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Consistency Of Endo-Rhizobacteria Formulation As Biocontrol Ingredients For Fusarium Disease In Shallots (Allium Ascalonicum L.)

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ABSTRACT

Fusarium oxysporum is a serious disease that affects shallots and can result in crop failure. Controlling fusarium disease requires a variety of strategies, including the development of microbial antagonists. Microbes are used sparingly in the field due to their instability, inconsistency, efficacy, and shelf life. The purpose of this study is to determine the efficacy of endophytic and rhizobacteria formulations in carrier media as biological control agents against fusarium disease in shallots.Protease, amylase, and inhibition tests were performed in vitro on three bacterial isolates formulated on 0.85 percent NaCl carrier, talc, bentonite, and husk charcoal powder. The variance of the data observations was determined, and those that revealed a significant effect were further examined using Duncan's Multiple Range Test at a 95% confidence level.The results indicate that rhizobacteria and endophytic bacteria formulated in the carrier medium are capable of inhibiting Fusarium oxysporum pathogen development and increasing the activity of protease and amylase enzymes. Protease and amylase enzymes exhibited the greatest inhibitory power and activity when used to treat the W2R06 isolate rhizobacteria formulation that was formulated in a carrier medium of husk charcoal powder. W2R06 isolate formulated in husk charcoal may be used as a biological controller for plant diseases, particularly fusarium disease, thereby reducing the need for synthetic pesticides.

Keywords: Endophytic bacteria, bentonite, Fusarium oxysporum, husk charcoal powder, rhizobacteria, talc

INTRODUCTION

Fusarium disease, also known as moler disease, is a significant disease of shallots, attacking at a rate of 5-60% and even causing crop failure (Hadiwiyono et al., 2020; Le et al., 2021; Mandal and Cramer, 2021). This disease is caused by the fungus Fusarium oxysporum, a soil-borne pathogen that survives without a host and spreads rapidly through the soil (Le et al., 2021).

Microbial antagonists are now being developed for the control of plant diseases in order to prevent the excessive use of pesticides, which have a detrimental effect on human health and the environment (Alori and Babalola, 2018; Sellitto et al., 2021). Microbial antagonists such as rhizobacteria and endophytic bacteria can suppress infections in a variety of plants by producing siderophores, salicylate enzymes, peroxidases, and HCN (Sutariati et al., 2019; Gupta and Gopal, 2008; Nithyapriya et al., 2021; Martnez-Viveros et al., 2010). Bacteria that generate peroxidase enzymes can aid pepper plants in resisting disease attacks caused by Phytophthora capsici (Shobha and Murthy, 2018).

In general, the ability of rhizobacteria and endophytic bacteria to prevent the growth of pathogenic fungi or bacteria is still restricted to the synthesis of HCN, salicylate enzyme activity, and peroxidase enzyme activity (El-Deeb et al., 2012; Sutariati et al., 2020). However, the capacity of microbial antagonists to produce amylase and prosthetic enzymes is being explored at the moment (Khan et al., 2017; Kim et al., 2009; Saboti and Kos, 2012). Bacteria that produce amylase, protease, or peroxidase have the ability to degrade bacterial or fungal cells (Bibi et al., 2017; El-Deeb et al., 2012; Chaiharn et al., 2008).

The carrier medium and shelf life of microbes have an effect on their superiority in inhibiting pathogen development and increasing plant growth (Sarin and Riddech, 2018). According to Muis et al. (2006), B. subtilis that has been formulated and stored for an extended period of time will lose viability. As a result, a solid carrier medium is required that aims to stabilize the bacterial population, meet the microbe's nutritional requirements, and maintain the bacteria's ability to promote growth and control plant diseases (Rekha et al., 2007). Rakian et al. (2018) reported on a study of solid carrier media in which they discovered that using solid carrier media improved bacteria's performance in the field.

Suriyadi et al. (2013) continued by stating that using bentonite as a carrier medium can help maintain the bacterial population while inhibiting pathogen development. As such, this study will assess the consistency of an endophytic-rhizobacteria formulation used as a biological control agent against fusarium disease (Fusarium oxyzporum) on shallots (Allium ascalonicum L.).

MATERIALS AND METHOD

From December 2018 to June 2019, this research was conducted at UniversitasHalu Oleo's Laboratory of Agricultural Agronomy. This study used a completely randomized design with endo-rhizobacteria treatment formulated in a carrier medium with 12 levels, including endophytic bacteria isolate BE03 in 0.85 percent NaCl carrier medium (B1/control), isolate BE03 in Talc (B2), isolate BE03 in bentonite (B3), isolate BE03 in husk charcoal powder (B4), rhizobacteria isolate TWB11 in 0.85 percent NaCl carrier medium (B5 (B12). Each treatment was repeated three times, resulting in a total of 36 treatment units.

Source of Endo-rhizobacteria Isolate

Three bacterial isolates were obtained from the Laboratory of Agrotechnology, Faculty of Agriculture, UniversitasHalu Oleo: BE03, TWB11, and W2RO6. Bacterial isolates were rejuvenated using the scratch method and incubated at room temperature for three days on TSA medium. The isolated strains were kept to investigate the activity of protease, amylase, and inhibitory enzymes.

Fusarium oxysporum isolation

Fusarium oxysporumwas isolated from shallot bulbs suffering from Fusarium symptoms disease. Symptomatic onion bulbs were washed under running water and allowed to dry naturally. The tubers were cut to a diameter of 1 cm and disinfected for two minutes with a 1 percent sodium hypochlorite solution. Desiccated tubers were rinsed and dried with sterile distilled water. The tubers were planted in a petri dish containing PDA media and incubated at 270C for 3-5 days until fungal mycelium developed and could be identified

under a microscope. To purify and test F. oxysporum mycelium, it was transferred to PDA media.

Formation of Indigenous Bacterial Biological Agent

Talc, bentonite, and husk charcoal powder were sterilized in an autoclave at a temperature of 121^0C and a pressure of 1.5 atm. The sterile carrier material was air-dried in laminar air flaw and mixed with endo-rhizobacteria suspension until it reached a moisture content of 50% (w/v) and the control treatment for each bacterium used 0.85% NaCl. The carrier and bacterial formulations were put in sterile scotch bottles and stored at room temperature to test the performance of inhibition, protease and amylase enzyme activity for four months.

Quantity Test of Protease Activity

The protease activity test was qualitatively determined using the method blank disc which has been modified. Indigenous bacterial biological agents were inoculated in nutrient broth (NB) medium and incubated on a rotary shaker for 24 hours. One ose of bacterial culture was placed in the middle of a petri dish containing 1% protease-skim milk agar medium with a pH of 7.0 and incubated at 28oC. The diameter of the clear zone formed around the bacterial colony was measured, and the activity was qualitatively determined based on the index value, namely the total zone diameter divided by the diameter of the bacterial colony.

Qualitative Test of Amylase Activity

Bacterial isolates were grown on tilted TSA agar media and transferred to YPS agar media and incubated for two days at room temperature. Selective media overgrown with bacteria dripped with iodine solution. Bacteria that have amylase activity will see a clear zone around the bacterial colony and the media around it will look blue.

Antagonism Ability of Endo-rhizobacterial Biological Agents

The ability of endo-rhizobacteria antagonists was tested using the dual test method on PDA media (Marwan et al., 2020). Antagonism was observed daily by measuring the radius of pathogen growth towards the edge of the petri dish (R1) and the radius of pathogen growth towards the bacteria (R2). The data obtained is calculated using the formula:

$$DH = \frac{R_1 - R_2}{R_1} x100\%$$

Note: DH= Inhibitory power

R1 = Radius of pathogen growth towards the edge of the petri

R2 = Radius of growth of the pathogen towards the isolate of the biological agent

Data analysis

Observational data were analyzed for variance, which showed a significant effect followed by Duncan's Multiple Range Test at 95% confidence level and the relationship between inhibition and activity of protease and amylase enzymes was described in a simple regression test.

RESULTS

Protease Enzyme Production

The activity of protease enzymes produced by bacteria on carrier media with a shelf life of 0, 8 and 16 MS, respectively, is presented in Table 1. In general, the highest amylase and protease enzyme activities were obtained in B12 treatment which was different from other treatments.

Table 1. The average activity of protease enzymes produced by bacteria on several carrier media with a shelf life of 0, 8 and 16 MS.

Treatment	Amylase Enzyme Activity Test (cm) Week		
	0	8	16
B1	1.45 a	1.50 d	1.50d
B2	1.55 a	1.55cd	1.65c
B3	1.60 a	1.60cd	1.70c
B4	1.65 a	1.65bc	1.65c
B5	1.55 a	1.60cd	1.60cd
B6	1.60 a	1.60cd	1.65c
B7	1.65 a	1.65bc	1.65c

B8	1.65 a	1.75ab	1.70c
B9	1.70 a	1.75ab	1.90b
B10	1.75 a	1.85 a	2.00ab
B11	1.75 a	1.80 a	1.95ab
B12	1.80 a	1.85 a	2.05a

Note: Numbers followed by the same letter in the same column are declared to be not significantly different based on Duncan's Multiple Range Test at a 95% confidence level.

Amylase Enzyme Production

The activity of the amylase enzyme produced by bacteria on carrier media with a shelf life of 0, 8 and 16 MS, respectively, is presented in Table 2. In general, the highest amylase enzyme activity was obtained in the treatment B12 treatment which is different from other treatments.

Table 2. The average activity of the amylase enzyme produced by bacteria on several carrier media with a shelf life of 0, 8 and 16 MS.

Treatment	Amylase Enzyme Activity Test (cm) Week		
	0	8	16
B1	1.10d	1.15d	1.40f
B2	1.15d	1.20d	1.45ef
B3	1.15d	1.15d	1.40f
B4	1.20d	1.20d	1.50def
B5	1.55c	1.60c	1.60cde
B6	1.60bc	1.65bc	1.65bcd
B7	1.65abc	1.70bc	1.65bcd
B8	1.65abc	1.65bc	1.70bc
B9	1.60bc	1.70bc	1.75abc
B10	1.70abc	1.70bc	1.80ab
B11	1.75ab	1.75b	0 1.90a

B12	1.80a	1.90a	1.90a

Note: Numbers followed by the same letter in the same column are declared to be not significantly different based on Duncan's Multiple Range Test at a 95% confidence level.

Bacterial Antagonist Ability and Its Relationship with Amylase and Protease Enzyme Activities

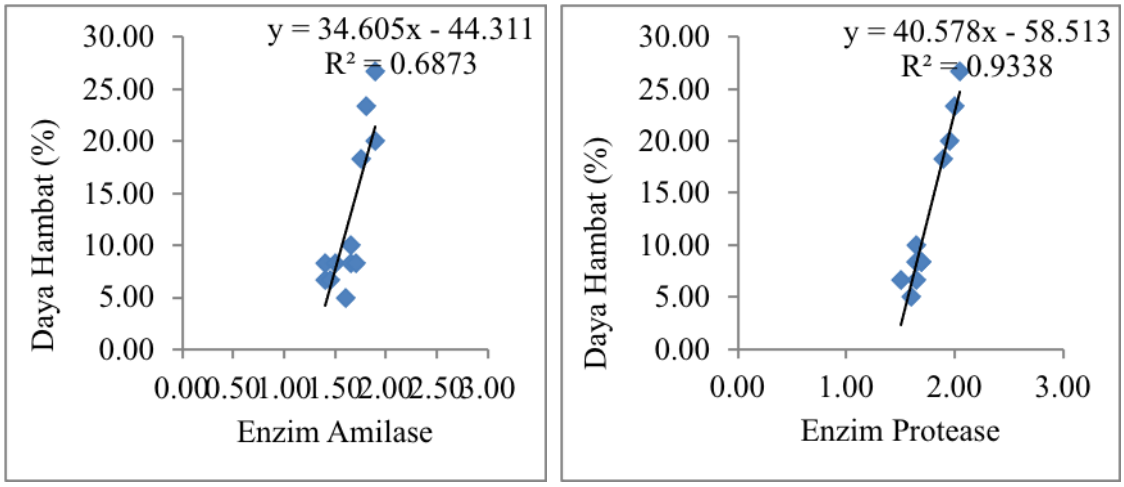
The inhibition zone of bacteria stored on several carrier media observed at 0, 8 and 16 weeks after the shelf life is presented in Table 3 and the relationship of inhibition with the activity of protease and amylase enzymes is presented in Figure 1.

Table 3. The average inhibition of bacteria stored on several carrier media were observed at 0, 8 and 16 weeks after the storage period.

Treatment	Rhizobacteria Inhibitory (%) Week		
	0	8	16
B1	5.00d	6.67 c	6.67 c
B2	6.67cd	8.33 c	6.67 c
B3	5.00d	6.67 c	8.33 c
B4	10.76bcd	6.51 c	8.33 c
B5	9.38bcd	6.67 c	5.00 c
B6	3.33abcd	8.33 c	8.33 c
B7	7.82cd	8.33 c	10.00 c
B8	3.33d	6.67 c	8.33 c
B9	20.00abc	16.67b	18.33b
B10	25.00a	21.67ab	23.33ab
B11	21.67ab	23.33a	20.00ab
B12	25.00a	26.67a	26.67a

Description: Numbers followed by the same letter in the column the same is declared to be not significantly different based on Duncan's Multiple Range Test at a 95% confidence level.

In general, the highest average inhibition of bacteria at week 0, 8 and 16 was obtained in treatment B12, which was significantly different from other treatments, especially bacteria treated with NaCl.



A **B**

Figure 1. The relationship between bacterial inhibition with amylase (A) and protease (B) enzymes.

The inhibition of bacteria against the fungus Fusarium oxysforum is closely related to the activity of amylase and protease enzymes produced by bacteria. The relationship between inhibition with amylase enzyme (Figure 1A) was 68.73% with a regression value of y= 34,605x - 44,311 while with protease (Figure 1B) of 93.38% with a regression value of 40.578x - 58,513.

DISCUSSION

Agriculture is currently developing the use of bacteria to boost productivity and control diseases that attack cultivated plants. The bacteria used in this study were isolated from bacteria found around healthy plant roots in plants infected with fusarium disease, referred

to as rhizobacteria, and from bacteria found in plant tissue, referred to as endophytic bacteria. Babalola, (2010) and Firdous et al., (2019) stated that the bacteria developed were investigated in the root zone (rhizobacteria) and in plant tissues (endophytic bacteria). Rhizobacteria and endophytic bacteria can exert control over disease by producing HCN compounds, siderophores, amylase enzymes, peroxidases, chitinases, proteases, and salicylic acid. (2013); Shobha and Murthy, 2018; Chaiharn et al., 2008). Sutariati et al. (2019) and Rashad et al. (2017) demonstrated that bacteria with multiple chitinase, salicylate, and peroxidase enzyme activities can inhibit the growth of fusarium pathogens.

The results indicate that treatment of bacterial isolates resulted in the formation of a clear zone surrounding the bacterial colony. The W2R06 treatment, which used husk charcoal as a carrier medium, had the highest protease enzyme activity when compared to the other treatments. Each bacterial isolate is suspected to have unique characteristics and capacities for producing protease enzyme activity. According to Chaiharn et al. (2008), a variety of bacterial isolates were investigated, each with a unique ability to perform an enzyme activity. Apart from the characteristics of the bacteria, it is believed that the storage media influence the enzyme activity produced (Rani et al., 2012). The results indicated that each bacterial isolate formulated in rice husk charcoal carrier medium could significantly produce more protease enzyme activity than the control bacteria formulated in NaCl. Rani et al. (2012) demonstrated that the nutrients present in the carrier media used can affect the activity of the enzymes produced. Variations in carbon/nitrogen, glucose, metal ions, and metabolizable nitrogen sources all influence the activity of a bacterium. According to Dhillon et al. (2016), carbon sources such as starch, maltose, and molasses can boost proteolonitic enzyme production during the storage process. that the nutrients present in the carrier media can influence the activity of the enzymes produced. Variations in carbon/nitrogen, glucose, metal ions, and metabolizable nitrogen sources all influence the activity of a bacterium. According to Dhillon et al. (2016), carbon sources such as starch, maltose, and molasses can boost proteolonitic enzyme production during the storage process. that the nutrients present in the carrier media can influence the activity of the enzymes produced. Variations in carbon/nitrogen, glucose, metal ions, and metabolizable

nitrogen sources all have an effect on the activity of a bacterium. According to Dhillon et al. (2016), carbon sources such as starch, maltose, and molasses can boost proteolonitic enzyme production during the storage process.

Furthermore, the results showed that all of the bacterial isolates tested were capable of generating the amylase enzyme. The amylase enzyme's activity was evaluated by monitoring the clear zone surrounding bacterial colonies. The clear zone reveals how active the amylase enzyme is in the starch degradation of the test media. W2R06 isolate bacterium had the highest amylase enzyme activity when compared to other bacteria. The treatment of bacterial isolates is thought to have resulted in the creation of the amylase enzyme's storage phase in a variety of carrier medium. Couto and Sanromán (2006) and Sajedi et al. (2005) found that numerous parameters, including carrier medium composition, temperature, inoculum age, and carbon and nitrogen sources, influence bacteria that manufacture amylase enzymes during storage.

Inhibition of bacteria against pathogens that cause disease in cultured plants demonstrates bacteria' antagonistic ability. The results indicated that while the treatment of rhizobacteria W2R06 formulated on husk charcoal powder (B12) had a greater inhibitory power than the others, it was not significantly different from the treatment of W2R06 formulated on talc (B10) or W2R06 formulated on the carrier medium. but significantly different from other treatments due to the use of a bentonite carrier (B11). Bacterial inhibition of the fungus F. oxysporum demonstrated antagonism activity, which occurred because of the bacteria competing for nutrients and secreting antibiotics that inhibited F. oxysporum growth. With a correlation coefficient of 93.38 percent and a regression coefficient of 40.578x - 58.513, the increase in inhibition was closely related to the protease enzyme activity. In comparison to other treatments, the results of this study indicate that W2R06 bacteria can produce 2.05 cm of protease enzyme activity. Proteases can exert a significant inhibitory effect on aspartate, serine, and cysteine proteinases, as Kim et al. (2009) demonstrated. Protease enzymes are capable of degrading the pathogen's proteins, which serve as the pathogen's building blocks.Additionally, the activity of the amylase enzyme produced by bacteria corroborated the study's findings. All bacterial isolates

exhibited amylase enzyme activity, with the best results obtained when rhizobacteria isolates W2R06 were formulated in husk charcoal media. The inhibition of the amylase enzyme was correlated with its activity. The correlation between inhibitory power and amylase enzyme activity was 68.73 percent with a regression coefficient of y= 34,605x - 44,311. These findings demonstrate that the inhibition is inversely proportional to the increase in amylase activity. Amylase enzymes are capable of hydrolyzing starch molecules into polymers composed of glucose, maltose, and maltotriose units (Paula and Magalhaes, 2010), which comprise the fungus body structure.

The results showed that the bacterial isolates formulated in the carrier medium had a wide range of inhibitory characteristics. In general, the results showed that rhizobacteria and endophytic bacteria isolate K2R06 were inhibited more successfully when treated with a formulation containing husk charcoal, bentonite, and talc as carrier media than when NaCl was employed as the control. It is thought that carrier media can provide the nutritional needs of microorganisms. Vassilev et al. (2020) demonstrated the importance of carrier media in supplying nutrients to microbes, preventing bacterial cell degradation during storage, and improving microbial performance. Bacteria mixed with alginate, bentonite, starch, and other carrier medium can boost bacterial cell storage and proline content, as well as peroxidase enzyme activity, according to Hee et al. (2017) and Liffourrenna and Lucchesi (2018). According to Regards et al. (2013), using bacteria made with wood grain can aid in the prevention of plant disease transmission. As a result, the utilization of microorganisms formulated in carrier media for the field control of plant diseases has the potential to be developed.

CONCLUSION

The rhizobacteria and endophytic bacteria formulated in the carrier medium were able to inhibit the growth of the pathogen Fusarium oxysporum and increase the activity of protease and amylase enzymes. The highest inhibitory power and activity of protease and amylase enzymes were obtained in the treatment of the W2R06 isolate rhizobacteria formulation which was formulated in the carrier medium of husk charcoal powder.

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REFERENCES

Alori ET, and B.OO. (2018). Microbial inoculants for improving crop quality and human health in Africa. Front. Microbiol.**Vol. 9 (2213): 1-12.**

Babalola AA. 2010. Beneficial bacteria of agricultural importance. Biotechnology Letters**Vol. 32(11):1559-70.**DOI:10.1007/s10529-010-0347-0.

Bibi F, Ullah I, Al-Ghamdi AAK, Akhtar S., Yasir M, Kensarah EA, and Azha EI. (2017). Diversity and bioprospecting potential of rhizo and endophytic bacteria from two mangrove plants in Saudi Arabia. **Vol. 11(19) : 729-739.** DOI: 10.5897/AJMR2017.8552.

ChaiharnM, Chunhaleuchanon S, Kozo A, and Lumyong S. (2008). Screening of rhizobacteria for their plant growth promoting activities. **KMITL Sci. Tech. J. Vol. 8 : 18-23.**

Couto SR, and Sanromán MA. (2006). Application of solid-state fermentation to food industry- A review. Journal of Food Engineering. **Vol. 76:291–302.**

Dhillon, SharmaK, Rajulapati V, GoyalA. 2016).Proteolytic Enzymes. In book: Current Developments in Biotechnology and Bioengineering Production, Isolation and Purification of Industrial Products (pp.149-173). http://dx.doi.org/10.1016/B978-0-444-63662-1.00007-5

El-Deeb B, Bazaid S, Gherbawy Y, and Elhariry H. (2012). Characterization of endophytic bacteria associated with rose plant (Rosa damascenatrigintipeta) during flowering stage and their plant growth promoting traits. Journal of Plant Interactions**Vol. 7 (3) : 248-253.** DOI: 10.1080/17429145.2011.637161.

Firdous J, Lathif NA, Mona R, and Muhamad N. (2019). Endophytic bacteria and their potential application in agriculture: A review. Indian J. Agric. Res. **Vol. 53(1) : 1-7.**DOI:10.18805/IJARe.A-366.

Geetha, K., Venkatesham E, Hindumathi A, and Bhadraiah B, (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on Vigna radita (L.). Int.J.Curr.Microbiol.App.Sci**Vol. 3(6) : 799-809.**

Gupta A. and Gopal M. (2008). Siderophore production by plant growth promoting rhizobacteria. Indian J. Agric. Res. **Vol 42 (2) : 153 -156.**

Hadiwiyono, Sari K, and Poromarto SH. (2020). Yields Losses Caused by Basal Plate Rot (Fusarium oxysporumf.sp. cepae) in Some Shallot Varieties.Journal of Sustainable Agriculture. **Vol. 35 (2) : 250-257**,DOI: http://dx.doi.org/10.20961/carakatani.v35i2.26916.

Khan AL,Shahzad R, and Al-HarrasiA. (2017). Endophytic microbes: a resource for producing extracellular enzymes. In Book Endophytes: Crop Productivity And Protection : 95-110. DOI:10.1007/978-3-319-66544-3_5.

Kim JY, ParkSC, Hwang I, and Cheong H. (2009). Protease inhibitors from plants with antimicrobial activity. International Journal of Molecular Sciences, **Vol. 10 (6): 60-72.** DOI:10.3390/ijms10062860.

Le D, Audenaert K, and Haesaert G. (2021). Distribution of major diseases of shallot in South Kalimantan, Indonesia.Asian Journal of Agriculture, **Vol. 3 (2) : 33-40.** DOI: 10.13057/asianjagric/g030201.Tropical Plant Pathology, 46 : 241–253. https://link.springer.com/article/10.1007/s40858-021-00421-9.

Liffourrena AS, and Lucchesi GI. (2018). Alginate-perlite encapsulated Pseudomonas putida A (ATCC 12633) cells: Preparation, characterization and potential use as plant inoculants. J Biotechnol**Vol. 20 (278) : 28-33.**doi: 10.1016/j.jbiotec.2018.04.019.

Mandal S, and Cramer CS. (2021). Improving Fusarium Basal Rot Resistance of Onion Cultivars through Artificial Inoculation and Selection of Mature Bulbs. Horticulturae**Vol. 7 (168) : 1-13.**https://doi.org/10.3390/horticultuae7070168.

Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, and Mora ML. (2010). Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr. **Vol. 10 (3): 293 – 319.**

Marwan, Mulyati S, Sarman S, and Hayati I. (2020). Fluorescent pseudomonads from plant rhizosphere as biological agent to control white root disease and growth-promoting on rubber plants. Biodiversitas**Vol. 21 (11) : 5338-5343.** DOI: 10.13057/biodiv/d211139.

Muis A, and. Quimio AJ. (2006). Biological control of banded leaf and sheath blight disease (Rhizoctonia solani Kuhn) incorn with formulated Bacillus subtilis BR23. Indonesia. J. Agric.Sci.**Vol. 7(1): 1–7.**

Nandal M. and Hooda R. (2013). Plant growth promoting rhizobacteria: a review article. International Journal of Current Research **Vol. 5 (12) : 3863-3871.**

Nithyapriya S, Lalitha S,Sayyed RZ, Reddy MS, Dailin DJ, and El Enshasy HA, Suriani NL, and Herlambang S. (2021). Production, Purification, and Characterization of Bacillibactin Siderophore of Bacillus subtilis and Its Application for Improvement in Plant Growth and Oil Content in Sesame. Sustainability**Vol. 13 (5394) : 1-18.**https://doi.org/10.3390/su1310539.

Rakian TC, Karimuna L, Taufik M, Sutariati GAK, Muhidin and Fermin U, (2018). The effectiveness of various rhizobacteria carriersto improve the shelf life and the stability ofrhizobacteria as bioherbicide. IOP Conf. Series: Earth and Environmental Science 122 (2018) 012032. doi :10.1088/1755-1315/122/1/012032.

Rashad Y, Al-Askar AA, Ghoneem K, and Sabe WIA. (2017). Chitinolytic Streptomyces griseorubens E44G enhances the biocontrol efficacy against Fusarium wilt disease of tomato. Phytoparasitica 45(2):227-237. DOI:10.1007/s12600-017-0580-3.

Ratnawati, Sjam S, Rosmana A, and Tresnaputra US. (2019). Impact of pesticides on the diversity of fungi at local shallot in Palu, Indonesia. Int.J.Curr.Microbiol.App.Sci **Vol. 8 (8) : 730-738.**

Rekha PD, Lai W, Arun AB, and Young C. (2007). Efect of free and encapsulated Pseudomonas putida CC-FR2-4 and Bacillus subtilis CC-pg104 on plant growth under gnotobiotic conditions. Bioresource Technology**Vol. 98 (2007) 447–451** Doi.10.1016/j.biortech.2006.01.009.

Sabotič J, and Kos J. (2012). Microbial and fungal protease inhibitors—current and potential applications. Appl Microbiol Biotechnol**Vol. 93:1351–1375**. DOI 10.1007/s00253-011-3834-x.

Sajedi RH, Naderi-Manesh H, Khajeh K, Ahmadvand R, Ranjbar B, Asoodeh A, and Moradian F. (2005). A Ca-independent α-amylase that is active and stable at low ph from the Bacillus sp. KR-8104. Enzyme Microb. Technol. **Vol. 36 : 666-671.**

Sellitto, VM; Zara S, Fracchetti F, Capozzi V, and Nardi T. (2021. Microbial biocontrol as an alternative to synthetic fungicides: boundaries between pre- and postharvest applications on vegetables and fruits. Fermentation**Vol. 7 (60).** https://doi.org/10.3390/fermentation7020060 .

Shobha MS and Murthy MS. Effect of Endophytic and Plant Growth Promoting Rhizobacteria against Foot Rot Disease of Piper nigrum L. International Journal of Environment, Agriculture and Biotechnology (IJEAB)**Vol. 3 (6) : 2001-2020**. http://dx.doi.org/10.22161/ijeab/3.6.7.

Sintayehu A, SakhujaPK, FininsaC, and AhmedS. (2011). Management of fusarium basal rot (Fusariumoxysporum f. sp. cepae) on shallot through fungicidal bulb treatment. Crop Protection**Vol. 30 (5) : 560-565**. DOI:10.1016/j.cropro.2010.12.027.

Souza PM, and Magalhães PO. (2010). Application of microbial α-amylase in industry-A review.Braz. J. Microbiol.**Vol. 41 (4) :850–861.**https://doi.org/10.1590/S1517-83822010000400004.

Suryadi Y, Susilowati DN, Priyatno TP, Samudra IM, Kadir TS and Mubarik NR. (2013). Prospect of using bacterial bio-formulation to suppress bacterial leaf blight of rice: a case study in Cianjur, West Java. PenelitianPertanianTanamanPangan**Vol. 32 (2) : 83-90.**

SutariatiGAK, Khaeruni A, dan Madiki A. (2020). BakteriAsalWakatobiMenghambatPertumbuhanKoloni Alternaria porri dan Fusarium oxyporumPenyebabPenyakit Pada Bawang Merah Secara in Vitro. JurnalFitopatologi Indonesia **Vol. 16 (3) : 105-111.** DOI: 10.14692/jfi.16.3.105–111.

SutariatiGAK, Khaeruni A, Madiki A, Mudi L, Aco A, Ramadhani SA, AdriRM, and Mantja K. (2019). Effectiveness of endo-rhizobacteria as growth promoters and biological control

of moler disease in shallots (Allium ascalonicum L.). IOP Conference Series: Earth and Environmental Science,Vol. 681, 30 March 2020, South Sulawesi, Indonesia.

VassilevN, Vassileva M, MartosV, Luis F. Garcia del Moral LFG, Kowalska J, Tylkowski B, and Malusá E. (2020). Formulation of Microbial Inoculants by Encapsulation in Natural Polysaccharides: Focus on Beneficial Properties of Carrier Additives and Derivatives.**Front. Plant Sci., 10** March 2020. https://doi.org/10.3389/fpls.2020.00270.