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Research Paper

ALGINATE-SEED ENCAPSULATION CONTAINING ENDOPHYTIC *Bacillus cereus* BTH21 FOR BIOCONTROLLING WILT DISEASE IN EGGPLANT

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ARTICLE HIGLIGHTS

Seed encapsulation using alginate and *B. cereus* BTH21 for biocontrol of wilt disease in eggplant.

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ABSTRACT

Bacterial wilt is a major plant disease caused by *Ralstonia solanacearum* that infects important crops, such as eggplant, causing wilt, stunted growth, and reduced yield. The biocontrol of the pathogen can be done by employing antagonistic bacteria, yet direct inoculation into the soils are often ineffective due to low population of the pathogen in the soils, which also easily washed away by watering activity. Seed encapsulation technique, using alginate, zeolite and peat, containing antagonistic bacteria *Bacillus cereus* BTH21 to control *R. solanacearum* is the novelty proposed in this research. Hence, this research aimed to develop alginate seed-encapsulation technique containing *Bacillus* cells as a mean to biologically control *R. solanacearum* in eggplant. Endophytic *Bacillus cereus* BTH21 strains was isolated from healthy eggplant tissue obtained from Kediri, East Java Province, Indonesia. Subsequent isolation, purification and molecular identification confirmed the identity of the strain. Three seed-encapsulation formulations were made: a) control (no encapsulation); b) alginate + zeolite + *Bacillus* (Al+Z+B); and c) alginate + peat + *Bacillus* (Al+P+B). A field experiment was designed in a Completely Randomized Design (CRD) where the alginate-encapsulated eggplant seeds were sown along with deliberate *R. solanacearum* inoculation (107 cfu/mL) into the soils. Observations were carried out every 7 days from 0 – 49 days after sowing (DAS) on several parameters, i.e., incubation period, infection rate, germination percentage, plant height, and number of leaves. The data were analyzed by using analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of $P < 0.05$. The results showed that both seed encapsulation formulations prolonged incubation period (15 and 13 DAS) compared to the control (10 DAS), reduced infection rate, increased germination rate, as well as higher agronomic performances (plant height, number of leaves) compared to the control at 7 – 49 DAS. Overall, this results indicated the potential of alginate seed-encapsulation techniques containing biocontrol agents to control plant diseases.

Keywords: alginate, *Bacillus*, bacterial wilt, biocontrol, endophytic, seed encapsulation

INTRODUCTION

Eggplant (*Solanum melongena* L.) is an important vegetable crops from the nightshade family of Solanaceae. Eggplant is used quite extensively in Indonesian cuisine due its nutritional content (protein, vitamins, minerals, phenols, flavonoids, and anthocyanin) (Sharma & Kaushik 2021) and unique bitter-astringent flavor that become tender once cooked (Emeribe & Ogbuehi 2024). Eggplant production and consumption in Indonesia is still lacking. Recent data showed that national per capita consumption of vegetables (40.66 kg/cap/year), including eggplant, is still lower than the recommendation from FAO (73.00 kg/cap/year) (Saliem & Ariningsih 2015). In 2022, Indonesia produced approximately 0.7 million

tonnes of eggplant, but this accounted for only 1.15% of global eggplant production, which was disproportionate to Indonesia's share of the world population (FAO 2025).

Bacterial wilt disease caused by *Ralstonia solanacearum* hampers eggplant production. Once attacked, the plants develop symptoms, such as wilted and yellowing leaves as well as the appearance of a milky white bacterial fluid which is a mass of bacteria which accumulates on the surface of the stem of the newly cut infected host. This pathogen is widely spread in many countries, harbored in multiple hosts, and capable to cause 100% harvest failure (Gbonamou *et al.* 2020). This pathogen also known to survive for quite a long time in soil or water when there are no host plants available. The pathogen also thrives in hot and humid environment, accumulates in the soil for extended periods of time, can also lead to disease outbreaks when environmental conditions become favorable for infection (Ascarrunz *et al.* 2011; Lie *et al.* 2016).

Conventional control by using chemical bactericides has been criticized for inconsistent results, residue buildups, and adverse effects on human health and environments. Therefore, alternative strategies to control the wilt disease bacteria must be developed to provide safe and high quality eggplant products (Sun *et al.* 2023).

Endophytic bacteria, such as *Bacillus* sp., can be used to biologically control *R. solanacearum*. This bacteria live within plant tissues in a mutualistic relationship without causing negative effects, while also provides protection from the pathogens and promote plant growth due to multiple mechanisms. This bacteria produce siderophore, antibiotics, as well as induce plant systemic resistance, to protect plants against the pathogens. Plant growth promotion is provided by producing phytohormones (auxin, cytokinin), fixing atmospheric nitrogen, and increasing the bioavailability of nutrients (phosphate, calcium) (Santos *et al.* 2018). For instance, B. subtilis R31 is shown to stabilize colonies in the rhizosphere and root tissues and to reduce the incidence of *R. solanacearum* wilt disease in tomato (Sun *et al.* 2023). A similar work also reported the efficacy use of four *Bacillus species,* e.g., *Bacillus subtilis*, *B. cereus, B. pumilus*, and *B. amyloliquefaciens* to control *R. solanacearum* in an in vitro condition (Singh *et al.* 2016).

Beside the bacterial strain, it is important to optimize the method for delivering the bacteria into the soils. Conventional methods, such as soaking roots in the bacterial suspension, drenching the soils with the suspension, or mixing the bacteria with fertilizers, are often considered ineffective because the suspension is easily washed away by water, hence fail to maintain high population of active bacterial cells in the rhizosphere. Another consideration is that bacterial strains are often isolated from other environments that are different from the target soil, which may reduce their survival probability due to environmental discrepancies (Castro-Sowinski *et al.* 2007; Lopes *et al.* 2021). Hence, it is important to develop an alternative strategy to deliver the bacterial strain, ensuring high survival and population.

Alginate seed encapsulation containing bacterial cells is a recent technology for delivering active bacterial cells into the soil. Alginate is a biocompatible and biodegradable natural polymer derived from seaweed that forms hydrogels when crosslinked with calcium ions (Ca^{2+}) (Rohman *et al.* 2021). Active bacterial cells can be mixed with an alginate solution, entrapping and immobilizing them within the encapsulation matrix to provide a supportive microenvironment for containing and delivering the cells into the soil (Riseh *et al.* 2021).

To produce encapsulated seeds, plant seeds can be dipped into the solution to form alginate-coated seeds containing the cells (Berninger *et al.* 2016). Additionally, other carrier materials, e.g., zeolite and peat, can be blended with alginate to enhance capsule properties, such as increasing structural integrity, improving controlled-release mechanisms, and creating a more supportive microenvironment for the entrapped cells (Hurtado *et al.* 2022). Zeolite is an inorganic crystalline materials that provides better structural integrity and stability, moisture retaining ability, and ion exchanges capability, improving capsules properties and microenvironments for the cells (Ciarleglio *et al.* 2023). In a similar fashion, peat has porous structure, high water-holding capacity, and nutrients content which will improve bacterial survivability for a long time (Reddy 2012; Novinscak & Filion 2020). Several works documented the success of seed-encapsulation technique to entrap active cells. For instances, *Trichoderma harzianum* was immobilized in alginate capsule to control *Bipolaris oryzae* causing brown spot disease in rice (Anuar *et al.* 2019),

Pseudomonas putida was encapsulated in alginate/gelatin beads to control *Fusarium solani* on potato (Pour *et al.* 2019), and *Bacillus megaterium* was immobilized in alginate microcapsules to control *Rhizoctonia solani* rice sheath blight disease (Wiwattanapatapee *et al.* 2013).

Therefore, the aims of this research were: a) to isolate and identify endophytic *Bacillus* strains from eggplant tissue; b) to develop an alginate seed encapsulation formulation blended with zeolite and peat to immobilize and deliver *Bacillus* cells into the soil; and c) to evaluate the in vivo efficacy of the *Bacillus*-embedded seed encapsulation technique for biologically controlling bacteria causing wilt disease and improving eggplant growth. The results of this research will provide a scientific contribution to the potential of bacterial-containing seed encapsulation technology for controlling plant diseases, particularly bacterial wilt caused by *R. solanacearum*.

MATERIALS AND METHODS

Isolation and Rejuvenation of *Bacillus cereus* BTH21 and *Ralstonia solanacearum*

The endophytic bacterial strain *B. cereus* BTH21 was obtained from a previous exploration study conducted in Kediri, East Java Province (Purnawati & Nirwanto 2021). The strain was isolated from healthy eggplant plants. Meanwhile, *R. solanacearum* was also collected from infected eggplants at the same location. Both bacterial stocks were stored long-term on nutrient agar (NA HiMedia Laboratories Pvt.Ltd. Maharasthra, India) overlaid with paraffin oil. For daily use, the stocks were rejuvenated by streaking them onto fresh NA media.

Molecular Identification of *Bacillus cereus* BTH21

Species identification was done molecularly by sequencing 16S rRNA gene of BTH21 isolate. The DNA was isolated by using QIAamp DSP DNA FFPE Tissue Kit (Qiagen N.V., Jerman) while following the instructions from the manufacturer. The DNA was further amplified using primer pairs 27F (5'-AGAGTTTGATCMTGGCTCAG-3') as the forward primer and 1492R degenerate primer (3'-TACGGYTACCTTGTTACGACT-5', Y = C or T) as the reverse primer (Isik *et al.* 2014).

Sequence amplification was carried out with a 2720 Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Massachusetts, USA). The PCR cocktail was prepared using RTG beads (GE Healthcare, UK), which contained BSA, dATP, dCTP, dGTP, dTTP, 2.5 units of pureTaq DNA polymerase, and a reaction buffer. Each bead was sufficient for a final reaction volume of 25 µL, providing a concentration of 200 µM for each dNTP in 10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl2. The composition of the reaction for a final volume of 25 µL included 1 RTG bead, 3 µL of primers (5 pmol/µL), 3 µL of DNA sample (± 5 ng/µL), and 19 µL of ddH_2O.

The PCR program was set as follows: pre-denaturation at 95 °C for 5 minutes; denaturation at 94 °C for 1 minute; annealing at 55 °C for 1 minute; extension at 72 °C for 1.5 minutes; and a final extension at 72 °C for 5 minutes, for a total of 35 cycles. The PCR products were confirmed by electrophoresis on a 1% (w/v) agarose gel. To check the quality and quantity of each PCR product, a mixture of 5 µL of amplicon (PCR product solution) and 1 µL of 10x BPB was prepared. The mixture was loaded onto the gel and run for 30 minutes at 100 V. The bands were observed and documented using a gel documentation system. The extracted DNA was then sequenced by using Next-Gen sequencing method and a phylogenetic tree was constructed by using neighbor joining (unrooted tree) BLAST Tree method.

Preparation of Alginate-Seed Encapsulation Containing *Bacillus cereus* BTH21

B. cereus BTH21 was first cultured in nutrient broth (NB HiMedia Laboratories Pvt.Ltd. Maharastrhra, India) medium by means of shaking for 3 days to prepare the inoculant. Subsequently, the culture's absorbance was measured on a UV-vis spectrophotometer and the density of the culture was set to 108 cfu/mL. The bacterial cells were then harvested by centrifugation at 8,000 rpm for 15 minutes The cell pellet was then diluted by using normal saline solution (NaCl 0.85% b/v) as inoculant to be used in the next step.

Two eggplant seed encapsulation formulations were prepared, i.e., a) alginate + zeolite + *B. cereus* BTH21 cells (Al+Z+B) and b) alginate + peat + *B. cereus* BTH21 cells (Al+P+B). Non-encapsulated seeds were also prepared as a control, resulting in three treatments tested in this study. To prepare the encapsulated seeds, a 3% (w/v) sodium alginate (Na-alginate) solution was first prepared as the primary matrix. Then, 1% (w/v) zeolite or peat was added and thoroughly mixed into the alginate solution, serving as the additional carrier material. Subsequently, a *B. cereus* BTH21 cell suspension

(10^8 cfu/mL) was added as the inoculant. The mixture was stirred thoroughly to ensure even distribution of the bacterial cells, forming the final encapsulation matrix. Next, eggplant seeds were dipped into the matrix and gently dropped into a hardening solution containing 2% (w/v) calcium chloride ($CaCl_2$). Hydrogel capsules were immediately formed and left to harden for 10 minutes. The encapsulated seeds were then harvested, air-dried, and stored at room temperature for further use.

Field Experiment

A field experiment was conducted to evaluate the efficacy of alginate-encapsulated eggplant seeds containing *B. cereus* BTH21 in controlling *R. solanacearum* in vivo. The experiment was conducted at the experimental garden of the Faculty of Agriculture Faculty, UPN "Veteran" Jawa Timur, located at 7°9′ – 7°21′ S and 112°36′ – 112°54′ E. The field experiment was arranged in a Completely Randomized Design (CRD) with three treatments of seed encapsulation formulations: a) non-encapsulated seeds as the control; b) alginate + zeolite + *B. cereus* BTH21 (Al+Z+B); and c) alginate + peat + *B. cereus* BTH21 (Al+P+B). Each treatment was replicated three times.

The encapsulated eggplant seeds were sown at a depth of 3 cm in two rows per bed, with a planting distance of 70 × 90 cm. The seeds were allowed to germinate and grow under proper plant maintenance, which included watering, replanting, staking, and other agronomic practices. The plants were maintained until 49 days after sowing (DAS). A deliberate inoculation with *R. solanacearum* was carried out by drenching a bacterial suspension (10^8 cfu/mL) at 49 DAS, followed by incubation for 48 hours to evaluate the efficacy of *B. cereus* BTH21 in suppressing the pathogenic bacterium.

Parameters Observation and Data Analysis

The parameters observed included plant height, number of leaves, number of flowers, incubation period, infection rate, and seeds germination percentage. Agronomic performances were measured in terms of plant height (cm) and number of leaves. The incubation period (DAS) was defined as the number of days required for the first plant in each treatment to show visible bacterial wilt symptoms, such as wilting, yellowing and chlorosis of leaves, as well as soft, decayed stems and roots. Plant height and number of leaves were measured every 7 days from 7 to 49 DAS. Similarly, infection rates (%), germination percentage (%) were calculated using the following formulas:

Infection rates (%) = (number of infected plants/ total plants observed) x 100%

Germination percentages (%) = (number of seeds germinated/total seeds sown) x 100%

Analysis of variance (ANOVA) was conducted to evaluate the effects of different treatments. Duncan's multiple range test was applied when significant differences were detected by ANOVA. Statistical significance was set at $P < 0.05$. Data analysis was performed using the statistical software program SPSS version 20 (SPSS Inc.) (IBM Corp. US).

RESULTS AND DISCUSSION

Macroscopic and Microscopic Identification of Bacterial Strain

Our study showed that the macroscopic identity of bacterial strain BTH21 colonies on NA media are whitish cream, round in shape with irregular edges, the surface is flat, dry, not shiny; while microscopically, the form of the bacterial strain BTH21 is bacil (rod), Gram-positive, produces endospores (Table 1).

Logan and Vos (2015) stated that *Bacillus* sp. have various characteristics, such as flat and uneven, rough and non-slimy surfaces, dry and powdery, and not shiny. Puspita *et al.* (2017) stated that *Bacillus* sp. have rod-shaped cells, are gram-positive and have endospores.

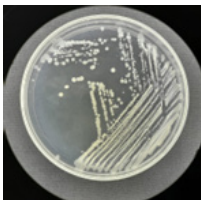
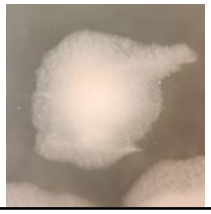
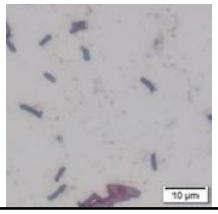
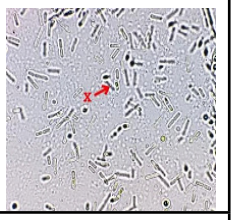
Molecular Identification of the Bacterial BTH21 Strain

The identity of bacterial BTH21 strain was assessed molecularly through sequencing the 16S rRNA gene and comparing the sequence to NCBI databases by using BLAST procedure. A primer pair of 27F and 1525R was used to amplify the gene. Further step was carried out to generate a phylogenetic tree of the isolates using neighbor joining (unrooted tree) BLAST Tree method. The results are displayed in Figure 1 below.

The band on gel electrophoresis of BTH21 indicated that the PCR product size was around 1,500 bp. Molecular identification on partial 16s rRNA gene sequence analysis indicated that the isolated BTH21 was affiliated with *Bacillus* genera

and grouped with other *Bacillus* spp., for example *Bacillus cereus*, *Bacillus* anthracis, *Bacillus* tropicus etc. obtained from NCBI nucleotide database. A 99% homology was obtained with *B. cereus* XJ-Q1XX-254B, indicated that the isolate belonged to *Bacillus cereus* species. Hence, the full identity of the isolate is *Bacillus cereus* BTH21.

Table 1 Macroscopic and microscopic identification of bacterial strain

Macroscopic	Microscopic		
			
Colony	Colony (stereo microscope, 40x)	Cell and Gram (compound microscope, 100x)	Endospore (stereo microscope, 100x)

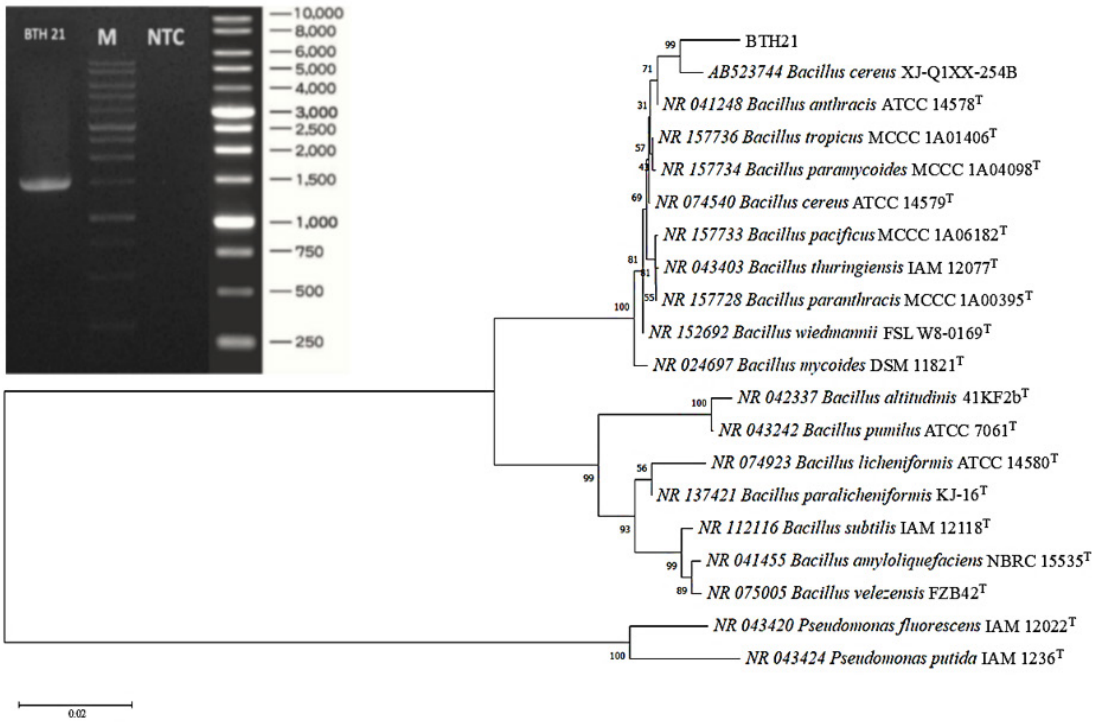


Figure 1 Visualized PCR product of BTH21 isolate 16s rDNA gene on agarose gel (inset) and phylogenetic tree of the isolate, generated using neighbor joining (unrooted tree) BLAST Tree method

Incubation Period

The incubation period is defined as the time required for the first visible emergence of bacterial wilt disease symptoms caused by *R. solanacearum* in eggplants grown from different seed encapsulation formulations. The inoculation of *B. cereus* BTH21 cells into the encapsulation matrices prolonged the incubation period of *R. solanacearum* compared to the control treatment. The data also indicated that the combination of alginate + zeolite + *Bacillus* cells (Al+Z+B) resulted in a longer incubation period compared to alginate + peat + *Bacillus* (Al+P+B) (Fig. 2).

The data indicated that the efficacy of *Bacillus*-embedded seed encapsulation techniques was able to prolong the incubation period of *R. solanacearum* in eggplants. The incubation period of *R. solanacearum* for *B. cereus* BTH21 treatment was 13 – 15 days with a standard error of ± 1 (Fig 2). The efficacy may have been due to synergistic association between the encapsulation matrices, i.e., alginate/zeolite and alginate/peat blend, and the *B. cereus* BTH21 cells embedded within the matrices. Alginate, as the primary material for seed encapsulation, provided a physical barrier that shielded the seeds from direct contact with outer environments (De Castro *et al.* 2020) including the pathogenic agents, such as bacteria and fungi (Luo *et al.* 2019), thereby delaying infection and extending the incubation period. Alginate is extensively used recently and deemed as a suitable material for bacterial encapsulation due to its biodegradability, biocompatibility and low-cost (Riseh *et al.* 2021; Martínez-Cano *et al.* 2022).

Moreover, the addition of either zeolite and peat to blend with alginate further improve the encapsulation performance, prolonging their activity in biocontrolling the pathogen as shown in Figure 2. Single use of alginate has its own drawback, such as mechanical instability, rapid losses of moisture and limited control over release profile (Berninger *et al.* 2016; Hurtado *et al.* 2022). The use of zeolite provided structural function to ensure the durability and longer shelf live (Ciarleglio *et al.* 2023; Ilinskaya *et al.* 2023), while also providing conducive environment for living cells, such as bacteria (Xiong *et al.* 2023). Meanwhile, peat which containing organic matter provides nutrient and moisture for the entrapped cells, protecting the cells from environmental stresses and ensuring survival of the cells during storage and application (Novinscak & Filion 2020).

The data also suggested the efficacy of *B. cereus* BTH21 in biocontrolling *R. solanacearum*. *Bacillus* is among genera that is extensively studied as antagonist for biocontrol of plant pathogens, in particular *R. solanacearum*. Several works reported the use of *B. cereus*, *B. amyloliquefaciens*, and *B. subtilis* to control *R. solanacearum*. Those studies also unraveled the responsible direct or indirect mechanisms of action including production of biocontrol agents (antibiotics, enzymes, volatile organic compounds, etc.) and inducing plant systemic resistance (Raza *et al.* 2016; Wang *et al.* 2019; Sun *et al.* 2023). Moreover, the use of encapsulated bacteria to control plant disease is among the most recent technology that is deemed better than conventional direct inoculation into the soils. The efficacy has been proven in many instances for seed-encapsulation containing bacteria (Wiwattanapatapee *et al.* 2013; Pour *et al.* 2019;) or fungi (Anuar *et al.* 2019; Coninck *et al.* 2020).

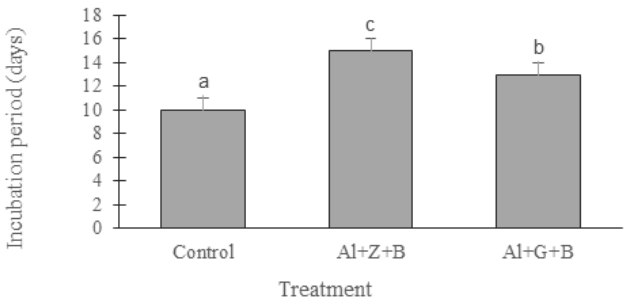


Figure 2 Incubation periods of *R. solanacearum* leading to visible wilt disease symptoms in eggplants grown from different encapsulation formulations

Infection Rate

The infection rate of *R. solanacearum*, which caused wilt disease in eggplants grown from different *Bacillus*-embedded encapsulated seeds (Fig. 3). The inoculation of *B. cereus* BTH21 cells into the encapsulation matrices reduced the infection rate of *R. solanacearum* in eggplants compared to the control treatment. In the control treatment, the infection rate peaked at 28 DAS (23%) and remained stagnant until 49 DAS. Meanwhile, in the *Bacillus*-embedded treatments, the infection rate peaked at 21 DAS (17% for Al+Z+B; 18% for Al+P+B) and showed a declining trend over time until 49 DAS. Standard error of infection rate in control ± 0 – 1; in A+Z+B treatment ± 1; in Al+P+B treatment ± 0 – 1 (Fig. 3).

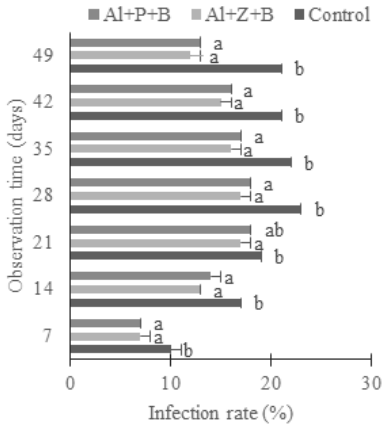


Figure 3 Infection rate of *R. solanacearum*-caused wilt disease in eggplants grown from different encapsulation formulations observed from 0 – 49 DAS

The data further support the previous finding regarding the potential use of alginate-based seed-encapsulation techniques to immobilize and deliver *B. cereus* BTH21 into the soil through a slow-release mechanism. The efficacy of *Bacillus* species in biologically controlling wilt disease caused by *R. solanacearum* was demonstrated in this research, as well as in numerous other reports with similar findings (Raza *et al.* 2016; Wang *et al.* 2019; Sun *et al.* 2023).

Our study also highlights the effectiveness of delivering the bacterium through a slow-release mechanism, due to alginate seed encapsulation, which prolongs disease protection. Alginate is a natural and biodegradable polymer, which, when applied to soil, is gradually degraded, releasing the bacterial cells entrapped within the matrix (He *et al.* 2015). This ensures a slower and more sustained delivery of bacterial cells into the soil, thereby extending the protective effect against *R. solanacearum* (Fig. 3), where infected plants showed decreasing infection rates compared to the control.

Germination Percentage

The inoculation of *B. cereus* BTH21 into the encapsulation matrices increased the germination percentage of eggplant seeds compared to the control treatment (Fig. 4). In both *Bacillus*-embedded seed treatments, i.e., Al+Z+B and Al+P+B, the germination rates reached as high as 15% by 49 DAS, whereas in the control treatment, the germination rate remained stable at 10% until 49 DAS. Standard error of germination percentage in control ± 0.5 - 1; in A+Z+B treatment ± 0.25 – 0.5; in Al+P+B treatment ± 0 – 0.05 (Fig. 4).

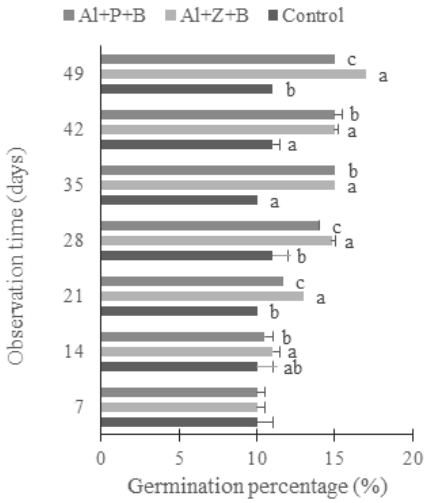


Figure 4 Germination percentage of *R. solanacearum*-caused wilt disease in eggplants grown from different encapsulation formulations observed from 0 – 49 DAS

Plants sown outside often face multiple biotic and abiotic stresses including soil-borne pathogenic bacteria, such as *R. solanacearum*. The presence of pathogens makes the soil a sub-optimal environment for seed germination process, where seed vigor is often compromised, causing lower seedling germination percentages, delayed seedlings emergence, or less vigor plants. The use of biocontrolling agents is a promising strategy to improve seed germination process and seed vigor on sub-optimal condition such as on the presence of plant pathogen (Lamichhane *et al.* 2018). Loliam *et al.* (2012) demonstrated the biocontrol of seedling damping off disease in several economic crops by using *Streptomyces rubrolavendulae*. Carvalho *et al.* (2011) reported

the use of *Trichoderma* to biocontrol *Aspergillus, Cladosporium*, and *S. sclerotiorum* in common beans seedling. Similarly, Zhu *et al.* (2020) displayed the ability of B. pumilus to prevent seedling damping off disease caused by *R. solani*.

In our study, the efficacy of *B. cereus* BTH21 in biocontrolling *R. solanacearum* in eggplant seed germination is demonstrated. In alginate-encapsulated eggplant seed containing *Bacillus* cells, the germination rate was higher compared to the non-encapsulated control seeds. *Bacillus* ability to control *R. solanacearum* has been demonstrated in this study, as well as in other similar studies (Raza *et al.* 2016; Wang *et al.* 2019; Sun *et al.* 2023). Nonetheless, immobilization of bacterial cells within alginate/zeolite and alginate/peat matrices provided a safe, non-toxic, and supportive environment for the cells to survive, while released in a slowly-manner, prolonging the duration of the protection (He *et al.* 2015; Pour *et al.* 2019). Our study also indicated the increase of the number of seedling emerged in the encapsulated seed treatment compared to that in the control, which otherwise shown constant germination rate until 49 DAS. The presence of the pathogen was indeed constraining the plants to exercising optimal vigor, yet upon the protection of the antagonistic *Bacillus*, this constraint was somewhat alleviated to a lower degree.

Agronomic Performances

In terms of agronomic performances, eggplants grown from encapsulated seeds containing living cells of *B. cereus* BTH21 displayed better results compared to the growth in the control treatment (Fig. 5). Standard error of plant height in control ± 0.115 – 0.17; in A+Z+B and Al+P+B treatments ± 0.058 – 0.12 (Fig. 5A).

The plant height of eggplants grown from encapsulated seeds (Al+Z+B, Al+P+B) were consistently increasing up to 28 cm at 49 DAS. In contrast, the height in control treatment was remained low up to 49 DAS. Similarly, in terms of the number of leaves, eggplants grown from *Bacillus*-embedded encapsulated seeds were consistently higher compared to that in the control treatment. Standard error of leaves number in control, A+Z+B, and Al+P+B treatments was ± 0 - 1 (Fig. 5B).

The results shown in Figure 5 indicate the plant growth-promoting effects of *B. cereus* BTH21, in addition to its previously demonstrated protective effects. Many beneficial bacteria commonly exhibit multiple mechanisms of action that support plant growth and alleviate both biotic and abiotic stresses. These mechanisms include biostimulation (production of plant phytohormones such as auxin, cytokinin, and gibberellin), bioprotection (synthesis of antibiotics, siderophores, and lipopeptides), biofertilization (nitrogen fixation and phosphate solubilization), and bioremediation (uptake of heavy metals and degradation of xenobiotics) (Mohanty *et al.* 2021). Although not specifically assessed in this study, *Bacillus* species have been shown to possess these capabilities, including the production of extracellular Indole Acetic Acid (IAA) (Shao *et al.* 2015), phosphate and zinc solubilization (Ahmad *et al.* 2021), and atmospheric nitrogen fixation (Xu *et al.* 2014). Therefore, it can be suggested that these plant growth-promoting activities, combined with the antagonistic effects of *B. cereus* BTH21 against *R. solanacearum*, result in a synergistic effect that enhances eggplants growth, as evidenced by increased plant height and number of leaves.

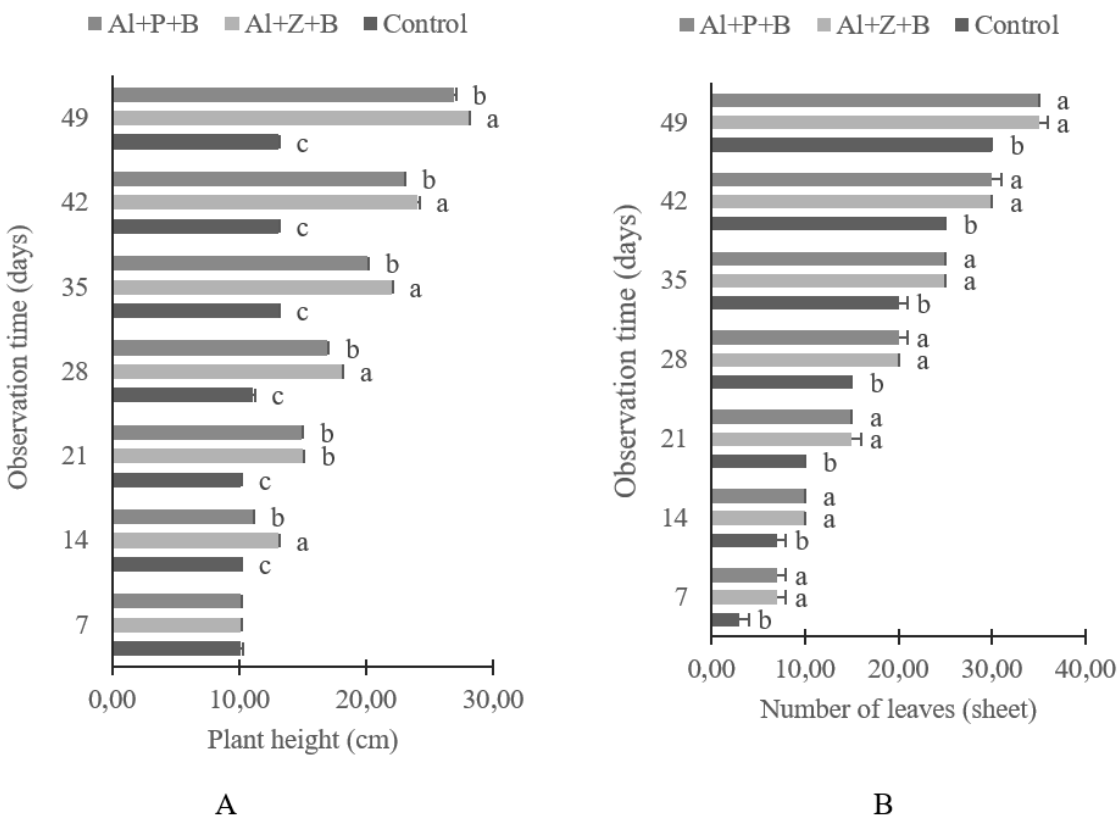


Figure 5 Agronomic performances of eggplants grown from different encapsulation formulations, while under deliberate *R. solanacearum* inoculation, observed from 0 – 49 DAS

Notes: A = plant height; B = number of leaves.

CONCLUSION

The endophytic bacterial BTH21 strain was isolated from healthy eggplant tissues. Molecular identification confirmed that the strain belonged to *B. cereus* species. Alginate seed encapsulation, blended with zeolite and peat, was successfully developed to immobilize and deliver active cells of *B. cereus* BTH21 into the soil. A field assay of sowing *Bacillus*-embedded encapsulated eggplant seeds inoculated with *R. solanacearum*, demonstrated significant biocontrol efficacy against the pathogen in terms of incubation period, infection rate, and germination percentage compared to the control treatment. The immobilized bacteria also exhibited positive plant growth-promoting effects, as indicated by higher plant height and number of leaves compared to that in the control treatment. Overall, the results highlight the potential of alginate seed encapsulation as an alternative method for delivering antagonistic organisms, providing a suitable microenvironment for the immobilized bacterial cells, while slowly releasing them into the soil, thereby prolonging the biocontrol effect against plant pathogens.

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