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Bio-efficacy of Nanosilicon in Regulating Oxidative Activity to Control Rice Seedlings Rot Disease Caused by *Burkholderia glumae*

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Bacterial panicle blight and seedling rot diseases in rice plants (*Oryza sativa* L.) are caused by the pathogenic bacterial *Burkholderia glumae.* The nanosilicon treatment is gaining attraction but its effectiveness towards *B. glumae* infection in rice seedlings through regulating enzymatic activities remains largely unexplored. This study aimed to evaluate the bio-efficacy of nanosilicon in controlling seedling rot disease through regulation of peroxidase and polyphenol oxidase enzymes after challenge infected with *B. glumae* in rice variety MR297 and PadiU Putra. Nanosilicon was applied as seed priming in germination testing at 0, 300, 600, and 900 ppm on both rice varieties before *B. glumae* inoculation. Both rice seed varieties primed with nanosilicon at 600 ppm exhibited a significant increase in seedling germination performances over control. The rice seedling of MR297 was more responsive to nanosilicon at 600 ppm with only 17.78% of disease severity index over 26.67% in PadiU Putra and was therefore selected for the enzymatic activity screening. The results showed that the foliar spray of nanosilicon rice plants (MR297) significantly increased both peroxidase (POX) at 24 h and polyphenol oxidase (PPO) at 48 h after *B. glumae* inoculation with 20.44/min/g and 7.46/g activities, respectively. In addition, the plant growth performances were significantly increased compared with control under the same treatment. This demonstrates nanosilicon's potential to control rice seedling rot disease by regulating POX and PPO activities and hence promote plant growth. The application of nanosilicon is an environmentally friendly approach for controlling *B. glumae* infection at the early rice growing stage.

Keywords **:** *Burkholderia glumae*, enzymatic activity, nanosilicon

In Asian countries, rice serves as a staple food, with Myanmar, Cambodia, Laos, and Vietnam deriving 60% of their daily calories from rice (Khush, 2003). According to the Department of Statistics Malaysia, the average Malaysian consumes 82.3 kg of rice annually. Governments and scientists have primarily focused on increasing rice production to ensure food security (Alexandratos and Bruinsma, 2012). However, achieving rice self-sufficiency remains a significant concern, particularly with the rapid growth of the human population especially in Asia (Wang and Li, 2005). Therefore, countries like China, Thailand, Vietnam, Malaysia and Indonesia are intensifying efforts to drastically increase rice production.

Unfortunately, various pathogenic diseases including fungal, bacterial and viral infections pose significant threats to the rice industry, leading to a reduction in global rice

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production. Bacterial panicle blight (BPB) disease, which disrupts both vegetative and pre-reproductive stages, exhibits symptoms such as panicle and leaf discoloration, grain rot, floret sterility, grain abortion, reduced grain weight and inhibition of seed germination (Jeong et al., 2003; Sayler et al., 2006) and causing improper maturation of grains within the panicles during grain-filling phase (Cui et al., 2016).

In Malaysia, symptoms of BPB were first observed in Sungai Aceh, Penang, in 2017 (Ramachandran et al., 2021). By 2022, over 121 ha of rice fields in Penang were affected, resulting in a high proportion of empty grains. Moreover, a significant 90% incidence of BPB disease has been documented in Sekinchan, Selangor (Zali et al., 2023). In highly infested fields, BPB can cause yield reductions of up to 75% (Jeong et al., 2003). This disease is prevalent in tropical and subtropical regions, thriving under temperatures ranging from 30-35°C (Syahri et al., 2018) to 38-40°C (Nandakumar et al., 2009), exacerbated by prolonged heat waves and high humidity during the rice growing season (Lee et al., 2015; Syahri et al., 2018). This disease poses a significant threat to national food production due to yield loss (Chompa et al., 2022), thereby jeopardizing food security sustainability. Consequently, urgent measures are required to manage this disease in rice crops, motivating plant pathologists to explore various strategies aimed at enhancing rice production.

Silicon has garnered considerable attention in agriculture for its potential to alleviate both biotic and abiotic stresses (Etesami and Jeong, 2023). Also, nanosilicon is used to mitigate stresses of heavy metals (Li et al., 2020), salinity (Naguib and Abdalla, 2019), water deficit (Aqaei et al., 2020), ultraviolet-B (Tripathi et al., 2017), and high temperature (Younis et al., 2020). Silicon application was reported to enhance the activity of defensive enzymes such as peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia-lyase, chitinase, and glucanase which play crucial roles in combating *Rhizoctonia solani*, the causal agent of leaf blight disease in rice (Schurt et al., 2014). POX activity was associated with cell wall reinforcement and lignin formation (Brisson et al., 1994) and PPO has been reported to acquire antibacterial effects in host plants through lignin formation (Song et al., 2016). Additionally, silicon application stimulates the production of jasmonic acid, enzymatic and non-enzymatic antioxidants, and secondary metabolites essential for plant defense under stress conditions (Ranjan et al., 2021). Silicon contributes to indirect disease resistance in rice plants by regulating osmotic potential, improving photosynthesis, facilitating mineral nutrient uptake, and promoting suberization and lignification in roots (Chen et al., 2011; Fleck et al., 2011; Khattab et al., 2014).

To enhance the capability of silicon the nanosilicon particle was introduced. This Si nanoparticle offers several advantages over bulk silicon fertilizers. Nanotechnology has enabled the commercial production of silicon nanoparticles (SiNPs) using various methods like sol-gel methods, precipitation, pyrolysis, laser ablation, flame synthesis, chemical vapour deposition, microemulsion, hydrothermal and plasma synthesis (Bhat et al., 2021). The production of two chemical precursors: tetraethyl orthosilicate and tetramethyl ortho silicate are high cost, toxic and unsafe to the environment and human health (Bhat et al., 2021). Alternatively, green synthesis of SiNPs using natural agricultural waste, such as rice husk to synthesize SiNPs is cost-effective, eco-friendly, and non-toxic to minimize the adverse effects associated with conventional methods. Green synthesis of SiO_2 involves various extraction methods that produce different characterization for agricultural applications in exerting physiological and biochemical effects on crops (Ibrahim et al., 2022).

Despite numerous studies on the beneficial effects of nanosilicon in promoting rice plant growth and mitigating fungal diseases (Elamawi et al., 2020; Ng et al., 2020; Sathe et al., 2021) and bacterial diseases (Abdallah et al., 2023). Its impact on reducing bacterial diseases, particularly BPB disease remains unexplored. Additionally, while much research has focused on the BPB disease development during the reproductive phase of rice plants (Pedraza et al., 2018), studies on in infection on vegetative phase are limited, despite reports of *Burkholderia glumae* infection during the early growth stages of the rice plants (Kim et al., 2014b; Syahri et al., 2018). Therefore, this study aimed to evaluate the bio-efficacy of nanosilicon in reducing rice seedling rot during germination and to elucidate the enzymatic activity response of nanosilicon-mediated rice seedlings to *B. glumae* inoculation in the early growth stages.

Materials and Methods

Maintaining and preparation of *B. glumae*. The pure culture of *B. glumae* was obtained from the Laboratory of Pest, Disease and Microbial Biotechnology (LAPDiM), Faculty of Fisheries and Food Science, University Malaysia Terengganu, Malaysia. The pure culture was maintained on King's B agar and incubated in an inverted position at 28 ± 2°C as described by Singh and Vishunavat (2015).

The inoculum of *B. glumae* was prepared using nutrient broth and incubated in an orbital shaker at 28 ± 2°C at 150 rpm for 24 h according to Pedraza et al. (2018). The bacterial biomass was then centrifuged at 6,000 rpm for 10 min and washed with sterilized distilled water before being adjusted to 1.0 × 10^8 cfu/ml at 600 nm using a UV spectrophotometer (22331, Eppendorf, Hamburg, Germany).

Rice seed priming with nanosilicon. Rice seed varieties MR297 and PadiU Putra were used in this experiment. Rice MR297 is one of the popular locally cultivated rice varieties in Malaysia (Khazanah Research Institute, 2019) and the PadiU Putra rice variety is a newly developed rice line with high-yielding properties and resistance to blast disease (Universiti Putra Malaysia, 2017) however, its susceptibility to *B. glumae* infection is yet to be determined. All rice seeds were surface sterilized through immersion in a 6% hypochlorite solution for 6 min, followed by rinsing with sterile distilled water 6 times. Subsequently, rice seeds undergo dormancy breaking by soaking in distilled water for 24 h. The surface sterilized rice seeds were primed by immersing into 50 ml of respective nanosilicon solutions at 300, 600, and 900 ppm for another 24 h as described by Hussain et al. (2019). Rice seeds immersed in 50 ml of sterile distilled water served as control. After priming, the rice seeds were further inoculated with *B. glumae*.

Inoculation of *B. glumae* on rice seeds. The rice seeds for both varieties (MR297 and PadiU Putra) were inoculated with *B. glumae* inoculum (Pedraza et al., 2018). Rice seeds were immersed into 25 ml of *B. glumae* inoculum (1.0 × 10^8 cfu/ml) and incubated in a rotary shaker for 1 h at 150 rpm. Subsequently, the *B. glumae* inoculum was removed and 15 rice seeds were randomly selected and placed on the moistened sterile filter paper in the petri dish. The Petri dishes were incubated in darkness for 3 days before transferring into a growth chamber maintained at 28 ± 2°C under a 12 h photoperiod of 12 h light and 12 h darkness commenced from 3 days after germination (DAG) (Ke et al., 2017). Rice seeds without *B. glumae* inoculation served as a control and was subjected to identical germination conditions.

Evaluation of rice seed germination. Rice seed germination was assessed by monitoring the emergence of the radicle and plumule at 7 DAG. The lengths of both plumules and radicles of the rice seedlings were measured using a digital calliper. The percentage of rice seed germination was determined using the formula reported by Mia et al. (2012), while the calculation of seed germination vigor index (GVI) was conducted using the following formula noted by Vibhuti et al. (2015).

$$\text{Seed germination \%} = \frac{\text{Number of germinated seedlings}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Germination vigor index (GVI)} = \frac{(\text{Mean shoot} + \text{Root length}) \times \text{Germination (\%)}}{100}$$

Infected disease caused by *B. glumae* was assessed at 14 DAG using developed symptom scoring (Table 1). The rice seedling rot severity scale was adapted from Devescovi et al. (2007), and the disease severity index (DSI) of the seedlings was calculated according to the formula recommended by Chiang et al. (2017).

$$\text{Seedlings disease severity index (\%)} = \frac{\sum(\text{Score} \times \text{Frequency})}{\text{Highest score} \times \text{Number of seedlings}} \times 100$$

Experimental design and nanosilicon foliar sprayed in the glasshouse. The glasshouse experiment was conducted with three replicates and repeated twice. Only the rice variety (MR297), which exhibited enhanced responsiveness to nanosilicon application with a lower DSI from a previous study was selected for further investigation of its biochemical responses to *B. glumae* inoculation. At 7 DAG, rice seedlings of MR297 were transplanted into the plastic pot

Table 1. Rice seedling rot disease scoring of nanosilicon-primed rice seedlings assessed at 14 days after germination with *Burkholderia glumae* inoculation

Scale	Disease symptom description
1	Greenish, healthy plumule and radicle (as control)
2	Greenish, less vigorous of roots and aerial shoot
3	Partially discolored shoot and root (less than 50%, respectively)
4	Partially discolored shoot and root (more than 50%, respectively)
5	Limited seedling growth or completely decolorated
6	Seedling death or root and shoot completely macerated

(13 cm in height and 9.5 cm in length), filled with 350 g of topsoil and placed under controlled glasshouse conditions, with temperatures maintained at 28 ± 2°C, light intensity at 4,650 ± 100 lux, relative humidity at 75%, and 12 h photoperiod of light and darkness. One week after transplanting, fertilizer with N:P:K (14:14:14) was applied weekly.

MR297 rice seedlings (21 days after transplant [DAT]) were foliar sprayed with nanosilicon at various concentrations (0, 300, 600, and 900 ppm) at 2 days intervals for 3 times. Nanosilicon used in the experiment was previously extracted from rice husks using green synthesis methods (unpublished data). The foliar spraying of nanosilicon on the rice seedling was conducted as described by Du et al. (2022) with slight modifications by adding Tween-80 at a concentration of 0.05% to enhance silicon adhesion to the rice leaves surface. In control (0 ppm), rice plants were foliar sprayed with sterile distilled water added with Tween-80. Each treatment was conducted with three replications.

After 7 days of the first nanosilicon foliar sprayed, rice seedlings (28 DAT) were inoculation with *B. glumae*. Rice seedlings without nanosilicon application served as control. The experimental units were arranged in a completely randomized block design to evaluate the enzymatic activity response in the nanosilicon-applied rice plants.

***B. glumae* inoculation on rice seedlings.** The *B. glumae* inoculum was prepared at a concentration of 1.0×10^8 cfu/ml before inoculation onto rice leaves. Inoculation was performed in the fourth week after transplanting (28 DAT) using the leaf clipping technique, following the method described by Chen et al. (2002). A pair of sterilized scissors were used to cut the rice leaf tips before dipping into the inoculum *B. glumae*, allowing the bacteria to enter through the resulting wounds. This method facilitates bacterial entry by creating openings at the leaf tips. To keep the uninoculated plants facing a similar treatment, another pair of sterilized scissors was used to cut the leaf tips before dipping the leaf into sterile distilled water.

Enzyme assays. The leaves were harvested at 24, 48, 72, and 96 h after being inoculated with *B. glumae* for the enzymatic activity assay of PPO and POX. Upon harvesting, the leaves were promptly placed into 45 ml centrifuge tubes and sealed to prevent desiccation. Leaf collection was carried out in the early morning (8 AM) before the commencement of the following experiments.

The protein extraction for the POX and PPO enzymes followed the procedure outlined by Liang et al. (2005) with minor adjustments. Fresh leaf (0.3 g) was randomly selected and homogenized in an ice bath using mortar and pestle. The extraction solution was 5 ml of 50 mM phosphate buffer (pH 8.7) supplemented with 5.0 mM sodium hydrogen sulphite and 0.1 g polyvinyl pyrrolidone. The extract was centrifuged at 6,000 ×*g* for 20 min at 4°C, the supernatant was utilized for enzyme assays.

POX activity was determined as described by Cai et al. (2018). Specifically, 0.05 ml of the enzyme extract was added to a substrate mixture containing 1.0 ml of 0.1 M phosphate buffer (pH 5.4), 0.95 ml of guaiacol (0.2% (v/v)), and 1.0 ml of 0.3% (v/v) H_2O_2. After one minute of reaction, the changes in the mixture were measured based on the absorbance at 470 nm using a spectrophotometer. POX activity was calculated according to the formula below (Cai et al., 2018).

$$\text{POX activity} = \frac{(\text{Change in abs per min}) \times (\text{Total volume of mixture [ml]})}{(2.8/\text{mM/cm} \times \text{Enzyme volume [ml]})}$$

PPO activity was determined according to Cai et al. (2018). The reaction mixture consisted of 0.05 ml of the supernatant mixed solution containing 1.95 ml of 50 mM potassium phosphate buffer (pH 6.5) and 1 ml of 0.1 mM catechol. The increase of absorbance at 410 nm, for 2 min at 30°C, was measured. PPO activity was calculated based on the following equation (Cai et al., 2018).

$$\text{PPO activity} = \frac{(\text{Abs at 2 min} - \text{Abs at 0 min} \times \text{Total reaction volume (ml)}}{\text{Time interval} \times \text{Enzyme volume (ml)}}$$

Plant growth parameters. At 45 DAT, the rice plants were collected for growth parameters assessment which included plant height, number of tillers, and fresh and dry root biomass. Plant height was measured according to the method described by Berahim et al. (2021) using a measuring tape. The rice plants were washed to remove any soil residue, and the roots were excised and measured for fresh root biomass. Subsequently, the roots were dried in an oven at 65°C for 24 h until the constant weight was achieved to obtain dry root biomass.

Statistical analysis. All data obtained from the *in vitro* and glasshouse experiments were analyzed using analysis of variance (ANOVA). The mean comparison between treatments was examined using Tukey's honestly significant difference test, with a significance level of $P < 0.05$. Statistical analyses were performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA).

Results

Germination performance of MR297 and PadiU Putra rice seedlings. Germination attributes of *B. glumae* inoculated and *B. glumae* non-inoculated seedlings exhibited significant variation in response to nanosilicon concentrations. The mean shoot length (MSL), mean root length (MRL), and GVI of both inoculated and non-inoculated rice seedlings showed a pronounced significance when treated with 600 ppm nanosilicon at 7 DAG (Table 2). Among the treatments, *B. glumae* inoculated seedlings demonstrated the highest value for MSL, MRL, and GVI at 600 ppm nanosilicon concentration, followed by 300 ppm, 900 ppm, and 0 ppm nanosilicon concentrations.

In contrast, the germination performance of *B. glumae* non-inoculated seedlings exhibited variable responses to nanosilicon seed priming. Notably, seedlings treated with 600 ppm nanosilicon exhibited the highest GVI, indicating superior development and health of seedlings at 7 DAG, irrespective of bacterial inoculation. While nanosilicon concentrations significantly ($P < 0.05$) influenced the germination parameters, the interaction effect between nanosilicon concentrations and bacterial inoculation was found to be not significantly different.

The bio-efficacy of nanosilicon significantly affected the growth parameters of PadiU Putra rice seedlings (Table 3). PadiU Putra rice seedlings inoculated with *B. glumae* inoculated and treated with 600 ppm nanosilicon exhibited the highest MRL, MSL, and GVI followed by 300 ppm, 900 ppm, and 0 ppm nanosilicon concentrations used. Conversely, *B. glumae* non-inoculated PadiU Putra rice seedlings showed the highest MRL, MSL, and GVI at 300 ppm nanosilicon, followed by 600 ppm, 0 ppm, and 900 ppm. There was no significant difference observed between the 300 ppm and 600 ppm nanosilicon concentrations ($P > 0.05$).

There is a variation of rice seedlings (both varieties) with and without *B. glumae* inoculation in responding to nanosilicon seed treatment. This result indicates that *B. glumae* inoculation potentially influences the response of rice seedlings to nanosilicon seed treatment. Thus, the interaction between *B. glumae* inoculation and nanosilicon concentration was not significant ($P > 0.05$) affecting the germination parameters.

Disease severity of *B. glumae* inoculated rice seedlings. Significant concentration-dependent effects were observed in enhancing resistance against *B. glumae* infection in both MR297 and PadiU Putra rice seedlings. Nanosilicon treat-

Table 2. The germination performances of nanosilicon-primed rice seedlings variety MR297 at 7 days after germination

Nanosilicon concentrations (ppm)	Mean shoot length (cm)		Mean root length (cm)		Germination vigor index (%)	
	Burkholderia glumae inoculated	*B. glumae* non-inoculated	*B. glumae* inoculated	*B. glumae* non-inoculated	*B. glumae* inoculated	*B. glumae* non-inoculated
0	1.59 ± 0.80 d	2.17 ± 1.06 d	3.04 ± 1.06 d	3.56 ± 1.70 c	5.03 ± 0.18 d	5.55 ± 0.89 d
300	2.96 ± 1.07 b	2.88 ± 1.58 c	3.90 ± 1.27 b	3.86 ± 1.99 b	6.58 ± 1.11 b	6.43 ± 0.60 b
600	4.32 ± 1.14 a	4.23 ± 0.91 a	5.78 ± 1.33 a	6.49 ± 1.35 a	9.81 ± 0.16 a	10.45 ± 0.49 a
900	2.55 ± 1.19 c	3.11 ± 1.39 b	3.55 ± 1.65 c	3.54 ± 1.52 d	5.66 ± 0.19 c	6.10 ± 0.51 c

Values are the means of three replicates ± standard deviation. Means within the same column with different letters indicate significant differences at a 5% level of probability ($P < 0.05$).

Table 3. The germination performances of nanosilicon-primed rice seedlings variety PadiU Putra at 7 days after germination

Nanosilicon concentrations (ppm)	Mean shoot length (cm)		Mean root length (cm)		Germination vigor index (%)	
	Burkholderia glumae inoculation	Without *B. glumae* inoculation	*B. glumae* inoculation	Without *B. glumae* inoculation	*B. glumae* inoculation	Without *B. glumae* inoculation
0	0.92 ± 1.11 d	1.35 ± 1.73 cd	1.84 ± 2.26 c	2.56 ± 3.20 c	2.24 ± 0.53 c	3.15 ± 0.58 c
300	1.61 ± 1.88 ab	2.58 ± 2.01 ab	2.81 ± 3.17 ab	4.30 ± 3.39 a	3.78 ± 0.39 ab	5.88 ± 0.39 a
600	1.73 ± 2.00 a	2.27 ± 2.08 a	3.17 ± 3.63 a	4.11 ± 3.78 ab	4.09 ± 0.13 a	5.32 ± 0.36 ab
900	0.97 ± 1.31 cd	0.95 ± 1.20 d	1.13 ± 1.55 d	1.55 ± 1.97 d	1.72 ± 0.35 d	1.92 ± 0.22 d

Values are the means of three replicates ± standard deviation. Means within the same column with different letters indicate significant differences at a 5% level of probability ($P < 0.05$).

Table 4. The disease severity index of nanosilicon-primed rice seedlings varieties MR297 and PadiU Putra assessed at 14 days after germination with *Burkholderia glumae* inoculation

Rice variety	Disease severity index (%)			
	Control	300 ppm	600 ppm	900 ppm
MR297	66.67 ± 4.39 ab	35.56 ± 1.70 bc	17.78 ± 3.40 d	86.67 ± 2.93 a
PadiU Putra	72.22 ± 9.88 ab	41.11 ± 1.23 c	26.67 ± 1.82 d	78.89 ± 4.63 a

Values are the means of three replicates ± standard deviation. Means within the same raw with different letters indicate significant differences at a 5% level of probability ($P < 0.05$).

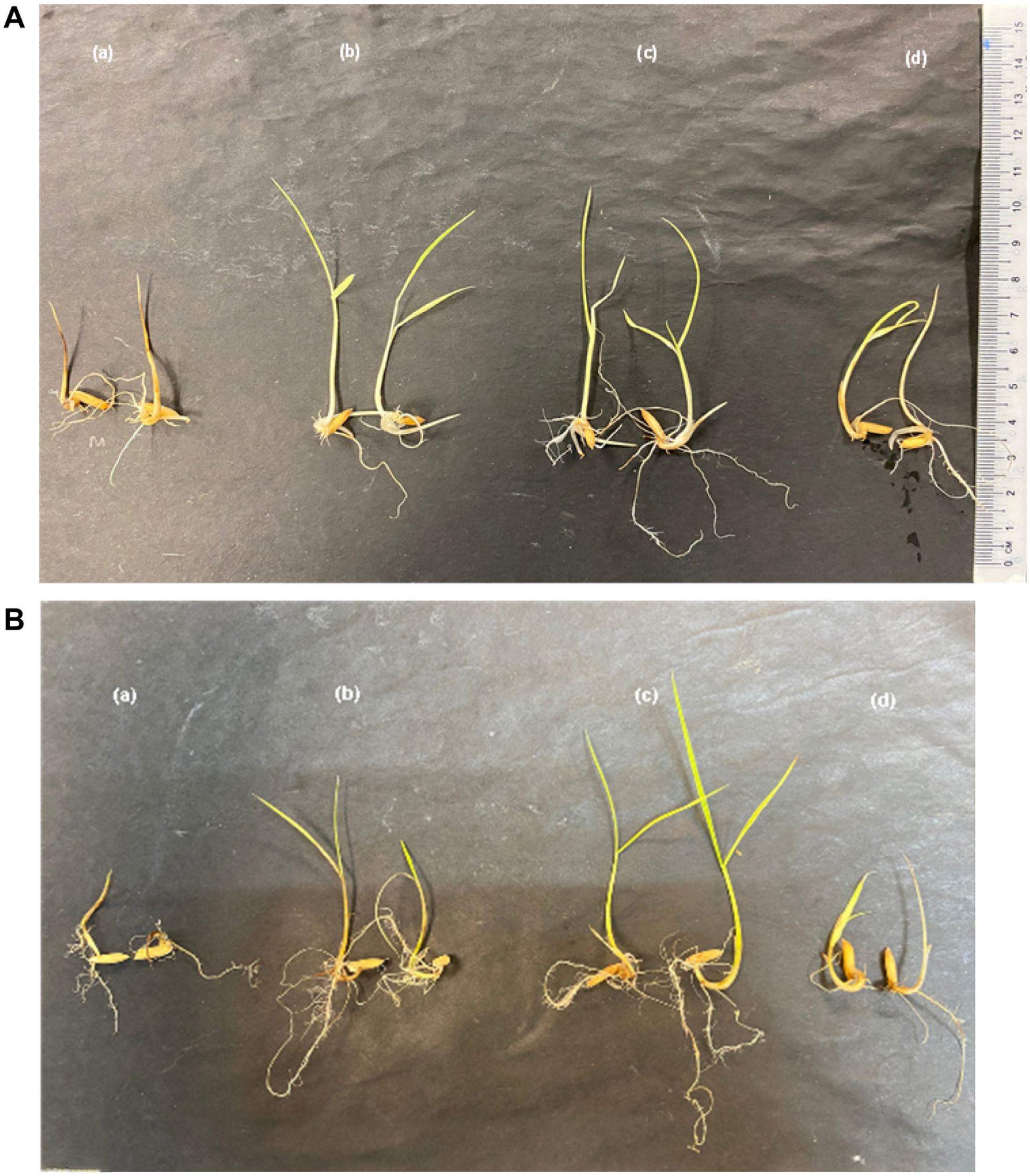

Fig. 1. Disease severity of *Burkholderia glumae* inoculated MR297 seedlings (A) and PadiU Putra (B) seedlings at 14 DAG with nanosilicon priming: 0 ppm (a), 300 ppm (b), 600 ppm (c), and 900 ppm (d), respectively. Rice seedlings with severe browning shoot and maceration roots were observed in control, without nanosilicon seed priming (a). While, in nanosilicon priming rice seedlings (b), (c), and (d) indicated different severity levels responding to the nanosilicon concentrations.

ment at 600 ppm notably increased resistance in both rice varieties, resulting in the highest number of healthy, green and well-developed seedlings compared to other treatments (Table 4). Conversely, the nanosilicon untreated seedlings of both MR297 and PadiU Putra rice varieties exhibited severe infection by *B. glumae* during germination and caused browning and complete root maceration (Fig. 1).

The efficacy of nanosilicon in controlling seedling rot varied with concentration. The highest nanosilicon concentration (900 ppm) tested did not effectively mitigate seedling rot compared to 600 ppm nanosilicon, resulting in DSI values of 86.67% and 78.89% for MR297 and PadiU Putra rice varieties, respectively. In contrast, the application of 600 ppm nanosilicon exhibited the lowest DSI values, with 17.78% for MR297 and 26.67% for PadiU Putra, indicating healthier seedlings compared to other concentrations. Furthermore, compared to the non-silicon applied control, 600 ppm nanosilicon reduced disease severity by 48.89% and 45.55% for MR297 and PadiU Putra rice varieties, respectively.

POX and PPO activities. Rice variety MR297 was identified as the most responsive variety toward nanosilicon application over PadiU Putra based on the seedling rot dis-

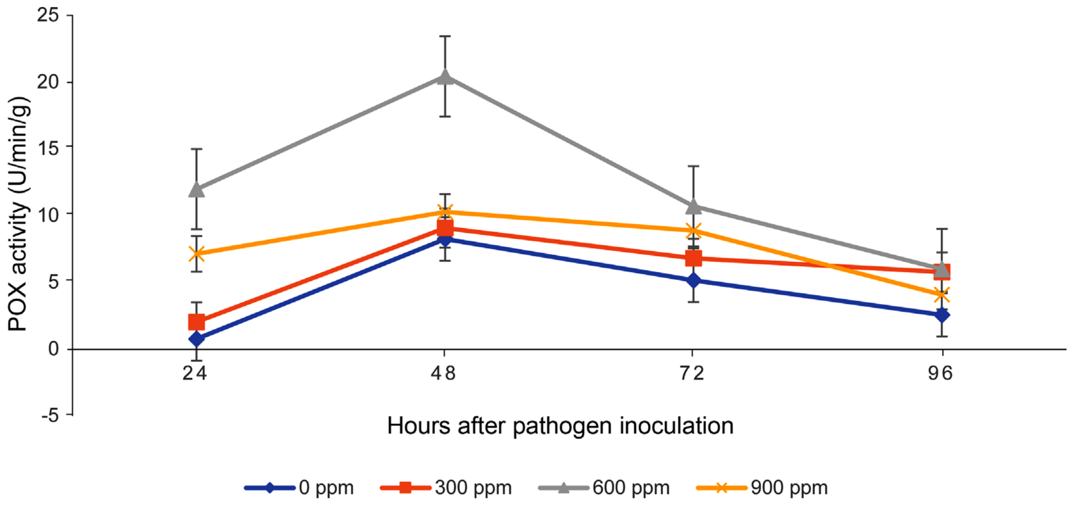

Fig. 2. Peroxidase (POX) enzyme activity of nanosilicon-foliar sprayed rice plants (MR297) after *Burkholderia glumae* inoculation at 28 days after transplant. Vertical bars indicate standard errors.

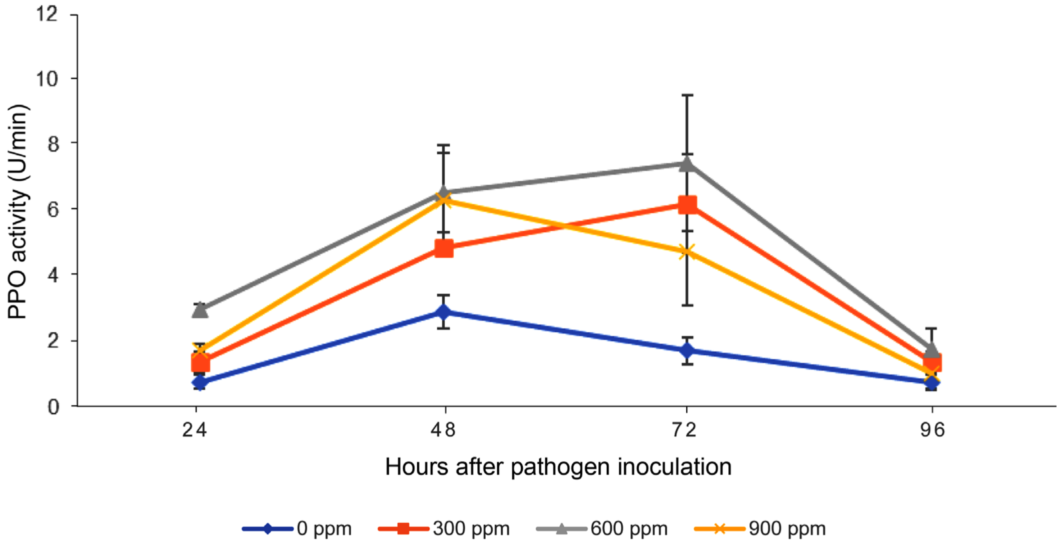

Fig. 3. Polyphenol oxidase (PPO) enzyme activity of nanosilicon-foliar sprayed rice plants (MR297) after *Burkholderia glumae* inoculation at 28 days after transplant. Vertical bars indicate standard errors.

ease severity. Therefore, rice variety MR297 was selected for the subsequent enzymatic activity assay under glasshouse conditions. Rice plants (28 DAT) were harvested at 24, 48, 72, and 96 h post-*B. glumae* inoculation (hpi). Significant changes in POX enzyme activity were noted at different time points (24, 48, 72, and 96 h) across all tested concentrations of nanosilicon (Fig. 2). The highest level of POX enzyme activity was detected in rice plants treated with 600 ppm of nanosilicon, regardless of the time point. Specifically, the peak activity of POX was at 48 hpi, reaching 20.436 U/min/g. However, this activity level declined to 10.693 U/min/g by 72 hpi and further dropped to 5.986 U/min/g at 96 hpi.

Rice plants foliar sprayed with a nanosilicon concentration of 600 ppm demonstrated the highest levels of PPO enzyme activity, while the control rice plants without silicon foliar application displayed the lowest PPO activity (Fig. 3). The peak PPO activity in plants treated with 600 ppm nanosilicon occurred at 72 hpi, reaching 7.46 U/min. PPO activity progressively rose from 24 to 48 hpi and reached its maximum at 72 hpi, after which it declined by 96 hpi.

The two-way ANOVA analysis indicated no significant interaction between the effects of nanosilicon concentrations and the timing of post-inoculation (hpi) on PPO enzyme activity. Nonetheless, both nanosilicon concentrations and hpi individually significantly influenced PPO activity ($P < 0.05$). Interestingly, the highest nanosilicon concentration tested (900 ppm) in this study, exhibited lower enzyme activity compared to the 600-ppm concentration.

The foliar application of nanosilicon did not significantly affect the height of the rice plants (Table 5). Nonetheless, the tallest rice plants were observed with the 600 ppm nanosilicon treatment, reaching a height of 58.33 cm, closely followed by the 300-ppm treatment at 58 cm. Rice plants not treated with nanosilicon (0 ppm) reached a plant height of 51 cm, while those treated with 900 ppm nanosilicon recorded the shortest height, at 49.33 cm. Similarly, the application of 600 ppm nanosilicon significantly increased the number of tillers compared to the non-nanosilicon application in control (Fig. 4). Moreover, foliar application of nanosilicon at 600 ppm and 300 ppm, significantly increased both fresh and dry root weights over those none silicon-treated rice plants, indicating a beneficial effect on root growth.

Fig. 4. The bio-efficacy of nanosilicon as a foliar spray in growth promotion of rice plant variety MR297 at 45 days after transplanting. Rice plants (MR297) foliar sprayed with nanosilicon at 0 ppm (control) (a), 300 ppm (b), 600 ppm (c), and 900 ppm (d).

Discussion

Rice is a vital crop with significant economic value, vulnerable to numerous diseases caused by pathogens including *B. glumae*. Silicon plays a crucial role in enhancing crop growth, offering protection against both biotic and abiotic stresses (Ma, 2004), mitigating the impact of heavy metals

Table 5. The plant growth parameters of nanosilicon-foliar sprayed rice seedlings variety MR297 at 45 days after transplanting

Treatments	Plant growth parameters			
	No. of tillers	Plant height (cm)	Fresh root weight (g)	Dry root weight (g)
Control	5.67 ± 0.58 c	51.00 ± 3.61 bc	3.33 ± 0.15 cd	1.03 ± 0.06 cd
300 ppm	8.33 ± 0.58 ab	58.00 ± 5.29 ab	15.30 ± 0.44 b	5.13 ± 0.59 b
600 ppm	9.67 ± 2.52 a	58.33 ± 3.79 a	18.18 ± 3.08 a	6.77 ± 1.54 a
900 ppm	5.00 ± 1.00 cd	49.33 ± 7.02 cd	10.47 ± 1.61 bc	2.20 ± 0.98 bc

The value indicates the means of three replicates ± standard deviation. Values with different letters indicate significant differences at a 5% level of probability ($P < 0.05$).

such as arsenic (As), silver (Ag) (Karunakaran et al., 2013), cadmium (Cd) (Kim et al., 2014a), chromium (Cr) (Hasanuzzaman et al., 2017), and manganese (Mn) (Maksimović et al., 2016) through apoplastic detoxification (Ranjan et al., 2021) and improving water efficiency and photosynthetic activity in various crops (Du et al., 2022).

Consequently, silicon application has been adopted for rice cultivation, showing effectiveness in managing diseases like sheath blight (Schurt et al., 2014), bacterial leaf blight (Patel et al., 2020), and blast diseases (Cai et al., 2018; Rahman and Udin, 2017). A rice plant is a silicon accumulator, that readily absorbs silicon through specific transporters, depositing it in leaf cells as a silica layer. Despite silicon's abundance in soil, its natural form is not easily accessible to plants, leading to the exploration of various silicon sources like bulk, commercial, and nanosilicon for rice benefits.

Nanosilicon, with its high reactive surface area, has garnered attention for agricultural use, enhancing crop yield and growth by supplying essential nutrients. In this study, nanosilicon treatments ranging from 0 to 900 ppm on MR297 and PadiU Putra rice seedlings have demonstrated their potential to boost resistance through improved germination metrics. Specifically, 600 ppm nanosilicon treatment was the most effective in reducing *B. glumae* infection, showing the best germination rates and development of shoots and roots.

The impact of SiNP concentration on plants varied based on their tolerance levels. In this study, the nanosilicon concentration chosen was previously screened on rice seedlings MR297 and PadiU Putra (our unpublished data). The uptake of silicon particles by plant cells and their organelles is prominent when applied in lower concentrations (below 1,000 ppm) (Hussain et al., 2013) and subsequently enhances the cells' capacity to control several biochemical processes including oxidative defense systems (Suriyaprabha et al., 2012).

However, higher concentrations of nanosilicon (900 ppm) demonstrated less effectiveness, leading to adverse effects like seed maceration and brownish rot symptoms in rice seedlings. This aligns with findings by Jiang et al. (2022) that lower nanosilicon concentrations favor germination by enhancing water permeability without harmful effects (Nath et al., 2019), while higher concentrations of nanosilicon supplied in a short time can cause ionic rivalry that impedes the uptake of other essential elements and reduces germination rates due to excessive ion leakage (Zhang et al., 2021). Higher nanosilicon concentration caused phytotoxic effects on seed germination and plant growth due to an increase in pH level and adherent of mineral nutrients to the surfaces of the SiNPs (Asgari et al., 2018). A higher concentration of nanosilicon suppressed plant growth by affecting enzymatic and non-enzymatic activities such as reduced photosynthetic pigment, lipid peroxidation and enhanced antioxidative enzymatic responses (Karimi and Mohsenzadeh, 2016) In addition, SiNP application at 1,000-3,000 ppm was reported to cause massive accumulation of silica nanoparticles around the stomata and subsequently might destroy the stomatal function (Du et al., 2022). Also, high concentrations of SiNPs (over 2,000 mg/l) demonstrated inhibition of root growth and reduced leaf numbers in *Arabidopsis thaliana* seedlings (Lu et al., 2020). In this study, the bio-efficacy of nanosilicon application on the GVI of rice seedling MR297 is notably higher compared to rice variety PadiU Putra, particularly at a concentration of 600 ppm. However, the response of nanosilicon in rice plants to inoculation challenges by *B. glumae* remains unexplored.

The subsequent study on the bio-efficacy of nanosilicon as a foliar application on MR297 rice plant post-*B. glumae* inoculation under greenhouse conditions showed that nanosilicon, especially when foliar was applied, increased the enzymatic activities, thereby activating defense mechanisms against pathogen stress. Nanosilicon was foliar applied at 3 weeks after transplanting, at which the rice plants started to produce tillers. It was said that the uptake of Si by rice plants rises at the tillering stage (Kheyri, 2022). The absorption of Nano-Si through rice leaves might be associated with the formation of silica cells at the epidermis of leaf blades. Foliar application of nanosilicon was conducted alternately at 2-day intervals for one week, thus allowing for Si absorption and transportation to the whole plant before *B. glumae* inoculation on the leaves.

Generally, plants have developed a range of strategies to deal with biotic threats, which are oxidative radicals, defense molecules, chemical compounds pathogenesis-related proteins (PR proteins) produced within the plants and structural barriers like lignified cell walls that hinder the entry and spread of pathogens within host cells (Naaz et al., 2022). The role of Si in alleviating pathogen stress through activation of defense systems such as antioxidant enzymes (PPO, POX, catalase, and superoxide dismutase) and the non-enzymatic antioxidants (polyphenols and proline) and production of osmolytes such as proline were reported in several studies (Huang et al., 2020; Kheyri, 2022; Ng et al., 2020; Tripathi et al., 2013). The beneficial effects of foliar-applied nanosilicon were particularly noticeable with the application of 600 ppm nanosilicon, which significantly elevated the activities of antioxidant enzymes and contributed to the plant's defense against pathogens.

The silicon-induced antioxidant enzyme activities could have induced the production of salicylic acid, which is a signaling hormone that activates systemic acquired resistance (SAR) in rice plants (Mahawar et al., 2023). SAR further promotes resistance within the host plant by alerting plant cells in non-pathogen-invaded areas as the signaling molecules travel from infected to uninfected parts of the plant. In response to these signals, cells from the uninfected parts of the plant activate a range of defense genes. This includes the production of PR proteins, antimicrobial compounds, and the strengthening of cell walls. The activation of these defense mechanisms makes the plant more resilient to subsequent infection as in the case of encountering a pathogen again, it can respond more quickly and effectively. Foliar-applied nanosilicon helps in the activation of SAR, thus, rice plants can better withstand various stresses, increase photosynthetic efficiency, boosting growth and biomass. Furthermore, reduced pathogen stress allows rice plants to mature and produce grain, increasing overall yield.

Schurt et al. (2014) reported that the POX and PPO of rice plants supplied with Si were higher when compared to the control plants. The active role of Si in activating POX rapidly after pathogen inoculation was also reported by Rodrigues et al. (2005). Similarly, the enzyme activities of PPO and POX also increased rapidly at 48 hour after inoculation (hai) of *Rhizoctonia solani* in rice plants (Schurt et al., 2014). Moreover, the activities of PPO and POX were also found to be highest at 2 days after inoculation (48 hai) and then declined starting from day 3 after inoculation in Si-applied *Brassica napus* (Zhang et al., 2023). The decrement in the POX activity is attributed to the enzyme's role in the plants, where it oxidizes phenols and produces hydrogen peroxide within the plant cells, creating a toxic environment for pathogens. As a result, POX activity diminishes over time as the pathogens cease to multiply actively in response to the toxic conditions. Rice plants that were not treated with silicon showed lower POX activity levels compared to those treated with various concentrations of nanosilicon. This observation is consistent with findings by (Shasmita et al., 2019), who noted that the activities of enzymes such as POX, PPO, and superoxide dismutase (SOD) were significantly lower in plants not treated with silicon.

The observed trend in the enzyme activity is linked to the role of PPO, which oxidizes phenolic compounds into quinones, substances that are more harmful to pathogens. The elevated levels of PPO activity observed at 48 and 72 hpi enhance the plant's resistance against the infection caused by *B. glumae*. PPO enzyme activity declines at 96 hpi, coinciding with a reduction in pathogen activity within the plant. The rise in reactive oxygen species level, which functions as a signalling molecule to boost the activity of the antioxidant system, maybe the cause of the increase in antioxidant enzyme activity because of pathogen stress (Sharma et al., 2012).

It was reported that the activities of antioxidant enzymes (POX, PPO, catalase, and SOD) remain non-significantly high under normal conditions at which without pathogen introduction to silicon-applied plants (Hegazy et al., 2015). POX and PPO activities in non-Si applied plants were relatively lower compared to other Si treatments. Although the enzymatic activities of Si foliar-applied plants were higher at 48 hpi, over time, the activities completely declined at 96 hpi. This could be explained by the fact that increased stress causes the proteolytic enzymes to become more active, which destroys the antioxidants (Gomathi et al., 2013). Furthermore, according to Gill and Tuteja (2010), biotic stress results in the destruction of the membrane and the cell wall which causes oxidative damage to membrane proteins, that further causes a destabilization of the enzyme's structure and a decrease in the activity of POX and PPO.

Despite the observed enzymatic activities of POX and PPO due to *B. glumae* inoculation, the rice plants did not produce any symptoms even after 2 weeks. This could be due to the method of inoculation employed in this study as the rice plants did not develop any symptoms even at 2 weeks after inoculation. It could be due to *B. glumae* remaining endophytically viable and not actively causing any symptoms during the vegetative phase (Pedraza et al., 2018). The development of symptoms took place only when rice plants entered the reproductive phase at which flowering and panicle initiation occurred. *B. glumae* begins to divide and colonize the rice spikelet actively, causing panicle discoloration. As the high night temperatures favor *B. glumae* colonization, the bacteria attack the panicles causing empty filled or with grains with less weight.

Overall, nanosilicon application not only contributes to disease management in rice cultivation but also promotes growth by improving plant height, tiller number, and root biomass. The efficacy of nanosilicon exhibited concentration-dependent effects, where the optimal levels (600 ppm) enhance growth parameters. The findings in this study are in line with Badawy et al. (2021) where nanosilicon foliar applied between 400 to 600 mg/l significantly enhanced the production of tillers. In addition, the application of nanosilicon at an optimal rate to rice plants was said to increase root volume and as per Mathur and Roy (2020), nanosilicon supplied at higher concentrations promoted less growth rate in terms of height, number of tillers and fresh and dry plant biomass. Also, higher concentrations of nanosilicon (1,000 mg/l and 2,000 mg/l) application indicated no significant enhancement in rice plant resistance to fungal pathogens. Higher concentrations of nanosilicon could have been associated with inducing cytotoxic effects in *Allium cepe* (Silva et al., 2017). Nanosilicon with particle sizes of 50 and 200 nm was reported to cause chlorosis due to inadequate synthesis of chlorophyll II (Slomberg and Schoenfisch, 2012). Additionally, the binding of nutrients to the negative-charged nanosilicon particles can impede nutrient uptake (Sollins et al., 1988).

The application of nanosilicon at 600 ppm managed to improve the seedling's growth of the two varieties and boost the resistance to rice seedling rot disease caused by challenge-inoculated *B. glumae* during the germination stage. The nanosilicon foliar application at 600 ppm also notably increased the activity of POX and PPO after 48 h of inoculation with *B. glumae* in rice seedlings MR297. Nanosilicon applied at 600 ppm exhibited effectiveness in controlling seedling rot caused by *B. glumae*.

This study demonstrates that the application of nanosilicon at an optimal concentration is crucial for promoting robust root and tiller development during the vegetative phase of rice plants. This might be due to the advantage of nanosilicon over bulk silicon with its superior ability with the presence of silanol group on the nanosilicon surface that makes it highly versatile to improve their targeted delivery and easily interact with phospholipid bilayer and facilitates endocytosis (Karimi et al., 2016). The significant increase in dry matter, leaf area index, grain quality and productivity and chlorophyll content of rice plants after nanosilicon application was also reported by Elshayb et al. (2021).

The increased tolerance of nanosilicon-treated rice seedlings to *B. glumea* is mediated by the enzymatic activities of POX and PPO. This underscores the critical need to precisely determine the appropriate dosage of nanosilicon for maximizing its benefits in rice plant cultivation. Furthermore, the mechanisms underlying nanosilicon uptake through foliar application and its physiological interactions at different growth stages, particularly in combating *B. glumae* need to be investigated. Research has extensively studied SiNPs' impact on plant growth and resilience towards biotic and abiotic stresses from both morphological and physiological perspectives. However, the molecular mechanisms by which nanosilicon influences the expression of specific plant proteins, growth regulators, defensive compounds, and stress-related genes remain unclear are require further investigation (Mahawar et al., 2023). Additionally, further research is required to confirm the long-term effects of nanosilicon on the growth, health, and productivity of rice plants.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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