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Application of PGPR and Antagonist Fungi-based Biofungicide for White Rust Disease Control and Its Economyc Analysis in Chrysanthemum Production

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) application in combination with other antagonist microbes as biopesticide have been considered in many crops. Our research was conducted to evaluate the efficacy of these useful combinations with the carrying agent for growth promotion, thus lowering white rust incidence in chrysanthemum production. The experiment was carried out at three cooperative farmer sites located in Cipanas, Cianjur, West Java, Indonesia from January to December 2016. The production process was arranged in a paired treatment; a combination of PGPR and antagonist fungi (without supplemental chemical fertilizers and fungicide), furtherly called biofungicide and common farmer practices. The results showed that the application of biofungicide promoted equal plant growth quality as common practices. White rust incidency was lower at biofungicide treatment sites, thus increased the markertable flowers quantity. The production cost was considered more efficient in biofungicide sites, due to cheaper price of biofungicide than chemical fertilizers and fungicide. The increase of marketable stalks and cost efficiency led to an increase of net income of biofungicide based production as also viewed from higher Revenue Cost Ratio (R/C) than common farmer practices.

Keywords: biological agent; Chrysanthemum (*Dendrathema grandiflora*); cost efficiency; disease control; *Puccinia horiana*

INTRODUCTION

Chrysanthemum (*Dendrathema grandiflora* Tzvelev syn. *Chrysanthemum morifolium* [Ramat.] Kitam) in form of cut flower and potted plants is one of the top marketed ornamentals in the world. In international trade, the commodity ranked first or about 35 % of the world's market request for cut flowers (Market News Service, 2012), second only to roses. Netherlands, Italy, Columbia, Spain, Germany and USA are the main producers which supply more than 60 % of the world trade for chrysanthemums. In Indonesia, chrysanthemum has replaced roses as the most marketed cut flowers since 2006. The demand for flowers has increased significantly in line with the increase of production in the country in the last decade from 108 million stems in 2009 to 387 million stems in 2013. The increase was to the increment of productivity from 9.92 % to 42.64 % respectively, thereby gave higher contribution to the export values of the total exported floriculture products from 8.0 % to 23.3 % in 2012 (Indonesian Ministry of Agriculture, 2014).

The efforts to make chrysanthemum production become more efficient and profitable, however, are still constrained by several factors, one of them is a disease called white rust. The disease was caused by *Puccinia horiana* P. Henn, an obligate parasitic fungus (*Basidiomycetes*) and in many chrysanthemums in many countries, is known to be the most disastrous problem. The fungus infects 12 species including chrysanthemum, *Nipponanthemum* and *Leucanthemella* (Alaei et al., 2009). *P. horiana* attacks the plant by enzymatic digestion to penetrate the leaf cuticle. The fungus then colonizes both inter- and intracellularly the mesophyll tissue (Bonde et al., 2015). The production lost due to the fungus may reach 80 % in India, during the outbreak seasons (Dheepa, Renukadevi, Kumar, & Nakkeeran, 2015).

According to Yusuf, Budiarto, Djatnika, & Suhardi (2017), so far there is no synthetic fungicide that is formally recommended to control white rust. Since the physical quality is very important, the growers then tend to use various kinds of fungicide with unapropriate dosages expecting the reduction of damages. These practices might spend 13 – 32 % of the total production cost (Suhardi, 2009) and can make the business uncompetitive.

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The use PGPR to substitute chemical fertilizers, pesticides and supplements in plant production has been reported to have better impact on growth, yield and disease control in some plants (Joko et al., 2012). The direct effects of PGPR on plant growth are through supplying certain compounds produced by the bacterium that is needed by the plant, like phytohormones. The bacterium also promotes the absorption of certain nutrient/element from the environment (Ahemad & Kibret, 2014). The indirect effects of PGPR on plant growth include the defend mechanism against one or more phytopathogenic organisms. The mechanism is through producing antagonistic substances and/or by inducing resistance againts pathogens (Beneduzi, Ambrosini, & Passaglia, 2012). A particular PGPR may stimulate the regulation of plant growth and development by using any one, or more, of these mechanisms. As biocontrol agents, the bacterium may act through various mechanisms, regardless of their effect on the promotion of plant growth, like auxin production (Miransari & Smith, 2014), ethylene levels decrement (Liu & Zhang, 2015) or nitrogen fixation in association with roots (Reed, Cleveland, & Townsend, 2011). It is about only 1 to 2 % of bacteria promote plant growth originated from the rhizosphere (Beneduzi, Ambrosini, & Passaglia, 2012). Bacteria from wide range of genera have been identified as PGPR, of which *Bacillus* sp. and *Pseudomonas* spp. are predominant (Raaijmakers, de Bruijn, Nybroe, & Ongena, 2010).

Certain strains of *Bacillus sp*. and *Pseudomonas flourescens* have been proven to be effective in suppressing leaf soft rot caused by *P. viridiflava* in Phalaenopsis (Nuryani et al. 2012), white rust (*Puccinia horiana*) in chrysanthemum (Hanudin et al. 2010), cucumber mosaic virus (CMV) in pepper (G. H. Lee & Ryu, 2016). Another genera, free living diazothrops *Azotobacter* sp. and *Azospirillum* sp. have been known to have capacity in non symbiotic fixation of nitrogen. While *Aspergillus* sp., *Bacillus megatherium* and *Penicillium* sp. were able to solubilize phosporus (P) and pottasium (K) (Karakurt & Aslantas, 2010).

These useful bacteria have been combined with antagonist fungi in a formulation to widen their beneficiaries as biopesticides and biofertilizers. The formulation was expected to have better impact not only in preventing the plant from disastrous diseases and also reducing the chemical fertilizers as well. The combination of PGPR and antagonist fungi would be mixed with their carrying substances and furtherly called as biofungicide. Before procceding to commercialization, the biopesticide should be tested for their effectiveness, applicability and efficiency in technical and economic perspectives. The experiment was conducted to evaluate the biofungicide in chrysanthemum production. These expected that the biofungicide would increase the quality of plant growth and marketable flowers as a result of lesser disease attacks and better nutrition supply compared to chemical fertilizers.

MATERIALS AND METHODS

The research was conducted from January to December 2016 covering laboratory and green house studies for isolates preparation at the Indonesian Ornamental Crops Research Institute (IOCRI) and the field work under plastic house conditions at the cooperative grower sites.

Preparation of PGPR and Antagonist Fungi Propagules

Several PGPR, namely *Azotobacter chroococcum* (Ac), *Bacillus substilis* (Bs) and *Pseudomonas flourescens* (Pf) served as the active ingredient of the formulation. The pure isolates of these bacteria collected from the laboratory of bacteriology and mycology, IOCRI. Ac was cultured using Asby medium, which was in per litre medium solidified with 20 g agar composed of 5 g glucose, 0.25 g K_2HPO_4, 0.1 g $MgSO_4.7H_2O$, 0.1 g NaCl, 2.5 g $CaCO_3$, 0.1 g $CaSO_4$, 0.001 g Na_2MoO_4. Bs were grown under Pikovkaya medium, consisting 10 g glucose, 5 g $Ca_3(PO_4)_3OH$, 0.2 g NaCl, 0.2 g KCl, 0.1 $MgSO_4.7H_2O$, 0.0025 g $MnSO_4.H2O$; 0.0021 g $FeSO_4.7H_2O$, 0.5 g yeast extract and 0.5 g $(NH_4)_2SO_4$. While Pf was grown on King's B medium, containing 20 g proteose peptone no. 3, 10 g glycerol, 1.5 g K_2HPO_4, 1.5 g $MgSO_4.7H_2O$ and 0.01 M $FeCl_3$. The cultured were then incubated under the temperature of 30 ± 2 ^{0}C for 24 h.

Each isolate was taken for about three bacterial loops and diluted in 10 ml sterile water and homogenized until it reached the density of 10^{12} cfu ml^{-1}. One ml isolate suspension was put into an elenmeyer containing 500 ml nutrient broth (NB) medium. The flask was then shaken in 30 ^{0}C waterbath at 3 rpm for 24 h. The bacterial cells were then suspended in the culture medium with the concentration of 1 %.

The antagonist fungi i.e. *Trichoderma* sp. and *Paecilomyces* sp. were included as the constituent of the formula aside from the mentioned PGPR. The method of culture and multiplication for antagonist fungi were similar as described in PGPR set up, only the mediun used was potato dextrose agar (PDA) medium.

Manufacture of PGPR in Granular Form

PGPR composed of Pf, Ac and Bs was massively multiplicated in EKG-plus medium in which per litre, the medium was consisted of 200 g potato extract, 50 g sugar, 50 g grinded green tea leaves, 50 g vermicompost, 10 ml molase and other additional organic substances. PGPR suspension was taken and put into the multiplication medium with the ratio of 1:100 (v/v) and aerobicly fermented using air pump biofermentor (Resun model LP.40.3501E with mini compressor) for 21 days or until the density of cells reached 10^{12} cfu ml^{-1}. The cell suspension was then sprayed into zeolite as the carrying agent in granule form with the ratio of 1:1 (v/w). The sprayed granules were then air-dried until the moisture content reached 15 %. The product has been registered with patent No. P00201605271, 10 August 2016 at the Directorate General of the Intellectual Property Right, Ministry of Justice and Human Right, The Republic of Indonesia.

Manufacture of Antagonist Fungi in Powdery Form and Mixture of PGPR + Antagonist Fungi as the Biofungicide

The corn medium was prepared by washing the corn grains with water and drained for several minutes until they were air-dried. The grain was then put into 20 x 20 cm plastic bags and sterilized using autoclave at 1.5 psi kg^{-1} and 120 ^{o}C for 20 minutes. The sterilized grain, furtherly called corn medium was used for the antagonist fungi, *Trichoderma* sp. and *Paecilomyces* sp. cultures. For about three oose needle-pure cultures of the respected fungus were put into the sterilized corn medium and then, incubated in dark room under the temperature of 25-29 ^{0}C for 15 days. After the fungus was properly grown (indicated by the excessive growth of the miselia covering the medium), the isolate were then mixed with Pf and Bs that previously had been cultured on EKG-plus medium with the ratio 1:25 (w/v). Within the ratio, the density of PGPR and antagonist fungi ranged 10^{11} – 10^{12} cfu spores per ml. The suspension was then, homogenously mixed with 400 meshed-diatomic powder/Kaolin (SiO_4) as the carrying substances with ratio of 1 : 1 (v/w).

Experimental Sites

The experiment was conducted at cooperative farmer sites, namely Agus (Co-Famer 1), H. Teten (Co-Famer 2) and Muhiddin (Co-Famer 3), located at the chrysanthemum production centre of Cianjur, West Java, Indonesia. All cooperative farmers planted similar varieties, but different in numbers of cuttings depended on the area of their plastic houses. The varieties used were Yellow Fiji, White Fiji and other white rust-susceptible varieties.

The area inside the plastic houses was hoed to clean the planting sites from weeds and other substances. The soil was then properly mixed with 30 t ha^{-1} manure. The planting sites were then organized into 1 x 2 m beds and the distance among beds was 1 m. The planting beds were then poured with water to facilitate humidity before planting. Rooted cuttings of the mentioned varieties were planted in beds with the density of 100 plants m^{-2}. After planting, the young plants were maintained under standard cultural practices until flowering.

All the plants were sprayed with recommended dosage of Abamectin (Syngenta Co. Ltd, Indonesia) once a week for insect pest prevention. On the control treatment, the additional fertilization was applied using NPK (15-15-15) at 7, 30 and 50 days after planting. On the biofungicide treatment, 50 kg ha^{-1} biofungicide were gently mixed with the top soil of the beds one day before the planting day. One day after planting, the treatment of biofungicide were applied by pouring the bed with biofungicide (the biofungicde was diluted with water at the concentration of 5 g l^{-1}) with the volume of 2 l m^{-2}. Additional biofungicide treatments were given weekly through foliar application at the same concentration until 50 DAP.

Data Gathering and Analysis

The observation of white rust intensity was conducted every week from the day of planting until 65 DAP. The observation of white rust intensity was conducted every week from the day of planting until 65 DAP. The disease development was determined based on Suhardi (2009) criteria as presented in Table 1.

Table 1. Scale and damage criteria of white rust (*Puccinia horiana* Henn) infection on chryasanthemum

Scale	Damage Criteria
0	Not infected (symptomless)
1	Very low, infection detected only on lower plant leaves and the intensity not exceed than 5 % from total leaf area.
2	Low, infection detected on lower plant leaves and the intensity ranges 5-10 % from total leaf area.
3	Medium damage, infection detected on middle and lower plant leaves and the intensity ranges 10-20 % from total leaf area.
4	Heavy damage, infection detected on upper, middle and lower plant leaves and the intensity ranges 20-40 % from total leaf area.
5	Very heavy damage, infection detected on upper, middle and lower plant leaves and the intensity was more 40 % from total leaf area.

The disease intensity was calculated using the following formula:

$$I = \frac{\Sigma(v \times n)}{(Z \times N)} 100\%$$

Where,

I = Intensity of white rust infection (%)
v = Scale of the observed damage
n = number of infected plants categorized in the respected damage scale
Z = highest scale of the observed damage
N = total number of observed plant samples

Percentage of suppression was calculated using the following formula for the consideration of biofungicide efication.

$$PS = (C \times T/C) \times 100\ \%$$

Where,

- PS = Percentage of suppression (%)
- C = Disease intensity of control plants
- T = Disease intensity of the the treated plants

The plant height and stem diameter were measured at 57 DAP while the quantity of marketable flower was observed at the harvesting time (90 DAP). The data gathered were classified into two group, i.e production procces using biofertilizer and biofungicide (X_1) and farmer common practice (X_2). The data were then analyzed using T-test (5 %) parametric pairing group. The standard deviation (Sd) of the data of each group as the basis of efication criteria was calculated based on D. K. Lee, In, & Lee (2015).

$$Sd = \sqrt{\frac{1}{n-1}\left\{\sum (X_1 - X_2)^2 - \frac{(\sum X_1 - X_2)^2}{n}\right\}}$$

Where,

- Sd = Standard deviation
- X_1 = Average value from treated plants
- X_2 = Average value from control plants
- n = Replication

Economic Analysis of the Biofungicide Application in Chrysanthemum Production

For economic feasibility assesement of the biofertilizer and biofungicide application on chrysanthemum production, the data gathered were (a) production cost, including labors during the production process and production input, such as planting material, organic/inorganic fertilizers, and other chemicals, (b) toal valuse of marketable product, (c) benefit and revenue: cost ratio (R/C ratio). The effects of biofertilizer and biofungicide on chrysanthemum production were formulated using linear production function Cobb Douglas (Ahmad & Khan, 2015).

$$\text{Log } Q = \text{Log } A + \Sigma \beta j \log_{j=1}^{n} Xj + e \quad \ldots\ldots\ldots\ldots (1)$$

Where,

- Q = harvestable cut flower product (bundle, @ = 10 stalks)
- X_1 = planting material (cuttings)
- X_2 = NPK fertilizers (kg)
- X_3 = organic substances/manure (m^3)
- X_4 = biofertilizers (kg)
- X_5 = chemical/syntethic pesticide (pack)
- X_6 = biofungicide (kg)
- X_7 = planting area (m^2)
- X_8 = labor (working days)
- A = constant
- e = error

The formula for production elasticity was derived from the derivation of the above equation.

$$\frac{\delta Q}{\delta X_j} = \beta j \frac{Q}{X_j} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

thus, simplify to :

$$\beta j = \frac{\delta Q}{\delta X_j} \frac{X_j}{Q} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The profit of the production process was calculated using financial benefit-cost ratio (B/C ratio) analysis as described by Prasetia *et al.* (2016).

$$\pi = P.Q - Tc = P.Q - (Fc + \sum_{I=1}^{n} P_i X_i)$$

Where,

- π = Profit
- Q = Flower production (bundle, @ = 10 stalks)
- P = Price of product/bundle
- Tc = Total cost
- Fc = Fixed cost
- Pi = Input cost of i^{th} component
- Xi = Amount of i^{th} input

RESULTS AND DISCUSSION

Plant Growth Improvement and White Rust Incidence

The data on Table 2 showed that the differences in growth performance of chrysanthemum maintained by common farmer practices and using biofungicide were negligible. In some locations, however, chrysanthemum plants grown under common farmer practice were taller and had bigger stems compared to those using biofungicide, though the values were insignificant.

The negligible differences on plant height and stem diameter of chrysanthemum plants treated by biofungicide and common farmer practices indicated that the application of biofungicide was able to provide equal niche to support plant growth as those common practices. These meant that the existence of beneficial microbes in biofungicide were able to induce conducive environment for optimal plant growth, though additional fertilizers and chemical fungicide were not applied during the production process. The positive impact of biofungicide on plant growth was related to certain traits of rhizobacteria (PGPR). According to several reports, PGPR might mediate plant growth promotion through the production of various substances by the alteration of the whole microbial community in rizhospere niche (de Souza, Ambrosini, & Passaglia, 2015). PGPR had direct mechanisms through facilitating nutrient uptake or increasing nutrient availability by nitrogen fixation, solubilization of nutrients, mineralization of organic compounds and production of phytohormones (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014). PGPR supported plant growth also through production of siderophore (low molecular weight iron-chelating compound) (Rahmoune et al., 2017), of which the plant can take up iron in large number (Sujatha & Ammani, 2013) and phytohormone (Zahedi & Abbasi, 2015). Siderophoer as a sole source allowed the plant to take up iron in large number (Sujatha & Ammani, 2013) and enhanced plant chlorophyll content (Sharma & Johri. 2003). Some PGPR are able to produce phytohormones such as auxins, cytokinins, gibberellins and ethylene that can induce cell ploriferation in the root by increasing lateral root growth and root hair development with a subsequent increment of nutrient and water uptake (Sukumar et al., 2013).

The improvement of plant performance on the chrysanthemum treated by biofungicide was also resulted form the less disease attacks on the plants. Table 3 showed that white rust index was observed lower in chrysanthemum 'White Fiji' treated by biofungicide compared to common farmer practices after 65 DAP. The disease index at chrysantemum treated by biofungicide ranged 25.33-75.33 %, while those maintained under common practice had 37.33-76.67 %. The average of white rust suppression due to biofungicide application compared to common farmer practices was 18.37 %. The values were found higher on cooperative farmers 1 and 2 at 32.15 % and 21.82 %, respectively and minimal in cooperative farmer Muhiddin (1.75 %).

The mechanisms of biofungicide in protecting the plant from pathogenic disease occurs by (a) induction of plant systemic resistance, (b) siderophore (iron chelate) production that made the ion unavailable for the pathogen, (c) synthesis of secondary metabolites like enzymes or cyanide that fungctioned as antifungal agent, degrading the cell wall and inhibiting the growth of pathogen, and (d) competitive abilty from space and nutrition againts the pathogen (Tariq, Hameed, Yasmeen, Zahid, & Zafar, 2014). Chrysanthemums treated by Pf were reported have higher ethylene compared to untreated plants with lower white rust infections (Hanudin, Budiarto, & Marwoto, 2016). Production ethylene and other substances like jasmonate is one signaling mechanism within the plant and these hormones stimulate the host's plant defense responses against the pathogen attacks (Naznin, Kimura, Miyazawa, & Hyakumachi, 2013).

Table 2. Plant height and stem diameter of chryasanthemum plants maintained under common farmer practice and biofungicide treatment at 57 DAP

Cooperative Farmer	Height of plants grown by farmer practice (X_1) and biofungicide (X_2)				Stem diameter of plants grown by farmer practice (X_1) and biofungicide (X_2)			
	X_1	X_2	$X_1 - X_2$	$(X_1-X_2)^2$	X_1	X_2	$X_1 - X_2$	$(X_1-X_2)^2$
Co-Farmer 1 (Rep 1)	101.40	101.30	0.10	0.01	0.65	0.61	0.04	0.0016
Co-Farmer 1 (Rep 2)	101.50	101.40	0.10	0.01	0.59	0.60	-0.01	0.0001
Co-Farmer 1 (Rep 3)	101.20	101.30	-0.10	0.01	0.64	0.63	0.01	0.0001
Co-Farmer 2 (Rep 1)	84.80	85.10	-0.30	0.09	0.53	0.54	-0.01	0.0001
Co-Farmer 2 (Rep 2)	90.20	90.20	0.00	0.00	0.52	0.52	0.00	0.00
Co-Farmer 2 (Rep 3)	89.90	89.90	0.00	0.00	0.53	0.54	-0.01	0.0001
Co-Farmer 3 (Rep 1)	87.40	88.40	-1.00	1.00	0.55	0.56	-0.01	0.0001
Co-Farmer 3 (Rep 2)	92.50	90.50	2.00	4.00	0.55	0.56	-0.01	0.0001
Co-Farmer 3 (Rep 3)	99.70	97.70	2.00	4.00	0.57	0.55	0.02	0.0004
Total	747.20	845.80	2.80	9.12	5.13	4.5238	0.02	0.0026
Average	93.40	93.9778	0.3111	1.0133	0.57	0.5026	0.0022	0.0003
Standard deviation (Sd)				1.02				0.02
Calculated t				1.04				0.37
t-table (df = 8)								2.30 (1 %), 3.36 (5 %)

Table 3. White rust *P. horiana* index at chrysanthemum 'White Fiji' treated by biofungicide and common practices at 65 DAP

Cooperative Farmer	White Rust Index (%)			
	Common Farmer Practice (X_1)	Biofungicide Treatment (X_2)	$X_1 - X_2$	$(X_1 - X_2)^2$
Co-Farmer 1 (Rep 1)	37.33	25.33	12.00	144.00
Co-Farmer 1 (Rep 2)	39.33	27.33	12.00	144.00
Co-Farmer 1 (Rep 3)	35.33	23.33	12.00	144.00
Co-Farmer 2 (Rep 1)	73.33	55.33	18.00	256.00
Co-Farmer 2 (Rep 2)	71.33	59.33	12.00	144.00
Co-Farmer 2 (Rep 3)	75.33	57.33	18.00	324.00
Co-Farmer 3 (Rep 1)	76.62	75.33	1.29	1.66
Co-Farmer 3 (Rep 2)	72.33	71.33	1.00	1.00
Co-Farmer 3 (Rep 3)	80.91	79.33	1.58	2.50
Total	561.84	473.97	87.87	1161.16
Average	62.43	52.66	9.76	129.02
% white rust suppression under biofungicide application to common practices in cooperative farmer Agus				32.15
% white rust suppression under biofungicide application to common practices in cooperative farmer H. Teten				21.82
% white rust suppression under biofungicide application to common practices in cooperative farmer Muhiddin				1.75
Average of white rust suppression under biofungicide application to common practices in all locations				18.57
Standard deviation (Sd)				6.81
Calculated t				4.30
t table (df = 8, α = 5 %)				2.30

According to several findings, Pseudomonads also had the capability in producing antibiotic (Molina-Santiago, Udaondo, & Ramos, 2015). Pf was reported to have capability in producing antibiotics such as *phenazine-1-carboxylic acid, pyoluteorin, fenazines*, dan *viscocinamide* (Mavrodia et al., 2012), *pyrrolnitrin* dan *viscocinamide* (Kim, Mele, & Crowley, 2013), while Bs released *mycosubtilins, basillomycin, fengimycin, mycobacillin, mycoserein,* dan *xanthobasidine* (Velho, Medina, Segalin, & Brandelli, 2011). Pf strain A 506 was reported effective to control fire blight on apple (Mikiciński, Sobiczewski, Puławska, & Malusa, 2016). While other strains have been accounted on controlling *Gaeumannomyces graminis* var. *tritici* on wheat (Thomashow and Weller 1988), *Ralstonia solanacearum* on tomato (Barret et al., 2009), stem rot disease caused by *Phytium* spp on cotton (Erdoğan, Bölek, & Göre, 2016). The combination of Pf and *B. subtilis* was able to reduce *Plasmodiophora brassicae* on chinese cabbage (Zhao et al., 2016) and *Phytopthora capsici* in pepper (Özyilmaz & Benlioglu, 2013).

Effect of Biofungicide on Marketable Flower

A better growth performance of chrysanthemum treated by biofungicide as viewed from taller plants with bigger stem and less white rust infection (Table 2 and Table 3) reflected to the more plants having qualified physical appearance. These expected conditions led to the more stalks passed the grading system and increased the marketable quantity of flowers. The higher marketable flowers from the plants treated by biofungicide (X_2) compared to common farmer practice (X_1) was presented in Table 4. The marketable flowers stalks produced by cooperative farmers using biofungicide reached 75-91 %, while those applied common practices ranged 70-88 %.

Aside from the main role of PGPR for soil-plant improvements, the carrying agent of biofungicide formulation, zeolite was also known to be microporous aluminosilicate minerals that can accommodate a wide variety of cations, such as K^+, Na^+, Ca^{2+}, Mg^{2+} and others that can be interchangeable in the soil absorption complex, thus increase the avalailability of these cations for the plant (Ghazavi, 2015). Zeolite also improved soil aggregate, prevented moisture content, induced soil remedy from heavy metal-acidic soil pollutant (Jakab, S. & Jakab, A., 2010). The application of zeolite for vegetative growth and yield improvement has also been reported in apple (Milosevic & Milosevic, 2009), corn (Ippolito, Tarkalson, & Lehrsch, 2011) and kale (*Brassica albograbra*) (Li, Wee, & Sohn, 2013).

Economic Analysis of Biofungicide Application

The agroinputs used by cooperative farmers for biofungicide based and common practices were similar, i.e. type of planting material (cuttings), varieties, dosage and type of organic fertilizer. The differences were only on the use of chemical fertilizers and fungicide. On common practice sites, the production process used NPK for the supplementary fertilizers and commercial fungicide chemicals. While, in biofungicide sites, supplementary chemical fertilizers and fungicide were not applied. The plants were maintained under standard cultural practices by providing long day treatment for 30 days from planting date and other activities, such as watering, weeding and pruning. The detail agroinputs during the chrysanthemum production process using biofungicide and common practice were presented in Table 5.

Table 4. Marketable chrysanthemum flowers produced by cooperative farmers using biofungicide and common practices

Cooperative Farmer	Marketable flower (bundle = @ 10 flower stalks)			
	Common Farmer Practice (X_1)	Biofungicide Treatment (X_2)	$X_1 - X_2$	$(X_1 - X_2)^2$
Co-Farmer 1 (Rep 1)	375	350	25	625
Co-Farmer 1 (Rep 2)	370	349	21	441
Co-Farmer 1 (Rep 3)	380	351	29	841
Co-Farmer 2 (Rep 1)	455	441	14	196
Co-Farmer 2 (Rep 2)	453	437	16	256
Co-Farmer 2 (Rep 3)	457	445	12	144
Co-Farmer 3 (Rep 1)	110	104	6	36
Co-Farmer 3 (Rep 2)	107	97	10	100
Co-Farmer 3 (Rep 3)	113	109	4	16
Total	2820	2683	137	2655
Average	940	894.34	45.67	6256.33
% increase of marketable flowers due to biofungicide application to common practices in cooperative farmer Agus				7.14
% increase of marketable flowers due to biofungicide application to common practices in cooperative farmer H. Teten				3.17
% increase of marketable flowers due to biofungicide application to common practices in cooperative farmer Muhiddin				6.45
Average increment of marketable flowers due to biofungicide application to common practices in all locations				5.59
Standard deviation (Sd)				71.2
Calculated t				5.41
t table (df = 8), α = 5 %)			3.36 (1 %)	2.30 (5 %)

Table 5. Type and amount of agroinputs during chrysanthemum production process using biofungicide and common practices in all cooperative farmers

Agroinput	Common Farmer Practice				Biofungicide Treatment			
	Co-Farmer 1	Co-Farmer 2	Co-Farmer 3	Average	Co-Farmer 1	Co-Farmer 2	Co-Farmer 3	Average
Planting area (m^2)	200	200	75	166.67	200	200	75	166.67
Number of cuttings	15000	15000	4000	11340	15000	15000	4000	11340
Supplementary fertilizer/NPK (kg)	50	50	15	23	0	0	0	0
Biofertilizer-biofungicide (kg)	0	0	0	0	10	10	2	11
Manure (m^3)	2	2	1	1.67	2	2	1	1.67
Chemical pesticide(s)								
• 200 g/l Azoxystrobin + 125 g/l Difenoconazole (250 ml)	1	1	0.5	0.83	0	0	0	0
• 250 g/l Difenoconazole (200 ml)	1	1	0.5	0.83	0	0	0	0
• 18 g/l Abamectin (200 ml)	1	1	0.5	0.83	0	0	0	0
• 5% Emamectin benzoate (200 g)	1	1	0.5	0.83	0	0	0	0
• 500 g biopesticide (pack)	0	0	0	0	2	2	1	1.67
• 60 ml organic insecticide (pack)	0	0	0	0	2	2	1	1.67
Workers (conctracted)								
• Soil tillage and planting bed	1	1	1	1	1	1	1	1
• Fertilizer application	1	1	1	1	1	1	1	1
• Pesticide application	1	1	1	1	1	1	1	1
• Irrigation	1	1	1	1	1	1	1	1
• Weeding	1	1	1	1	1	1	1	1
• Prunning	1	1	1	1	1	1	1	1
• Harvesting	1	1	1	1	1	1	1	1

In all the agroinput elements, the planting material (cuttings) was the highest expense, with the share at 57-72 % of the total production cost, followed by synthetic pesticides in common farmer practices as also stated by Pratomo & Andri (2013). On the other hands, the use of biofungicide reduced the production cost due to the decrement of pesticide and fertilizer expenses. The detail cost agroinput elements during the chrysanthemum production procces was presented in Table 6.

Total revenue is considered as the gross income and calculated through multiplying the total product (output) with the unit price of product, while productivity was measured as total marketable product (output) per unit planting area. Percentage of marketable product was calculated by comparing the harvestable and qualified stalks by total planted cuttings (Darwis, 2014). In chrysanthemum cut flower, the product was usually sold in form of 'bundle', in which in one bundle consisted of 10 flower stalks. From the field observations, the marketable products in each location of cooperative farmer were varied. In general, the planting sites using biofungicide produced more marketable products (75-91 %) than the common practices (70-75.5 %) (Table 7).

The higher product prices in location 1 compared to location 2 and 3 (Table 7) were due to the different harvesting periods. In location 1, the flowers were harvested nearly prior to 'led Fitr' (moslem religious festivity) when the price are usually higher. In location 2 and 3, the flowers were harvested 2 weeks after the festivity day, when the price was lower as also observed in some consumable agricultural products (Abidin, Suhadak, & Hidayat, 2016).

Higher marketable product on biofungicide treatment compared to common practices contributed to higher total revenue (Table 7). The total cost of biofungicide-based production was also lesser due to the cost reduction from the competitive price of biopesticide. These conditions led to the higher net income compared to common practices. The feasible biofungicide application in chrysanthemum production was also viewed form the production efficiency, in which R/C ratio of biofungicide-based production was higher (1.61) compared to farmer common practice (1.31). Higher R/C ratio represented to the more profitable production process (Setyono, 2016).

Table 6. Expense of agroinput elements of chrysanthemum production process using biofungicide and common practices in all cooperative farmers (in thousand rupiahs).

Agroinput	Common Farmer Practice				PGPR Treatment			
	Co-Farmer 1	Co-Farmer 2	Co-Farmer 3	Average	Co-Farmer 1	Co-Farmer 2	Co-Farmer 3	Average
Cuttings (125/cutting)	1,875	1,875	400	1,383.33	1,875	1,875	400,000	1,383.33
Supplementary fertilizer/NPK (10,000/kg)	500	500	150	316.67	0	0	0	0
Biofertilizer-biofungicide (25,000kg)	0	0	0	0	250	250	50	41.67
Manure (100,000/m^3)	200	200	100	166.67	200	200	100	166.67
Chemical pesticide(s)								
• 200 g/l Azoxystrobin + 125 g/l Difenoconazole (135,000/250 ml)	135	135	75	115,00	0	0	0	0
• 250 g/l Difenoconazole (94,000/200 ml)	94	94	50	79.33	0	0	0	0
• 18 g/l Abamectin (276,000/200 ml)	276	276	120	224	0	0	0	0
• 5% Emamectin benzoate (220,00/200 g)	220	220	125	188.33	0	0	0	0
• Biopesticide (pack) (75,000/500 g)	0	0	0	0	225	225	75	125
• Bioinsecticide (75,000/60 ml)	0	0	0	0	225	225	75	125
Workers (conctracted)								
• Soil tillage and planting bed	300	200	100	166.67	300	200	100	166.67
• Fertilizer application	160	200	80	133.33	80	120	40	66.67
• Pesticide application	280	280	240	266.67	280	280	240	266.67
• Irrigation	380	280	120	266.67	380	280	120	226.67
• Weeding	120	120	80	106.67	120	120	80	106.67
• Prunning	120	120	80	106.67	120	120	80	106.67
• Harvesting	80	80	40	66.67	80	80	40	66.67
Total Expense	4,640	4,480	1,760	3,546.67	4,135	3,975	1,375	2,806.67

Tabel 7. Productivity, marketable products and total revenue of chrysanthemum production based on common farmer practices and biofungicide treatment in all cooperative farmers

Parameter(s)	Common Farmer Practice				PGPR Treatment			
	Co-Farmer 1	Co-Farmer 2	Co-Farmer 3	Average	Co-Farmer 1	Co-Farmer 2	Co-Farmer 3	Average
Productivity (bundle/m^2)	1050	1323	310	894.34	1125	1365	330	940.00
Marketable products (%)	70.00	88.00	77.50	78.50	75.00	91.00	82.50	82.84
Price per unit product (in Rp)	7000	5000	5000	5670	7000	5000	5000	5670
Revenue (in thousand Rp)	**7,350**	**6,615**	**1,550**	**5,171**	**7,875**	**6,825**	**1,650**	**5,450**
Production Cost **(in thousand Rp)**								
• Agro Inputs	3,300	3,300	1,020	2,473	2,775	2,775	700	1,800
• Contratced worker(s)	1.44	1.28	740	1,073.35	1,360	1,200	700	1,006.69
Total Cost	**4.64**	**4.48**	**1.76**	**3.546**	**4.135**	**3.975**	**1.375**	**2.807**
Net Income **(in thousand Rp)**	2.71	2.135	210	1,545	3,740	2,850	275	2,288.33
Net Income/Cost (%)	58.41	47.66	11.93	31.38	90.4	71.70	20.00	47.38
R/C ratio *)	1.58	1.48	0.88	1.31	1.90	1.72	1.20	1.61

Remark: *) Items considered as fixed costs such as plastic houses, production tools (hose, knapsack sprayer, etc) were not included in the calculation of R/C ratio.

CONCLUSION

The application of PGPR in combinations with antagonist fungi as biofungicide in chrysanthemum production was able to support plant growth equally to chemical fertilizer and pesticide as practiced by common farmers, viewed from the plant height and stem diameter. Biofungicide also gave greater suppression to white rust development compared to fabricated chemicals. These lower diseases incidency contributed to higher marketable flowers stalks in the PGPR production site of all cooperative farmers. Aside from the higher productivity, the higher profit margin was gained from the production cost efficiency from the use of PGPR. The feasible use of PGPR in chrysanthemum production could also be drawn from the production efficiency with the higher R/C ratio (1.61) than farmer common practices (1.31).

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