Research article

The Effectiveness of the Organic Fertilizer Formula of the PGPR and Biocontrol Agents Consortium on the Growth of Leeks and Reduction of Soft Rot Disease

Hendri Bustamam*, Hartal Hartal, Hesti Wahyuni, and Herry Gusmara

Universitas Bengkulu, Bengkulu, Indonesia

ORCID
Hendri Bustamam https://orcid.org/0000-0002-0786-2987

Abstract.
The growing demand for organic leeks (*Allium fistulosum* L.) in the lowlands necessitates the introduction of organic fertilizers and disease-controlling biological agents. *Erwinia* sp. causes soft rot disease, which results in the loss of 12-40% of the plant's weight. Because soil-infected pathogens are difficult to control, biological agents that live in the same niche as pathogenic bacteria are used to combat them. This study chose to examine *Streptomyces* sp. isolate CS1 and *Trichoderma viride* isolate TTA1 for their potential antagonistic abilities against *Erwinia* sp. Organic fertilizers and biological agents can be combined in a single-use formulation for maximum efficiency. The study's primary objective was to see how effective the decomposer microbial consortium's organic fertilizer formula and biological agents were at promoting leek growth and reducing soft rot disease. The five treatments used in the study were B0 (no pathogen control), B1 (PGPR consortium organic fertilizer = BIOMEG), B2 (BIOMEG + *Streptomyces*), B3 (BIOMEG + *Trichoderma*), and B4 (BIOMEG + *Streptomyces* + *Trichoderma*). Each experiment was carried out five times. The consortium fertilizer was made in the following manner: the organic matter was fermented for four weeks with a suspension of *Bacillus megatrium, Bacillus ap., Pseudomonas sp.,* and *Aspergillus niger*, then for two weeks with a suspension of *Streptomyces*-CS1 and *Trichoderma* viride-TTA1. The leek seedlings were inoculated with *E. caratovora* and planted in a 1:4 mixture of consortium fertilizer and soil. The use of biological agents and the PGPR microbial consortium organic fertilizer formula reduced the incidence of soft rot disease in leeks better than the use of the PGPR microbial consortium organic fertilizer formula alone. The organic fertilizer formula of the microbial consortium PGPR + *Streptomyces* + *Trichoderma* produced the best results in terms of disease reduction and leek yield.

Keywords: Organic-fertilizer, PGPR, Leeks, Softrot-diseases

Corresponding Author: Hendri Bustamam; email: hendribustamam@unib.ac.id

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1. Introduction

The demand for organic leek (Allium fistulosum L) as a vegetable ingredient in Indonesia is getting higher with the increasing population and the level of welfare of the Indonesian people. So far, leek cultivation is done conventionally with chemical and organic fertilization. Leeks production in Indonesia in 2019 was 590,596.00 tons, with an area of 60,358 and productivity of 9.78 tons/ha [1]. The demand is not only for organic leeks, but the freshest leeks possible. This can be obtained through cultivating organic farms that are close to where consumers are or grown from far away and sent at a certain temperature to consumers. The Bengkulu lowlands along the Bengkulu Province coastline are 525 km, have high land potential for cultivating organic leeks, it is estimated that if 1 km from the coast can be cultivated, there will be 525 km2 of lowland land. The problem of developing organic leek in the Bengkulu lowlands is the low fertility of the land and the attack of soft rot disease. For that it is necessary to enter organic fertilizers and organic pesticides.

Leeks in Indonesia are suitable for growing in the lowlands and highlands with an altitude of 250-1500 m above sea level, but so far they are cultivated in the highlands above 700 m above sea level. Areas with rainfall of 150-200 mm/year and a daily temperature of 18-25oC are suitable for growing leeks. This plant requires a neutral pH (6.5-7.5) with Andosol soil type (former volcanic land) or sandy loam soil (Puslitbanghorti, 2021). Cultivation in the highlands with plant spacing of 25x25 cm2 and fertilization of 250 kg Urea (Nitrogen), 50 kg SP36 (Phospate), 50 kg KCl (Potassium), 7.5 tons of organic fertilizer and 1.5 tons of lime per hectare produced 15 tons of leeks [2] , but Indonesia's national productivity is only 9.78 tons/ha [1].

The main disease in leeks is soft rot caused by the bacterium Erwinia caratovora a (Jones) Bergey *et al.* [3]. Symptoms of rot develop in the field at times of high soil and air humidity. Bacteria that are in the soil and rhizosphere, enter tubers, leaf bases or roots through mechanical wounds or other wounds caused by soil organisms such as wounds caused by maggot larvae, which are the initial entry points that often occur. Wounds The initial attack of bacteria causes necrosis in the tissue and accumulation of bacterial mass along with mucus, then invasion of the surrounding tissue causes soft rot and emits a characteristic odor. Attacks on one tuber progress to the next bulb quickly. Disease progresses rapidly in warm and wet weather conditions. Bacterial growth in the field can occur in the temperature range of 6 to 32oC and the optimum temperature between 18 and 27oC; whereas in laboratory culture the optimal temperature is between 24 and 32°C. Bacteria can continue to multiply in storage or seed at temperatures greater than 3°C. The relatively high humidity and liquid water also promote the reproduction and spread of soft rot bacteria when temperatures are favorable. [4], [5],

Organic leek cultivation requires a biological plant disease control approach. The experimental results of leek cultivation in the lowlands and soft rot disease control showed that with low soil fertility conditions (pH 4.2), leeks cultivation was carried out with organic fertilizer enriched with Streptomyces at a dose of 4 tons/ha, fermented rice bran at a dose of 500 kg/ha. and lime at a dose of 4.6 tons/ha can produce leek production equivalent to 18.48 tons/ha and a decrease in soft rot disease from 100% to 12.5% [6].

Several biocontrol agent are potential to control soft rot diseases on some crops [7][8][9][10][11] [12]. Experimental results on papaya nurseries showed that the application of the consortium of bacteria *Weissella cibaria* PPKSD19 and *Lactococcus lactis* subsp. lactis PPSD39 can reduce the severity of dieback disease from 69% to 19%. The effectiveness of the biocontrol occurred after 18 days of application. *W. cibaria* PPKSD19 and *L. lactis* subsp. *lactis* PPSD39 has the potential as a biocontrol agent against E. mallotivora, the cause of papaya dieback disease. [7]. The results of in vivo experiments (in pots), the application of *Streptomyces, B. subtilis, Pseudomonas fluorescence,* and *P. aeruginosa*, were able to reduce soft rot disease of potatoes infected with 2 isolates of *E. caratovora* Ecc1 and Ecc2. The lowest disease severity value was obtained from the *Streptomyces* application; while *P. fluorescence, B. subtilis, and P. aeruginosa* each gave low disease severity scores. [8]. In vitro test using several media with different compositions, yeast strains *Metschnikowia pulcherrima* MSK1 and *Aureobasidium pullulans* CF10 and CF40 showed an antibiotic reaction against E. amylovora. The antimicrobial activity of the *M. pulcherrima* MSK1 strain is greater than other strains [9]. Inoculation of *B. subtilis* and *P. fluorescens* alone or both in potato plantations was able to inhibit the growth of *E. carotovora* (syn. *Pectobacterium carotovorum*)[10]. Disinfection of potato tubers with *S. diastatochromogenes* sk-6 can kill pathogenic infections on the surface and internal tubers, as well as protect tubers from attacks by *E. carotovora* ssp. *carotovora* (Ecc) in the early reproductive period. [11]. *Trichoderma* spp. application not only inhibits the growth of the pathogen E. carotovora, subsp *carotovora*, but also increases the growth and yield of potatoes [12].

The biological control of leek soft rot disease is not widely known. The use of organic fertilizers enriched with rice husks and *Streptomyces* biological agents can increase the growth and yield of leeks and reduce the incidence of soft rot disease[6]. The results of the selection of 10 isolates of Trichoderma and 10 isolates of Streptomyces showed that the isolates of Streptomyces sp.CJ1 and Trichoderma sp. TTA1 has excellent antagonistic ability against *E. caratovora* which causes soft rot of leeks [13]. These two isolates together with selected decomposer microbes were formulated in organic fertilizers to become fertilizer for a consortium of fertilizing microbes and biological agents. The test results are expected to obtain a consortium fertilizer that is effective in increasing yields and reducing the incidence of leek soft rot disease. For efficient use, organic fertilizers and biological agents are formulated together for single use. The aim of the study was to test the effectiveness of the organic fertilizer formula of the PGPR and biolocal agents microbial consortium on the growth and reduction of leek soft rot disease.

2. Methodology

2.1. Isolation of Erwinia carotovora pv. Carotovora

Isolation of bacterial pathogen was made from diseased bulb collected from field by streaking method. The nutrient agar (NA) medium was used as basal medium for the in-vitro studies and maintenance of pure culture of Erwinia carotovora pv. carotovora in slants [14] Growth of organism was observed regularly and maintained on NA slants.

2.2. Microbial Isolate Cultures and Plant Growth Promoting properties

Four microbial decomposers of *Bacillus megatrium, Azotobacter, P. fluorescens, and Aspergillus niger* sourced from the test results by Marwanto et al. 2020 [20]; two isolates of *Streptomyces* TTA1 and *Trichoderma* TTA1, were the result of in vitro selection which had excellent antagonists against *E. caratovora*[13]

Four bacterial isolates of *B. megatrium*, Azotobacter, *P. fluorescens*, and *Streptomyces* were sub-culturing several times on the yeast peptone glucose agar agar plate. Two fungal isolates of *A. niger* and *Trichoderma* CJ1 were sub-culturing several times on the Potato Dextrose agar plate. The 6 pure microbial isolates were screened in vitro for different PGP traits such as N fixation indicated by ammonia (NH3) production, phosphate (P) and potash (K) solubilization, IAA production, and biocontrol property by checking cellulase enzyme production ability. Microbial ability in fixing atmospheric N asymbiotically. Ammonia production indicated by ammonia production of each isolate was tested and performed according to Dobereiner method (1995) using a semisolid medium of Nfb (nitrogen-free bromthymol blue) without N [15]. The ability of the 12 microbial isolates to solubilize insoluble P was identified using the plate method [16] using Pikovskaya (PVK) agar medium containing KH3(PO4)2 as an insoluble inorganic form of P source. The appearances of the clear zone around bacterial or fungal growth (phosphate solubilization zone) were indicated a positive result for phosphate solubilization [17]. Isolates were tested for potassium solubilizing activity on Aleksandrov agar medium, The halo size produced by the respective strain was measured to calculate

K solubilization index. The solubilization index was measured according to the ratio of total diameter (the sum of colony diameter and the halo zone diameter and the colony diameter [18]. IAA production was detected on Luria-Bertani agar medium (LB), Bacteria or fungi producing IAA were characterized by the appearances of a specific red halo within the membrane immediately surrounding the colony, indicating a positive result for IAA production Bacteria or fungi producing IAA were characterized by the appearances of a specific red halo within the membrane immediately surrounding the colony, indicating a positive result for IAA production [21]. The 6 microbial isolates were confirmed for the ability to produce cellulose enzyme on a medium containing low viscosity carboxymethyl cellulose (CMC) agar as the sole carbon source (22); The formation of a clear pale halo zone with orange edge circling around each microbial colony indicated an area of cellulose hydrolysis by cellulase enzyme. This halo area was measured for subsequent calculation of the enzymatic index (EI) The EI was measured according to the ratio of total diameter (the sum of colony diameter and the halo zone diameter and the colony diameter [23][24]

2.3. Organic fertilizer decomposition

Isolates of the decomposer bacteria of *B. megatrium, Bacillus* sp. and *P. fluorescens* each propagated on 0.8% liquid nutrient medium, were incubated for 3 days on a shaking machine at 100 ppm speed. Bacterial cells were filtered and suspended in sterile water with a density of 10^8 cfu.ml. *A. niger* isolates were propagated each on 0.8% liquid nutrient medium, incubated for 3 days on a shaking machine with a speed of 100 ppm. Fungal spores were filtered and suspended in sterile water with a density of 10^6 cfu.ml.

600 kg of semi-finished and dry manure mixed with 12 kg of rice bran and 1.2 kg of CaCO3, then doused with a solution of 900 g of molasses in 30 l of water. This manure mixture is semi-aerobic fermented for 14 days with turning every 3 days. This process produces a microbial consortium organic fertilizer named BIOMEG [19)

2.4. Biocontrol agents consortium organic fertilizer formulation

BIOMEG is divided into 4 sections. The first part was inoculated with 100 g of *Streptomyces* CJ1 inoculum, the second part was inoculated with 100 g of *Trichoderma* TTA1 inoculum, the third part was inoculated with 100 g of Streptomyces CJ1 inoculum and 100 g of *Trichoderma* TTA1 inoculum; while the fourth part as a control. The organic fertilizers of the consortium of biological control agents were fermented again for 2 weeks. Next, the fertilizer is dried for 1 week and sifted so that it becomes a granular formula.

2.5. Testing the effectiveness of consortium fertilizer on leeks

The experiment was arranged in a randomized block design with 5 treatments and 5 replications. The treatments consisted of B0=control without pathogen, B1 = BIOMEG, B2 = BIOMEG + *Streptomyces*, B3 = BIOMEG + *Trichoderma*, and B4 = BIOMEG + *Streptomyces* + *Trichoderma*. 5 kg of the mixture of consortium fertilizer and soil (1:4) was put into a 40x30cm2 polybag, inoculated with 10 ml of suspension of E. caratovora at a density of 10^7 cfu/ml and incubated for a week. One stem of leek-Plumpung variety was planted in each polybag and maintained for 8 weeks. Effectiveness observations were made on growth, yield and incidence of soft rot disease.

3. Result and Discussion

3.1. PGPR ability isolate

Six isolates had different qualitative abilities in nitrogen (N) fixation, , phosphate (P) and potash (K) solubilization, IAA production, and cellulase enzyme production (Table 1.)

Table 1: Plant growth promoting properties of decomposer and biocontrol agent isolates.

Isolat	N Fixation	P Solubilization	PSI	K Solubilization	KSI	IAA Production	Cellulase Production	EI
Bacillus megatrium	+	+	1.46	+	1.42	+	+	1.35
Bacillus sp.	+	+	1.96	+	1.65	+	+	1.61
Pseudomonas fluorescens	+	+	1.41	+	1.83	+	+	1.53
Aspergillus niger	-	+	1.38	+	1.28	+	+	1.08
Streptomyces CJ1	-	+	1.28	+	1.10	+	+	1.32
*Trichoderma*TTA1	-	-	-	+	1.25	+	+	1.08

Remark: + = activity detected; -= no activity detected; PSI=Phosphate Solubilization Index;

KSI=Potash Solubilization Index; EI= Enzymatic Index

The role of the above microbial isolates as plant growth promoting has been shown by many previous researchers, but the difference in property quality is caused by differences and types of isolates [25][26][27][28][29][30][31].

Nutrient content of consortium fertilizer

From the examination of the nutrient content of the consortium fertilizer showed that the application of biological control agents could increase the nutrient content. Both fertilizers decomposed with microbial decomposers consortium, or added with biocontrol agents showed higher nutrient content (Table 2).

Table 2: Nutrient content of consortium fertilizer and soil.

Formulation	N-Total (%)	C (%)	P (ppm)	K (me/100g)	pH
Soil	0.22	2.68	7.06	0.29	4.21
Manure	1.40	16.42	0.76	0.80	6.86
BIOMEG	1.52	21.63	0.80	0.85	7.12
BIOMEG+*Streptomyces*	1.69	19.38	0.96	1.13	6.53
BIOMEG+*Trichoderma*	1.73	18.45	0.86	1.17	6.65
BIOMEG+*Streptomyces*+*Trichoderma*	2.14	24.62	1.17	1.47	6.70

The table 2 shows that the soil used for the study has a low nutrient content of Nitrogen, Carbon, Potassium and pH. For good growth needs, organic fertilizer and CaCO3 can be added. The increase in the nutrient content of the consortium's organic fertilizers was caused by the role of microbes added as decomposers by decomposing organic matter into nutrient elements. Biocontrol agents that added also act as decomposers. The more the number and types of microbes given, the faster the decomposition process and more. This has been shown by several previous researchers, the diversity of decomposer microbes influences the decomposition rate of soil organic matter [32]. PGPR inoculation can accelerate the decomposition of soil organic compounds, provide substrates for enzymatic reactions, and promote microbial growth, thereby improving soil fertility [33].

3.2. The effectiveness of consortium fertilizer on leeks

Observations on healthy plants, application of the microbial decomposers consortium fertilizer and the biocontrol agent consortium fertilizer showed the same results for plant growth. However, its growth is not as good as growth in healthy soil(Table 3). The development of plant growth also shows the same (Figure 1, 2, and 3)

Table 3.

Note: Numbers followed by the same letter in the same column mean that they are not significantly

different according to the DMRT test at the 5% level.

Table 3: Effects of application of consortium fertilizer to growth and yield of leeks in infected soil.

Formulation	Plant height (cm)	Number of shoot	Number of leaves	Weight (g)				
				Fresh crop	Fresh root	Fresh shoot	Dry root	Dry shoot
Kontrol no pathogen	44,1a	5,8a	19,4a	121a	11,2a	109,8a	1,431a	23,002a
BIOMEG	**45,8a**	2,9a	9,5a	44,9b	4,2b	40,7b	0,881b	7,009b
BIOMEG+Streptomyces	46a	3,8a	12,4a	64,1b	5,5b	58,6b	1,103ab	11,402b
BIOMEG+Trichoderma	45,5a	3,1a	10,9a	**73,9b**	**6,2b**	**67,7ab**	**1,156ab**	**11,06b**
BIOMEG+Streptomyces +Trichoderma	44,1a	**4,3a**	**11,2a**	56,2b	5,2b	51b	0,833b	8,401b

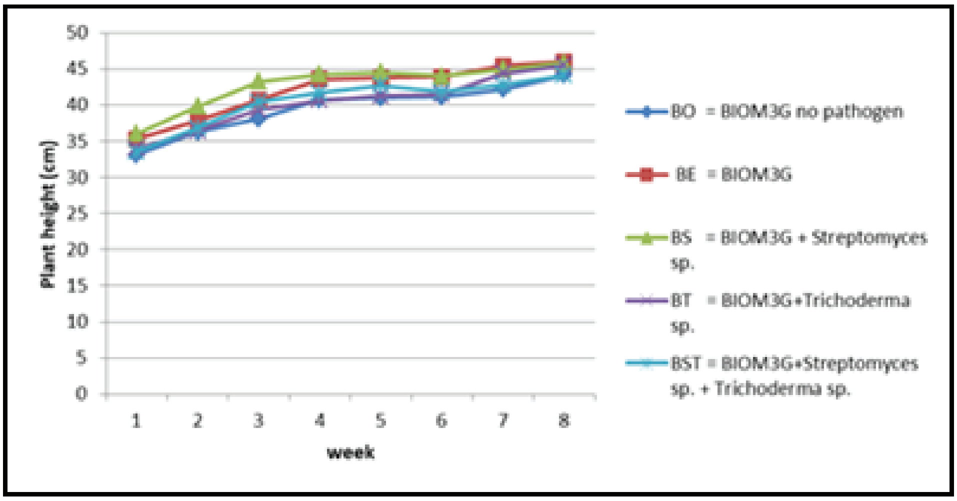

Figure 1: Effect of microbes consortium fertilizer to plant height of leeks.

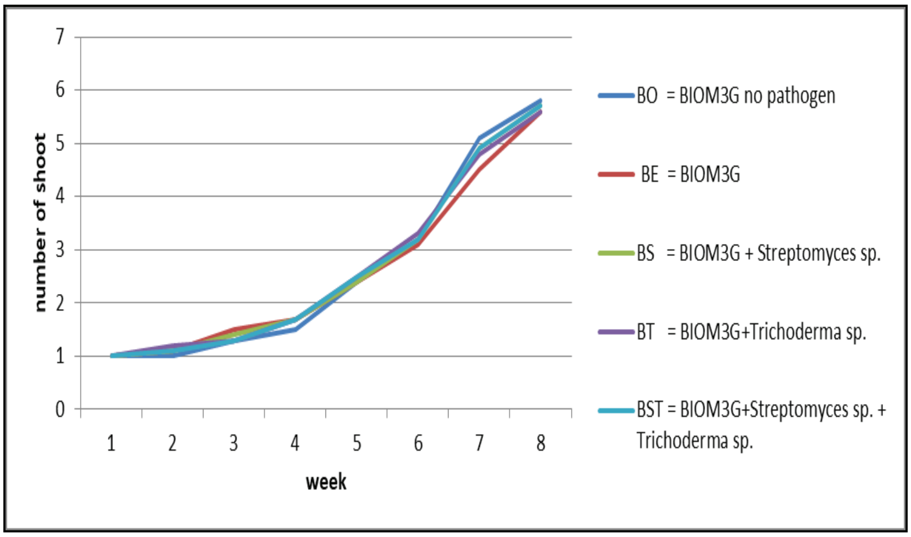

Figure 2: Effect of microbes consortium fertilizer to shoot number of leeks.

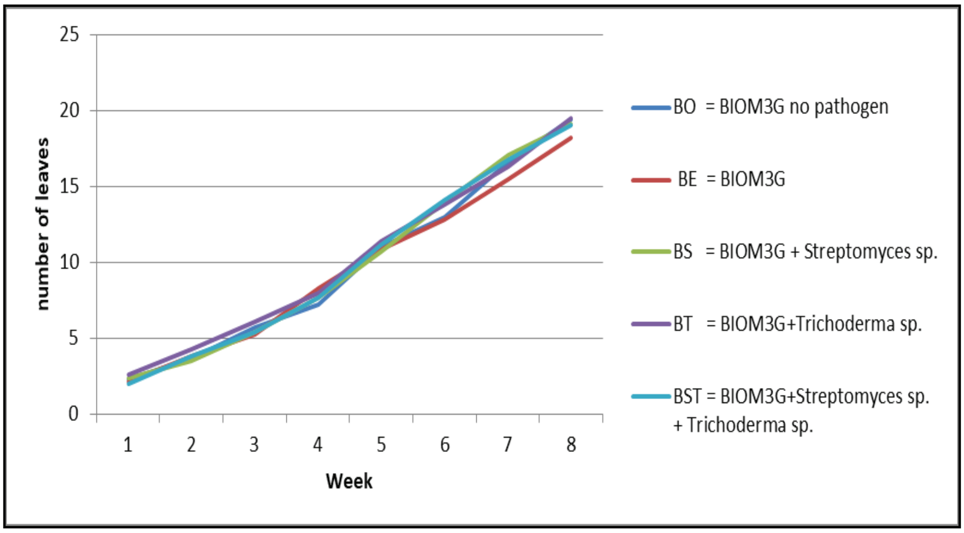

Figure 3: Effect of microbes consortium fertilize to leaves number of leeks.

It is suspected that, in the infected soil, the consortium of microbes in the soil tries to block or kill the pathogen first, before degrading the organic matter present in the soil. This condition affects the speed of plants in absorbing nutrients from the soil.

3.3. Disease incidence

The use of organic fertilizers from the consortium of biocontrol agents reduced the incidence of soft rot disease in leeks better than the use of organic fertilizers by the microbial consortium of PGPR (BIOMEG) (Figure 4). The formula that best reduces the incidence of disease is the organic fertilizer consortium BIOMEG+*Streptomyces*+*Trichoderma*.

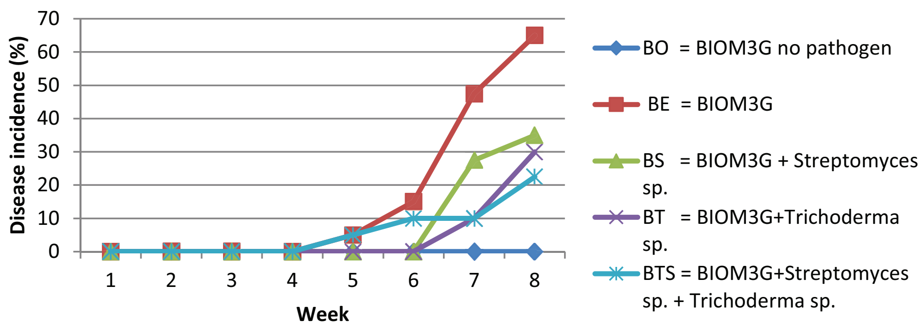

Figure 4: Effect microbes consortium fertilizer to disease incidence of leeks soft root.

The reduction in disease incidence is associated with yield, so the organic fertilizer formula of the BIOMEG+Streptomyces+*Trichoderma* consortium is very effective in reducing the incidence of disease and the yield of leek. The mechanism for reducing the incidence of disease can occur because *Trichoderma* and *Streptomyces* found in the soil through the application of organic fertilizer consortia develop in the soil and colonize around plant roots. Streptomyces and Trichoderma produce antibiotics and chitin-degrading enzymes and always to inhibit or kill the growth of pathogenic bacteria in the soil and in the rhizosphere. This has been shown by Streptomyces research to reduce wilt disease in tomatoes [25] and the application of indigenous PGPR and *Trichoderma* to tomatoes wilt [26], *Trichoderma* on potato softrot [12].

4. Conclusion

Various organic fertilizer formulas enriched with PGPR microbial consortium and bio-control agent were effective in growing onions in soil infected with soft rot pathogens, but the results were not as good as plant growth in healthy soil. The application of the PGPR microbial consortium organic fertilizer formula and biological agents reduced the incidence of soft rot disease of leeks better than the PGPR microbial consortium organic fertilizer formula. Application of the organic fertilizer formula of the microbial consortium PGPR+*Streptomyces*+*Trichoderma* reduced the incidence of disease the best and gave the highest yield of leek.

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