Evaluation of Biological and Chemical Fungicides to Control White Rust in Chrysanthemum Grown Under Open Condition

Indijarto Budi Rahardjo*, Kurniawan Budiarto and Budi Marwoto

Indonesian Ornamental Crops Research Institute (IOCRI)
Jl. Raya Pacet-Ciherang, PO Box 8 SDL, Cianjur(43253), West Java Indonesia

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* Corresponding author:
E-mail: ib_rahardjo@yahoo.com

ABSTRACT

The effort to reduce chemicals has become an important issue in floriculture agribusiness. The chemicals included fungicides for white rust control in chrysanthemum. The combined application of synthetic fungicide with biological agent and botanical fungicide were expected not only to reduce synthetic fungicide application, but also suppressed the white rust attacks. The research was conducted at the Indonesian Ornamental Crops Research Institute (IOCRI). A factorial randomized complete blocked experiment was designed to facilitate combined applications of hexaconazol-50 (SFH-50), antagonist microbe Corynebacterium sp. (Cor-5) and formulated botanical fungicide CEES 50EC (ES-50). The results showed that the combined SFH-50 with Cor-5 and ES-50 merely affected the disease intensity, yet less on white rust incidence, period of disease emergence, plant growth, flower production and quality. All combined applications of SFH-50 with Cor-5 and ES-50 showed lower disease intensity than single SFH-50. Higher suppressions were detected on 1 ml/l SFH-50 + 5 ml/l Cor-5-3 and 1 ml/l SFH-50 + 2 ml/l ES-50-3. The use of antagonist microbe and botanical fungicide singly or in combination with synthetic fungicide could reduce with the higher affectivity than single synthetic fungicide application.

INTRODUCTION

Chrysanthemum (Dendranthema grandiflora Tzvelev syn. Chrysanthemum morifolium [Ramat.] Kitam) is one of the most important ornamentals grown all over the world. They are typically used as cut flowers or potted forms. The existing commercial varieties have various colors like white, yellow, red, purple and pink, but lack of bright red and blue flowers (He, Ke, Keting, Qiaoyan, &Silan, 2013; He et al., 2013). In international market, the commodity positioned the first occupying more than 35% of the world’s marketed cut flowers, second only to roses. Several European and American countries like Netherlands, Italy, Columbia, Spain, Germany and USA have been known to be the main chrysanthemum producers which supplied more than 60% of the world market request (Van Rijswick, 2015). In Indonesia, chrysanthemum was also positioned as the top marketed ornamentals in domestic market. The production increased from 305.9 million in 2011 to 442.7 million stalks in 2015 as a result from the increase of harvested area from 881.2 ha in 2011 to 1,087.2 ha in 2015 (Indonesian Ministry of Agriculture, 2016).

The production process, however, was still constrained by several factors, particularly at traditional farmers when planting in open conditions. Planting under open conditions have been studied by Budiarto & Rosario (2005) on stock plant to produce cutting for planting materials. White rust caused by fungal pathogen Puccinia horiana Henn was the most common disease under such conditions (Bonde et al., 2014). Several authors have reported that these pathogenic fungus attacks resulted in less selling price up to 30% and harvest postponement (Suhardi, 2009). While during outbreak season, the production lost due to white rust might reach 80% as reported in Turkey (Göre, 2008). The pathogen is an obligate parasite,
which hosted restrictedly in 12 plant species including chrysanthemum (Bonde et al., 2015). The fungus attacks the leaf directly by enzymatic digestion degrading the cuticle and then colonizes the mesophyll tissues (O’Keefe & Davis, 2015). The attack intensity fluctuates depending on cultivars resistance and environmental conditions, such as temperature and humidity. Warmer temperature accompanied by high humidity especially during rainy season may trigger the rapid development and spread of the disease (Sriram, Chandran, Kumar, & Reddy, 2015).

The use of resistant varieties and chemicals are the most common practices in handling the disease up to this moment. In Indonesia, however, no synthetic fungicide has been officially registered to control the disease (Yusuf, Djatnika, & Suhardi, 2014). Since the physical performance is very important, the growers prone to put high inputs such as chemicals and other costly substances, expecting the reduction of damages thus promote optimal plant growth and development to produce high quality cut flowers. In long and frequent applications, these practices would induce pathogen resistance and make the chemicals no longer effective (Torres et al., 2017). These practices were often applied even the symptoms were still absent, and these made the business uncompetitive (Suhardi, 2009).

The use of antagonist bacteria and botanical fungicides has been reported successful in reducing or even substituting chemicals for disease control in many crops. Corynebacterium sp., an aerobic, positive gram and polarly flagellated rod bacterium, is also known to have antagonistic activity with several important soil-borne fungal diseases in corn (Dhinakaran, Rajasekaran, & Jayalakshmi, 2012), leaf spot caused by fungal pathogen Cycloconium oleaginum in olive (Al-Khatib, Alhussaen, El-banna, & Zyadeh, 2010) and leaf blight by Xanthomonas oryzae pv. oryzae in rice (Serdani, Aini, & Abadi, 2017). The use of Corynebacterium sp. in controlling white rust disease in chrysanthemum has been studied by Hanudin, Nuryani, Silvia Yusuf, Djatnika, & Soedarjo (2011) and they found that certain Corynebacterium isolate effectively suppressed the disease development. The reduction of disease intensity contributed to the improvement of plant growth and harvestable cut flowers.

Botanical fungicide is also an alternative way out for disease control in several plants. Extract or oil of clove buds (Syzygium aromaticum L.) that contained essential oil of eugenol (Rana, Rana, & Rajak, 2011), was reported to have antifungal activity against Phytophthora, including P. capsici on pepper, P. drechsleri on cucumber and P. melonis on melon (Amini, Farhang, Javadi, & Nazemi, 2016), leaf rust caused by Puccinia triticina on wheat (Shabana et al., 2017) and several seed-borne diseases caused by Aspergillus flavus, A. niger, A. terrens, A. oryzae, A. fumigatus, Fusarium moniliforme and Penicillium sp. on maize (Shirurkar & Wahegaonkar, 2012). Lemon grass extracts that also reported containing citronella (Rana et al., 2011) was also shown antagonistic effect on several diseases caused by Fusarium sp. (Gawai, 2015), Aspergillus flavus on rice (Paranagama, Abeyskerika, Abeywickrama, & Nugaliyadde, 2003), Curvularia leaf spot on maize (Mourao et al., 2017) and late leaf spot and crown rot in peanut. The successful utilization of biological agent Corynebacterium sp. and botanical extracts of clove and lemon grass in controlling fungal pathogens on many crops has raised the possibility to use these potential biofungicides to control white rust in chrysanthemum. The application of these substances were combined and compared with common synthetic fungicides in respect to reduce the frequency and substitute the chemical application in chrysanthemum production.

MATERIALS AND METHODS

The research was conducted from January to July 2014 at the experimental field of the Indonesian Ornamental Crops Research Institute (IOCRI). The experiment was carried out based on a randomized completely block design with 3 replications and facilitating the combined applications of biological control using Corynebacterium sp., synthetic and botanical fungicides. The chrysanthemum variety used was Puspita Nusantara that is known to be susceptible to white rust. The synthetic fungicide used was 50 g/l hexaconazol which is formulated with the commercial name of Anvil 50SC (Syngenta Co Ltd.). The synthetic fungicide treatment was further named as synthetic fungicide hexaconazol-50 (SFH-50). The botanical fungicide used was formulated biofungicide with commercial brand of CEES 50EC with active component of eugenol and citronella, produced by Indonesian Spice and Medicinal Crops Research Institute. Furtherly, this biofungicide treatment was named as ES-50.

Preparation of Fungicide Treatments

The antagonist microbe Corynebacterium sp. isolate was collected from the pure culture collection of biological control laboratory, the Indonesian
Ornamental Crops Research Institute. The treatment of Corynebacterium sp. with the concentration of 5 ml/l was called as Corynebacterium-5. The preparation of Corynebacterium sp. propagule was prepared in the following processes.

The pure culture of Corynebacterium sp. were transferred and grown under King’s B medium containing 0.001 M FeCl₃. The culture were then incubated for 24 h under the constant temperature of 30±2 °C. Three looped-suspension were diluted in 10 ml aquadest and homogenized until the suspension had the density of 10¹² cfu/ml. For about 100 μl isolate suspension were taken and put into petridishes containing 15 ml solidified SPA medium. The cultures were then incubated for another 24h under the constant temperature of 30±2 °C. The bacterial cells were then suspended into sterile aquadest and the solution were then diluted into the carrier media.

The carrier media were made from boiled mixtures of 10% organic worm feces and 30% potato. The boiled water was filtered and 1.5% sucrose and 10 % molase was added into the water. The suspension was then stirred until homogen. The suspension was fermented for 21 days inside the biofermentor instruments. The pH of suspension was then adjusted using 1N KOH until it reached 7.4.

Land Preparation, Planting and Plant Maintenance

The soil under open condition was tilled and the weeds were disposed outside from the experimental sites. After the soils were mixed with 30 tons/ha manures and 10 tons/ha bamboo humus, for about 52 planting beds with the size of 1 x 2 m each were constructed. The distances between planting beds were arranged in 50 cm. The planting bed had 25 cm in height with the distance between planting beds was 60 cm. For about 40 g/m² NPK (16:16:16) were mixed gently with the top soil and the planting beds were then irrigated with water to keep the humidity. Long day instruments were provided by the installment of 11 watt LED lamps that were arranged 1.5 m above the planting bed and the distance between lamps were 2 x 2 m.

The planting material used was rooted cutting after 18 days in rooting process. The cutting was planted with the density of 64 plants/m². After planted, the cuttings were poured with water to facilitate humidity and avoid plant stress. The water supply was given using sprinkle system every 2-3 days until harvesting period. The long day conditions were applied for 30 days starting from the day of planting for 4 h during night time from 10.00 pm to 02.00 am. After 30 days, the long day treatment was terminated and the plants were forced to flower in neutral day length. Additional fertilizers using NPK (16:16:16) were applied after 30 and 60 days planting. Half dosage of insecticide was applied twice a week together with foliar fertilizers to prevent disease attacks.

Application of Fungicide Treatments

The fungicide (biological, botanical and synthetic) treatments were applied since the first week of planting until one week before harvesting period. Depending on the application period, each type of fungicide was sprayed twice a week with the volume of 2 l/m². The application of combined bio-, botanical and synthetic fungicides treatments are presented in Table 1.

Table 1. Application period and concetration of combined biological agent and botanical fungicides with synthetic fungicide treatments used in the study

| No. | Treatment               | Application period*) (at ...." week) |
|-----|------------------------|-------------------------------------|
| 1.  | 1 ml/l SFH-50          | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 2.  | 1 ml/l SFH-50 + 5 ml/l Cor-5-1 | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 3.  | 1 ml/l SFH-50 + 5 ml/l Cor-5-2 | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 4.  | 1 ml/l SFH-50 + 5 ml/l Cor-5-3 | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 5.  | 5 ml/l Cor-5            | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 6.  | 1 ml/l SFH-50 + 2 ml/l ES-50-1 | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 7.  | 1 ml/l SFH-50 + 2 ml/l ES-50-2 | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 8.  | 1 ml/l SFH-50 + 2 ml/l ES-50-3 | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 9.  | 2 ml/l ES-50            | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |
| 10. | Control (water)         | 01 02 03 04 05 06 07 08 09 10 11 12 13 14 |

Remarks : *) Synthetic fungicide (SFH-50) treatment was denoted as biological agent Corynebacterium (Cor-5) as , botanical fungicide containing eugenol and citronella as and control (water) as .
Data Gathering and Analysis

The observations were conducted at 10% sample plants from the population per plot. The emergence of white rust disease was determined from the appearance of the symptom on each treatment combination. The disease intensity was measured using the following formula:

\[ I = \frac{\sum (v \times n)}{(Z \times N)} \times 100\% \tag{1} \]

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

The disease development was determined according to Suhardi (2009) criteria as presented in Table 2.

Disease incidences were observed weekly and measured using the formula:

\[ IP = \frac{n}{N} \times 100\% \tag{2} \]

Where:
- \( IP \) = Disease incidence (%)
- \( n \) = Number of infected plants
- \( N \) = Total number of observed plants

The effect of fungicide treatments were observed through the agronomical properties such as plant height, flowering uniformity, flower diameter and number of marketable flower.

Flowering uniformity were determined using the following categories.
1 = 75 - 100% flowers within a plant were fully opened
2 = 50 - 74% flowers within a plant were fully opened
3 = 25 - 49% flowers within a plant were fully opened
4 = 1 – 24% flowers within a plant were fully opened

RESULTS AND DISCUSSION

Disease Emergence and Incidence

The white rust disease symptoms appeared at 3 weeks after planting (WAP) in all treated plants and there was no difference in all treatments related to the period of symptom emergence. The symptom was characterized by light green to yellow spots up to 5 mm in diameter appeared on the upper surface of the leaf. These spots become brown and necrotic with age (Zeng et al., 2013). The pustule actually contained teliospores that enabled to germinate and form infectious basidiospores. The development of the disease were influenced by several factors, such as warm, high humidity and susceptible varieties might induce a higher the disease incidence (Bonde et al., 2014).

The disease incidence was considered inferior at 3 WAP with the values were < 35%, except in 1 ml/l SFH-50+2 ml/l ES-50-3 (Fig. 1). The incidences then sharply increased at 4 WAP. During these period, control treatment showed the highest (100%). While the rest had 73-99% incidences. The increament of disease incidence flattened at 5 WAP with the highest at 1 ml/l SFH-50+5 ml/l Cor-5-1 and control treatments. From 6 WAP until termination of growing period, the incidences were mostly above 95% with negligible differences among the treatments.

Table 2. Scale and damage criteria of white rust (Puccinia horiana Henn) infection on chrysanthemum (Suhardi, 2009)

| Scale | Damage Criteria |
|-------|-----------------|
| 0     | Not infected (symptomless) |
| 1     | Very low, infection was detected only on lower plant leaves and the intensity was not exceeding more than 5% from total leaf area |
| 2     | Low, infection was detected on lower plant leaves and the intensity ranged between 5-10% from total leaf area |
| 3     | Medium damage, infection was detected on middle and lower plant leaves and the intensity ranged between 10-20% from total leaf area |
| 4     | Heavy damage, infection was detected on upper, middle and lower plant leaves and the intensity ranged between 20-40% from total leaf area |
| 5     | Very heavy damage, infection was detected on upper, middle and lower plant leaves and the intensity was more 40% from total leaf area |
The disease incidences in all treatments during the early planting period were in accordance with the disease intensity. The disease intensity in all treatments were <15% at 3 WAP (Fig. 2). The intensity increased slightly at 4 WAP and then flattened at 5 WAP. Sharper increase of disease intensities were detected in most treatments at 9 WAP except in 1 ml/l SFH-50 and 1 ml/l SFH-50 + 2 ml/l ES-50-2 that had the values of 33.53 and 58.5%, respectively. The suppression effect of single application synthetic fungicide, however, was not persistent. The disease intensity on 1 ml/l SFH-50 was observed to be the 2nd highest after the control after 11 WAP. These findings were not in accordance with (Lam & Lim, 1993) which stated that hexaconazole was effective to control white rust in chrysanthemum. High environmental humidity (RH > 90%) during 8 to 11 WAP was predicted to have correlation with these situation. High environmental humidity might reduce the concentration and adhesive potential of the chemical on targeted sites. The chemical killed the existing telia, yet the viable teliospores might be still exist (Radha & Chattannavar, 2017). Under these situations, the intensity were then increase more sharply until the end of the growing period. Unlike in control and 1 ml/l SFH-50 treatments, the disease intensities at single 5 ml/l Cor-5, 2 ml/l ES-50 and combined 1 ml/l SFH-50 with 5 ml/l Cor-5 and 2 ml/l ES-50 were decrease after 10 WAP (Fig. 2). These conditions inferred that the applications of single application of biological agent Corynebacterium and botanical fungicides eugenol+citronella were more effective in reducing the disease intensity than SFH-50. The chemical SFH-50 was also compatible with these biofungicides when applied in combinations, especially with ES-50 (1, 2 and 3) and Cor-5 (3). These four combined treatments had disease intensity of 45.1, 45.5, 39.27 and 38.13%, respectively and differed significantly from other treatments. The successful control of white rust using biological agent had also been reported by Torres et al., (2017). While essential substances eugenol and citronella had also been reported to have antifungal effects on pathogenic fungi on several plants (Pereira, Lucas, Perina, Ribeiro Junior, & Alves, 2012).
The more positive effects of *Corynebacterium* and botanical fungicide ES-50 (eugenol and citronella) on decreasing disease intensity than single application of synthetic fungicide (SFH-50) indicated that certain properties of these agents had additional protective mechanism against white rust. *Corynebacterium* has capability to produce antibiotic-like bacteriocine, *corynecin-linocin* type (Salamiah & Wahdah, 2015). While eugenol is also known as allyl chain-substituted guaiac, a naturally occurring phenolic compound and has been used as registered biofungicide in several countries (Harni, Amaria, & Supriadi, 2013). The mode of action was through membrane binding and permeability alteration of pathogen hyphae. The substance induced the increase of potassium ion concentration and other material within the cell thus revealed to morphological alteration, such as cytoplasmic coagulation, vacuolation and hyphal shivering (Abd-Elsalam & Khokhlov, 2015). The substance also induced the generation of H$_2$O$_2$ leading to destabilization and disruption of the plasma membrane (Wang, Zhang, Chen, Fan, & Shi, 2010). While citronella has citronellol, geraniol and d-limonene as the main components and been reported to have inhibitory effects on pathogenic fungi in several crops (Pereira et al., 2012) with putative mechanism of suppression on spore germination (Enyiukwu, Ononuju, Awurum, & Nwaneri, 2016). Eugenol and citronella also induced indigenous plant resistance through certain enzyme and lignin production (Pereira et al., 2012).

The lower decrement of disease intensity on combined SFH-50 with Cor-5 and ES-50 than single application on SFH-50 indicated that the reduction of synthetic pesticide uses in controlling white rust was actually possible. The combined application SFH-50 with Cor-5 and ES-50 reduced the use synthetic pesticides up to 25-100% with the comparative effectivity suppression of 34-58% compared to single application of synthetic fungicide SFH-50 (20.8%) from control treatment. These findings were in accordance with (Hanudin, Budiarto, & Marwoto, 2017) that confirmed the successful control of white rust in chrysanthemum using PGPR on the effort on reducing synthetic fungicide application.

**Fig. 2.** White rust disease intensity on chrysanthemum treated by combination of biological, botanical and synthetic fungicides
### Table 3. Effects of the combination of biological, botanical and synthetic fungicides on plant height, flower diameter, uniformity and number of marketable flowers

| Fungicide treatments                  | Plant height\(^a\) (cm) | Flower diameter\(^a\) (cm) | Flowering uniformity\(^a\) (%) | Number of marketable flowers/plot\(^b\) |
|--------------------------------------|-------------------------|----------------------------|-------------------------------|---------------------------------------|
| 1 ml/l SFH-50                        | 104.60                  | 7.13                       | 86.23 ab                      | 84.63 a                               |
| 1 ml/l SFH-50 + 5 ml/l Cor-5-1       | 102.90                  | 7.27                       | 86.50 ab                      | 90.46 a                               |
| 1 ml/l SFH-50 + 5 ml/l Cor-5-2       | 98.37                   | 7.31                       | 90.12 a                       | 84.06 a                               |
| 1 ml/l SFH-50 + 5 ml/l Cor-5-3       | 94.07                   | 7.21                       | 81.64 ab                      | 86.46 a                               |
| 5 ml/l Cor-5                         | 95.80                   | 7.40                       | 87.47 ab                      | 85.17 a                               |
| 1 ml/l SFH-50 + 2 ml/l ES-50-1       | 97.47                   | 7.12                       | 84.63 ab                      | 86.65 a                               |
| 1 ml/l SFH-50 + 2 ml/l ES-50-2       | 100.40                  | 7.31                       | 79.42 b                       | 81.15 a                               |
| 1 ml/l SFH-50 + 2 ml/l ES-50-3       | 101.40                  | 7.43                       | 87.53 ab                      | 89.12 a                               |
| 2 ml/l ES-50                         | 94.43                   | 7.29                       | 85.17 ab                      | 83.87 a                               |
| Control (water)                      | 99.87                   | 7.06                       | 85.57 ab                      | 80.57 a                               |

*Values in the same column followed by different letters were significantly different under HSD (α = 5%)*

**Agronomic and Flower Yield Properties**

The effects of biological, botanical, synthetic fungicides applications and the combination among them on plant height, flower diameter, uniformity and number of marketable flowers are presented on Table 3. The final plant height, flowering uniformity and number of marketable flowers seemed were not affected by the combination of biological, botanical and synthetic fungicides treatments. While, the highest flowering uniformity was detected at 1 ml/l SFH-50 + 5 ml/l Cor-5-2, 5 ml/l Cor-5, 1 ml/l SFH-50 + 2 ml/l ES-50-3 and 1 ml/l SFH-50 + 5 ml/l Cor-5-1 that were in accordance with the less disease intensities at flowering periods (Fig. 2).

The decrement on disease intensity induced the plants to recover and retain their potential growth. The development of white rust disease was inhibited and unable to infect the newly developed leaves. With more uninfected leaves, the more physiological and biochemical processes within the plants might undergo more conducive (Shabana et al., 2017). Less infection reflected the more potential photosynthetic area leading to higher photosynthetic rates. These resulted to higher assimilates for the plant into reproductive stages and organ formation (Yusuf et al., 2012), including flowering period uniformity.

**CONCLUSIONS**

The application of combined synthetic fungicide (SFH-50) with antagonist microbe *Corynebacterium* (Cor-5) and botanical fungicide (ES-50) slightly affected white rust incidence, the period of disease emergence, plant growth, flower production and quality. All combined application of SFH-50 with Cor-5 and ES-50 had lower disease intensity than single SFH-50 with higher suppressions were detected on 1 ml/l SFH-50 + 5 ml/l Cor-5-3 and 1 ml/l SFH-50 + 2 ml/l ES-50-3. The use of antagonist microbe Cor-5 and botanical fungicide ES-50 singly or in combination with SFH-50 might reduce the application of SFH-50 up to 25-100% with the effectivity suppression of 34-58% compared to single SFH-50 and control treatment.

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