by fungal infection [51]. Proline plays a role in preserving chlorophyll content and leaf turgor while also improving stomatal conductance, all of which are linked to drought tolerance [52].

Paclobutrazol (PBZ) can indeed increase the activity of various antioxidant enzymes, i.e., catalase (CAT), peroxidase (POD), polyphenol-oxidase (PPO), etc., in plants [53,54]. These enzymes break down harmful reactive oxygen species (ROS) like H_2O_2 , protecting cells from damage. This might be due to enhanced energy interception by photosynthetic pigments [55]. When plants are inoculated with *F. fujikuroi*, it triggers a defense response in the plants. As part of this defense response, the plants may increase the activity of antioxidant enzymes. Therefore, observed increase in antioxidant enzyme activity under *F. fujikuroi* inoculation suggests that the plants are mounting a defense response to counteract oxidative stress caused by the pathogen. This increase in enzyme activity helps to protect the plants and may contribute to their overall resistance against *F. fujikuroi* infection [56,57]. Under stress conditions, the activity of polyphenol oxidase (PPO) increases in plants. PPO is an enzyme involved in the oxidation and degradation of phenolic compounds, which often accumulated in plants as a response to stress [58]. The increased activity of PPO allows for oxidation of phenolic compounds, converting them into quinones. Quinones can then undergo further reactions, including polymerization, which helps to detoxify and degrade the phenolic compounds [55]. These enzymes mitigate ROS, protecting cells from damage. As a result, chlorophyll content tends to rise.

The presence of the fungal inoculation (*F. fujikuroi*) did not have a noticeable effect on CO_2 around the leaf levels in the absence of PBZ treatment. However, significant increases in leaf CO_2 exchange rates were observed when PBZ was applied at concentrations of 50 and 100 mg/L in the absence of fungal inoculation. The increased leaf CO_2 exchange rates under PBZ treatment suggest that PBZ may have influenced the stomatal conductance of the rice seedlings. Stomatal conductance refers to the regulation of the stomata, which control the exchange of gases, including CO_2 . The relationship between gibberellins (GA) and abscisic acid (ABA) in regulating stomata opening and transpiration is well-established in plants. ABA signals the guard cells surrounding the stomata to close, reducing water loss through transpiration [59].

When environmental conditions are favorable, stomata open wider, allowing for increased gas exchange. This leads to higher stomatal conductance; Conversely, when unfavorable conditions, such as biotic and abiotic stress, stomata close partially or wholly to reduce water loss through transpiration. This results in lower stomatal conductance. The interaction between spraying time and paclobutrazol concentration significantly influences stomatal conductance in rain tree leaves [59]. In most plants, higher CO_2 concentration inside the leaves typically increases stomatal activity [59]. It's important to note that increasing transpiration under infection conditions can lead to dehydration and stress in plants. It's important to note that while stomata conductance and transpiration rate are generally positively correlated, there can be exceptions depending on specific plant species, environmental conditions, and the plant's physiological responses to different stress factors [59].

Hydrogen peroxide is reactive oxygen species (ROS) that is produced as a byproduct of various plants' metabolic processes, including photosynthesis and respiration. Various abiotic and biotic stresses can trigger hydrogen peroxide (H_2O_2) accumulation in leaves. Under salinity conditions, it is common for the concentration of (H_2O_2) to increase significantly in leaf tissues. Salinity stress can disrupt the balance between reactive oxygen species (ROS) production and detoxification in plant cells, leading to an accumulation of ROS, including H_2O_2 [56]. The increased levels of H_2O_2 can activate defense mechanisms and antioxidant systems to mitigate the damage caused by stress factors. While hydrogen peroxide is a ROS, it may also cause oxidative stress if present in excess.

Both spraying time and paclobutrazol concentration significantly influenced the photosynthesis rate of raintree leaves. While higher stomatal conductance could indeed lead to increased CO₂ diffusion and potentially boost photosynthesis [58]. PBZ-increased CO₂ diffusion due to higher stomatal conductance can lead to a more active carboxylation reaction in the mesophyll cells, potentially boosting the photosynthesis rate [60]. PBZ can lead to increased ABA (abscisic acid) content in plants; the products of photosynthesis are directed toward roots, leading to dominant root growth [39]. A positive correlation between the significant decrease in CO₂ concentration in rice leaves and a higher growth rate [61].

Triazole compounds, specifically triazole-based fungicides like triadimefon and propiconazole, are commonly used in agriculture to control fungal diseases in crops. This could be due to the potential influence of PBZ on plant defense mechanisms or the pathogen's ability to overcome the plant's natural resistance. However, their mode of action against pathogens is primarily through inhibiting synthesis of ergosterol, a key component of fungal cell membranes, rather than directly increasing the content of antioxidants in plants [62,63]. Application of paclobutrazol (PBZ), a plant growth regulator, can lead to increased concentrations of abscisic acid (ABA) in leaves. ABA is phytohormone that plays crucial role in different physiological processes in plants, including stomatal regulation, and responses to environmental stresses [15]. ABA is also associated with plant responses to various environmental stresses, such as salinity and cold temperatures. Increased ABA concentrations can trigger stress-related signaling pathways, leading to the activation of stress-responsive genes and the production of protective compounds. Additionally, certain triazole compounds may have indirect effects on plant defense mechanisms. For example, they might induce the production of defense-related hormones like jasmonic acid or enhance the activity of defense-related enzymes, which can indirectly contribute to plant resistance against biotic stresses [62]. The activity of antioxidant enzymes in plants is a key indicator of their ability to resist diseases and environmental stresses [55]. Elevated antioxidant enzyme levels often correlate with enhanced plant health, reduced disease severity, and greater resilience under stress conditions [64,65]. Therefore, improving antioxidant defenses through breeding or biotechnological approaches is an important strategy for enhancing plant resistance to diseases.

5 Conclusion

PBZ, whether used alone or in combination with *F. fujikuroi* inoculation, results in reduced seedling height but enhances several growth traits, including chlorophyll and proline levels, enzyme activity, and net photosynthesis. Additionally, low concentrations of PBZ are effective at inhibiting fungal growth. Moreover, a 100 mg/L concentration of PBZ notably reduces the rate of bakanae disease infection in rice seedlings. The findings emphasize the importance of paclobutrazol in reducing bakanae disease and enhancing growth traits under greenhouse conditions. However, a key question remains: Will paclobutrazol as a seed priming treatment provide substantial benefits for managing bakanae disease in field conditions? Additional research is required to answer this question.

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Metwaly, Dina Abdulmajid; analysis and interpretation of results: Metwaly Mahfouz Salem Metwaly, Dina Abdulmajid, Hossam S. El-Beltagi, Adel A. Rezk, Mohammed I. Al-Daej, Mohamed M. El-Mogy, Elsayed Abd Elmaksoud Abomarzoka, Sherif Mohamed El-Ganainy; draft manuscript preparation: Mohamed Fathi El-Nady, Abdelaziz M. Tahoon, Metwaly Mahfouz Salem Metwaly, Dina Abdulmajid, Hossam S. El-Beltagi. All authors reviewed the results and approved the final version of the manuscript.

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