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Optimization of Bioencapsulation of *Bacillus* sp. to Increase Resistance to Bacterial Wilt and Growth of Cayenne Pepper

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ABSTRACT

Cayenne pepper has a high production demand. The high demand does not match the amount of production produced. One of the factors for decreased production is the presence of bacterial wilt disease caused by Ralstonia solanacearum. Bacterial wilt control can be done with the application of antagonistic bacteria, namely Bacillus sp. Bacillus sp. application requires an optimal formulation to maintain bacterial activity in the soil, one of which is Bacillus sp. encapsulation. This study aims to determine the effectiveness of Bacillus sp. encapsulation as a formulation of biological agent bacteria for the control of bacterial wilt disease and cayenne pepper plant growth. This study used the encapsulating material concentration and dosage factors for application to plants. Encapsulation was made using the extrusion method. The results showed that the concentration of 2% sodium alginate and 1.5% gelatin gave a significant difference in disease intensity of 18.89% and in the growth parameters of cayenne pepper plants were able to reach a height of 30.58 cm and a root length of 17.18 cm. The right concentration and high dose provide the ability to suppress intensity and encourage maximum growth.

1. INTRODUCTION

Cayenne pepper is one of the horticultural commodities that has high economic value. Chili is commonly used as a raw material for the food and medicine industry (Lede *et al*., 2018). Data from the Central Statistics Agency shows cayenne pepper production in East Java Province in 2022 was able to reach 39% of the entire territory of Indonesia, with a total of 612,409 tons. However, the total production of cayenne pepper decreased in 2023 by 562,816 tons (BPS, 2023).

One of the factors in the decline in cayenne pepper production was due to attacks by plant pests (OPT). Damage to cayenne pepper plants can reach 25-100% until farmers experience crop failure (Hasyim *et al*., 2015). One of the OPTs that causes damage to cayenne pepper plants is the presence of *Ralstonia solanacearum* which causes bacterial wilt. Raihanah *et al*. (2023) explained that the intensity of disease severity in cayenne pepper plants can reach 90%. As a result, the plants will wilt and the plants will die as a whole. *R. solanacearum* will infect plants through natural holes or wounds from pest attacks such as nematodes (Kurabachew & Ayana, 2017).

Control in inhibiting *R. solanacearum* infection is still commonly done using conventional methods, namely the use of chemical bactericides. Continuous use of chemical bactericides will reduce the quality of agricultural areas both in the long and short term (Arif, 2015). So that a synthetic bactericide replacement is needed, one of which is the use of antagonistic bacteria. Antagonistic bacteria will produce compounds to suppress pathogen activity in plants (Haryani & Tombe, 2017). One of the antagonistic bacteria commonly used for control is *Bacillus* sp.

Application to plants requires a formulation. The formulation is intended to maintain the population and biocontrol activity of antagonistic bacteria in the soil (Bashan *et al*., 2014). One of the formulations used is encapsulation.

Encapsulation is the trapping of active ingredients in certain encapsulating materials (Ezhilarasi *et al*., 2013). Encapsulation has the advantage of being able to protect biological agents from damage and being able to control the release of biological agents. So that biological agents applied in the field are able to adapt in the soil. The encapsulating material commonly used in the encapsulation of biological agents is sodium alginate. An oligosaccharide biopolymer derived from brown algae with environmentally friendly and easily degradable characteristics. Sodium alginate can be added with gelatin to improve the encapsulation pores so that *Bacillus* sp. is released slowly (Tomić *et al*., 2023). This study aims to determine the effect of sodium alginate and gelatin concentrations as encapsulation materials with the active ingredient *Bacillus* sp. isolate Bcz 30 on the controlling of bacterial wilt and plant growth of cayenne pepper.

2. MATERIALS AND METHODS

2.1. Research Location and Materials

The research was carried out from June to July 2024. The implementation location was at the Greenhouse in Wage Village, Taman District, Sidoarjo Regency, East Java with coordinates -7.362038 S and 112.708890 E.

Materials for the encapsulation constituted sodium alginate and gelatin, $CaCl_2$, *Bacillus* sp. isolate Bcz 30, soil, compost, and 35 HSS cayenne pepper seeds. Whereas the tools included sterile bottles, syringes, polybags, rulers, scalpels, test tube, Osem needles, beaker glasses.

2.2. Research Design

This study used a completely randomized design (CRD) factorial with two factors, namely three compositions of materials with the best encapsulation efficiency obtained from in vitro tests (K1 = Sodium alginate 2%, K2 = Sodium alginate 2% and gelatin 1%, and K3 = Sodium alginate 2% and gelatin 1.5%) and two doses of beads application (D1 = Encapsulation dose of 2 grams/plant and D2 = Encapsulation dose of 4 g/plant). So that 6 treatments and 3 replications were obtained. Each unit has 6 plants. There is a control factor with *R. solanacearum* inoculation without giving *Bacillus* sp. bioencapsulation. A total of 114 cayenne pepper plants were used for *in vivo* tests.

2.3. Encapsulation Preparation

Sodium alginate and gelatin were dissolved in 100ml of distilled water at hot temperature and stirred until homogeneous. Biopolymer was sterilized using an autoclave for 1 hour. *Bacillus* sp. isolate Bcz 30 with a population of 108 CFU/ml was added to a mixture of sterile sodium alginate and gelatin biopolymer with a ratio of 1:10 (v/v) and stirred until homogeneous. The bioencapsulation solution was slowly dripped into a sterile 3% $CaCl_2$ solution using a syringe. The encapsulation formed was soaked in a 3% $CaCl_2$ solution for 30 minutes (Khimmakthong *et al*., 2020).

2.4. Encapsulation Application

The soil used as a planting medium was sterilized by adding 5% formalin at a dose of 2.5 ml/kg for 1 week. The planting medium used was sterile soil and compost with a ratio of 1:1. The application of encapsulation and suspension of *R. solanacearum* was carried out 7 days before the seedlings were transplanted into polybags (Choliq *et al*., 2020). Suspension of *R. solanacearum* as much as 10 ml with a population of 10^8 CFU/ml and encapsulation of *Bacillus* sp. was poured into the planting hole according to the treatment. After 7 days of incubation in the soil, the cayenne pepper seedlings were transplanted into polybags.

2.5. Incubation Period

The incubation period of *R. solanacearum* was observed after planting the cayenne pepper seedlings until the plants showed initial symptoms marked by wilting of the leaves.

2.6. Length of Vessel Decoloration

Decoloration of the stem vessels was carried out by cutting the stem vertically from the base of the stem to see the color change in the vessels marked by a brownish color. Measurements were taken after 30 days of observation of disease intensity. The length of decoloration was measured using a caliper.

2.7. Disease Intensity

Observations were made every day starting from one day after inoculation of *Ralstonia solanacearum* on the plants. For 30 days, the intensity of the disease was checked every day with an interval of every five days. Disease intensity measurement is done using the following formula:

$$IP = \frac{\sum_{i=1}^{k} k \times Nk}{Z \times N} \times 100 \quad (1)$$

where k is number of plants attacked at each scale value, Nk is number of plants with severity scale k (k = 0, 1, 2, 3), N is number of plants used in the experiment, and Z is the highest disease severity scale as detailed in Table 1.

Table 1. Score for attacking intensity based on wilted leaves

Score	0	1	2	3	4	5
Mark	all leaves are healthy	1 - <10% leaves wilted	10 - <30% leaves wilted	30 - <60% leaves wilted	60 - <100% leaves wilted	100% wilted leaves (dead plant)

Source: Arwiyanto & Hartana (1999).

2.8. Plant Growth

Growth observation was carried out with two parameters, namely plant height and root length. Plant height observation was carried out starting 7 days after treatment with a five-day interval until the 30th day. Plant height and root length were measured using a ruler.

2.3. Data Analysis

The data obtained will be analyzed using ANOVA to see the effect of treatment on each parameter. If it is known that there is a difference, it will be continued with the DMRT test with a level of 5%.

3. RESULTS AND DISCUSSION

3.1. Bioencapsulation of *Bacillus* sp.

The encapsulation of *Bacillus* sp. produced is round like a ball (Figure 1a). The interaction between sodium alginate and gelatin forms a solid matrix to maintain *Bacillus* sp. in it. The pores produced by sodium alginate are large so that they cannot maintain *Bacillus* sp. in them so that *Bacillus* sp. easily exits through diffusion activity between the pores of sodium alginate. The addition of gelatin improves the pores of sodium alginate and is able to maintain *Bacillus* sp. in it because gelatin increases the number of interaction points with sodium alginate (Skopinska-Wisniewska, *et al*., 2024).

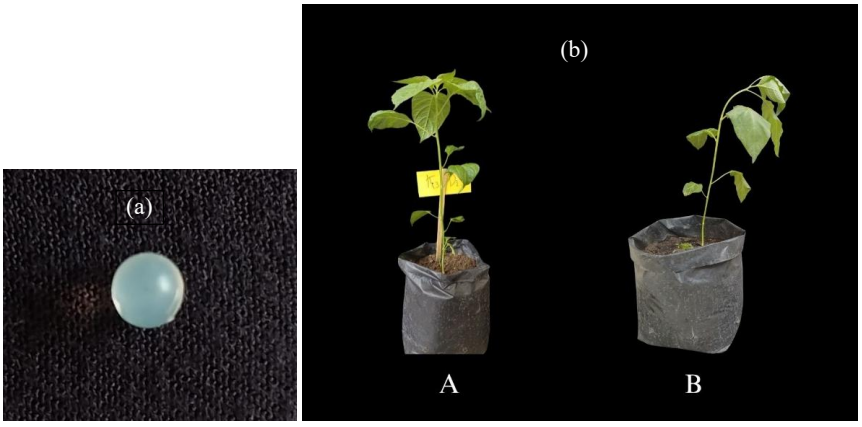

Figure 1. (a) Encapsulation of *Bacillus* sp., and (b) Plants symptomatic due to withered bacteria disease: (A) Healthy plant; (B) Withered plant.

3.2. Disease Intensity

Figure 1b depicts visual feature of plants symptomatic due to withered bacteria disease. The application of *Bacillus* sp. encapsulation with various concentrations of encapsulating materials and doses has a significant effect on suppressing the intensity of bacterial wilt (Table 2). Based on the analysis of variance test, the concentration of the encapsulating material and the dose in the plant gave a significant interaction (ANOVA <0.05) on the intensity of bacterial wilt disease in cayenne pepper plants on the 30th day. In the six treatments, the lowest intensity was given at the Sodium Alginate coating concentration of 2% and gelatin 1% with the active ingredient *Bacillus* sp. isolate Bcz 30 with a dose of 4 grams/plant with an intensity of 18.89% and an inhibition effectiveness (EP) of 36.61%. In a study conducted by Saputra *et al*. (2024), the application of *Bacillus* sp. isolate bcz 30 encapsulation with a dose of 1 gram/plant was able to suppress *R. solanacearum* infection by up to 61%. It is suspected that this concentration is able to form good pores so that the release of bacteria occurs regularly so that it can maintain the survival of bacteria in the soil. In addition, at higher doses, the number of bacterial populations also increases so that the activity in inhibiting pathogens increases. Low intensity indicates that the combination of factors between 2% Sodium Alginate and 1% gelatin with the active ingredient *Bacillus* sp. isolate Bcz 30 at a dose of 4 grams/plant is effective in inhibiting the growth of *R. solanacearum* which is indicated by the high value of inhibition effectiveness (EP). This encapsulation application has been tested on Pseudomonas fluorescens bacteria to control Fusarium solanii in potato plants up to an intensity of 24% with a concentration of 2% sodium alginate and 1.5% gelatin (Pour *et al*., 2019). At the same concentration of encapsulating material, Pour *et al*. (2021) used the pathogenic bacteria *Bacillus* velezensis to control damping off of up to 3.67%.

Table 2. Disease intensity withered bacteria in cayenne pepper plants

Treatment	Intensity Disease (%)						EP (%)
	5 DAP	10 DAP	15 DAP	20 DAP	25 DAP	30 DAP	
Control	0.00	11.11	15.56	17.78	22.22	28.89	-
K1D1	0.00	7.78b	17.78b	17.78bc	20.00a	25.56c	11.53
K1D2	0.00	10.00b	13.33a	14.44ab	16.67a	22.22abc	23.09
K2D1	0.00	6.67ab	12.22a	13.33a	20.00a	23.33bc	19.25
K2D2	0.00	5.56ab	10.00a	14.44ab	17.78a	21.11ab	26.93
K3D1	0.00	5.56ab	12.22a	18.89bc	21.11a	22.22abc	23.09
K3D2	0.00	4.44a	11.11a	16.67abc	16.67a	18.89a	34.61

*Numbers followed by different letter notations indicate differences between treatments

Description: K describes the encapsulation composition in term of sodium alginate and gelatin concentration (%), while D describes dose of application (g/plant). K1 = sodium alginate 2%, K2 = sodium alginate 2% and gelatin 1%, and K3 = sodium alginate 2% and gelatin 1.5%. D1 = application dose 2 g/plant, and D2 = application dose 4 g/plant.

The ability of *Bacillus* sp. is also a factor causing low intensity. When *Bacillus* sp. has come out of encapsulation, *Bacillus* sp. will colonize the root area. *Bacillus* sp, will produce a compound to inhibit the rate of *R. solanacearum* infection, namely the siderophore compound (Istiqomah *et al*., 2017). This compound will inhibit the growth of pathogens by binding iron so that iron is not available to pathogens (Wulandasari *et al*., 2022). *R. solanacearum* will fail to reproduce in the root area and is inhibited from entering plant tissue. In addition to producing compounds to inhibit the activity of *R. solanacearum* in the vessels themselves (Setiaji *et al*., 2023). This is in accordance with the short color changes in the vessels and the absence of exopolysaccharides in the infected area.

3.3. Incubation Period

The virulence ability of pathogens can be measured through the incubation period of *R. solanacearum*. Based on the analysis of variance test, the concentration of the encapsulant material and the application dose in the plant as well as their interaction did not provide significant on the incubation period of *R. solanacearum* in cayenne pepper plants with *p*-value of 0.747 for material composition, 0.728 for dose, and 0.734 for the interaction. Table 3 shows the K3D1 treatment showed the fastest average incubation period of 11.67 days to appear initial symptoms, while the K3D2 treatment showed the longest average incubation period of 13.64 days to appear initial symptoms. This difference in incubation period indicates a delay in the appearance of initial symptoms, such as wilting of leaves. The concentration

Table 3. Effect of encapsulation treatment and application dose on the incubation period of *R. solanacearum* on cayenne pepper plant

Treatment Code	Treatment	Incubation Period (days)
K1	Sodium alginate 2%	11.50a
K2	Sodium alginate 2% and gelatin 1%	12.08a
K3	Sodium alginate 2% and gelatin 1.5%	12.15a
Treatment Code	**Treatment**	**Incubation Period (day)**
D1	Dose application 2 g/plant	11.25A
D2	Dose application 4 g/plant	12.05A

Description: Numbers followed by the same letter indicate that there is no significant difference in the 5% DMRT table.

of 2% sodium alginate and 1.5% gelatin can delay the incubation period because *Bacillus* sp. survives longer in the soil. The slow release ability at this concentration is thought to help *Bacillus* sp. in maintaining its activity. In addition, a dose of 4 g/plant produces a higher population of *Bacillus* sp. compared to a dose of 2 g/plant. Overall, average incubation period is 11.81 days.

The incubation period delay is caused by the antagonist activity of *Bacillus* sp. *Bacillus* sp. isolate Bcz 30 is bactericidal against pathogens. So *R. solanacearum* takes longer to colonize the root area and infect plants (Zinidin, 2022). In addition, in the root area there is a competition for nutrients between *Bacillus* sp. and *R. solanacearum*. Available nutrients can accelerate colonization in the root area. *Bacillus* sp. obtains nutrients from the root area as well as from encapsulation biopolymers that help maintain the population of *Bacillus* sp. in the soil.

3.4. Discoloration Symptoms

Bacterial wilt symptoms can be observed through discoloration or color changes in the vascular tissue of the base of the stem as can be observed in Figure 2. Based on the ANOVA test, the concentration of the encapsulating material and the application dose in the plant as well as their interaction gave a significant impact on the length of the discoloration length of the stem vessels in cayenne pepper plants on the 30th day with p-value is 0.001 for composition, 0.004 for dose, and 0.020 for the interaction (all p>0.05). The length of discoloration in the vessels showed significant differences in each treatment (Table 3.) In the treatment of 2% Sodium alginate and 1.5% gelatin with a dose of 4 g/plant, it was able to suppress *R. solanacearum* infection with discoloration symptoms of 1.6 mm.

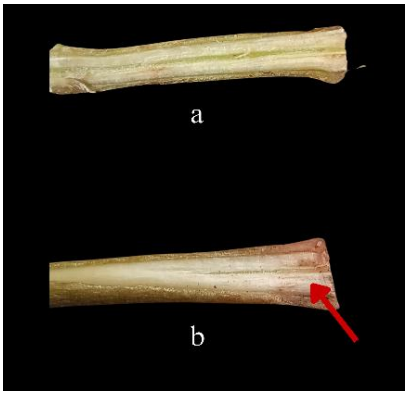

Figure 2. Discoloration in stem vessels: (a) Healthy stem vessels, (b) Infected stem vessels

The short discoloration length indicates the activity of *Bacillus* sp. in inhibiting the activity of *R. solanacearum*. The inhibitory activity by *Bacillus* sp. is thought to be influenced by the concentration of encapsulation material and dose. At a concentration of 2% sodium alginate and 1.5% gelatin, it is able to release *Bacillus* sp. consistently and maintain the survival of *Bacillus* sp. in the soil. Based on Table 3, at a dose of 4 g/plant, the discoloration in the vessels is shorter, namely 1.6 mm. This is thought to be a relationship between the population of *Bacillus* sp. in root colonization and the dose of encapsulation used. The higher the dose of encapsulation applied to the plant, the higher the population of *Bacillus* sp., the more resistant the roots of cayenne pepper plants are to *R. solanacearum* infection.

Table 3. Effect of encapsulation treatment and application dose on the discoloration length in stem vessels of cayenne pepper plant

Treatment Code	Treatment	Discoloration Length (mm)
K1D1	Sodium alginate 2% with dose 2 g/plant	3.45d
K1D2	Sodium alginate 2% with dose 4 g/plant	2.55c
K2D1	Sodium alginate 2% and gelatin 1% with dose 2 g/plant	3.22d
K2D2	Sodium alginate 2% and gelatin 1% with dose 4 g/plant	1.88ab
K3D1	Sodium alginate 2% and gelatin 1.5% with dose 2 g/plant	2.3bc
K3D2	Sodium alginate 2% and gelatin 1.5% with dose 4 g/plant	1.6a

Description: Numbers followed by the same letter indicate that there is no significant difference in the 5% DMRT table.

Factors affecting the intensity is the activity of *R. solanacearum* in infecting plant tissue. *R. solanacearum* will form colonies in the area of the stem vessels. One sign of infection in the stem vessels is characterized by decolorization of the stem vessels (McGarvey, 1999; Dalsing & Allen, 2014). Diyasti & Lizarni (2021) explained that during infection in plants, *R. solanacearum* safely forms a fluid called exopolysaccharide in the stem vessels which will inhibit anchorage activity. So that the plant will slowly experience leaf drooping and will experience irreversible wilting.

Figure 3. Growth of cayenne pepper plants: (a) Plant height, (b) Root length

3.5. Plant Growth and Root Length

Plant growth can be observed in terms of plant height and root length as presented in Figure 3. Based on the ANOVA test results, the interaction of encapsulating material concentration and dose in plants gave a significant effect on plant height and root length in cayenne pepper plants on the 30th day with p-value of 0.017 for plant height and 0.020 for root length (Table 4). The concentration of sodium alginate coating is 2% and gelatin 1% with the active ingredient *Bacillus* sp. isolate Bcz 30 with a dose of 4 g/plant gave a significant difference in plant height and root length in both parameters with a plant height of 30.58 cm and a root length of 17.13 cm (Table 5).

Differences in encapsulation materials and doses affect the growth of cayenne pepper. This difference is thought to be due to the addition of gelatin which affects the release of *Bacillus* sp. into the soil. In the encapsulation materials of 2% sodium alginate and 1.5% gelatin, *Bacillus* sp. is able to come out regularly so that *Bacillus* sp. is able to adapt and form colonization in the root area. Gelatin will keep *Bacillus* sp. trapped and able to adapt so that viability in the soil is maintained. The dose given to plants also affects plant growth. At a dose of 4 grams/plant, it can trigger plant heights of up to 30.58 cm and root length of 17.13 cm. with a higher dose, the population of *Bacillus* sp. that comes out will be

Table 5. Summary of ANOVA test for plant height and root length

	Dependent Variable: Plant Height					Dependent Variable: Root Length				
Source	SS	df	MS	F	Sig.	SS	df	MS	F	Sig.
Corrected Model	5.181[a]	5	1.036	3.018	0.054	41.231[b]	5	8.246	9.895	0.001
Intercept	15856.24	1	15856.24	46175.76	0.000	3901.39	1	3901.39	4681.67	0.000
Dosage	0.139	1	0.139	0.404	0.537	10.276	1	10.276	12.331	0.004
Composition	0.999	2	0.500	1.455	0.272	21.754	2	10.877	13.053	0.001
Dosage * Composition	4.043	2	2.022	5.887	0.017	9.201	2	4.601	5.521	0.020
Error	4.121	12	0.343			10.000	12	0.833		
Total	15865.545	18				3952.62	18			
Corrected Total	9.302	17				51.231	17			
a. R Squared = 0.557 (Adjusted R Squared = 0.372)						b. R Squared = 0.557 (Adjusted R Squared = 0.372)				

Table 4. Growth of Cayenne Pepper plants

Treatment Code	Treatment	Plant height (cm)	Root Length (cm)
K1D1	Sodium alginate 2% with dose 2 grams/ plant	29.90b	12.73 a
K1D2	Sodium alginate 2% with dose 4 grams/ plant	30.36b	15.83 cd
K2D1	Sodium alginate 2% and gelatin 1% with dose 2 grams/ plant	28.66a	13.83 ab
K2D2	Sodium alginate 2% and gelatin 1% with dose 4 grams/ plant	30.11ab	13.47 a
K3D1	Sodium alginate 2% and gelatin 1.5% with dose 2 grams/ plant	29.29ab	15.33 bc
K3D2	Sodium alginate 2% and gelatin 1.5 % with dose 4 grams/ plant	30.58b	17.13 d

Description: Numbers followed by the same letter indicate that there is no significant difference in the 5% DMRT table.

more abundant than a dose of 2 g/plant. Research conducted by Saputra *et al.* (2024) at a dose of 1 g/plant without the addition of gelatin, optimal growth for chili plants only reached a height of 16.3 cm and a root length of 10.3 cm.

The difference in plant height and root length is suspected of the formation of different colonization when released into the soil. So that at high doses and concentrations of encapsulation materials it is more optimal for the activity of *Bacillus* sp. to stimulate plant growth. Optimal plant growth also occurred in the study of Pour *et al.* (2019), the use of encapsulation formulations on *P. flurosescens* isolates with 25% sodium alginate and 1.5% gelatin encapsulation materials had an effect on potato growth with a plant height of 52+0.57cm and was able to form long roots with dense branching.

Various types of microorganisms that are classified as Plant Growth Promoting Rhizobacteria (PGPR), one of which is *Bacillus* sp. PGPR has an important role in plant growth. *Bacillus* sp. will produce Indole 3-Acetic Acid (IAA) one type of auxin for plant growth (Husna *et al*., 2019). This hormone will increase plant productivity in the elongation of plant roots so that plants are able to reach nutrients to areas that have not been reached by roots (Djereng *et al*., 2017). *Bacillus* sp. has a role in the soil to provide nutrients that are ready to be absorbed by plants. *Bacillus* sp. will provide nitrogen by converting dinitrogen (N2) into ammonia (NH3). In addition to Nitrogen, phosphate is also available by bacteria by converting organic phosphate compounds into inorganic ones (Istiqomah *et al*., 2017). High growth is thought to be the production of more abundant hormones and nutrients. This is in line with the opinion of Wartono *et al.* (2014), that the increasing population of *Bacillus* sp. will increase plant growth activity which is indicated by an increase in plant height and root length.

4. CONCLUSION

The application of encapsulation has a significant impact on controlling bacterial wilt caused by *R. solanacearum* and helps the cayenne pepper plants to grow optimally. At a material composition with sodium alginate concentration of 2% and 1.5% gelatin, it can suppress the intensity of bacterial wilt disease up to 18.89% inhibition effectiveness of 34.61%. As comparison control treatment reveals a higher disease intensity of 28.89. At the same dose, it also provides optimal growth in plant height and root length so that plants are more productive during their vegetative period. The next research need to determine the effect of encapsulation application time and antagonist tests on other soil-borne pathogens.

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