

ENCAPSULATION OF SOYBEAN SEEDS WITH *Actinomyces* AS A BIOCONTROL AGENT AGAINST *Sclerotium rolfsii* Sacc

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ABSTRACT

This study evaluates the effectiveness of *Actinomyces* in encapsulating soybean seeds to control damping-off disease caused by *Sclerotium rolfsii*. With national soybean demand reaching 22 million tons per year and domestic production only 1 million tons, increasing production is essential. One major constraint is the attack of plant pathogens such as *S. rolfsii*, which can cause significant losses. *Actinomyces* were isolated from the rhizosphere of weeds and tested for their antagonistic activity in vitro. Seed encapsulation using various carriers (kaolin, talc, and zeolite) was conducted to determine the most effective formula. In vivo tests showed that the *Actinomyces* isolate PM2 exhibited the best antagonistic activity, with the zeolite formulation reducing pre-emergence damping-off disease incidence to 12.50% and post-emergence damping-off to 8.25%, compared to the positive control. These results indicate that the use of *Actinomyces* in soybean seed encapsulation is an effective and sustainable method for controlling damping-off disease. Zeolite as a carrier proved to provide a supportive environment for antagonistic microbes, maintaining moisture and supplying necessary nutrients. This research reveals the great potential of *Actinomyces* as a biocontrol agent in plant disease management strategies, which can significantly enhance soybean production and support national food security.

Keywords: fungi, germ, plant, seed, soil.

INTRODUCTION

The soybean plant (*Glycine max*) is one of the popular food crops in Indonesia. This plant is an essential part of the daily diet of the Indonesian population. Many processed products are derived from soybean seeds, which contain various essential nutrients such as proteins, fats, vitamins, and minerals like calcium, phosphorus, and iron. Commonly utilized soybean products include tofu, tempeh, soy milk, soy sauce, soybean flour, and tauco [1]. The high nutritional content of soybeans makes them a valuable source of protein, significantly improving the nutritional status of the population. Soybean seeds contain approximately 35% protein, 35% carbohydrates, and 15% fat. Additionally, soybean seeds also contain important vitamins A and B [2].

The national demand for soybeans reaches 2.2 million tons per year, while domestic production only amounts to 920 thousand to 1 million tons per year. This gap can become problematic as demand continues to rise. One of the factors limiting the increase in soybean production is the attack of plant pests and diseases (PPD) that can reduce soybean yield [3].

Attacks by PPD are a major constraint in efforts to increase soybean production, leading to suboptimal yields. One significant PPD is pathogens, which are organisms that cause diseases in soybean plants. One important pathogen in this regard is *Sclerotium rolfsii*, which causes diseases such as damping-off or wilting in soybean plants [4]. The intensity of disease attacks caused by *S. rolfsii* can reach up to 80%, severely disrupting plant growth and potentially leading to plant death [5]. Therefore, to ensure sufficient soybean availability for the population and to increase soybean

production, effective measures must be taken to address the factors causing a decline in soybean production, especially pathogen attacks such as those from *S. rolf sii*.

Several control techniques can be used to manage diseases caused by the *S. rolf sii*. One effective method is the use of antagonistic microorganisms, such as *Actinomyces* bacteria. These bacteria compete with *S. rolf sii*, inhibit its growth, and help fight infections [6]. The advantage of using antagonistic microorganisms is that they are environmentally safe and can persist in the soil for several growing seasons. Additionally, the use of disease-resistant soybean varieties is an effective option. Resistant varieties have natural resistance to fungal infections, thereby reducing the risk of disease attacks. Mechanical control techniques, such as removing infected plants or clearing plant residues after harvest, can also help reduce disease spread. However, the use of chemical fungicides should be avoided if possible due to the need for knowledge about soil properties that can absorb these chemicals and their negative impact on the environment and health. Therefore, the use of antagonistic microorganisms such as *Actinomyces* is considered a safer and more sustainable method for the environment and effective in combating soil-borne pathogens like *S. rolf sii* [7].

The use of biological agents such as *Actinomyces* is considered highly effective in controlling damping-off disease caused by *S. rolf sii*. *Actinomyces* have the ability to produce antibiotics that can damage the cell walls and plasma of pathogens. The antibiotics produced by *Actinomyces* can inhibit and even kill the pathogens [8]. The presence of *Actinomyces* in fertile soil often indicates the level of soil fertility. Moreover, *Actinomyces* have a broad spectrum, meaning they can combat various types of pathogens. This makes *Actinomyces* a potential choice in preventing damping-off disease caused by *S. rolf sii* [9].

Utilizing antagonistic bacteria to suppress and control disease can be done through various methods, one of which is seed treatment with the addition of antagonistic bacterial coatings [10]. The aim of adding these bacteria is to protect plants from pathogen attacks and also to enhance seed storage durability. The selection of appropriate carrier materials plays a crucial role in ensuring that the antagonistic bacteria can survive on the seed coating and effectively perform their function in controlling pathogens [11].

In this study, the potential use of *Actinomyces* through soybean seed encapsulation is evaluated to control damping-off disease caused by *S. rolf sii*. Additionally, the research aims to identify the most effective carrier materials in enhancing the ability of *Actinomyces* bacteria as biological agents. Thus, this study will provide essential information on methods that can be used to improve the effectiveness of using antagonistic bacteria in disease control in soybean plants, specifically damping-off disease caused by *S. rolf sii*.

MATERIALS AND METHODS

Time and Place of Research

The research was conducted from January to June 2019. Testing was carried out in the Plant Disease Laboratory and the Greenhouse of the Faculty of Agriculture, University of Jember.

Isolation and Purification of *Actinomyces*

Actinomyces were isolated from the rhizosphere of *Mimosa pudica* and *Cyperus rotundus* at a depth of 20 cm from the soil surface. A 1 g soil sample was placed into a test tube containing 9 mL of sterile water, followed by serial dilution up to 10^{-9} . Soil suspensions at dilutions of 10^{-7} , 10^{-8} , and 10^{-9} were cultured on yeast peptone glucose agar (YPGA) medium using the pour plate method and incubated at room temperature for 7 days. *Actinomyces*, characterized by slow growth, wrinkled, powdery texture, and adherence to the medium, were purified and grown on fresh media [12].

Hypersensitivity Reaction Test

This test was conducted to confirm the pathogenicity of the isolated *Actinomyces*. *Actinomyces* isolates were grown in yeast peptone glucose broth (YPGB) for 48 hours. The resulting suspension was infiltrated into tobacco leaf laminae and incubated for 48 hours. Hypersensitivity reaction was indicated by necrosis symptoms within 48 hours. Isolates showing positive hypersensitivity reactions were potential pathogens and were not used in further testing. Isolates without hypersensitivity reactions were used for subsequent tests [13].

Gram Staining Test

The Gram test was performed by taking a loopful of *Actinomyces* isolate from a petri dish and mixing it with 3% KOH solution, stirring for 15 seconds. After stirring, the *Actinomyces* were lifted. Gram-positive was indicated by the absence of mucous formation, while Gram-negative was indicated by the formation of mucous [12].

In Vitro Antagonism Activity Test of *Actinomyces*

The inhibition test was performed using the dual culture method. A 5 mm diameter fungal culture was grown in the center of a 9 cm diameter YPGA petri dish, then a 5 mm diameter *Actinomyces* culture was placed opposite the *S. rolfsii* culture at a distance of 3 cm and incubated at room temperature for 7 days. Inhibition was detected by the presence of a barrier between *S. rolfsii* and *Actinomyces*. Observations were made after 5-7 days to determine the potential of *Actinomyces* in inhibiting the growth of *S. rolfsii* pathogen. The test was repeated four times following a completely randomized design. As a control, *S. rolfsii* was grown on media without *Actinomyces*. Inhibition percentage data were tabulated and analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level. The best treatment from this test was used in further testing [14].

Seed Encapsulation Formulation

The encapsulation materials used were a mixture of compost, carrier materials (kaolin, talc, zeolite), and adhesive materials (gum arabic). CaCO_3 was added to lower pH and glucose as a bacterial nutrient to match the bacterial environmental conditions. Fifty grams of compost mixed with 2 g of gum arabic as an adhesive were used per treatment. Inorganic carrier materials such as talc, kaolin, and zeolite were added at 28 g each, along with 5 g of glucose and 15 g of CaCO_3 per encapsulation treatment. The materials used for encapsulation were filtered using a 20 μm sieve. Before use, the mixed materials were sterilized using an autoclave to avoid contamination [15].

Soybean seeds and encapsulation formulation were placed into an encapsulator. For the application of biological agents, a suspension of *Actinomyces* bacteria at a density of 10^8 CFU ml⁻¹ was used. The *Actinomyces* bacterial suspension at the same density was sprayed using a hand sprayer while the encapsulator was operating to ensure all encapsulation materials and added bacteria adhered to the soybean seeds. The encapsulation process was carried out until the coating materials were evenly distributed on the seed surface, followed by air drying for 30 minutes [16].

In Vivo Antagonism Activity Test of *Actinomyces*

The ability of *Actinomyces* to control *S. rolfsii* in vivo was tested by encapsulating soybean seeds with various carrier materials. This study involved 5 treatments and 4 replications, with each replication consisting of 30 seeds, resulting in a total of 20 germination trays and 600 soybean seeds. The treatments were arranged as follows:

- P0: Seeds not coated and without pathogen (negative control).
- P1: Seeds not coated and inoculated with the pathogen (positive control).
- P2: Seed coating with a formulation of compost, gum arabic, kaolin, glucose, CaCO₃, and *Actinomyces* bacteria.
- P3: Seed coating with a formulation of compost, gum arabic, talc, glucose, CaCO₃, and *Actinomyces* bacteria.
- P4: Seed coating with a formulation of compost, gum arabic, zeolite, glucose, CaCO₃, and *Actinomyces* bacteria.

A total of 150 ml of *S. rolfsii* pathogen was inoculated into the planting medium in the germination trays. The pathogen suspension was inoculated into the planting medium after the encapsulated soybean seeds were planted. The germination trays containing the planting medium, soybean seeds, and pathogen suspension were covered with black netting for 24 hours, after which the netting was removed. Observations were made on each germination tray containing soybean seeds with specific treatments to assess seed growth, disease incidence, and the effectiveness of disease control in vivo.

This test was conducted following a completely randomized design. Observed variables included germination percentage, percentage of infected seeds, percentage of seedlings infected after emergence, and control effectiveness. Inhibition percentage data were tabulated and analyzed using ANOVA followed by DMRT at a 95% confidence level [16].

RESULT AND DISCUSSION

***Actinomyces* Isolates**

The isolation of *Actinomyces* was carried out by sampling soil from the roots of two types of weeds: *Mimosa pudica* (isolate code PM) and *Cyperus rotundus* (isolate code RT). From this exploration process, six *Actinomyces* isolates were identified: four isolates originated from *M. pudica* and two isolates from *C. rotundus*. These isolates exhibited typical *Actinomyces* characteristics (Figure 1), consistent with the study by Rathore *et al.* [17]. According to their research, *Actinomyces* can be distinguished from other bacteria based on colony morphology. Characteristics of *Actinomyces* colonies include a round shape with raised and convex elevation, smooth and

irregular edges, and surfaces that can be powdery, smooth, rough, or wrinkled. This finding is supported by another study, which stated that *Actinomyces* colonies macroscopically adhere firmly to the agar surface, appear powdery, are dispersed in small colonies, and are white in color. These *Actinomyces* isolates were successfully isolated from the roots of *M. pudica* and *C. rotundus*, which have different root systems in the same area (Table 1).

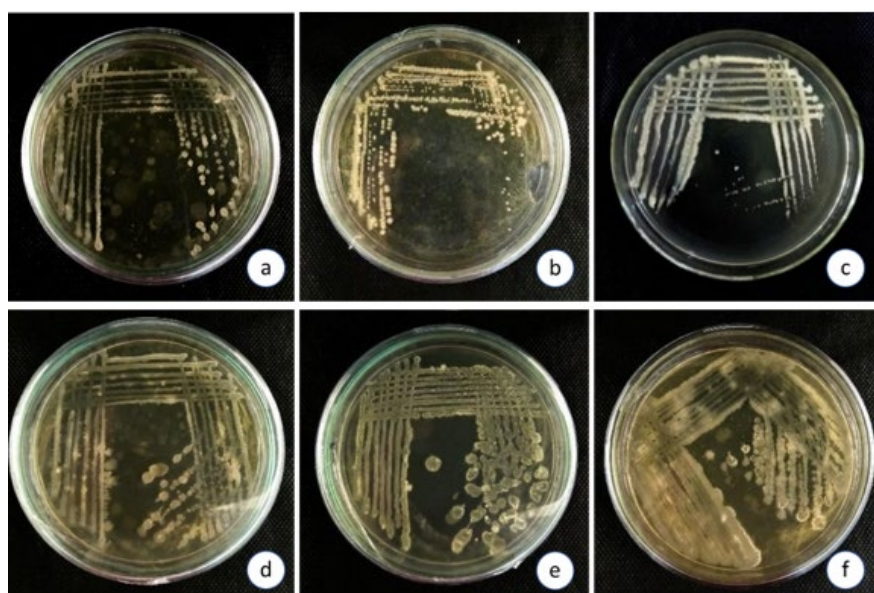


Figure 1. *Actinomyces* isolation results (a) *Actinomyces* isolate PM1; (b) *Actinomyces* isolate PM2; (c) *Actinomyces* isolate PM3; (d) *Actinomyces* isolate PM4; (e) *Actinomyces* isolate RT1; (f) *Actinomyces* isolate RT2.

Table 1. Characteristics of *Actinomyces* isolates from exploration

Isolate Code	Source of Isolate	Isolate Characteristics	Mycelium Color	Aerial Mycelium Color
PM1	Rhizosphere of <i>M. pudica</i>	Irregular edges, wrinkled surface	White	White
PM2	Rhizosphere of <i>M. pudica</i>	Irregular edges, dry, wrinkled, and powdery surface	Gray	White
PM3	Rhizosphere of <i>M. pudica</i>	Irregular edges, dry, wrinkled, and powdery surface	Gray	White
PM4	Rhizosphere of <i>M. pudica</i>	Irregular edges, dry, wrinkled, and powdery surface	Gray	Gray
RT1	Rhizosphere of <i>C. rotundus</i>	Irregular edges, dry, wrinkled, and powdery surface	Gray	White
RT2	Rhizosphere of <i>C. rotundus</i>	Irregular edges, dry, wrinkled, and powdery surface	Gray	White

Microscopic observations of the six *Actinomyces* isolates were conducted using a microscope with 1000× magnification. These observations revealed typical *Actinomyces* characteristics, most notably the presence of branched mycelium, similar to the mycelium structures found in fungi (Figure 2). These features are consistent with the findings of Putri and Nurkanto [18], where

Actinomycetes, when observed under a microscope, display the formation of branched mycelium. This mycelium structure is unique because its spores form spiral-shaped chains.

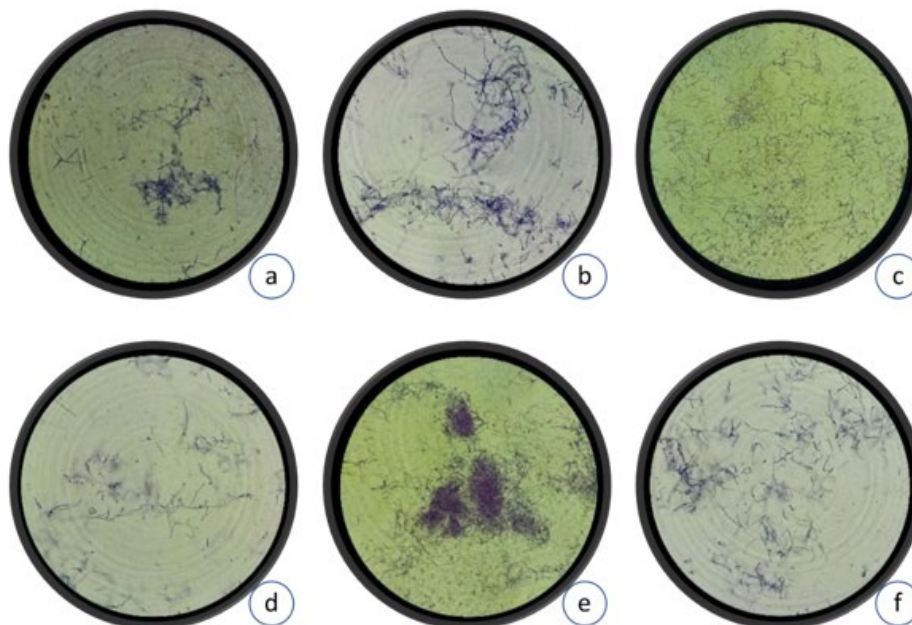


Figure 2. Microscopic Observation of mycelium from six *Actinomycetes* isolates at 1000× Magnification (a) Mycelium of *Actinomycetes* PM1; (b) Mycelium of *Actinomycetes* PM2; (c) Mycelium of *Actinomycetes* PM3; (d) Mycelium of *Actinomycetes* PM4; (e) Mycelium of *Actinomycetes* RT1; (f) Mycelium of *Actinomycetes* RT2.

Actinomycetes are a unique group of bacteria that exhibit microscopic characteristics resembling fungi. This can be explained through several aspects. First, *Actinomycetes* develop in filamentous forms similar to fungal hyphae. Hyphae are thread-like structures that are the main components of fungi. Second, *Actinomycetes* have the ability to produce spores, a process also common in fungi. Although bacterial and fungal spores have biological differences, this similarity reflects a common strategy for survival and dissemination in the environment [19].

Actinomycetes are prokaryotes, meaning they lack a true cell nucleus, unlike fungi which are eukaryotes with a defined nucleus. These structural and genetic differences emphasize that the resemblance between *Actinomycetes* and fungi is more a result of convergent evolution, where genetically unrelated organisms develop similar physical traits as adaptations to similar environmental conditions [20, 21].

Biosafety and Characteristics of *Actinomycetes* Isolates

The hypersensitivity activity test was conducted to determine the pathogenic nature of the purified *Actinomycetes* bacterial colonies. In this test, all *Actinomycetes* isolates were found to be non-pathogenic. This was evidenced by the absence of necrosis symptoms on tobacco leaves, indicating a negative reaction to pathogenicity. Furthermore, based on the Gram staining test results, all six identified *Actinomycetes* isolates exhibited Gram-positive characteristics. This was corroborated by the fact that no mucous formation occurred when the bacteria were mixed with 3% KOH solution, a typical indicator for Gram-positive bacteria.

From a molecular perspective, the hypersensitivity response (HR) in plants involves interactions between specific proteins within plant and bacterial cells [22]. In plants, proteins called NLRs (nucleotide-binding leucine-rich repeat receptors) play a crucial role in recognizing pathogens. When NLRs recognize virulence factors secreted by pathogens, they activate the plant's defense mechanisms, including HR [23].

HR itself is a form of rapid and programmed cell death at the infection site. This mechanism aims to prevent the spread of the pathogen to other parts of the plant. When NLRs recognize pathogen effectors, they can form structures called resistosomes. Resistosomes interact with the cell membrane, creating pores that lead to ion leakage and ultimately cell death [24]. This mechanism demonstrates how plants can specifically recognize and react to pathogens. In the hypersensitivity test using tobacco, if pathogenic bacteria are inoculated onto tobacco leaves, they will trigger HR if they possess virulence factors recognized by the plant's defense system. Consequently, the area around the infection site will die, which can be seen as lesions or burned areas on the leaves [25].

Effectiveness of *Actinomyces* Against *S. rolfsii* In Vitro

The antagonistic test was conducted using the dual culture method, wherein *Actinomyces* and the pathogen *S. rolfsii* were co-cultured on a single YPGA growth medium. The results of this test indicated a significant antagonistic capability of *Actinomyces* in inhibiting the hyphal growth of *S. rolfsii*. This antagonistic ability was reflected in the reduced growth of *S. rolfsii* hyphae, causing them to become abnormally shaped or even cease growing altogether. In the visual illustration, the antagonistic test using the dual culture method shows that *Actinomyces* bacteria, acting as antagonistic agents, are placed in the lower isolate position, while the pathogen isolate *S. rolfsii* is placed in the upper isolate position. This position can be identified by the presence of the white fungal hyphae of the pathogen in Figure 3.

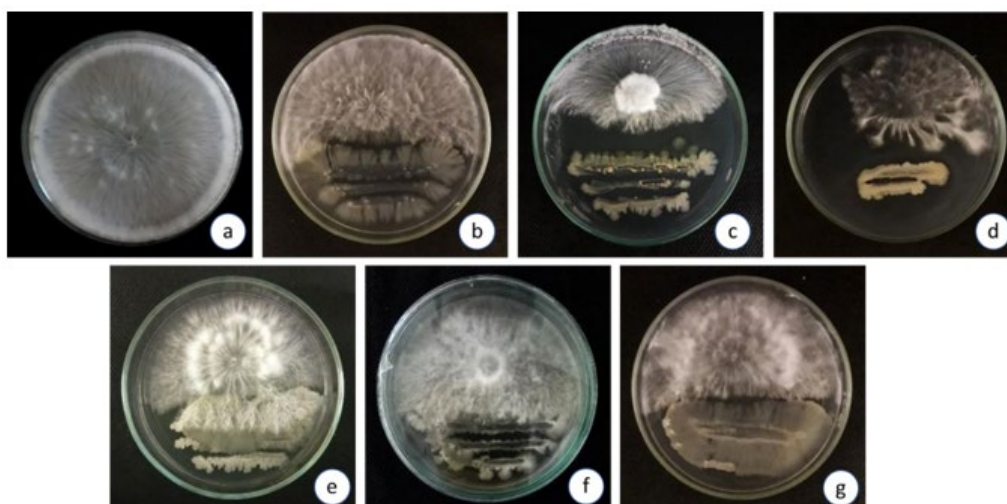


Figure 3. Results of the antagonistic test of *Actinomyces* against *S. rolfsii* in vitro (a) Control; (b) Antagonistic test of *Actinomyces* isolate PM1; (c) Antagonistic test of *Actinomyces* isolate PM2; (d) Antagonistic test of *Actinomyces* isolate PM3; (e) Antagonistic test of *Actinomyces* isolate PM4; (f) Antagonistic test of *Actinomyces* isolate RT1; (g) Antagonistic test of *Actinomyces* isolate RT2.

The *in vitro* inhibition ability test on the six *Actinomycetes* isolates showed varying levels of consistency and different inhibition percentages. This test was performed with four repetitions for each *Actinomycetes* isolate. The *Actinomycetes* isolates that demonstrated consistent inhibition of *S. rolfsii* hyphal growth in each repetition were the isolates with codes PM1, PM2, and PM3. Meanwhile, the *Actinomycetes* isolates PM4, RT1, and RT2 did not show consistent inhibition of *S. rolfsii* hyphal growth *in vitro*.

Based on the data in Table 2, it can be concluded that the *Actinomycetes* isolate with the code PM2 is the best at inhibiting the hyphal growth of *S. rolfsii*. This isolate had the highest inhibition percentage, which was 25.73%, and showed consistency in inhibiting the hyphal growth of *S. rolfsii* *in vitro* compared to the other *Actinomycetes* isolates. Therefore, *Actinomycetes* PM2 will be used as a biological agent in the seed encapsulation formula for the subsequent *in vivo* test.

Table 2. Results of the antagonistic test of *Actinomycetes* against *S. rolfsii*

Isolate Code	Average Pathogen Diameter Growth (cm)	Inhibition Percentage (%)	Inhibition Consistency
Kontrol	4.40 ± 0.35	0.00 c	-
PM1	3.52 ± 0.51	20.66 b	Consistent
PM2	3.29 ± 0.23	25.73 a	Consistent
PM3	3.31 ± 0.40	25.28 a	Consistent
PM4	3.53 ± 0.53	20.16 b	Not consistent
PM5	3.66 ± 0.32	17.45 b	Not consistent
PM6	3.56 ± 0.43	19.65 b	Not consistent

Note: Numbers followed by the same letter do not show significant differences in Duncan's multiple range test at a 95% confidence level.

According to Errakhi et al. [26], differences in the ability of *Streptomyces* isolates, which are a species of *Actinomycetes*, to inhibit the hyphal growth of *S. rolfsii* are influenced by the presence of bioactive compounds in addition to antimicrobial compounds that can inhibit the growth of *S. rolfsii*. The study by Takahashi and Nakashima [27] also stated that these differences in inhibition ability are due to the different mechanisms of action of the antibiotics produced. Antibiotics such as Streptomycin are known to inhibit protein synthesis by disrupting ribosome function, and Nystatin works by binding to ergosterol in the fungal cell membrane, causing pores that lead to leakage of intracellular components, illustrating how *Streptomyces* inhibits fungal growth. Additionally, Actinomycin D interferes with the DNA transcription process, while Chloramphenicol and Tetracycline inhibit protein synthesis through different mechanisms [28, 29].

Effectiveness of *Actinomycetes* Encapsulation Formula

Isolate PM2 was further used in the process of soybean seed encapsulation in this study (Figure 4). The encapsulated seeds were then tested for their performance in growth and disease inhibition. Based on the data presented in Table 3, the germination percentage of seeds in the control group reached 95%. Furthermore, the results showed that the germination percentage of seeds in the control group was higher compared to the seeds that underwent the encapsulation process with the use of *Actinomycetes* bacteria and several additional carrier materials. The treatment results involving seed encapsulation with *Actinomycetes* bacteria and the addition of carrier materials such

as kaolin, talc, and zeolite did not result in a significant increase in the germination percentage of soybean seeds.

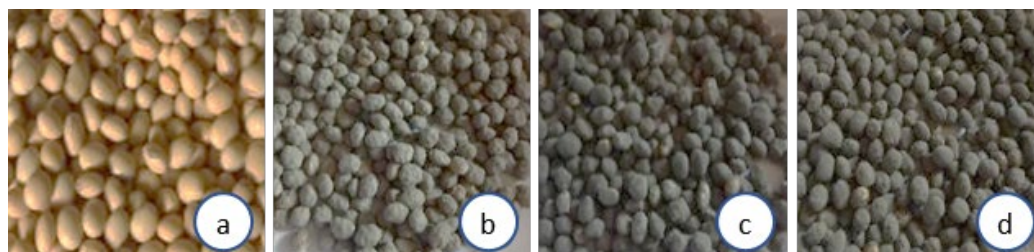


Figure 4. Encapsulation of soybean seeds (a) Seeds without encapsulation; (b) Seeds encapsulated with *Actinomyces* bacteria + kaolin; (c) Seeds encapsulated with *Actinomyces* bacteria + talc; (d) Seeds encapsulated with *Actinomyces* bacteria + zeolite.

Table 3. Germination percentage of soybean seeds with *Actinomyces* bacteria encapsulation

Treatment	Germination Percentage (%)
Control	95.00 ^{ns}
Kaolin	90.83 ^{ns}
Zeolite	91.67 ^{ns}
Talc	89.17 ^{ns}

The results of Duncan's Multiple Range Test (DMRT) at a 5% significance level for each treatment tested, including the control group and seeds encapsulated with *Actinomyces* bacteria and the addition of carrier materials such as kaolin, talc, and zeolite, showed that the germination percentage of the seeds did not significantly differ among these groups.

Two main factors influencing the germination percentage of encapsulated seeds are the adhesive used and the thickness of the encapsulation layer. The selection of the adhesive material is a crucial step in the seed encapsulation formulation because an incorrect choice can affect the final germination percentage. In this study, gum arabic, derived from the sap of leguminous trees and known for its strong adhesive properties, was used as the adhesive. Gum arabic has been frequently used in seed encapsulation research [30].

The study by Rostamabadi *et al.* [31] showed that using gum arabic as an adhesive resulted in higher germination percentages compared to other adhesives like CMC and Chitosan in tomato and cucumber seed coating treatments. Similarly, Al-Hamayda *et al.* [32] reported that using 3% gum arabic + 1% talc increased the germination percentage to 87%, higher than the control group, which only reached 80%. However, in this study, using gum arabic as an adhesive did not increase the germination percentage of soybean seeds. In fact, the germination percentage of seeds encapsulated with gum arabic tended to be lower than the control group.

This phenomenon is suspected to be caused by the strong adhesive properties of gum arabic, which can inhibit the seed imbibition process, negatively impacting the germination percentage of soybean seeds. Therefore, adjustments in the seed encapsulation formulation using gum arabic are necessary to minimize these negative effects and improve the germination percentage of soybean seeds.

In Table 4, the results of Duncan's Multiple Range Test (DMRT) at a 5% significance level show that the seed encapsulation treatment using *Actinomyces* bacteria with additional carrier materials like kaolin, talc, and zeolite significantly reduced the incidence of pre-emergence damping-off disease compared to the positive control treatment. The incidence of pre-emergence damping-off disease in the positive control treatment (P1) reached 23.33%. In contrast, in the treatments where seeds underwent the encapsulation process (P2, P3, and P4), the incidence of pre-emergence damping-off disease was lower, below 15%. These results indicate that seed encapsulation treatment using *Actinomyces* bacteria with the addition of carrier materials effectively reduces the incidence of pre-emergence damping-off disease in soybean plants.

Table 4. Percentage of pre-emergence damping-off in soybean seeds

Treatment	Incidence Percentage (%)
P0 (Control without pathogen)	0,00 c
P1 (Control with pathogen)	23,33 a
P2 (<i>Actinomyces</i> + kaolin)	13,33 b
P3 (<i>Actinomyces</i> + talc)	14,17 b
P4 (<i>Actinomyces</i> + zeolite)	12,50 b

Note: Numbers followed by the same letter indicate no significant difference in Duncan's Multiple Range Test (DMRT) at a 5% significance level.

The results of the progression of pre-emergence damping-off disease show that the positive control group (P1) had a higher disease progression rate compared to the treatment groups P2, P3, and P4, which were seeds that had undergone the encapsulation process. Specifically, the P4 seed treatment demonstrated the most beneficial results in suppressing the progression of pre-emergence damping-off disease, as it had the lowest disease progression percentage compared to the other seed treatments (Figure 5).

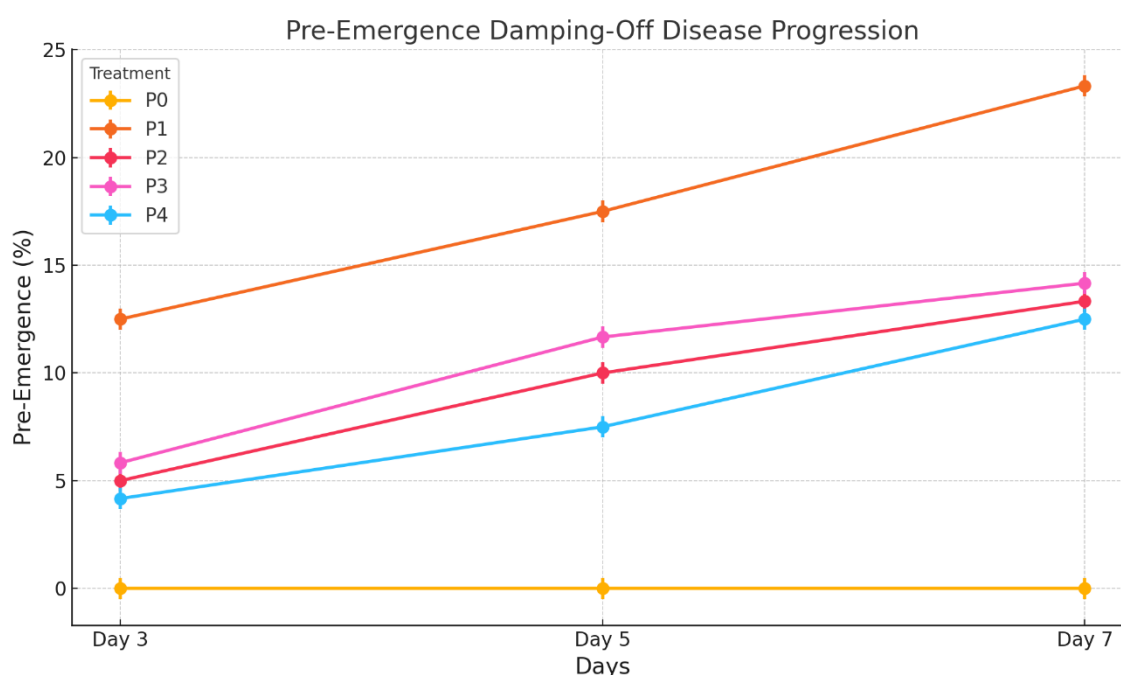


Figure 5. Progression of pre-emergence damping-off disease in soybean seeds.

In this study, the control of post-emergence damping-off disease in soybean plants found that seed treatments that underwent the encapsulation process (P2, P3, and P4) had a significant impact in reducing this disease compared to the control group (P1), as shown in Table 5. Seed encapsulation treatments proved effective in reducing the disease incidence to a level considered mild, which is less than 10%. Although there were no striking differences among the various seed encapsulation methods, P2 showed the most effective results with the lowest disease incidence percentage compared to P1, P3, and P4 (Figure 6).

Table 5. Percentage of post-emergence damping-off in soybean seeds

Treatment	Incidence Percentage (%)
P0 (Control without pathogen)	0,00 c
P1 (Control with pathogen)	45,59 a
P2 (Actinomycetes + kaolin)	8,25 b
P3 (Actinomycetes + talc)	9,01 b
P4 (Actinomycetes + zeolite)	8,70 b

Note: Numbers followed by the same letter indicate no significant difference in Duncan's multiple range test at a 5% significance level.

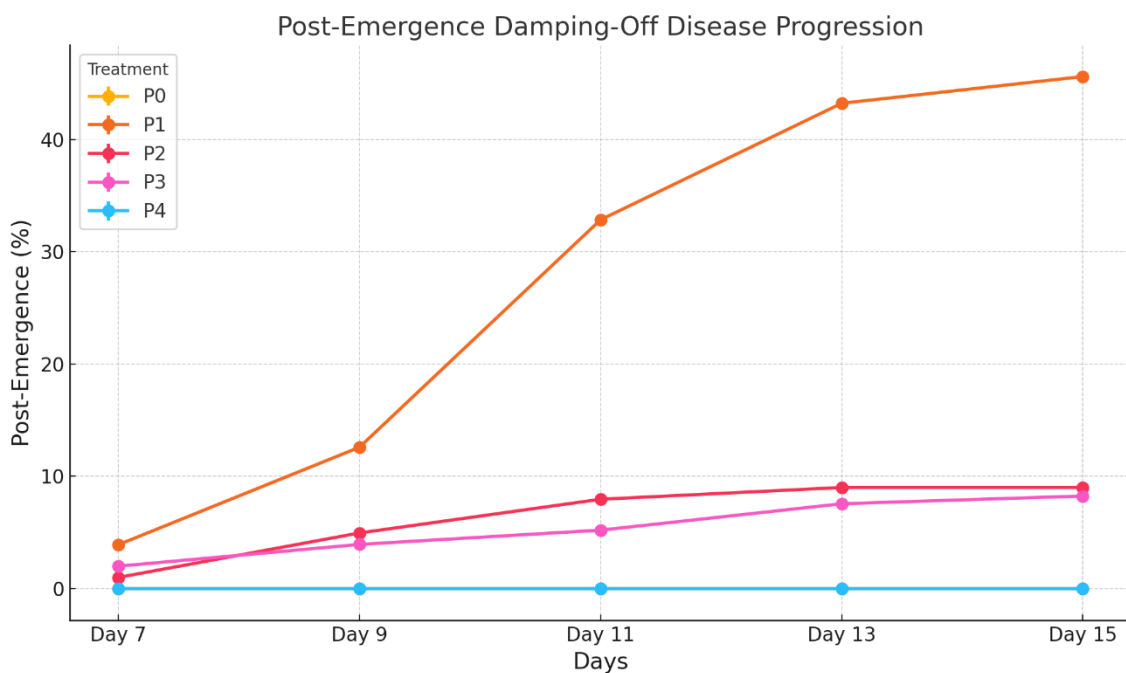


Figure 6. Progression of post-emergence damping-off disease in soybean seeds.

Based on the results of the percentage of pre-emergence damping-off disease incidence in treatments P2, P3, and P4, it is evident that these treatments significantly influenced the reduction of damping-off disease caused by *S. rolfisii* in the pre-emergence phase compared to treatment P1 (positive control). The incidence of *S. rolfisii* damping-off disease before seedling emergence in treatment P1 had a higher attack rate than the seed encapsulation treatments, namely P2, P3, and P4.

As mentioned by Riseh *et al.* [33], soybean seed encapsulation combined with biological agents can provide protection against soil-borne pathogens such as *S. rolf sii*. The addition of carrier materials in the seed encapsulation formula containing biological agents aims to provide the necessary nutrients for these agents, thereby enhancing their antagonistic role and protecting the seeds from soil-borne pathogens like *S. rolf sii*, which often attack soybean plants during the germination phase, causing damping-off disease.

Treatment P4, involving soybean seed encapsulation, showed the most effective impact in suppressing pre-emergence damping-off disease. The incidence of pre-emergence damping-off disease in treatment P4 was 12.50%, the lowest percentage compared to treatments P1, P2, and P3. Treatment P2, involving seed encapsulation using *Actinomyces* enriched with zeolite as a carrier material, showed excellent results in controlling *S. rolf sii* damping-off disease (pre-emergence) compared to other treatments. Zeolite has the advantage of absorbing water and retaining moisture, which is crucial for microbial survival. Moreover, zeolite contains various minerals such as Si, Al, Ca, Fe, K, Mg, and Na, which are essential nutrients for microbial growth and development. The moist conditions created by zeolite also enhance the seed imbibition process, allowing seeds to germinate well before being infected by *S. rolf sii* hyphae. Additionally, zeolite helps increase cation exchange capacity, provides micronutrients, and maintains a neutral pH, thereby enhancing nutrient availability for plants.

The best treatment for controlling damping-off disease during post-emergence is treatment P2. Treatment P2 showed the highest disease control effectiveness, reaching 81.90%, the highest percentage compared to other seed treatments. Treatment P2 involves the use of kaolin as a carrier material, which has been studied in various research. According to Widiastuti *et al.* (2019), kaolin can be used as a carrier for the bioinsecticide product *B. thuringiensis* because it can maintain a *B. thuringiensis* population of 10^6 . Based on these findings, the seed encapsulation treatment using *Actinomyces* with the addition of kaolin as a carrier showed a positive interaction in suppressing the development of *S. rolf sii* damping-off disease in the post-emergence phase.

The use of *Actinomyces* bacteria in seed treatment has also been examined in previous research by Rahim *et al.* [34]. Their study indicated that *Actinomyces* have the potential as an agent for controlling bacterial leaf blight in rice plants because they can reduce the population of the pathogen *Xanthomonas oryzae* pv. *oryzae*. Therefore, the combination of *Actinomyces* and kaolin in treatment P2 provided effective results in controlling damping-off disease in soybean plants.

CONCLUSION

In this study, encapsulating soybean seeds with *Actinomyces* bacteria combined with carrier materials like kaolin and zeolite was effective in controlling damping-off disease caused by *S. rolf sii*. Encapsulation significantly reduced the incidence of both pre-emergence and post-emergence damping-off diseases compared to untreated controls. Zeolite enhanced the effectiveness by retaining moisture and providing essential minerals, while kaolin maintained microbial populations and created a conducive environment for microbial activity. These results align with previous research, demonstrating that the combination of biological agents and specific carrier materials can

protect plants from soil-borne pathogens, thereby enhancing seed germination and plant health, and offering a promising strategy for sustainable agriculture.

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REFERENCES

- (1) Yulifianti R, Muzaiyanah S, & Utomo JS. 2018. Kedelai sebagai bahan pangan kaya isoflavan. *Buletin Palawija*, 16(2): 84-93.
- (2) Bantacut T. 2017. Pengembangan kedelai untuk kemandirian pangan, energi, industri, dan ekonomi. *Jurnal Pangan*, 26(1): 81-96.
- (3) Aldillah R. 2015. Proyeksi produksi dan konsumsi kedelai Indonesia. *Jurnal Ekonomi Kuantitatif Terapan*, 8(1): 44324.
- (4) Dwivedi S, & Prasad G. 2016. Integrated management of *Sclerotium rolfsii*: an overview. *European Journal of Biomedical and Pharmaceutical Sciences*, 3(11): 137-146.
- (5) Bhamra GK, & Borah M. 2022. A review on collar rot disease of soybean caused by *Sclerotium rolfsii* sacc. *International Journal of Economic Plants*, 9(3): 235-239.
- (6) Li Y, He F, Lai H, & Xue Q. 2017. Mechanism of in vitro antagonism of phytopathogenic *Scelrotium rolfsii* by *Actinomycetes*. *European Journal of Plant Pathology*. 149(2): 299-311.
- (7) Zhan J, Thrall PH, Papaix J, Xie L, & Burdon JJ. 2015. Playing on a pathogen's weakness: using evolution to guide sustainable plant disease control strategies. *Annual Review of Phytopathology*, 53(1): 19-43.
- (8) Singh S, & Gaur R. 2016. Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against *Sclerotium rolfsii* in chickpea. *Journal of Applied Microbiology*, 121(2): 506-518.
- (9) Chaurasia A, Meena B, Tripathi A, Pandey K, Rai A, & Singh B. 2018. *Actinomycetes*: an unexplored microorganisms for plant growth promotion and biocontrol in vegetable crops. *World Journal of Microbiology and Biotechnology*, 34: 1-16.
- (10) Chen Q, Bai S, Zhang T, Duan C, Zhao J, Xue Q, & Li Y. 2021. Effects of seed-coating preparations of living streptomyces globisporus on plant growth promotion and disease control against *Verticillium* wilt in cotton. *Sustainability*, 13(11): 6001.
- (11) Mattei V, Motta A, Saracchi M, Kunova A, Cortesi P, Pizzatti C, & Pasquali M. 2022. Wheat seed coating with *Streptomyces* sp. strain DEF39 spores protects against *Fusarium* head blight. *Microorganisms*, 10(8): 1536.
- (12) Sapkota A, Thapa A, Budhathoki A, Sainju M, Shrestha P, & Aryal S. 2020. Isolation, characterization, and screening of antimicrobial-producing *Actinomycetes* from soil samples. *International Journal of Microbiology*, 20: 7-16.
- (13) Wahyudi AT, Priyanto JA, Afrista R, Kurniati D, Astuti RI, & Akhdiya A. 2019. Plant growth promoting activity of *Actinomycetes* isolated from soybean rhizosphere. *Online Journal of Biological Science*, 19: 1-8.
- (14) Singh C, Parmar RS, Jadon P, & Kumar A. 2016. Characterization of *Actinomycetes* against phytopathogenic fungi of *Glycine max* (L.). *Asian Journal of Pharmaceutical and Clinical Research*, 9(1): 216-219.

- (15) Morsy EM. 2015. Efficiency of free and encapsulated yeast strains as PGRs producers on faba bean plants. *Middle East Journal of Applied Science*, 5(1): 534-542.
- (16) Saputri E, Lisnawita L, & Pinem MI. 2015. Enkapsulasi beberapa jenis *Trichoderma* sp. pada benih kedelai untuk mengendalikan penyakit *Sclerotium Rolfsii* Sacc. *Jurnal Agroekoteknologi Universitas Sumatera Utara*. 3(3): 105478.
- (17) Rathore DS, Sheikh M, Gohel S, & Singh SP. 2019. Isolation strategies, abundance and characteristics of the marine *Actinomycetes* of Kachhighadi, Gujarat, India. *Journal of the Marine Biological Association of India*, 61(1): 71-78.
- (18) Putri AL, & Nurkanto A. 2017. Keragaman Aktinomisetes asal serasah, sedimen, dan tanah Pulau Enggano, Bengkulu. *Berita Biologi*, 15(3): 217-225.
- (19) Bhatti AA, Haq S, & Bhat RA. 2017. *Actinomycetes* benefaction role in soil and plant health. *Microbial Pathogenesis*, 111: 458-467.
- (20) Javed Z, Tripathi GD, Mishra M, & Dashora K. 2021. *Actinomycetes*–the microbial machinery for the organic-cycling, plant growth, and sustainable soil health. *Biocatalysis and Agricultural Biotechnology*, 31: 101893.
- (21) Silva GdC, Kitano IT, Ribeiro IAdF, & Lacava PT. 2022. The potential use of *Actinomycetes* as microbial inoculants and biopesticides in agriculture. *Frontiers in Soil Science*, 2: 30-38.
- (22) Balint-Kurti P. 2019. The plant hypersensitive response: concepts, control and consequences. *Molecular Plant Pathology*, 20(8): 1163-1178.
- (23) Tang J, Wang Y, Yin W, Dong G, Sun K, Teng Z, Wu X, Wang S, Qian Y, & Pan X. 2019. Mutation of a nucleotide-binding leucine-rich repeat immune receptor-type protein disrupts immunity to bacterial blight. *Plant Physiology*, 181(3): 1295-1313.
- (24) Baker CJ, Mock NM, Smith JM, & Aver'Yanov AA. 2015. The dynamics of apoplast phenolics in tobacco leaves following inoculation with bacteria. *Frontiers in plant science*. 6(2): 649.
- (25) Kvitko BH, & Collmer A. 2023. Discovery of the Hrp type III secretion system in phytopathogenic bacteria: how investigation of hypersensitive cell death in plants led to a novel protein injector system and a world of inter-organismal molecular interactions within plant cells. *Phytopathology*, 113(4): 626-636.
- (26) Errakhi R, Lebrihi A, & Barakate M. 2009. In vitro and in vivo antagonism of *Actinomycetes* isolated from Moroccan rhizospheric soils against *Sclerotium rolfsii*: a causal agent of root rot on sugar beet (*Beta vulgaris* L.). *Journal of Applied Microbiology*, 107(2): 672-681.
- (27) Takahashi Y, & Nakashima T. 2018. *Actinomycetes*, an inexhaustible source of naturally occurring antibiotics. *Antibiotics*, 7(2): 45.
- (28) Das P, Ghosal K, Jana NK, Mukherjee A, & Basak P. 2019. Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent. *Materials Chemistry and Physics*, 228: 310-317.
- (29) Guarner J. 2018. Buruli ulcer: review of a neglected skin mycobacterial disease. *Journal of Clinical Microbiology*, 56(4): 17-25.
- (30) Gantait S, Kundu S, Ali N, & Sahu NC. 2015. Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. *Acta Physiologiae Plantarum*, 37: 1-12.
- (31) Rostamabadi MM, Falsafi SR, Nishinari K, & Rostamabadi H. 2023. Seed gum-based delivery systems and their application in encapsulation of bioactive molecules. *Critical Reviews in Food Science and Nutrition*, 63(29): 9937-9960.
- (32) Al-Hamayda A, Abu-Jdayil B, Ayyash M, & Tannous J. 2023. Advances in microencapsulation techniques using Arabic gum: A comprehensive review. *Industrial Crops and Products*, 205: 117556.

- (33) Riseh RS, Hassanisaadi M, Vatankhah M, & Kennedy JF. 2023. Encapsulating biocontrol bacteria with starch as a safe and edible biopolymer to alleviate plant diseases: A review. *Carbohydrate Polymers*, 302: 120384.
- (34) Rahim I, Zamzam S, Suherman S, Syamsia S, Meriem S, Yunarti Y, & Nasruddin A. 2019. Enhance content of leaf chlorophylls and the primary root diameter of shallot (*Allium cepa* L.) with seed coating by rot fungi. *International Journal of Agriculture System*, 7(1): 18-26.