Research article

# Nano-enhanced defense: Titanium-enriched Alginate–Bentonite coating augments *Bacillus amyloliquefaciens* D203 efficacy against *Magnaporthe oryzae* in Kenyan rice cultivation

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ABSTRACT

Rice blast disease, caused by *Magnaporthe oryzae*, poses a significant threat to global rice production, necessitating the development of effective and sustainable management strategies. Biological control using beneficial microbes like *Bacillus amyloliquefaciens* has emerged as a promising approach due to its ability to enhance plant resistance and reduce disease incidence. Nano-encapsulation of bacteria, which involves embedding beneficial microbes within nano-materials, offers a novel method to improve the stability, survival, and efficacy of these biocontrol agents. This study evaluated the capacity of encapsulated *Bacillus amyloliquefaciens* D203, embedded within an alginate-bentonite coating infused with titanium nanoparticles (TNs), to stimulate defense responses in rice seedlings challenged by the *Magnaporthe oryzae* the causal agent of rice blast disease. Encapsulation was achieved using the extrusion technique, with some modifications. Using a completely randomized design, the experiment was conducted in a greenhouse, with four treatments replicated four times. The experiment used the popular Kenyan rice variety "BASMATI 370". The study investigated the impact of strain D203 on the incidence, severity, and area under disease progress curves related to *M. oryzae*, as well as the expression of defense-related enzymes. The results demonstrated that rice plants derived from seeds coated with the D203 encapsulated *B. amyloliquefaciens* strain exhibited higher levels of defense-related enzyme expression, including peroxidase (POD), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD) and catalase (CAT), compared to controls. In addition, the incidence and severity of the disease were markedly lower in plants treated with encapsulated *B. amyloliquefaciens* compared to controls, sometimes paralleling the efficacy of hexaconazole treatment. These findings suggest that the encapsulation of strain D203 has the potential to enhance resistance against rice blast disease by inducing systemic resistance through the production of antioxidant enzymes.

## 1. Introduction

Biocontrol of plant diseases, using microorganisms or biologically derived compounds, is increasingly seen as a promising

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alternative to chemical pesticides [1]. Biocontrol provides effective protection against numerous diseases and is environmentally sustainable [2]. By engaging multiple modes of action to inhibit the growth of microbial pathogens, biocontrol strategies can help reduce the development of resistance mechanisms in these pathogens [3].

Research by different scholars has shown that interactions between plant hosts and beneficial microorganisms mitigate the effects of harmful pathogens. Plants release a variety of substances from their roots, including glucose, proteins, and acidic compounds, which encourage the growth of beneficial and harmful microbes. Plant growth-promoting rhizobacteria (PGPR), plant growth-promoting fungi, bacteria, nitrogen-fixing rhizobia, and mycorrhizal fungi are all important in the nutrient cycle and pathogen control (Mousa and Raizada, 2015). Biocontrols in turn protect plants from plant pathogens and also induce resistance in the plants [4]. The timely induction of systemic resistance (ISR) in plants by biological control agents (BCAs) can improve the effectiveness of pathogen control through complementarity. Nevertheless, the BCA often encounters a lot of problems due to the variability of the environment, too harsh leading to death before they can colonize their new environment (Attitalla et al., 2001).

Additionally, despite the numerous discoveries of new microbials with the capacity to manage plant pathogens, only a few are available in the market [5], and this is attributed to the fact that the efficacy of these biocontrol agents is only efficient in gnotobiotic and controlled environments, which is attributed to the inability of the bacteria to maintain the initial minimum threshold levels ($1 \times 10^6$-$1X10^7$) of heterogeneous soil conditions among competitors and antagonists [6].

The use of antagonistic bacteria to biologically control soil-borne plant pathogens can be unstable [7]. One main reason for the instability in biological control using antagonistic bacteria is their inability to effectively and adequately colonize plant roots. This can be attributed to factors related to the bacteria themselves as well as environmental conditions, including both biotic and abiotic elements [8]. Utilizing free bacteria for colonizing plant roots is not a natural process, as microbial agents are sensitive to changes like temperature changes, pH variations, humidity levels, and environmental stresses [9]. Therefore, it is necessary to improve the survival and resilience of these biocontrol agents for use under all conditions. Given that they are susceptible to harsh environmental conditions, biocontrols can be effectively protected from adverse conditions by coating them [5,10]. Microencapsulation is a technique for encasing cells within an encapsulating matrix or membrane. It is widely used in the pharmaceutical and food industry and recently in agriculture [11]. The effectiveness of biocontrol agents depends on their timely and localized application because their secondary metabolites are produced in limited quantities and cannot move long distances. Therefore, biocontrol agents must be in direct contact with pathogens [9].

The survival of encapsulated cells is influenced by the type and concentration of coatings, capsule size, initial cell count, and bacterial type (Young et al., 2006). Encapsulation in polymers is a key aspect of bacterial carrier technology [9].

Biodegradable and biocompatible polysaccharides such as alginate, chitosan, gums, and starch are safe for human use and widely applied in agriculture [12]. Recent studies on the use of chitosan have revealed that it is compatible with other materials, has easy digestion and dilution, non-toxicity, high adsorption, and also biodegradable [12]. A hybrid blend of chitosan and silicon nanoparticles has been discovered to trigger stronger and more efficient defense responses, offering a promising approach to managing plant pathogens and pests [13]. Similar findings by Saberi Riseh et al. [13] also found that carboxymethyl cellulose (CMC) had a high water absorbance potential, and high efficacy in removing pollutants, such as pesticides and heavy metals. Cellulose nanofibrils (CNFs) and CNF-based hydrogels have also been found to hold significant potential as controlled-release fertilizer (CRF) matrices due to their biodegradability, environmental friendliness, and excellent controlled-release and improved soil health [14].

Polymers like β-Glucans, which are composed of linked glucose units, can induce Systemic Acquired Resistance (SAR) in plants. This is achieved by triggering downstream signaling pathways, leading to the accumulation of various pathogenesis-related (PR) proteins, reactive oxygen species, antioxidant defense enzymes, phytoalexins, modifications to cell wall composition, activation of defense enzymes, and stimulation of secondary metabolite production. These responses enhance plant immunity, strengthening defenses against pathogens and oxidative stress, ultimately improving disease resistance [15]. Most gums are natural polysaccharides that readily dissolve in water. When they come into contact with water, they form thick, viscous solutions and gels. These properties have made them widely used in encapsulation hence they are biocompatible with other substances, colorless, tasteless, edible, flexible to use, easily available, and biodegradable (Riseh. et al., 2022; [16]) Encapsulation protects microorganisms from biotic and abiotic stresses, stabilizing and maintaining cells against soil stresses [9].

Microencapsulation of the *Bacillus velezensis* using Alginate-Gum polymers enriched with Titanium nanoparticles and silicon dioxide produced tiny capsules containing *B. velezensis* with the capacity to hold moisture, swell, and protect the bacteria. Tests confirmed that these ingredients blended well together without any unwanted chemical reactions proving encapsulation is an excellent method to conserve biocontrols [17]. Nanoencapsulation of *Pseudomonas fluorescens* VUPF5 and *Bacillus subtilis* VRU1 nanocapsules were found to enhance root and upper biomass of pistachio while the highest root length was reported in VUPF5 metabolite nanoformulation [10]. Moradi Pour et al. [18] demonstrated that *Bacillus velezensis* encapsulated in sodium alginate, carbon nanotubes, and silicon dioxide exhibited electrostatic interactions. Proton High Nuclear Magnetic Resonance (HNMR) and X-ray Diffraction (XRD) analyses confirmed that the microcapsules were stable, and globular-shaped, with a bacteria survival rate of $10^7$ CFU/ml over one year of storage. The results showed that the encapsulated unique formulation containing *B. velezensis* and nanoparticles was effective in controlling *Phytophthora drechsleri* in pistachio. Encapsulation of biocontrols such as *Methylobacterium oryzae* in chitosan which contains randomly distributed β-(1 → 4)-linked D-glucosamine and N-acetyl-D-glucosamine residues was found to alleviate drought stress in barley and pearl millet underscoring the importance of this technique [19].

Rice (*Oryza sativa*) is a crucial staple food in sub-Saharan Africa [20]. In Kenya, more than 40,000 ha of land are dedicated to rice cultivation [21], and globally, about 158 million hectares produce over 750 million metric tons [22]. According to Uma [21], rice production accounts for 29 % of the total global grain crop production, with Africa contributing 10–13 %. Approximately 800 million people depend on it for their livelihood [23]. In Kenya, rice demand exceeds production and this shortfall is met through imports. Atera

et al. [24] asserts that only 20 % of the demand is met by local production, and estimates suggest that the demand will increase due to population growth. Farmers face various challenges in rice production, including biotic and abiotic factors, as well as the lack of modern farming techniques.

The blast of rice disease, caused by *Magnaporthe oryzae*, whose anamorphic form is *Pyricularia oryzae*, is a significant biotic constraint worldwide, especially in small-scale farming. This pathogen affects not only rice but also other members of the Poaceae family, such as wheat and other grass families. The impact of the pathogen on global rice production and productivity is substantial, with 10–30 % of annual rice harvest being lost [25].

Among the numerous pathogens that affect rice, ranging from bacteria, fungi, and viral pathogens, *M. oryzae* is believed to be the most destructive rice pathogen [25], causing significant decrease in rice yields, typically ranging from 70 % to 100 % [26,27]. Factors such as temperatures exceeding 25 °C, humidity levels between 85 % and 89 %, the presence of dew, drought conditions, and excessive nitrogen fertilization contribute to the spread of the disease [27].

According to Ref. [28], in 2008 alone, Rice blast disease is estimated to have destroyed about 5600 ha, or 13,840 acres, of rice in Kenya's Central Province, which represents the majority of the country's rice production. This represented 10–12 % of Kenya's annual production and, as a result, the country needed to increase its rice imports to meet the deficit. *Magnaporthe oryzae* infects various parts of aerial plants, including leaves, internodes, nodes, neck, panicles, and seeds. Among these, leaves are the most commonly affected by rice blast disease, but the disease becomes more destructive when it spreads to the plant nodes, internodes, and neck. When rice leaves are infected with the rice blast pathogen, the photosynthetic area is reduced, leading to limited carbohydrate assimilation. As a result, the yield of grain per plant is reduced [27].

Because the main source of inoculum source is the seeds [25],the spread and dissemination of *M. oryzae* in SSA has been facilitated by trade and exchange of contaminated germplasm across borders due to the high demand for varieties that yield without adhering to the phytosanitary standards [20]. This has had far-reaching consequences for the food security situation in Kenya due to the introduction and reintroduction of new races of the *M. oryzae* pathogen. Data by Mutiga et al. [20] shows that about 490 isolates have been characterized in the SSA, while 232 isolates were from Kenya, as shown in Fig. 1. This highlights the importance of trade regulations in Kenya and the SSA.

Due to its polycyclic nature, small inoculum of *M. oryzae* in seeds under the right conditions can multiply in nurseries and later in the field, leading to the development of endemic disease [29]. Therefore, it is necessary to strengthen, harmonize and implement seed policies and phytosanitary standards among trade blocks in the SSA to prevent further spread of this pathogen [30].

Agrochemicals such as hexaconazole and tricyclazole fungicides have been effective in the management of rice blast disease. However, their continuous and non-selective use has led to several problems, including residual toxicity [4], resistance development due to point mutations in the nucleotide sequence of the prospective gene sequence [31,32], environmental pollution, health risks to humans and animals, higher costs of plant protection, produce residues, negative impacts on natural enemies, and the emergence of new physiological strains of *M. oryzae*. The fungus is highly versatile to rapid mutations in its DNA, enabling it to adapt to different rice varieties and develop resistance by producing AVR-pita genes that counter host resistance [33]; [25,34]. Despite various strategies available for managing rice blast disease, conventional breeding and chemical methods have proven ineffective in adequately addressing the issue, that is, the durability and effectiveness of resistance conferred by the R gene (Pi-ta), which is dependent on alterations in the functional segment of the corresponding AVR gene (AVR-Pita) in *M. oryzae*.

Integration of the transposon within the coding region of the AVR-Pita gene is believed to be responsible for the loss of resistance to rice explosion in rice varieties carrying the R (Pi-ta) gene [25,34]. In response, plant pathologists have shifted their focus towards the development of eco-friendly, durable, and efficient biocontrol methods to effectively manage plant diseases. Biocontrol offers a safe and sustainable approach under field conditions.

Agbowuro [35] and Zhu et al. [4] respectively in their independent reviews, noted that biological controls especially *Bacillus and pseudomonas* species sustained production where rice blast disease reduces yields. According to Aldayel et al. [32], *Bacillus amyloli-quefaciens* was able to suppress the mycelial growth of pathogens and positively influenced the growth of plants. Saberi Riseh et al. [36] also reiterated that Streptomyces bacteria could promote plant growth through a two-pronged approach. Directly facilitating nitrogen fixation, siderophore-mediated iron acquisition, phytohormone synthesis, and mineral solubilization, and indirectly, they suppress harmful microorganisms through antibiotics, competition for iron through siderophore, hydrogen cyanide acting as a toxin, and
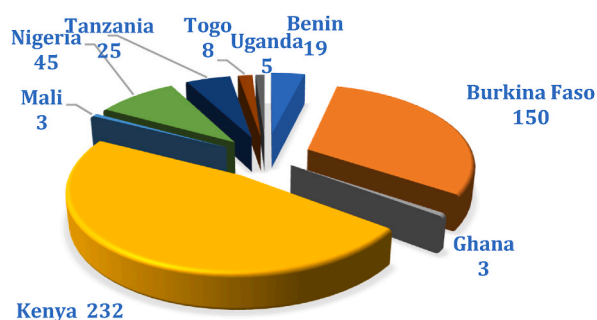


**Fig. 1.** M. oryzae isolates in sub-Saharan Africa. Source Mutiga et al. [20].

hydrolytic enzymes like protease, lipase, chitinase, and cellulase enzymes. Cellulose-based mulches provide a solution that can optimize agricultural productivity while minimizing negative impacts. These mulches are produced from renewable bio-based materials rich in cellulose. Compared to plastic mulches, cellulose-based alternatives demonstrate promising capabilities in enhancing nutrient retention, soil health, weed control, water conservation, and erosion prevention [37].

Using biopolymers such as chitosan, alginate, xanthan resin, gelatin et al., encapsulation of bacteria is a technology that aims to create a shield-like wall around the bacteria colony, offering protection against harsh environmental conditions and for periodic release [5]. Furthermore, microcapsules can be created by bursting and diffusing them, allowing for the gradual or controlled release of key ingredients [6,38]. Nanotechnology is a cutting-edge approach in plant science that aims to reduce the dependence on traditional chemicals and fertilizers. Its primary objective is to improve seed germination, promote plant growth, and increase total biomass [5]. Seed coating agents can improve cell survival and protect microorganisms from various environmental stresses, whose immobilization in the coating agent has been proven to be advantageous [6].

Coating *Bacillus amyloliquefaciens* and bentonite alginate by extrusion has shown improved survival under greenhouse conditions since biodegradable and eco-friendly solvents are used in this method and can be used under other conditions. Encapsulation of biocontrol seed coating agents (ESCA) by coating *B. subtilis* Sl-13 showed good characteristics in cotton, for example, the ability to improve germination and vigor [38]. Encapsulation of Bacteria *Streptomyces fulvissimus Uts2*2 by Alginate–Arabic Gum was found to counter soil microbe diversity and harsh conditions that challenge PGPR establishment. These effective formulations ensured sufficient dispersion, delivery, and survival of PGPR, providing physical protection to create favorable niches, and ensuring long-term plant growth promotion, and were found effective against *Pythium aphanidermatum* in cucumber [39].

According to research by Aldayel et al. [32] the use of *B. amyloliquefaciens* IKMM induced systemic resistance in tomatoes against early tomato blight. Studies by Sha et al. [40] showed that the appressorial formation, and conidia development of *M. oryzae,* were affected in rice when plants were treated with *B. subtills,* proving that Bacillus strains could be good candidates for biocontrols for rice blast disease. Studies by Moradi Pour et al. [7], demonstrated that encapsulation of *Bacillus velezensis* with alginate or gelatin and nanoparticles enhanced its efficacy against *Rhizoctonia solani* in beans. The protective layer ensures gradual release, prolonging activity. Nanoparticles induce plant resistance, regulate pathogen penetration, and increase plant growth factors further aiding in disease control, and offering a sustainable biological control method.

This research was aimed at developing an encapsulated seed coating agent (ESCA) containing *B. amyloliquefaciens* D203 in alginate and bentonite enriched with Titanium nanoparticles (TNs) by extrusion technique that exhibits desirable qualities, such as the capacity to tolerate rice blast disease through eliciting defense-related genes. The incidences and severities of disease and area under disease progress curves of different treatments were studied against the controls. To the best of our knowledge, no research on the use of encapsulated Seed coating agent (ESCA) containing biocontrols like *B. amyloliquefaciens* has been done or published in Kenya.

## 2. Materials and methods

### 2.1. Experimental setup

Sodium alginate was acquired from Inqaba Biotec East Africa Ltd. Bentonite clay was obtained from Fisher Scientific in Italy in this experiment, and only chemicals and reagents of analytical grade were used. The *B. amyloliquefaciens* D203 strain was obtained from Russell Biologicals IPM UK. The *M. oryzae* strain KE0002 isolates were obtained from the Kenya Agricultural Livestock Research Organization - Horticultural Research Institute (KALRO-HRI) for this study. The rice variety chosen for its susceptibility to rice blast disease in Kenya, named "BASMATI 370," was obtained from the Kenya Agricultural Livestock Research Organization (KALRO, Mwea), namely the Industrial Crops Research Institute.

To make encapsulated seed coating agent (ESCA), the technique described by Young et al. [6] was followed with some modifications. Eight grams (8 g) of sodium alginate were supplemented with a density of D203 cells of 1 % *B. amyloliquefaciens* and adjusted to $1 \times 10^7$ CFU/ml. Eight hundred milliliters (800 ml) of water were added, followed by stirring using a magnetic agitator while warming to 30 °C to ensure complete dissolution. Four grams (4 g) of sodium bentonite and 1 g of titanium dioxide were added to the sodium alginate solution and stirred until dissolved. Three hundred grams of calcium chloride were weighed and dispensed into 1000 ml of distilled water and then stirred using a magnetic stirrer. The reaction is an exothermic process; therefore, it was allowed to cool down. The solution was reconstituted to approximately 2 L by adding distilled water. The solution containing sodium alginate, *B. amyloliquefaciens* D203, and titanium dioxide was slowly dispensed into a calcium chloride solution using a syringe while stirring with a magnetic stirrer. White crystal-like beads were formed through ionic gelation of the two solutions by solubilization of the polymer. Agitation continued for 15 min until the capsules formed fully. Subsequently, the beads were rinsed three times using sterile distilled water to ensure that all the calcium chloride was washed away. Subsequently, the beads were dried for 1 min in the microwave using low heat to remove excess water. Rice seeds were placed in water and those that sank were selected for the experiment, while those that floated were removed. Seed sterilization was done following the procedure outlined by Anhar et al. (2019). The selected seeds were left in the open air to dry for 4 h. The beads and seeds were then mixed with 4 ml of glycerol to improve adhesion, and also provide an additional energy and energy source because it is oxidized to dihydroxyacetone phosphate (DHAP) by glycerol-3-phosphate dehydrogenase. DHAP is a key intermediate in glycolysis and gluconeogenesis, which can enter the central metabolic pathways of the bacterium cell [41,42]. Additionally, Bacillus species are known to utilize glycerol capable of synthesizing intracellular glycogen and polyhydroxybutyrate which it uses in the event of low carbon supply [43]. Three grams of sodium bentonite powder was added to the mixture followed by shaking the mixture leading to the formation of coated seeds.

The experiment was carried out in a completely randomized 4 × 4 design in a greenhouse. One week after germination, the

seedlings were transplanted into pots containing silty loam mixed with well decomposed manure homogeneously at a rate of 1: 4. Soil sterilization was carried out by steaming to prevent the growth of soil-borne pathogens such as *Fusarium graminearum*. A total of 16 pots by genotype were divided into 4 treatment groups, including blank controls without inoculation, hexaconazole treatment as the preventive group, plain seed with inoculation and tricyclazole treatment, and plants whose seeds were coated with encapsulated Seed Coating Agent containing strain D203. The greenhouse temperature was maintained at 25–28 °C under natural light conditions.

### 2.2. Inoculation

The plants were inoculated with a suspension of spores that were harvested earlier and suspended in double distilled water when the primary leaves were 21 days old, which is close to the heading stage. The development of the appressorium is initiated by specific conditions, such as a rigid and water-repellent surface and the absence of external nutrients. Certain substances, such as soluble cutin monomers or lipid monomers, can induce the formation of appressoria, even on surfaces that are typically not conducive. To aid in the adhesion of the spores to the plant surfaces, a few drops of Tween 20 were added to the suspension. The spore concentration was adjusted to $2 \times 10^5$ spores/ml using a hemocytometer as a standard practice. The leaves were moistened with water before inoculated with the suspension using an atomizer or hand sprayer to ensure uniform coverage. The entire growing area was then covered with a shade net and polybags to block direct light and maintain high humidity around the leaves at about 95 % for 48 h to create optimal conditions for spore germination. It was assumed that all plants received the same number of spores and that conditions were maintained uniformly.

### 2.3. Effects of ESCA B. amyloliquefaciens D203 on defense enzymes

To analyze the effects of the encapsulated strain D203 on the expression of defense-related enzymes, rice leaf samples were collected at various intervals after treatment, specifically on days 1, 3, 5, and after inoculation.

### 2.4. Peroxidase (POD)

Hammerschmidt et al. (1982) methodology was followed in evaluating the peroxidase activity. The reaction mixture consisted of 2.9 ml of a solution containing 0.25 % v/v guaiacol in 0.01 mol of phosphate buffer solution (PBS) at pH 7, along with 0.5 ml of 0.1 M hydrogen peroxide. Subsequently, 0.1 ml of the extract was introduced to initiate the reaction. A UV spectrophotometer was used to measure the absorbance at 470 nm. Then the activity of peroxidase (PO) was calculated from the increase in absorbance resulting from the guaiacol oxidation.

### 2.5. Catalase (CAT)

The activity of catalase was measured by the method described by Amin et al. [44] whereby 1 g of plant tissues ground in 4 ml of ethanol was added to 0.01mol of PBS (pH 7), 0.5 ml of hydrogen peroxide (0.2 mol) was amended in 2 ml of acetic acid and the absorbance was read at 610 nm with a spectrophotometer. Results were expressed as units per milligram of protein, where 1 mM hydrogen peroxide degraded per minute was equivalent to one unit of reaction.

### 2.6. Phenylalanine ammonia lyse (PAL)

PAL assessment was carried out according to Dickerson's method of Dickerson as described by Aldayel et al. [32] whereby to make the reaction mixture, 1300 µl of sodium borate buffer (0.1M, pH 8.7) was added to 200 µl of crude enzyme. Subsequently, 500 µL of L-phenylalanine (12 mM) was added to the mixture and incubated for 30 min at 37 °C. After incubation, the absorbance at a wavelength of 290 nm was measured using cinnamic acid (ranging from 0 to 5 mg, nmol) as a standard. The results were expressed in minutes per gram of polyphenol protein.

The rate of absorbance changes per minute and the specific activity in enzyme units per milligram of soluble protein, using an extinction coefficient of 6.39 mM$-$1 cm$-$1, were used to quantify the activity of peroxidase. The enzyme activity was measured in units per milligram of protein.

### 2.7. Superoxide dismutase (SOD)

The evaluation of SOD was done according to the method as described by Ref. [45]. One (1) gram of leaves from each treatment was homogenized separately in 5 ml of 50 mM phosphate buffer (pH 7.0) containing 1 % polyvinylpyrrolidone (PVP). The homogenate was filtered and centrifuged at 15,000 RPM for 10 min. The obtained supernatant was used as the enzyme extract. All steps in the preparation of the enzyme extract were carried out at 0–4 °C. An aliquot part of 2 ml of the enzyme extract was used to assess the protein content at 600 nm OD using the NBT method.

### 2.8. Effects of Bacterial Coating on the incidences and severity of M. oryzae

Disease incidences, which is the proportion of leaves that exhibit symptoms of *Magnaporthe oryzae* within a specified time period,

were evaluated by counting daily the proportion of infected leaves in a selected rice plant. The severity grade of the disease was evaluated as described by Ref. [46,47] with some modifications. The severity of the disease index (DSI) was calculated using the following formula.

$$\text{Disease Severity Index} = \sum \left( \frac{severity\ rating\ *\ plants\ per\ rating}{Total\ plants\ *\ highest\ severity\ rating} \right)$$

Blast symptoms were determined approximately 7 days after inoculation, when approximately 50 % of conidia were expected to have sporulated for 12 days. The disease evaluation was carried out on a scale, severity score as described by Manandhar et al. [46] as described in Table 1. The disease correlation was classified according to Fig. 2 where (1) is no lesions and score (9) means typical blast lesions and more than 75 % of the leaf area or the entire leaf dead. Data on disease incidence were used to calculate disease increase rates and plot disease progress curves. Area under disease progress curves (AUDPC) was given by formula given by Jeger and Viljanen-Rollinson [48] as follows:

$$AUDPC = \sum_{i=1}^{n} \frac{(yi + yi - 1)}{2} X (xi - xi - 1)$$

$$= \left[ y_1 X \frac{t_2 - t_1}{2} \right] + \sum_{i=2}^{n-1} X \frac{t2 - t1 - 1}{2} \Bigg]_+ \left[ y_n X \frac{t_n - t_{n-1}}{2} \right]$$

where $y_i$ is an assessment of a disease (percentage, proportion, ordinal score, etc.)

At the ith observation,

t is time (in days, hours, etc.) at the ith observation,

n is the total number of observations.

## 3. Results

### 3.1. Effects of Bacterial Coating on the incidence and severity of M. oryzae

The results of the disease incidence assessment shown in Table 2 demonstrate the assessment of the effectiveness of different treatments in the management of rice blast disease. Hexaconazole, administered as a preventive measure from the initial time point (D1) to the final assessment (D12), exhibited the lowest incidence (67.8 %). In contrast to the control group, encapsulation showed an increasing trend over time. The mean incidence was 7 % higher than that of hexaconazole, but 32 % lower than that of the control. This result demonstrates that while the incidence of encapsulation could be higher, possibly because of the slow release of the polymer, its efficiency is comparable to that of preventive treatment with hexaconazole. Tricyclazole, when applied after infection, initially exhibited a higher incidence of 12 % than hexaconazole. However, it stabilized at approximately 51.6 % from day 7 to day 12, as shown in Fig. 3. This indicates that tricyclazole was less effective in preventing the initial infection, but showed a suppressive effect on the further development of rice blasts once applied.

### 3.2. Disease severity

The severity index values after rice infection with *Magnaporthe oryzae* strain KE0002 were compared between different treatments, namely control, encapsulation, hexaconazole and tricyclazole (Fig. 4). The severity index values, measured at different time points (D1–D12), provided information on the effectiveness of each treatment in managing the disease. The control group exhibited relatively high severity index values (93.75 %) throughout the observation period (Fig. 4). The mean severity index increased gradually from 13.3 in D1 to 64.1 in D12. This indicates a significant and ongoing progression of the disease in the absence of any treatment.

On the contrary, the encapsulation of *B. amyloliquefaciens* D203 resulted in a significant decrease in disease severity, highlighting the potential of biocontrol strategies to mitigate the impact of *Magnaporthe oryzae*. The lower LSD and CV values (Table 3) compared to

**Table 1**
Score guide for rice blast.

|  | Rice blast disease severity rates |
|---|---|
| 1 | *No lesions, or small brown specks of pinhead size (0.1-1.0 mm), less than 1 % leaf area affected* |
| 2 | *Typical blast lesions covering 1–5% of leaf area covered* |
| 3 | *6-10 % leaf area covered by typical blast lesions* |
| 4 | *11-20 % of leaf area covered by typical blast lesions* |
| 5 | *21-30 %, leaf area covered by typical blast lesions* |
| 6 | *31-40 %, leaf area covered by typical blast lesions* |
| 7 | *41-50 %, leaf area covered by typical blast lesions* |
| 8 | *51-75 %, leaf area covered by typical blast lesions* |
| 9 | *Typical blast lesions covering >75 % leaf area or all dead leaves* |

Source: Manandhar et al. [46].
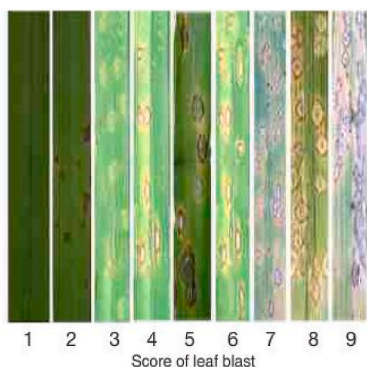
**Fig. 2.** Score guide for rice blast. Source: Manandhar et al. [46].

**Table 2**
Disease incidence score 12 DAI (days after inoculation).

| Treatment | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 22.1 ± 7.5[a] | 39.3 ± 7.1[a] | 60.7 ± 13.7a | 78.6 ± 8.2a | 85.7 ± 11.6 a | 85.7 ± 11.6a | 90.1 ± 6.7a | 93.2 ± 5.8a | 98.7 ± 2.5a | 100 ± 0.0a | 100 ± 0.0a | 100 ± 0.0a |
| Encapsulation | 7.1 ± 14.3[b] | 17.9 ± 13.7[b] | 24.9 ± 7.1b | 39.3 ± 7.1b | 44.7 ± 3.75b | 50.9 ± 5.5b | 54.6 ± 7.9b | 60.7 ± 7.1b | 60.7 ± 7.1b | 64.3 ± 8.2b | 67.8 ±7b | 67.8 ±7b |
| Hexaconazole | 0.0 ± 0.0[b] | 14.5 ± 2.5[b] | 23.5 ± 1.98b | 32.0 ± 3.26b | 42.4 ±4b | 47.4 ± 7.1b | 57.2 ± 0.8b | 63.7 ± 1.3b | 69.8 ± 2.8c | 73.4 ± 1.0c | 74.6 ± 1.8c | 74.7 ±2c |
| Tricyclazole | 10.7 ± 7.1[ab] | 25.0 ± 13.7[ab] | 42.85 ± 11.7b | 42.9 ± 11.66b | 46.4 ±7b | 48.0 ± 4.0b | 51.60 ± 90b | 51.6 ± 6.9c | 51.6 ± 6.9d | 51.6 ± 6.9d | 55.2 ± 3.9d | 74.7 ±2d |
| Mean | 10.0 | 42.9 | 38.01 | 66.4 | 54.8 | 58.0 | 63.4 | 67.3 | 70.2 | 72.3 | 74.4 | 74.4 |
| LSD | 13.6 | 15.9 | 14.97 | 12.6 | 11.4 | 12.5 | 9.6 | 8.9 | 8.1 | 8.3 | 6.4 | 6.4 |
| CV (%) | 88.2 | 42.99 | 25.57 | 16.9 | 13.5 | 13.9 | 9.9 | 8.6 | 7.5 | 7.4 | 5.6 | 5.6 |

Note: The data were the mean ± standard error of four independent replications. The means with the different letters represent significant differences ($p < 0.05$) with Fisher's multiple range tests; SE = Standard Error; LSD = least significant difference; CV = coefficient of variation.
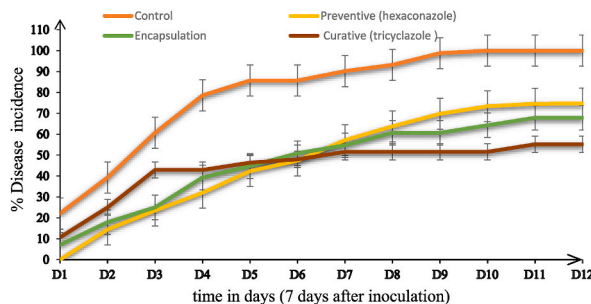


**Fig. 3.** Effects of bacterial coating on the incidence of *Magnaporthe oryzae*.

the control further support the consistency and effectiveness of the encapsulation treatment.

Hexaconazole, a preventive chemical fungicide, also exhibited a trend toward a lower severity index value compared to the control. The mean values for plants treated with hexaconazole were comparable to those of encapsulation treatment, indicating a similar level of efficacy in disease suppression. The LSD values suggest a reliable distinction between treatments, whereas the CV values imply a relatively stable and consistent response to hexaconazole.

Tricyclazole, a chemical fungicide of curative treatment, had a lower severity index (37.5 % values than the control (93.8 %) with 56 % lower severity and was higher than encapsulation and hexaconazole by 12.5 % and 31.3 %, respectively. The mean severity index increased from 12.5 at D1 to 37.5 at D12, indicating a weaker effect on suppressing the disease. This implies that the timing of the application of tricyclazole can determine the efficacy of treatment against blast disease. The severity index analysis highlights the effectiveness of both biocontrol (encapsulation) and chemical fungicides (hexaconazole) in reducing the severity of rice blast disease. Encapsulation of *Bacillus amyloliquefaciens* D203 is a particularly promising strategy, offering a sustainable and eco-friendly alternative for disease management.

The general mean severity index values for all treatments provide a comprehensive overview of the comparative efficacy.
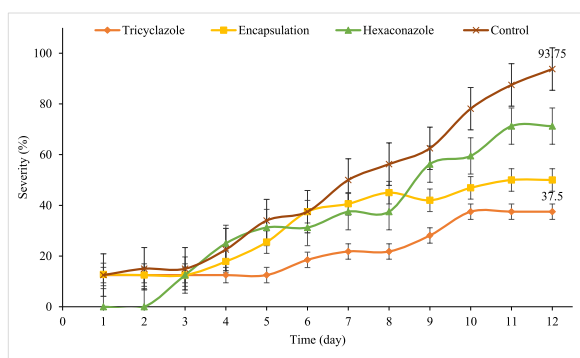
**Fig. 4.** *Effects of ESCA on the severity of Magnaporthe oryzae infection.*

**Table 3**
Comparison of means of treatment effect on rice blast disease severity index.

| Treatment | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 15.6 ± 6.3[a] | 15.6 ± 6.3[a] | 28.1 ± 6.3[a] | 34.4 ± 11.9[ab] | 46.9 ± 6.3[a] | 50.0 ± 0.0[a] | 53.1 ± 6.3[a] | 62.5 ± 10.2[a] | 71.9 ± 6.3[a] | 78.1 ± 6.3[a] | 87.5 ± 10.2[a] | 93.8 ± 7.2[a] |
| **Encapsulation** | 12.5 ± 0.0[a] | 12.5 ± 0.0[a] | 25.0 ± 0.0[a] | 25.0 ± 0.0[bc] | 34.4 ± 6.3[b] | 40.6 ± 6.3[b] | 40.6 ± 6.25[b] | 37.5 ± 0.0[b] | 40.6 ± 6.3[c] | 50.0 ± 0.0[c] | 50.0 ± 0.0[c] | 50.0 ± 0.0[c] |
| **Hexaconazole** | 12.5 ± 0.0[a] | 12.5 ± 0.0[a] | 25.0 ± 0.0[a] | 37.5 ± 0.0[a] | 37.5 ± 0.0[b] | 50.0 ± 0.0[a] | 50.0 ± 0.0[a] | 53.1 ± 6.3[a] | 50.0 ± 0.0[b] | 62.5 ± 0.0[b] | 68.8 ± 7.2[b] | 75.0 ± 0.0[b] |
| **Tricyclazole** | 12.5 ± 0.0[a] | 12.5 ± 0.0[a] | 15.6 ± 6.3[b] | 18.8 ± 7.2[c] | 21.9 ± 6.3[c] | 21.9 ± 6.3[c] | 21.9 ± 6.3[c] | 28.1 ± 6.3[b] | 34.4 ± 6.3[c] | 37.5 ± 0.0[d] | 37.5 ± 0.0[d] | 37.5 ± 0.0[d] |
| **Mean** | 13.3 | 13.3 | 23.4 | 28.9 | 35.2 | 40.6 | 41.4 | 45.3 | 51.4 | 57.0 | 60.9 | 64.1 |
| **LSD** | 4.8 | 4.8 | 6.8 | 10.8 | 8.33 | 6.8 | 8.3 | 10.4 | 8.3 | 4.8 | 9.6 | 5.6 |
| **CV (%)** | 23.5 | 23.5 | 18.9 | 24.2 | 15.4 | 10.9 | 13.1 | 14.9 | 10.3 | 5.5 | 10.2 | 5.6 |

Note: Data represent the mean ± standard deviation of four independent replicates. Means with different letters indicate significant differences ($p < 0.05$) with Fisher's multiple range test; SD = Standard Deviation; LSD = least significant difference; CV = coefficient of variation.

Encapsulation treatments have emerged as a promising approach to disease management, with consistent and low severity index values. The observed trends underscore the potential of biocontrol strategies as viable alternatives to chemical fungicides, aligning with the greater emphasis on sustainable and eco-friendly agricultural practices.

### 3.3. Area under disease progress curve (AUDPC)

The disease severity results for various treatments, ie control, encapsulation, hexaconazole, and tricyclazole (Table 4) provided understanding of the effectiveness of each approach. Starting with control treatment, the relatively high AUDPC value of about (695.50) suggests significant and sustained disease progression during the observed period. This underscores the natural tendency of the disease to escalate without intervention, thus emphasizing the importance of implementing protective measures, as shown in Fig. 5.

On the contrary, the encapsulation treatment resulted in a lower AUDPC by 30.6 % (Fig. 6) than the control. Encapsulation of *Bacillus amyloliquefaciens* D203 appears to have a positive impact on disease progression. The controlled release of the bacterium through encapsulation contributed to a more effective and sustained suppression of the disease than untreated control. These findings are promising for the potential use of biocontrol strategies in disease management.

Additionally, chemical treatment with hexaconazole demonstrated a lower AUDPC than control, indicating its effectiveness in controlling disease progression. Interestingly, the AUDPC values for hexaconazole (496.875) were comparable, but slightly higher by 14 %, to those of encapsulated *Bacillus amyloliquefaciens* D203 (369.375) as shown in Table 4. This suggests that if optimized, strain D203 could be effective in managing rice blast disease.

Similarly, tricyclazole exhibited a lower AUDPC (25.93 %) than the control, demonstrating its effectiveness as a curative agent.

**Table 4**
AUDPC values.

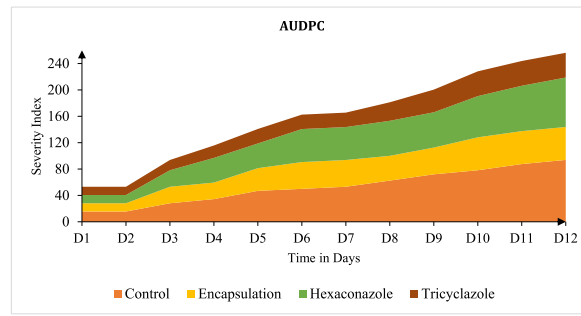| Treatment | AUDPC Values |
|---|---|
| Control | 695.50 |
| ESCA (*Bacillus amyloliquefaciens strain D203*) | 369.375 |
| Hexaconazole | 496.875 |
| Tricyclazole | 230.650 |

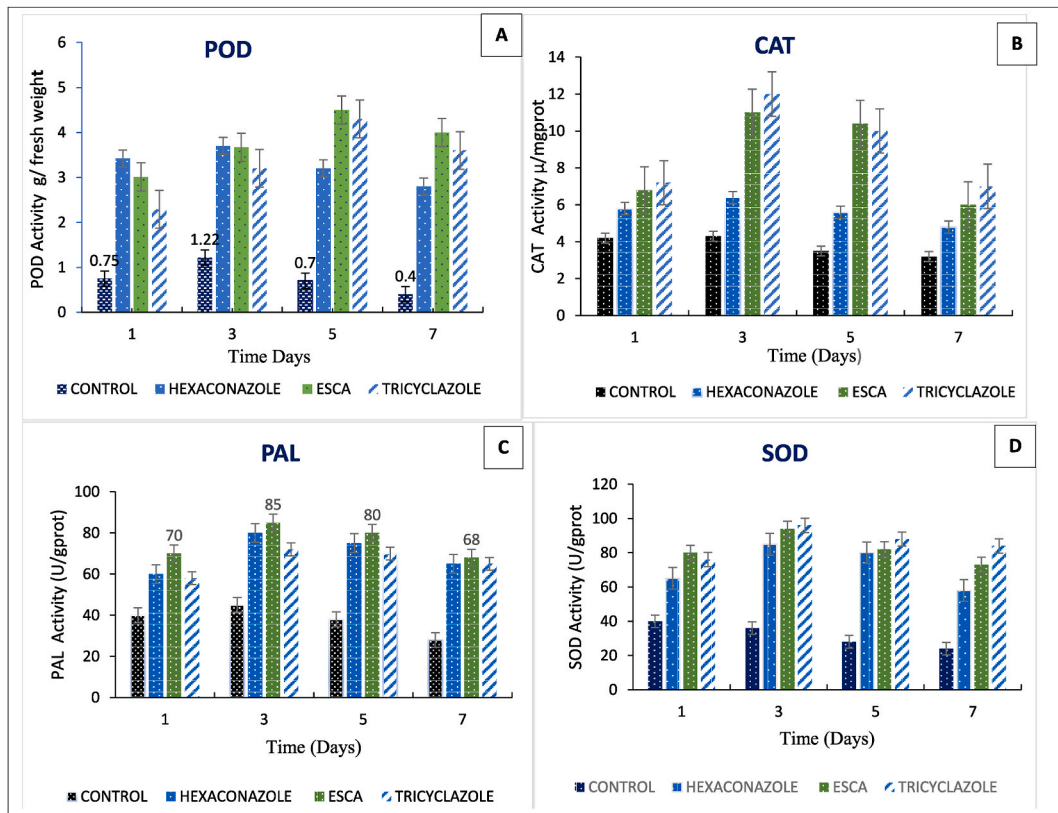**Fig. 5.** Area under disease progress curve (AUDPC).



**Fig. 6.** Quantative changes in defense enzymes (antioxidants)(A) Peroxidase (POD), (B) Catalase (CAT), (C) Phenyl alanine lyse PAL and (D) Superoxide Dismutase (SOD).

However, the AUDPC values imply that tricyclazole is not as effective in suppressing rice blast as some other treatments, such as hexaconazole.

### 3.4. Defense-related enzymes bioassay

From the assays and graphs, the activity of defense-related enzymes PAL, SOD, POD, and CAT increased significantly compared to the control after inoculation with the M. oryzae KE0002 strain. The antioxidant catalase activity increased drastically by 30 %, 48 %, and 52 % respectively in all climax treatments with climax on the third day with a sharp decline in levels on day 7 (38 %), as shown in Fig. 6B. It is, however, interesting that the ESCA treatment performed insignificantly different as compared to the chemical treatments elucidating its efficiency in promoting plant defense.

This enhanced the rice plant's ability to resist *Magnaporthe oryzae* infection by eliminating hydrogen peroxide from cells, reducing the biotic stress.

The evolution of SOD was not significantly different between treatment with hexaconazole, ESCA, and tricyclazole in the first five

days (Fig. 6D). There was a slight decrease in SOD levels on day 7 between chemical treatments i.e.; hexaconazole (5 %) and (4 %) tryclazole, compared to the ESCA treatment, which registered a 15.9 % drop. The evolution of peroxidase increased significantly after inoculating rice with M. *oryzae* in the presence of ESCA and hexaconazole (3.75 and 3.3 g/fresh weight, respectively) compared to the control (0.92 g/fresh weight). PAL levels (Fig. 6C) were consistently high with a slight decrease in treatment with 8 % ESCA, 3.7 % in Tryclazole, and 7 % in treatment with hexaconazole. While ESCA exhibits higher mean levels of POD (Fig. 6A), (3.75g/fresh weight) compared to treatment with chemical pesticides (3.28 and 3.34 g/fresh weight hexaconazole and tricyclazole, respectively) throughout the experiment. One peculiar finding is that POD activity was still high on day 7 compared to other enzymes such as SOD catalase CAT and PAL, which recorded a drastic decrease.

## 4. Discussion

Ascomycete- *Magnaporthe oryzae*, the causative agent of rice blast fungus, is the most important disease of rice in the world. It infects rice (*Oryza sativa),* potentially causing 70–100 % yield loss [26]. Severe epidemics occur in the tropics and subtropics because climatic conditions favor the spread and virulence of M. *oryzae,* Upland rice is the most susceptible due to the warm and high relative humidity of 80–85 %.

This research demonstrated that the encapsulation of *Bacillus amyloliquefaciens D203* as an alternative seed coating agent could sustain production where rice blast reduces yields. From the experiment, the potential of various treatments for managing rice blast disease is demonstrated. Encapsulation of D203 strain has emerged as a promising method for controlling rice blast, particularly when the encapsulation technique is optimized. Despite an increase in disease incidence, severity remains below economic threshold levels. This observation is crucial because it indicates that, even in the presence of the pathogen, the plant maintains its photosynthetic capacity, minimizing the impact on grain production. These findings are similar to the findings of [6] while working on the encapsulation of plant growth-promoting bacteria in alginate beads enriched with humic acid retaliated that encapsulation was an effective method to protect Plant growth-promoting inoculum against adverse environmental conditions.

While this is so, there is a need for extensive research as to whether there is a need to study the effects of supplementation of the encapsulated strain D203 with a spray of Bacillus *strains* D203 in the boosting of immunity. Harish et al. [49] while working on brown spot disease (*Bipolaris oryzae)* reported that the combination of plant extracts *Nerium oleander* and *Pithecolobium dulce* with *Trichoderma* sp biocontrol was effective in the management of *Cochliobolus miyabeanus* by up to 80 %.

Hexaconazole, on the other hand, effectively manages rice explosions as a preventive measure by maintaining low incidence levels. However, the fact that disease severity increases over time implies a potential loss of protection for the fungicide due to pathogen resistance or degradation caused by environmental factors. This aligns with studies that highlight the development of resistance in pathogens to chemical treatments over extended periods.Vinodkumar et al. [50], for instance, explains that *Scleretonia sclelotiorum* has already developed resistance to carbendazim.

Tricyclazole, a curative agent that acts after infection, demonstrates a suppressive effect on disease severity, stabilizing at around 37.5 %. However, the timing of application could influence the efficacy of tricyclazole, which is crucial. Studies emphasize the importance of precise timing in fungicide application for optimal disease control [51].

Chemical pesticides, as discussed by Vinodkumar et al. [50] pose a significant risk to the environment and human health. His views align with broader discussions on the environmental impact of chemical fungicides and the importance of sustainable, eco-friendly alternatives.

The D203 strain of *B. amyloliquefaciens* highlights the potential for biological control. The controlled release of these strains maintains protection, resulting in a lower disease severity index despite high levels of incidence. These findings correspond to the findings of Zhu et al. [4] while investigating the role of *Bacillus subtilis* in growth and control of *M. oryzae.* The studies revealed lower blast disease indexes in the group treated with B. *subtilis* JN005 at $1.58 \pm 0.13$ and $2.16 \pm 0.13$ during the seedling and maturity stages of rice plants, respectively, compared to $1.32 \pm 0.32$ and $1.64 \pm 0.25$ derived from the group treated with *B. subtilis* WP.

The AUDPC analysis provides a comprehensive assessment of the progression of the disease under different treatments. Tricyclazole exhibited a lower AUDPC compared to the control, demonstrating its effectiveness as a curative agent. However, the AUDPC values imply that tricyclazole is not as effective in suppressing rice blast as some other treatments, such as hexaconazole. These findings corresponds to the findings of [52–54] in their independent assessments of melanization inhibition of *Pyricularia oryzae* by tricyclazole found little or no toxicity to the pathogen in vivo experiments suggesting that the activity of tricyclazole is indirect. Tokousbalides & Sisler [52] while investigating the effects of tricyclazole on the growth and secondary metabolism of *Pyricularia oryzae,* found that tricyclazole was toxic to *P. oryzae* only in high concentrations. This finding suggests that only high doses are effective and, hence, expensive in production. The findings of Wolkow et al. [55] while investigating the effect of inhibitors of melanin biosynthesis on the structure and function of the *Colletotrichum lindemuthianum* appressoria in common beans also highlighted that tricyclazole prevented infection only of unwounded plant tissues but not those with wounded tissues and was not toxic to the pathogen at low levels.

Encapsulation of *Bacillus amyloliquefaciens* D203 emerges as a promising alternative, which could match the effectiveness of chemical fungicides. These findings underscore the importance of investigating eco-friendly alternatives, such as biocontrols and encapsulation, in developing sustainable strategies for disease management.

The evolution of reactive species in the ESCA plants in all the treatments after inoculation with the K0002 strain of M. *oryzae* demonstrates a higher peak i.e. SOD, PAL, POD, and CAT compared to the water control. The results suggest that strain D203 of B. *amyloliquefaciens* helped to reduce the oxidative stress by up-regulation of the SOD, CAT, PAL, and POD enzymes, which improved the defense apparatus of plants. Similar findings by Ref. [4], demonstrated that treatment of rice seeds with Bacillus Subtilis led to the evolution and accumulation of antioxidant enzymes SOD,PAL,POD and CAT in both leaves and roots. Similar studies by Ref. [38],

while working on the role of different concentrations of ESCA of *Bacillus subtills SL-13* ESCA in cotton increased the production of SOD, POD, and MDA enzymes that induced systemic resistance.

In a parallel study, Aldayel et al. [32] investigated the effects of *Bacillus amyloliquefaciens* IKMM and zinc nanoparticles as potential biocontrol agents, which induce systemic resistance in tomato plants against early blight pathogen. They observed that the activities of peroxidase (POD) increased notably 48 h post-inoculation. These findings correspond to our research in some way whereby POD activity increased after 3 days; however, peak activity was recorded on the 5th day. Interestingly as compared to the other enzymes, the activity of POD seemed to be extended since by the 7th day its activity was still high and hence its ability to sustain defense. Tu et al. [38] also retaliated that the increase of PAL and SOD not only protected plants from scavenging reactive oxygen species but also promoted the growth of shoots and radicles. The finding of Fathi et al. [56] also demonstrated that encapsulation of *Pseudomonas* strain (VUPF506) in alginate was able to promote root proliferation and reduce stress despite the presence of *Rhizoctonia solani* in potato plants. This result shows that encapsulation was able to induce resistance in the potato plants.

## 5. Conclusions

The study aimed to evaluate the effectiveness of *Bacillus amyloliquefaciens* D203 in the management of rice blast disease. The research provided valuable information on the symbiotic relationship between the bacterium and rice plants. *Bacillus amyloliquefaciens* has the potential to form beneficial relationships with various plant species in diverse environments, as demonstrated in previous studies.

The study proposes an alternative strategy that involves the encapsulation of Bacillus *amyloliquefaciens* D203, which has shown promising outcomes in the management of rice blast disease. This study explored a novel approach combining biological control methods with nanotechnology. The resulting formulation enhanced plant growth and protected rice plants against rice blast disease. Greenhouse experiments showed that encapsulating beneficial bacteria is crucial for controlling plant pathogens. Despite an increase in disease incidence, the severity remains below economic threshold levels, showcasing the potential of biological control agents and emphasizing the importance of eco-friendly alternatives in disease management strategies. Encapsulation technology, when properly optimized, proves to be effective in safeguarding beneficial bacteria against adverse environmental conditions.

Furthermore, research suggests that the encapsulated strain D203 contributes to enhancing the plant defense mechanisms, as evidenced by upregulation of antioxidant enzymes such as SOD, CAT, PAL, and POD. These enzymes play a crucial role in mitigating pathogen-induced oxidative stress, thus enhancing the plant defense system.

This discovery is consistent with previous studies on the ability of Bacillus strains to induce systemic resistance and stimulate plant growth. Although beneficial bacteria encapsulation presents a promising avenue, further investigation is necessary, including optimizing the encapsulation technique and exploring potential synergies with other biocontrol agents or supplements to enhance plant immunity.

The research emphasizes the importance of sustainable and eco-friendly approaches in the management of agricultural diseases and highlights the potential of biological control agents, such as encapsulated Bacillus strains, as viable alternatives to traditional chemical pesticides.

## Data availability

Data will be made available upon reasonable request.

## CRediT authorship contribution statement

**Francis Mirara:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Daniel Kwadjo Dzidzienyo:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Maina Mwangi:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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