

Conference Paper

Chili Plant Growth Promoted by Bio-Encapsulated *Bacillus* sp. Biological Control Agents in Different Coating Materials

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ABSTRACT

Bacillus sp. is a biological control agent widely used to enhance plant growth and control diseases, especially in suppressing *Fusarium* wilt pathogens. The effectiveness of *Bacillus* species in controlling diseases varies, with isolates Bcz20, Bcz14, and Bcz16 showing the highest inhibition. *Fusarium* sp. a fungal pathogen, isolated from infected chili plants, produces characteristic crescent-shaped spores. For bio-encapsulation, various coating materials such as sodium alginate, carrageenan, and chitosan were used. Sodium alginate proved to be the most compatible coating material, effectively maintaining the *Bacillus* population over time. The encapsulation efficiency was tested through a detailed process, which revealed *Bacillus* isolate Bcz20 as the most viable among the various coating materials, especially sodium alginate. In VIVO tests on chili plants showed that bio-encapsulation of *Bacillus* sp. did not show significant differences in height but showed better root development in the treatment using *Bacillus* Bcz20 with carrageenan. *Bacillus* sp. plays an important role in enhancing plant growth and promoting systemic resistance, making it a promising agent for sustainable agriculture.

Keywords: *Bacillus* sp., biological control, *Fusarium* wilt, bio-encapsulation

Introduction

Bacillus species have emerged as significant biological control agents in agriculture, particularly noted for their ability to enhance plant growth and combat diseases such as *Fusarium* wilt (Wuryandari et al., 2022). This fungal pathogen, notorious for affecting crops like chili plants, poses a substantial threat to agricultural productivity (Morales-Rodriguez et al., 2003). Recent studies have identified specific *Bacillus* isolates, including Bcz20, Bcz14, and Bcz16, which exhibit remarkable efficacy in inhibiting *Fusarium* sp. These isolates not only suppress the pathogen but also contribute to improved root development in treated plants, highlighting their dual role in promoting plant health.

The encapsulation of *Bacillus* sp. using various coating materials—sodium alginate, carrageenan, and chitosan—has been explored to enhance the stability and effectiveness of these beneficial microbes (Khan et al., 2013; Pour et al., 2022; Suryani et al., 2019; Uyen et al., 2020; Szczech & Maciorowski, 2016). Among these, sodium alginate has proven to be the most effective coating material, maintaining viable *Bacillus* populations over extended periods. The encapsulation process was rigorously tested, revealing that isolate Bcz20 demonstrated superior viability across different coating materials, particularly under conditions that favor pathogen proliferation.

In vivo experiments on chili plants have shown that bioencapsulated *Bacillus* significantly mitigates *Fusarium* disease symptoms (Zhang et al., 2008; Elad & Freeman, 2002) especially following wet weather that typically promotes fungal growth (Agrios, 2005; Munkvold, 2003).

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However, optimal application of these bioencapsulated agents necessitates careful adjustments regarding dosage and environmental factors to maximize disease control efficacy. While growth measurements indicated no significant height differences among treatments, enhanced root development was observed in plants treated with *Bacillus* isolate Bcz16 combined with sodium alginate.

Overall, the multifaceted benefits of *Bacillus* sp. underscore their potential as sustainable agricultural solutions. Their ability to boost plant growth while inducing systemic resistance against pathogens positions them as valuable allies in the quest for environmentally friendly agricultural practices. As research continues to unravel the complexities of *Bacillus* interactions with plants and pathogens, these microorganisms may play an increasingly pivotal role in sustainable crop production strategies.

Material and Methods

Study area and source of plant material

This research was conducted in Plumbungan sub-district, Sukodono district, Sidoarjo Regency with inceptisols soil type, at an altitude of 25 m above sea level, rainfall of 235 mm/year, and an average daily air temperature of 35°C. The seeds used in this study were chili seeds of the Ori 212 variety.

Experimental design

This study was conducted using a Randomized Block Design (RBD) factorial experiment with 2 factors and 3 replications. The first factor is a different isolate of *Bacillus* sp. (B), namely B1 (*Bacillus* sp. Bcz 14 isolate); B2 (*Bacillus* sp. Bcz 16 isolate); and B3 (*Bacillus* sp. Bcz 20 isolate). The second factor is the coating material (P) consisting of 3 types of coating materials, namely P1 (sodium alginate); P2 (chitosan); and P3 (carrageenan). While the control treatment (K) is without any treatment.

Bacillus sp. Beads Formulation

The encapsulation process using sodium alginate as a coating material involves dissolving 2% sodium alginate in distilled water (aquadest) while heating until a homogeneous solution is obtained, and 3% CaCl₂ is dissolved in distilled water and stirred until homogeneous. Both solutions are sterilized using an autoclave at a temperature of 120°C and a pressure of 1 atm for 20 minutes, then cooled to room temperature. 10 ml bacterial suspensions (*Bacillus* sp. isolates Bcz 12, Bcz 14, and Bcz 20 10⁶ CFU/ml) are added to the sodium alginate solution, and the mixture is put into a syringe. The solution is dripped into the CaCl₂ solution to form beads, which are then filtered and stored in a dry container at room temperature.

The encapsulation process involves dissolving 1 gram of chitosan into 95 ml of distilled water and 5 ml of acetic acid using a magnetic stirrer for 1 hour. Additionally, 2 grams of sodium tripolyphosphate (STPP) are dissolved in 100 ml of distilled water. Both the chitosan solution and the STPP solution are sterilized using an autoclave at 121°C for 20 minutes. A suspension of *Bacillus* sp. strains Bcz 14, 16, and 20 (10⁶ CFU/ml) is prepared in 10 ml. This *Bacillus* sp. suspension is then added according to the treatment to the sterilized chitosan solution and stirred using a glass rod. The STPP solution is then poured into the chitosan-bacteria suspension mixture. The resulting mixture is filtered and stored in a container at room temperature.

The encapsulation process involves dissolving 2% carrageenan in distilled water while heating on a stove until a homogeneous solution is achieved. The carrageenan solution and CaCl₂ solution are then sterilized using an autoclave at 120°C and 1 atm pressure for 20 minutes. The carrageenan solution is cooled to 30-40°C, after which the *Bacillus* sp. (isolates Bcz 12, Bcz 14, and Bcz 20 10⁶ CFU/ml) suspension is added to the carrageenan solution. The combined suspension of *Bacillus* sp. and carrageenan is poured into a 15 cm Petri dish and left to solidify and cool. The

solidified carrageenan is then cut into cubes of approximately 3 mm using a scalpel. The carrageenan cubes are stored in a container at room temperature.

Land preparation, seedlings, and transplanting

The land used is chili land with endemic *Fusarium oxysporum* disease. Plowing the field is carried out to loosen the soil, then beds are made with a height of 30 cm, and a width of 1 m and plowed following the shape of the land. The distance between beds is 30-40 cm wide. Then the land is left for one week. Basic fertilizer is given in the form of compost as much as 10 tons ha⁻¹. Chili seeds of the Ori 212 variety are sown by making rows (rows) on the seeding medium with a distance between rows of 5 cm and a depth of 1 cm. Seeds are sown with a distance between seeds of 2-3 cm. The shoots are covered with soil and watered sufficiently. Sprouts that grow approximately 5 cm or about 10 days after planting can be moved by making planting holes with a diameter of 5-7 cm. One bed has two rows of planting holes, the distance between rows is 40 cm and the distance between holes in one row is 50 cm, the depth of the planting hole is approximately 5-7 cm.

Treatments of Bioencapsulated-Bacillus sp. formula

The purpose of this experiment was to assess the impact of the bio-encapsulation formula on the growth and production of chili plants in the field. The procedure involved transplanting 30-day-old seedlings into the field, where 30 grams of the bioencapsulation formula was applied to each planting hole. Inoculation with *Fusarium oxysporum* occurred naturally, without any artificial inoculation.

Maintenance of chili plants

Watering practices are adjusted based on the weather; in periods of high rainfall, drainage systems are established to manage rainwater absorption. During the dry season, watering is performed in the morning to prevent soil from drying out and cracking. Fertilization in this study utilized urea, SP-36, and KCl, with application rates of 180 kg/ha for nitrogen (N), 150 kg/ha for phosphorus (P2O5), and 100 kg/ha for potassium (K₂O). This equates to 6.3 g of urea per plant, 6.7 g of SP-36 per plant, and 2.7 g of KCl per plant.

Data collection

Observations of the results were carried out for 35 days with a 7-day interval. Observations of the results included; (1) Plant height (cm) measured using a ruler; (2) Number of leaves/plant measured by counting the number of strands per plant; and (3) Root length (cm) measured using a destructive method using a ruler.

Data analysis

The observation data was then analyzed using analysis of variance, Analysis of Variance (ANOVA), and carried out with the F test at an error rate of 5%, to determine the effect of the treatment applied. If there is a real difference between the treatments, a further BNJ test is carried out at an error rate of 5%.

Results and Discussion

Bioencapsulated-Bacillus sp. Formulation

Bacillus sp. beads produced from sodium alginate, chitosan, and carrageenan coating materials are presented in Figure 1. There are differences in the shape of beads in the bioencapsulation of *Bacillus* sp. Beads from sodium alginate coating materials appear perfectly round. Beads from chitosan coating materials appear irregularly round. While beads from carrageenan coating materials are asymmetrical. However, each coating material has different properties in the formation of beads. Sodium alginate has the ability to form gels in aqueous

solutions, especially when combined with calcium ions (Ca^{2+}). This provides a stable matrix for the encapsulation of active compounds (Pour et al., 2022), so it is often used to protect active ingredients from harsh environmental conditions. Chitosan has polycationic properties and can form gels or membranes through electrostatic interactions with polyanions or with other polymers (Khan et al., 2013). Carrageenan is able to form strong and elastic gels, is compatible with various materials, and has the ability to form microcapsules with uniform sizes. Chitosan, although able to form beads, can dissolve in a weakly acidic environment, so slightly acidic pH changes can change the solid form of chitosan beads. Carrageenan, on the other hand, can survive in an acidic environment but is unstable when paired with calcium ions (Uyen et al., 2020).

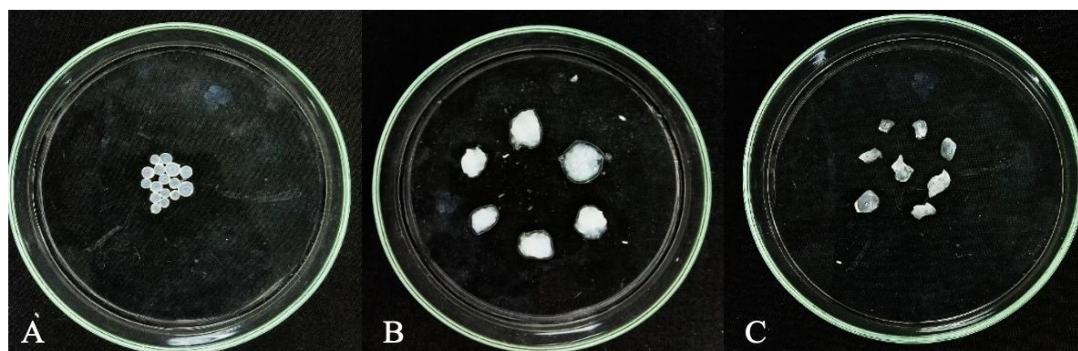


Figure 1. Bioencapsulated-*Bacillus* sp. formulation (A) sodium alginate-based beads; (B) chitosan-based beads; (C) carrageenan-based beads

Chili plant heights

Based on Table 1, there are differences in the height of chili plants between treatments of *Bacillus* sp. isolates encapsulated with several different coating materials. Observations of plant height for 35 days showed that the height of chili plants continued to increase from week to week according to the sigmoid pattern of plant growth. On the 35th day of observation, the highest chili plants were in the B3P3 treatment or *Bacillus* sp. Bcz 20 isolate encapsulated with carrageenan. While the lowest chili plants were in the B2P3 treatment or *Bacillus* sp. Bcz 16 isolate encapsulated with carrageenan.

Table 1. Chili plant heights in different treatments of bioencapsulated-*Bacillus* sp.

Treatment Code	Plant Height Observation day- (cm)				
	7	14	21	28	35
B1P1	15.21 a	16.38 ab	17.53 a	18.25 ab	19.11 ab
B1P2	15.53 b	16.96 ab	17.84 a	20.13 ab	20.88 abc
B1P3	15.28 ab	18.03 ab	19.57 ab	22.01 ab	23.55 abc
B2P1	14.25 ab	15.50 a	17.83 a	21.97 ab	24.65 bcd
B2P2	14.94 ab	15.46 a	16.12 a	17.51 ab	19.65 abc
B2P3	14.26 ab	15.49 a	16.55 a	17.08 a	17.17 a
B3P1	15.02 ab	16.27 ab	18.01 a	20.26 ab	22.17 abc
B3P2	13.45 a	16.31 ab	18.83 a	23.81 bc	26.94 cde
B3P3	14.94 ab	18.25 ab	22.57 b	29.54 c	33.20 e
K	16.14 b	18.93 b	22.55 b	28.84 c	31.55 de

Note: B1 = *Bacillus* sp. Bcz 14 isolate; B2 = *Bacillus* sp. Bcz 16 isolate; B3 = *Bacillus* sp. Bcz 20 isolate; P1 = sodium alginate encapsulation; P2 = chitosan encapsulation; P3 = carrageenan encapsulation; K = control

When viewed as a whole, the bioencapsulation formulation of *Bacillus* sp. with several coating materials was not significantly different from the control treatment (Figure 2). The results in Table 1 and Figure 2 show that the bioencapsulation formulation of *Bacillus* sp. with several

coating materials did not have any effect on increasing plant height. This is not in accordance with much literature on *Bacillus* sp. which is able to produce various phytohormones and dissolve phosphate so that plant height increases significantly (Prakash & Arora, 2019).

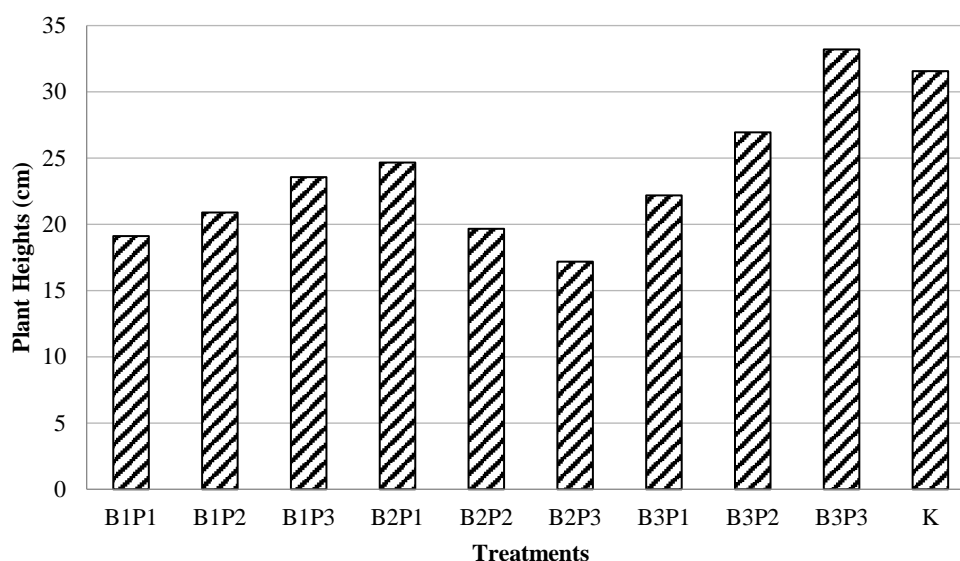


Figure 2. Average chili plant heights

Number of chili plant leaves

Based on Table 2, the treatment of *Bacillus* sp. bio encapsulation formulation has a significant effect on the number of leaves of chili plants. The highest number of leaves is in the B3P3 treatment or *Bacillus* sp. Bcz 20 isolate encapsulated with carrageenan and the B3P2 treatment or *Bacillus* sp. Bcz 20 isolate encapsulated with chitosan. While the lowest number of leaves is in the B1P1 treatment or *Bacillus* sp. Bcz 14 isolate encapsulated with sodium alginate.

Table 2. Number of chili plant leaves

Treatment Code	Number of Leaves Observation day-				
	7	14	21	28	35
B1P1	5.89 a	9.11 abc	10.44 a	12.67 a	14.22 a
B1P2	6.67 bcd	8.89 ab	11.11 a	14.00 a	17.78 a
B1P3	6.89 cd	9.44 abc	11.56 ab	15.89 a	23.11 a
B2P1	6.33 ab	9.22 abc	11.67 ab	21.33 ab	47.56 abc
B2P2	6.00 ab	9.33 abc	11.44 ab	15.89 a	27.89 ab
B2P3	6.67 bcd	8.33 a	10.44 a	12.56 a	12.44 a
B3P1	6.44 abc	9.78 abc	13.00 abc	19.56 ab	26.56 ab
B3P2	6.44 abc	10.67 c	14.56 bc	31.78 bc	74.44 cc
B3P3	6.44 abc	10.67 c	15.67 c	35.89 c	78.33 c
K	7.22 d	10.56 bc	15.67 c	32.22 bc	53.78 bc

Note: B1 = *Bacillus* sp. Bcz 14 isolate; B2 = *Bacillus* sp. Bcz 16 isolate; B3 = *Bacillus* sp. Bcz 20 isolate; P1 = sodium alginate encapsulation; P2 = chitosan encapsulation; P3 = carrageenan encapsulation; K = control

According to the statement (Prihatiningsih et al., 2015), *Bacillus* sp. can help the absorption of nutrients so that they are available to plants and produce phytohormones that are good for plant growth and development. Figure 3 shows that the B3P3 and B3P2 treatments have the highest results in increasing the leaf blades of chili plants. In the B3P3 and B3P2 treatments, chitosan and carrageenan were able to maintain the viability of *Bacillus* sp. Bcz 20. In addition, chitosan and carrageenan are easily hydrolyzed in a weak acidic environment or the presence of

calcium ions in nature, so that encapsulated *Bacillus* sp. can easily be released and colonized around the chili plant growth area.

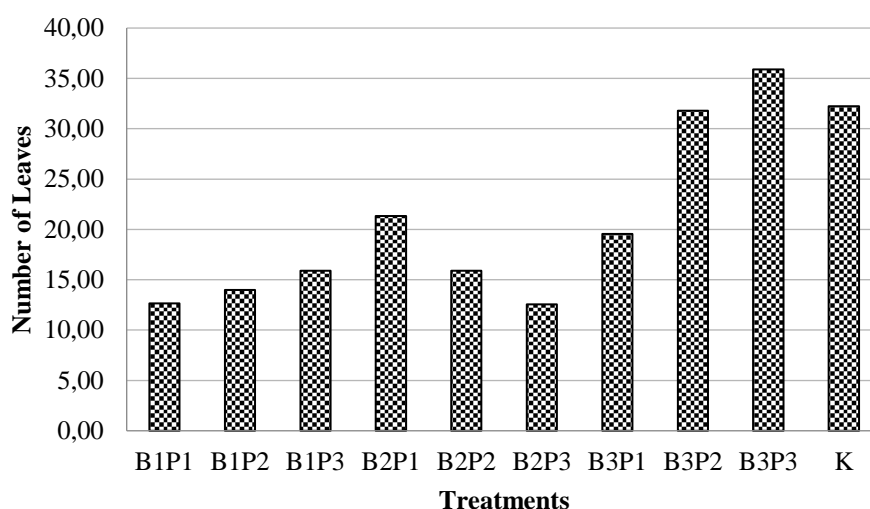


Figure 3. Average number of chili plant leaves

Chili plant root length

Based on Table 3, there are differences in root length in various treatments of *Bacillus* sp. bioencapsulation formulations. The longest chili plant roots were in the B3P3 treatment (*Bacillus* sp. Bcz 20 isolate encapsulated with carrageenan); and B3P2 treatment (*Bacillus* sp. Bcz 20 isolate encapsulated with chitosan). While the shortest chili plant roots were in the B2P1 treatment (*Bacillus* sp. Bcz 16 isolate encapsulated with sodium alginate).

Table 3. Root length of chili plants that received several *Bacillus* sp. bio encapsulation treatments

Treatments	Root Length
B1P1	19.30 a
B1P2	17.20 a
B1P3	14.30 a
B2P1	14.00 a
B2P2	19.60 a
B2P3	13.80 a
B3P1	20.00 a
B3P2	22.00 ab
B3P3	28.30 b
K	21.00 ab

Note: B1 = *Bacillus* sp. Bcz 14 isolate; B2 = *Bacillus* sp. Bcz 16 isolate; B3 = *Bacillus* sp. Bcz 20 isolate; P1 = sodium alginate encapsulation; P2 = chitosan encapsulation; P3 = carrageenan encapsulation; K = control

In Figure 4, it can be seen that the B3P3 and B3P2 treatments not only have the highest results in increasing the leaf blades of chili plants but also the highest in plant root growth. In the B3P3 and B3P2 treatments, chitosan and carrageenan were able to maintain the viability of *Bacillus* sp. Bcz 20. In addition, chitosan and carrageenan can survive in environments with neutral to hot temperatures (Saad et al., 2020), so they can survive on dry land. While sodium alginate can survive in environments that tend to be wet (Uyen et al., 2020).

Previous research has demonstrated that *Bacillus* sp. can produce phytohormones that have the potential to support the development of sustainable agricultural systems. These bacterial phytohormones indirectly inhibit pathogen activity in plants, while directly enhancing plant

growth and facilitating the absorption of nutrients from the environment. The mechanisms of biological control (Junaid et al., 2013) include (1) antibiosis, (2) competition, (3) mycoparasitism, (4) cell wall-degrading enzymes, (5) resistance induction, (6) growth promotion, and (7) rhizosphere colonization. The use of biological control agents for plant pathogens has become increasingly important as it does not leave harmful residues, are environmentally safe, and have positive effects on plants.



Figure 4. Root morphology (root length) of chili plants in all bio encapsulation treatments and controls

Studies indicate that *Bacillus* sp. can act as biofertilizers and biological control agents through mechanisms such as antibiosis, secretion of lytic enzymes, and induction of systemic resistance (Induced Systemic Resistance or ISR) (Choudhary & Johri, 2008). Additionally, these agents may indirectly promote plant growth and enhance systemic resistance. For example, *B. subtilis* B315 has been shown to reduce the severity of diseases such as leaf blight caused by *Phytophthora infestans* in potatoes, with disease intensity below 10% (Prihatiningsih et al., 2015). Other research has found that *Bacillus* sp. B.64 (Mugiastutu et al., 2019) has the potential for controlling Fusarium wilt in tomatoes due to its ability to suppress the disease, boost plant resistance, and improve plant growth and yield.

The ability of *Bacillus* sp. to induce plant resistance (Gond et al., 2015) is linked to its enhancement of peroxidase enzyme activity, production of chitinase and glucanase enzymes, and accumulation of salicylic acid, which collectively contribute to the reduction of disease intensity. Induced resistance is also associated with increased levels of phenolic compounds in plants (Malfanova, 2013). Research has shown that treatment of tomato plants with *Bacillus* sp. (Radhakrishnan, 2016) can enhance the content of phenolic compounds and positively affect plant growth and yield, as demonstrated by increased dry weight of shoots, roots, and fruits. Low disease intensity allows for better plant growth and development. Additionally, *Bacillus* sp. is known not only as a biocontrol agent but also as a PGPR (Plant Growth Promoting Rhizobacteria).

Conclusion

Bacillus sp. is a widely used biocontrol agent for promoting plant growth and controlling diseases, particularly effective in suppressing Fusarium wilt pathogens. The effectiveness of different *Bacillus* species in disease control varies, with isolates Bcz20, Bcz14, and Bcz16 showing the highest levels of inhibition. In vivo tests on chili plants demonstrated that while there was no significant difference in plant height, better root development was observed with the treatment

of *Bacillus* sp. Bcz20 with carrageenan. Overall, *Bacillus* sp. plays a crucial role in enhancing plant growth and promoting systemic resistance, making it a promising agent for sustainable agriculture.

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