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POTENTIAL OF ENCAPSULATION *Bacillus cereus* BTH-22 AGAINTS BACTERIAL WILT DISEASE ON EGGPLANT

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ABSTRACT

Endophytic bacteria are a group of bacteria that live in plant tissues, potentially as biological agents of plant diseases, especially wilt disease caused by Ralstonia solanacearum and as an inducer of eggplant (Solanum melongena) plant growth. One of these bacteria is *Bacillus cereus* BTH-22 which was isolated from healthy eggplant stems in Kediri. The purpose and formulation of the problem are to obtain an encapsulation formulation of B. cereus BTH-22 which has the potential as a biological agent against wilt disease caused by R. solanacearum and as an inducer of eggplant (S. melongena) plant growth. The novelty of the research is the encapsulation formulation of B. cereus which is applied to eggplant plants with wilt, because the application generally uses root soaking, watering to the soil surface, mixing with fertilizer, liquid and powder formulations but all are easily washed away by rain. The research method is as follows: (1) Making encapsulation formulation: 75% Na-alginate with 10% aloe vera extract (Na-1) and 75% Na-alginate, 0.875% zeolite and 0.875% sago flour (Na-2), (2) Sterile soil, inoculate R. solanacearum and prepare 1 month old plants, (3) Application of encapsulation formulation was carried out 3 days after inoculation of R. solanacearum (10^7CFU/mL) using a Completely Randomized Design with 3 replications. Observations were made on days 7, 14, 21, 28, 35, 42, 49 including : disease intensity, plant height, number of leaves, number of flowers. The results of the study showed: viability in the Na-1 and Na-2 treatments was higher than control at the 48th to 96th hours, disease intensity in the Na-1 and Na-2 treatments was lower than control at the 7th to 49th day, plant height in the Na-1 and Na-2 treatments was higher than control at the 14th to 49th day.

Keywords: *Bacillus cereus BTH-22, Encapsulation, Bacterial wilt, Biological control*

ABSTRAK

Bakteri endofit merupakan kelompok bakteri yang hidup di dalam jaringan tanaman, berpotensi sebagai agensia hayati penyakit tanaman utamanya penyakit layu yang disebabkan oleh *Ralstonia solanacearum* dan sebagai induktor pertumbuhan tanaman terung (*Solanum melongena*). Salah satu bakteri tersebut adalah *Bacillus cereus* BTH-22 yang diisolasi dari batang tanaman terung sehat dari Kediri. Tujuan dan rumusan masalah untuk memperoleh formulasi enkapsulasi *B.cereus* BTH-22 yang berpotensi sebagai agensia hayati terhadap penyakit layu yang disebabkan oleh *R. solanacearum* dan sebagai induktor pertumbuhan tanaman terung (*S. melongena*). Kebaruan penelitian adalah formulasi enkapsulasi *B. cereus* yang diaplikasikan pada tanaman terung sakit layu, karena aplikasi umumnya menggunakan perendaman akar, penyiraman ke permukaan tanah, pencampuran dengan pupuk, formulasi cair dan serbuk tetapi semuanya mudah tercuci hujan. Metode penelitian sebagai berikut : (1) Pembuatan formulasi enkapsulasi : Na-alginat 75% dengan ekstrak lidah buaya 10% (Na-1) dan Na-alginat 75%, zeolit 0,875% dan tepung sagu 0,875% (Na-2), (2) Steril tanah, inokulasi *R. solanacearum* dan menyiapkan tanaman umur 1 bulan, (3) Aplikasi formulasi enkapsulasi dilakukan 3 hari setelah inokulasi *R. solanacearum* (10^7CFU/mL) menggunakan Rancangan Acak Lengkap dengan 3 ulangan. Pengamatan

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dilakukan pada hari ke-7, 14, 21, 28, 35, 42, 49 meliputi : viabilitas, intensitas penyakit, tinggi tanaman. Hasil penelitian menunjukkan : viabilitas pada perlakuan Na-1 dan Na-2 lebih tinggi dibanding kontrol pada jam ke-48 sampai dengan ke-96, intensitas penyakit pada perlakuan Na-1 dan Na-2 lebih rendah dibanding kontrol pada hari ke-7 sampai dengan ke-49, tinggi tanaman pada perlakuan Na-1 dan Na-2 lebih tinggi dibanding kontrol pada hari ke-14 sampai dengan ke-49.

Kata kunci: *Bacillus cereus* BTH-22, Enkapsulasi, Layu bakteri, Pengendalian hayati

INTRODUCTION

Eggplant (*S. melongena*) is a plant that is easy to obtain in East Java with production in 2021 is 905,188 quintal and increasing to 1 026,387 quintal in 2022 (Badan Pusat Statistik, 2023). This increase was obstacle by *Ralstonia solanacearum* which causes wilt disease and resulted a decrease in production and economic is 68% (Kago *et al*., 2016). Control of this disease until now using bactericide and until now the results are not satisfactory so need alternative control using endophytic bacteria as a biocontrol agents.

Endophytic bacteria are group of bacteria that live in plant tissue, not cause sick of plants and have mutualistic relationship with the host. One of the endophytic bacteria that has potential as biological agent for bacterial wilt disease caused by *Ralstonia solanacearum* and a growth inductor for eggplant plants (*Solanum melongena*) is *Bacillus cereus* BTH-22. This bacteri is an endophytic bacteria that can be isolated from the stem tissue of eggplant (*S. melongena*) from healthy stem tissue of eggplant (*S. melongena*) and taken from Kediri (Purnawati and Nirwanto, 2021) and Purnawati *et al*. (2019) state that formulation of endophytic bacteria from tomato stem can elicit of tomato resistance to *R. solanacearum*. Beside that, in Johor, Selangor, Penang, Kedah, Perak, Pahang, and Negeri Sembilan and is able to inhibit the *R. solanacearum* that causes wilt *in vitro* with an antibiosis mechanism and producing metabolites (Tuhumury *et al*. 2021) and Ramires *et al*. (2022) stated that *B. cereus* MH778713 produces volatile compounds, capable of inhibiting *Fusarium oxysporum* and inducing tomato plant growth *in vitro* and *in vivo*.

Research about encapsulation of *B. cereus* as a plant disease agent has been conducted by Chen *et al*., (2013) about encapsulation using maltodextrin and gum arabic against the pathogen *Cochliobolus heterostrophus*. In general, encapsulation research has been widely conducted for several pathogenic biological agents, including encapsulation of *Pseudomonas fluorescens* using alginate combined with carboxymethyl cellulose (CMC), and peanut butter (PB) to control *Rhizoctonia solani* in potato plants (Fathi *et al*., 2021), encapsulation of *Pseudomonas fluorescens* using Na-alginate to control *Fusarium solani* in potato plants (Pou *et al*., 2019), and encapsulation of *Bacillus subtilis* using bentonite combine with alginate enriched with titanium nanoparticles to control *Rhizoctonia solani* in bean plants (Riseh and Pour, 2020). Application of *B.cereus* BTH-22 as biocontrol agents generally uses root soaking, watering to the soil surface, mixing with fertilizer, liquid and powder formulations but all are easily washed away by rain (Purnawati *et al*., 2019) so application techniques using the right formula are needed to obtain effective results from the application of *B.cereus* BTH-22 as a control agent and as a plant growth inductor, so need other technique use encapsulation formulation. Formula encapsulation using of them able to protect and provide nutrition for *B.cereus* BTH-22 so that its growth with stable colony number indicators. Dobrincic *et al*., (2020) state that Alginate is a significant polysaccharide that is present in abundance in the cell wall of *Macrocystis pyrifera*, and *Ascophyllum nodosum* that can facilitate the growth of microbes so viability *B. cereus* BTH-22 is stable, Fanucci and Seese (1991) state that alginate is capable of absorbing bacteria through the absorption process and retaining bacteria during the gel matrix formation process. Beside that, *Aloe vera* in powder form contains amylose and amylopectin as bacterial nutrients, so that encapsulation of microorganisms is not easily damaged and protects microorganisms (Nurlaeli, 2012). Zeolite as a nitrogen fixing material and sago is a

biopolymer starch that can be used as a packaging material, both are sources of nutrition for stable growth of *B.cereus* colonies (Styana, 2010; Muslim *et al*., 2015).

Research on encapsulation of *B. cereus* BTH-22 using Na-alginate combined with *Aloe vera* and Na-alginate combined with zeolite and sago flour as biological agents of *R. solanacearum* has not been widely available, so this encapsulation formulation in this research is a novelty.

The aim of the research was to assess of *B.cereus* BTH-22 encapsulation formulation using combination of Na-alginate with *Aloe vera* and combination of Na-alginate, zeolite and sago against the bacterial wilt disease *R. solanacearum* on eggplant.

MATERIAL AND METHODE

The research was done in Plant Health Laboratory, Faculty of Agriculture, UPN "Veteran" Jawa Timur at April until August 2023. The research using a Completely Randomized Design with 3 replications. Observations were made on days 7, 14, 21, 28, 35, 42, 49 including: bacterial viability, disease intensity, plant height.

The tools that used in this research are autoclave (All American 75x), laminar air flow (Vertical Laminar Air Flow 1350 LABTECH LCB-1121 VE), hot plate (HS 7 IKA C-MAG MAGNETIC STIRER), mikropipet 1 000 ul (Accumax), vortex (Maxi Mix II). Material that used in this research are *B. cereus* BTH-22 that taken from Kediri which is located is 111°47'05"- 112°18'20" EL and 7°36'12"-8°0'32" LS, *R. solanacearum* isolate that collection Dr. Arika Purnawati, 1.75% Na-alginate (Merck), 10% *Aloe vera* extract, 0.875% zeolite powder, 0.875% sago starch, distilled water, 1% calcium chloride ($CaCl_2$), Nutrient Agar (NA) (Merck), natrium chloride (NaCl), 70% alcohol, 5% sodium hypochlorite solution (5% NaOCl), 4% formalin, local eggplant variety aged 2 months, sterile soil, polybag 3 kg.

Methode

Isolation *B.cereus* BTH-22 and Rejuvenation *R. solanacearum*

B.cereus was isolated BTH-22 from stems of healthy eggplant then the surface was disinfected by 70% alcohol, planted in NA medium and was incubated at 37ºC for 24 hours. The growing bacteria then purified in new medium and was identified. The collection of *R. solanacearum* was rejuvenated on new NA medium and used for research (Purnawati and Nirwanto, 2021)

Preparation Encapsulation Using Combination of Na-alginate with *Aloe Vera*

The 1.75% Na-alginate solution had been already and still on the magnetic stirrer was added 50% *Aloe vera* extract. The *B. cereus* bacterial suspension (10^7 CFU/mL) was added to a mixture of Na-alginate and *Aloe vera* solution, stirred until homogen. This mixed solution was then extruded using 10 ml syringe into 1% $CaCl_2$ solution and stirred until homogen (Nurlaeli, 2012).

Preparation Encapsulation Using Combination of Na-alginate with zeolite and sago

The 1.75% Na-alginate solution had been already was mixed with 0.875% zeolite powder and 0.875% sago starch. The *B. cereus* bacterial suspension (10^7 CFU/mL) was added to a mixture of Na-alginate and *Aloe vera* solution, stirrer until homogen. This mixed solution was then extruded using 10 ml syringe into 1% $CaCl_2$ solution andstirred until homogen (Suci and Astar, 2022).

Application of Formula Encapsulation to Plants in Screen House

Soil was sterilized using 4% formalin then put it in polybag and was inoculated 10 ml of *R. solanacearum* (10^7 CFU/mL) into each polybag and incubate for 7 days. Plant Application of formula encapsulation at 3 days after inoculation *R. solanacearum* and each plant was applicated 5 g beads (Purnawati *et al.,* 2019).

Statistical Analysis

Analysis of variance (ANOVA) was used to analyze the effect of different treatments. Duncan's multiple range test was applied when ANOVA found significant differences between treatments. The statistical significance was set at $P<0.05$. Data analysis was performed using statistical software programs (SPSS 20, SPSS Inc.).

RESULT AND DISCUSSION

The Anova results for all observation parameters are not different so they cannot be further analyzed using the Duncan test. However, the presentation using the curves in Figures 1, 2, 3 can read the results of each observation parameter.

Viability of Bacteria

Figure 1. Viability encapsulation *B. cereus* was observed by counting the number of colonies and the number of colonies is an indicator of bacterial growth. The results for Na-1 and Na-2 were more higher than the control at 48th, 72nd, 96th day. Because Na-alginate is polysaccharide which is a source of nutrition for bacteria consisting of two monosaccharide units, namely β-D-mannuronic acid (M) and α-L-guluronic acid (G). The polysaccharide is composed of elements C, H, O which are needed by bacteria to grow with an indicator of viability or the number of bacterial colonies. The polysaccharide can be combined with aloe vera, zeolite and sago flour which contains C, H, O, N elements so that it supports bacterial viability. *Aloe vera* produces amylose and amylopectin as bacterial nutrients, so that encapsulation of microorganisms is not easily damaged and protects microorganisms, zeolite as a nitrogen fixing material and sago is a biopolymer starch that can be used as a packaging material, both of them are sources of nutrition for stable growth of *B.cereus* colonies. Proteins are made of amino acids, while polysaccharides are composed of sugar molecules that can be used as carbon sources for the growth of microbes Todar (2020) stated that bacteria require nutrients for growth, namely the main elements C, H, O, N, S, P which are found in the form of micromolecules and macromolecules in cells and supporting elements Mn, Co, Zn, Cu, and Mo. Natrium alginate is polysaccharide which is a source of nutrition for bacteria consisting of two monosaccharide units, namely β-D-mannuronic acid (M) and α-L-guluronic acid (G) and can combine with *Aloe vera*, zeolite, sago (Miskiyah *et al*., 2020; Suci and Astar, 2021). Encapsulation with biopolymers or polysaccharide can increase maximum cell viability and encapsulation of microbe cells with alginate enhances the cell survival rate, and extends the microbe cell release period. This reason is supported by the statement Sumanti *et al*. (2017), effect of alginate in microencapsulated bacteria *L. acidophilus* was significantly on cell viability.

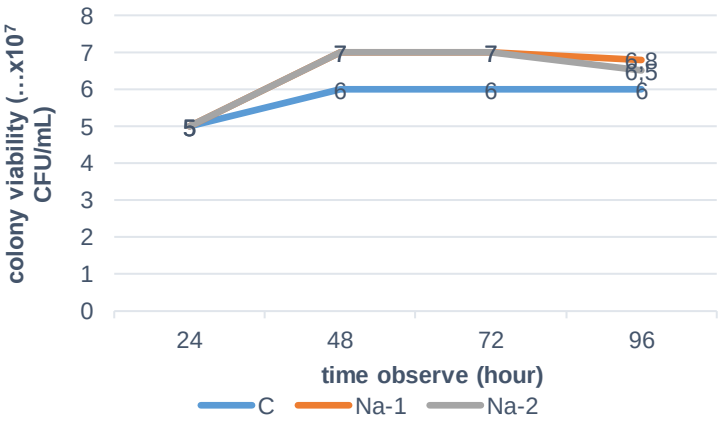

Figure 1. Colony viability of *B. cereus*

Disease Intensity

Figure 2. Disease intensity of bacterial wilt disease in the Na-1 and Na-2 treatments was generally lower than the control at 7th to 49th day. Because the viscosity of Na-alginate is low so that it is easily dissolved in the soil and causes *B. cereus* to be released faster, colonize eggplant plant roots faster and protect against *R. solanacearum* infection which causes wilt disease. In addition, the presence of aloe vera, zeolite, and sago supports the release of *B. cereus* from the capsule because these three ingredients affect the optimal balance between solubility and efficiency of bacterial release, which supports the suppression of bacterial wilt disease intensity. This is supported by the statement of Aoki *et al*., (2012) that the viscosity of Na-alginate affects its release in nature. Pitchaon *et al*., (2013) stated that high viscosity and thickness of the

solution affect the porosity of microparticles and prevent the diffusion of active ingredients to the surface of microparticles so that the lower the viscosity, the faster the release occurs. *Aloe vera*, zeolite, sago each function as Na-alginate adhesives against *B. cereus*, controllers of Na-alginate solubility in the soil, wrappers of *B. cereus* in Na-alginate so that its solubility in the soil becomes stable and the release of *B. cereus* in the soil becomes controlled. Encapsulation with biopolymers or polysaccharide can increase in bacterial colonization around the plant roots. This reason is supported by Nurlaeli (2012) statement that *Aloe vera* is a Na-alginate adhesive against *B. cereus*, Suci and Astar (2021) stated that zeolite is able to control the solubility of Na-alginate in the soil and Martínez-Cano *et al*., 2022) stated that sago wraps *B. cereus* in Na-alginate so that its solubility in the soil becomes stable and the release of *B. cereus* in the soil becomes controlled.

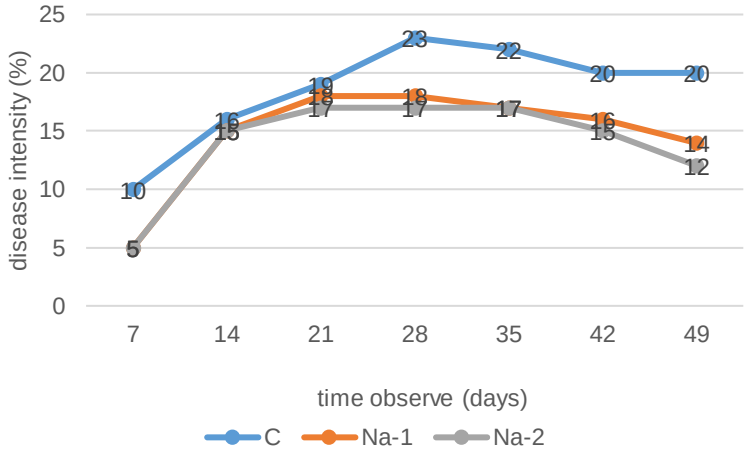

Figure 2. Disease intensity of wilt on *S. melongena*

Plant Height

Figure 3. Plant height in the Na-1 and Na-2 treatments was higher than the control at 14th to 49th day because encapsulation using biopolymers (alginate) can protect bacterial cells against environmental conditions and toxic compounds, increase the activity of *B. cereus* as Plant Growth Promoting Rhizobacteria (PGPR), resulting in maximum cell survival, increasing bacterial colonization around plant roots, can slowly release microorganisms into the soil and have longer effectiveness on plant growth through siderophore production, nitrogen fixation, cytokine auxin synthesis, vitamin and plant hormone production. This reason is supported by Nielsen *et al*., (2020) statement that polysaccharides have been used for the encapsulation of microbes, to enhance their shelf life, control their release, and to promote their bioactivities.

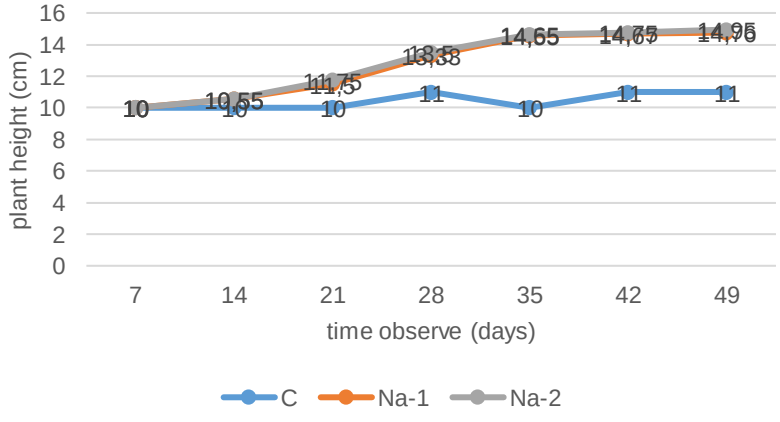

Figure 3. Plant height of *S. melongena*

CONCLUSION

Encapsulation of *B. cereus* Bth-22 has an effect on: (1) viability of bacteria more higher than control at 48th, 72nd, 96th hours, (2) decrease of disease intensity at 7th to 49th day and more lower than control, (3) plant heigh was higher than control at 14th to 49th day.

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