

## Effects of Varieties, Cutting Health and Fungicide Application on Chrysanthemum White Rust

Evi Silvia Yusuf <sup>\*)</sup>, Kurniawan Budiarto, I. Djatnika and Suhardi

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

<sup>\*)</sup> Corresponding author E-mail: evinugraha99@yahoo.com

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### ABSTRACT

White rust caused by fungal pathogen *Puccinia horiana* P. Henn. is one devastated disease that could make significant economic loss in chrysanthemum production. The study of effects of varieties, cutting health and fungicide application on chrysanthemum white rust was established. The treatments were arranged in split-split plot completely randomized design with three replications. Three chrysanthemum varieties i.e. cv. Puma White, Reagent Purple and Town Talk served as main plot. Seedlings with 20 % intensity of white rust infection and symptomless functioned as sub plot, while fungicide application on the transplanted cutting (no fungicide) and dithiocarbamate (Antracol<sup>R</sup> 75 WP, 2 g L<sup>-1</sup>) application served as sub-sub plot. Result showed genetic background of the cultivars significantly determined the degree of infection of white rust. Reagent Purple exhibited least disease intensities. Fungicide application was less effective in controlling white rust development, yet gave significant impact on the plant height and number of leaves of chrysanthemum plants. Cuttings selection based on the visual observation on the presence or absence of white rust pustules symptom did not gave significant differences on the further development of the disease. The symptomless cuttings were also infected with this fungal disease after the cuttings were planted under plastic house.

Keywords: chrysanthemum varieties; disease development; fungicide (dithiocarbamate); growth quality; white rust symptom

### INTRODUCTION

White rust disease is known to be the most common problem in chrysanthemum production in the world. It is caused by the obligate parasitic

fungi *Puccinia horiana* P. Henn (Basidiomycetes). In Indonesia, the introduction of the disease was reported in 1990's and it probably entered through imported symptomless seedlings (Djatnika, Kristina, & Sanjaya, 1994). Up to now, the disease has widespread to the most of the production centers in the world (Hanudin & Marwoto, 2012; Göre, 2008; O'Keefe & Davis, 2012).

The economic lost due to white rust damage in chrysanthemum has properly been evaluated in several reports. Suhardi (2009) observed the damages resulted in less selling price and harvest postponement and might reach 30 % of the total production values. While in Turkey and Poland, the production lost due to these pathogenic fungi grasped 80 % and 100 % respectively, during the outbreak seasons (Dordevic, 1988; Göre, 2008).

Efforts to increase the productivity of chrysanthemum have been carried out to make the production system become more efficient and profitable, especially reducing the production lost due to white rust incidences. Several practices have been accomplished solely or in combinations to reduce the damaging effect of white rust such as the use of tolerant/resistant varieties, cultural practices, i.e. infected leaves detachment and water management, antagonist microbes and pesticide applications (Zeng et al., 2013). The severity of the damages, plant growth stages and environmental conditions have also been determined the success of specific cultural treatments in reducing white rust. Due to the fast and unpredictable spreading of the disease, most recommended treatments have been dedicated to be preventive actions such as during land preparation, on seedling stages or before the emergence of the visual symptoms (Whipps, 1993; Sheroze, Rashid, Shakir, & Khan, 2003; Budiarto, Sulyo, Nugroho, & Maaswinkel, 2007; Yusuf et al., 2012).

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Plant resistance is an important component on disease control but information on resistance of chrysanthemum varieties to white rust is very lack. Djatnika, Kristina, & Sanjaya (1994) reported that cv. Phuma White, cv. Tiger, cv. Yellow West and cv. Rhino were highly resistant, while Phuma Suny was vulnerable. According to Suhardi (2009) cv. Phuma White was tolerant and more resistant than cv. Reagent Purple and Town Talk. Some farmers use their chrysanthemum seeds or bought from other farmers to suppress production cost of chrysanthemum. The seeds produced by farmers have been infected by rust diseases (28.5 %). Using healthy seeds is a strategic step to reduce inoculum source of white rust on chrysanthemum.

The use of synthetic chemicals dithiocarbamate (DTCs) have been considered to be the most practical and widely applied to control white rust in many commercial nurseries in west Java Indonesia. Considering the close relation of initial inoculum on further development of the disease and the possible action of these wide spectrum fungicides to control white rust in chrysanthemum, the experiment was then conducted to find out the effects of fungicide application and white rust symptoms at seedling stages on plant growth and development of white rust during greenhouse forcing of several chrysanthemum cultivars.

## MATERIALS AND METHODS

The experiment was conducted in plastic house conditions at The Indonesian Ornamental Crops Research Institute (IOCRI), at Pacet, Cianjur West Java, Indonesia (latitude 6° 45.5393 'S, longitude 107° 3.2193' E, 1100 m asl). The treatments were arranged using split-split plot design with three replications. Three chrysanthemum varieties i.e. cv. Puma White, Reagent Purple and Town Talk served as main plot. Seedlings in the form of rooted cutting with 20 % intensity of white rust infection and no visual symptom (symptomless) functioned as sub plot, while fungicide application on the transplanted cutting (no fungicide and dithiocarbamate (Antracol<sup>®</sup> 75 WP, 2 g L<sup>-1</sup>) application served as sub-sub plot.

### Planting Material and Experimental Site Preparations

Rooted cuttings of the varietal treatment were used for the planting material. The apical

cuttings of the respective varieties were collected from the mother stock plants which were maintained under standard cultural practices. The cuttings were selected for uniformity (vegetative apical flush + 2 fully expanded leaves). The stem base of cuttings was then quick dipped on Rooton F paste (root promoter). The cuttings were stored under ambient temperature for 2-3 minutes to let the paste a little bit dried. The cuttings were then planted in carbonized rice husk as a rooting media and maintained under long day conditions for 18 days. During the rooting process, the water supply was also given every 2 days by spraying.

Coincident with the rooting process, the land for the experimental sites was also prepared. The soil was being crumbled using hoe and the planting bed in the size of 100 cm in width, 150 cm in length and 20 cm in height was constructed. The distance between planting bed was 60 cm and each planting bed represented one treatment combination. During these soil tillage, 4.6 kg horse manure, 2 kg bamboo husk, 234.4 g NPK (15:15:15) per planting bed were also added and mixed with the soil. Supplemental light for long day conditions was provided by incandescent lamps with the intensity of 70 luxs. These was achieved by arranging the lamps 1.5 m high from the planting bed and 2 m between the lamp points.

### Treatment Arrangement

The treatments were arranged using split-split plot design with three replications. Three chrysanthemum varieties i.e. cv. Puma White, Reagent Purple and Town Talk served as main plot. Seedlings in the form of rooted cutting with 20 % intensity of white rust infection and no visual symptom (symptomless) functioned as sub plot, while fungicide application on the transplanted cutting (no fungicide and dithiocarbamate (Antracol<sup>®</sup> 75 WP, 2 g L<sup>-1</sup>) application served as sub-sub plot.

The rooted cuttings of the three varieties were selected based on the visual appearance of white rust symptoms. Symptomless seedlings were characterized by the absent of rust pustule in the leaf or stem. The infected seedlings, on the other hands, were recognized by the existence at least one pustule on the leaf or stem per cutting. The symptomless and rust infected cuttings were then separately planted on the

experimental sites based on the constructed design.

The fungicide treatments were applied using knapsack sprayer with the approximate volume of 30 ml fungicide solution per plant. The frequency of fungicide application was every 7 days until 2 weeks before the flower's harvest, while the plants under no fungicide treatment were sprayed with sterile water in the same volume, time and frequency with those under fungicide treatments.

### Planting and Cultural Practices

One hour before planting, for about 10-liter water per planting bed was gently poured to facilitate sufficient soil humidity for the planted cuttings. After the rooting process, the rooted cuttings were selected based on the treatment being applied and then planted in the sites with the density of 96 plants per planting bed. Same amount of water was given to reduce the stress and hinder the early death of the newly planted cuttings. The water supply was then provided by gently water pouring every day during the first 7 days after planting. After that, irrigation through drip system facilitated the water supply every 3 days or when necessary until the harvest period.

Long day conditions were provided with supplemental lightning for 4 h every night from 10 pm to 2 am until 30 days after planting. Supplemental fertilizers were applied through foliar spraying once a week and side dressing of 30 g NPK (15:15:15) per planting bed every 2 weeks. Bactericide and insecticide were also employed to the whole plants for preventive purposes or only when the symptom was appeared.

### Parameter Observations

Plant height and number of leaves per plant sample was measured at the harvest period. The intensity of white rust infection was recorded every week on 12 random plant samples per treatment combination. The severities of the attacks were categorized based on the following disease index (GDAI, 2013):

- 0 = no visual symptom
- 1 = 1-3 rust pustules per leaf and located only in the adaxial leaf surface
- 2 = more than 5 pustules per leaf, located only in the older leaves near the stem base, or on the whole plant leaves with the number of 1-3 pustules per leaf.

- 3 = the infection reached middle plant leaves, generally more than 5 pustules per leaf.
- 4 = the infection reached upper plant leaves, generally more than 5 pustules per leaf.
- 5 = the infection covered the whole plant part including primordial flower leaves, the death tissue and dried leaves as indication of further infection stages might be found.

The disease intensity in each treatment combination was calculated using the following formula (GDAI, 2013):

$$P = \frac{\sum (v \times n)}{(N \times Z)} \times 100\%$$

where,

- P = percentage of the rust infection
- v = disease index of the observed intensity
- n = number of plants categorized on the respective index
- Z = disease index of the highest infection intensity
- N = number of plant samples (12 plants)

The increase of disease intensity in line with the time of observations on the three varieties can be depicted on linear mode as:

$$Y = a + bX$$

Where:

- Y = Dependent Variable
- X = Independent Variable
- a = constant
- b = coefficient regression

## RESULTS AND DISCUSSION

### The Development of the White Rust Disease

The development of the rust disease under the plastic house was dynamic throughout the experiment. In general, the rust disease incidence increased since 15 days after planting and reached the highest at 49-63 days after planting (Figure 1). The intensity sharply decreased up to 77 days after planting and hit the lowest point at the harvest period (8 %).

The temporary increase of the disease intensity in first two months indicated that the infected seedlings might become the source of inoculum when planted in field. The rust fungi from the previous infected seedling were triggered to sporulate and produce viable spore, thus made

new infections. This condition was presumably bolstered by the high humidity supplied by the water irrigation during the early stage of planting. The high water supply through drip irrigation after planting was actually dedicated to reduce the death rate of newly planted cuttings because of transplanting stress. On the other hands, however, these led a conducive circumstance for rust spore development and spreading (Rahardjo & Suhardi, 2008).

The highest disease intensity was observed at 49 to 63 days after planting, in which for about 18 % of the plant populations were infected. These condition, however, was still categorized as moderately low according to Yusuf *et al.* (2012). These implied the carried inoculum in the infected seedling was the main factor of the successful infection, as also stated by Rademaker & Jong (1987) that the plant performance/characteristic and physical environment also played important roles in the process of sporulation, spreading of viable spores, germination of spore and further development of the germinated spore in the healthy tissue. Since rust infections had been found in the seedling stages and used as a basis for the symptom treatment, the rust pathogen might be classified as a virulent type.

These facts also indicated that all of chrysanthemum varieties used in this study were not categorized as resistant cultivars to this rust pathotype.

During the experiment, the maximum temperature inside the plastic was recorded at 32 °C while minimum at 17 °C and average of 52.7 % humidity especially after 63 days of planting. This unsuitable humidity seemed to be the key factor on the unsuccessful development of white rust during these stages. According to Ortega (1999), Sangeetha & Siddaramaiah (2007) and Barhate, Musmade, & Bahirat (2015), the minimum humidity of 65-90 % was required for the germination of teliospore, though these spores could withstand for more than 7 weeks when exposed to 50 % moisture. While basidiospores according to Gullino & Garibaldi (2007) were more fragile and would die in an hour if the environmental wetness dropped below 90 % or even less than 10 minutes if surrounded with 80 % humidity (Hanudin & Marwoto, 2012). These sub-optimal environment conditions were then, predicted preventing the maximum potential of the spore to release and germinate and contribute to the failure of widespread of the disease.



Figure 1. Development of white rust disease intensity on chrysanthemum plants grown under plastic house conditions

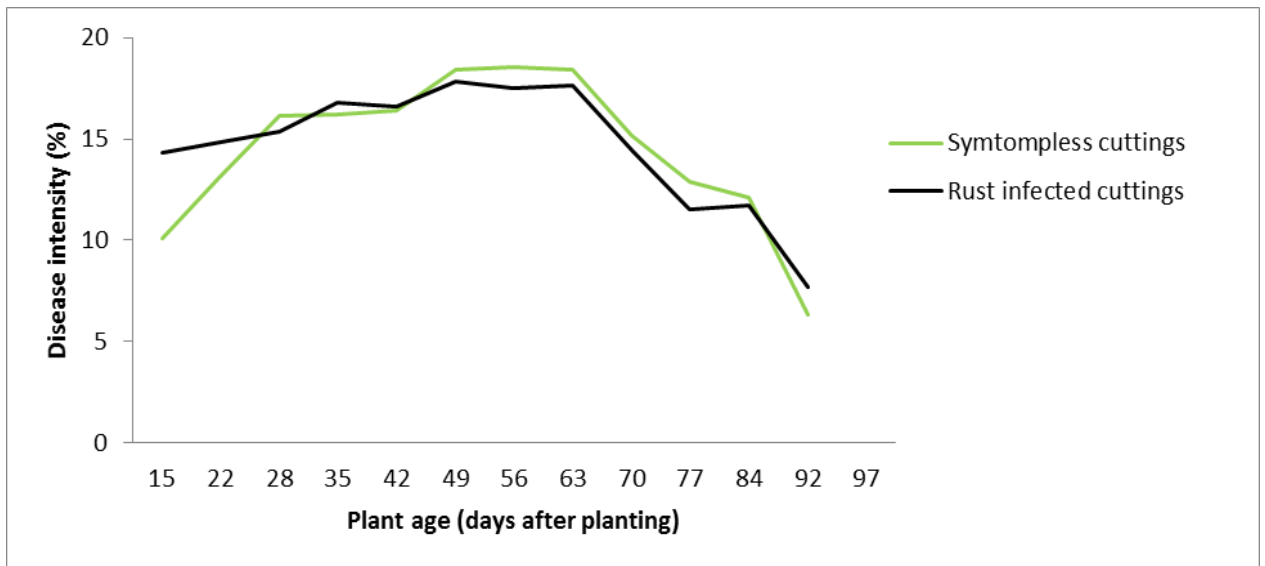


Figure 2. White rust disease intensity of chrysanthemum plants from different cutting sources under plastic house condition

#### Effect of Rust Symptom at Seedling Stage, Fungicide Application on the Development of the Disease on Three Chrysanthemum Varieties under Plastic House Conditions

Analysis of variance revealed that initial rust symptom at seedling stage, fungicide application and chrysanthemum varieties had significant impact on the development of the rust disease after the seedlings were planted under plastic house conditions. Among the three treatments, however, no interaction among others was detected in all parameters observed.

#### Effect of Rust Symptom on Seedling Stage

Rust infection as characterized by the appearance of pustules on the leaves had been detected in chrysanthemum plants grown under plastic house from both treatment i.e. symptomless and rust-infected cuttings. The intensity was increase from the planting day up to 35-42 days and reached the peak at 49-56 days after planting (Figure 2). In general, no significant differences on the disease intensity between the chrysanthemum plants derived from symptomless and infected cuttings. The variation existed only in the first 15 days in which plants from the infected cuttings affected more severely than those from the symptomless cuttings. As predicted earlier, a conducive environment (high humidity due to the high water supply in the early planting stage) would trigger further disease development and spreading in the chrysanthemum plants under

plastic house when a virulent white rust inoculum was existed (Sugimura & Okayama, 2000).

The lower disease intensity at the chrysanthemum plants derived from symptomless cuttings than those from infected cuttings in the first 15 days indicated the different level of rust infections. These different levels might designate to different stage in life cycles of the pathogenic fungi on the cuttings when they were planted on the plastic house. There was also possibility that the symptomless cuttings were already infected during the rooting process. The infection began with the germination of the spore in leaf surface and the mycelia penetrated into leaf surface through the natural cavity, like stomata. The successful infection was characterized by the appearance of pustule which spent 7-15 days' incubation period depending on the environmental conditions (Wang, Liu, & Dai, 2006). All the stages before the appearance of pustule were difficult to be seen by naked eyes (Zhu *et al.* 2010) thus, the visual determination on the absent of pustule in the leaf or stem would not directly indicate the cuttings were free from rust pathogen.

#### Effect of Fungicide Application

The rust infection under both fungicide treatments was not so divergent during plastic house forcing. The rust infection of fungicide-treated plants was found lower only on the early planting period and though the cumulative disease

intensity increased up to 49 days, the differences were not significant until the harvesting period (Table 1). These insignificant differences on the disease intensity after 15 days of planting were predictably due to a defined factor that contributed to the failure of further rust pathogen development, since the average of rust incidence during the plant life cycle was considered low according to Yusuf *et al.* (2012).

Several complex mechanisms might involve and affect the effectiveness of fungicide action and development of rust pathogen. The environmental condition had predictably affected not only by providing adverse circumstances for disease manifestation, but influenced the fungicide mode of action as well. Dithiocarbamate was known to be a systemic fungicide and acted by preventing the plant surface (leaf and stem) from the infection (Malik & Faubel, 1999). The mechanism was through multi-site contacts to the spore in which the active compound was toxic and prevented the germination of the spore. The action of the compound, however, was depended on the moisture condition of the preventive site. The compound would be active when the sites were in wet condition (Dutky, 2004). These inferred that high humidity due to water supply during the early period of newly planted cutting (15 days after planting) also supported the fungicide mode of action. The significant suppression

of rust infection was clearly shown in these period, though these condition was also favorable to rust development (Table 1). After 15 days, the water was supplied using drip system, wetted only the soil root area and least contacted/splashed to newly emerging leaves. In the low relative humidity, the effect of moisturized condition of the irrigation and fungicide spraying was temporary shorted especially in plant canopy, thus limited the effectiveness of the fungicide action.

Based on the mode of action, dithiocarbamate-based fungicide was recommended for preventive and less powerful for curative actions (Dutky, 2004), as also reported in several growers in Indonesia (Suhardi, 2009). These indicated that the dithiocarbamate action was effective only before the spore germination stages and less effective on the subsequent developmental stages after germination. Since not all rust developmental stages could be seen visually (especially during the incubation period), the existence of these invisible rust stages either could not be observed even on the symptomless cutting with naked eyes. These inferred that the appearance of rust pustules on the plants from the symptomless cuttings was resulted from the ineffective fungicide to prevent further rust development from the invisible inoculum during rooting.

Table 1. White rust disease intensity of chrysanthemum plants at different fungicide applications under plastic house condition.

Fungicide application	Percentage of rust disease intensity after... days after planting (%)											
	15	22	28	35	42	49	56	63	70	77	84	92
With fungicide	9.63 a	12.25 a	15.08 a	16.19 a	16.21 a	17.68 a	17.45 a	17.47 a	14.58 a	11.91 a	11.73 a	6.82 a
No fungicide	14.89 b	14.76 a	16.33 b	16.81 a	16.87 a	18.59 a	18.66 a	18.58 a	15.14 a	12.56 a	11.64 a	7.21 a

Remarks: \*) Values in the same column followed by the same letter were not significantly different under Duncan Multiple Range Test (DMRT) 5 %

Table 2. White rust disease intensity on three chrysanthemum cultivar under plastic house condition

Chrysanthemum Varieties	Percentage of rust disease intensity after... days after planting (%)											
	15	22	28	35	42	49	56	63	70	77	84	92
Reagent Purple	6.11 a	7.73 a	10.26 a	11.05 a	11.11 a	11.65 a	9.47 a	9.14 a	8.45 a	5.95 a	5.64 a	3.13 a
Puma White	13.02 b	14.32 b	15.93 b	16.71 b	16.65 b	17.32 b	15.48 b	17.57 b	16.71 b	13.53 b	13.21 b	7.63 b
Town Talk	18.12 c	18.45 c	20.11 c	20.95 c	21.02 c	22.57 c	20.31 c	21.29 c	19.28 c	17.11 c	16.81 c	10.44 c

Remarks: \*) Values in the same column followed by the same letter were not significantly different under Duncan Multiple Range Test (DMRT) 5 %

Table 3. Plant height and number of leaves of chrysanthemum under plastic house conditions based on varieties, rust symptom at seedling stage and fungicide treatments

Treatments	Plant growth parameters <sup>*)</sup>	
	Plant height <sup>**) (cm)</sup>	Number of leaves <sup>**) )</sup>
<i>Chrysanthemum cultivar</i>		
Puma White	109.01 a	57.80 a
Town Talk	129.19 b	59.76 b
Reagent Purple	134.88 c	74.01 c
<i>Rust symptom at seedling stage</i>		
Symptomless	125.74 a	64.41 a
Rust infected cuttings	122.98 a	63.31 a
<i>Fungicide application</i>		
No fungicide	121.18 a	62.01 a
With fungicide	127.42 b	65.71 b

Remarks: <sup>\*)</sup> Plant growth parameters were observed on the harvest period (92 days after planting); <sup>\*\*) )</sup> Values on the same parameter column and under the same treatment category followed by the same letter were not significantly different under Duncan Multiple Range Test (DMRT) 5 %

#### White Rust Infections among the Chrysanthemum Varieties

White rust development as reflected by the rust intensity was varied among the chrysanthemum cultivars tested. Chrysanthemum plants cv. Town Talk exhibited the most susceptible viewed from the highest intensity of white rust infection from the early planting until the harvest period, while cv. Puma White showed better characteristic which showed less infection than Town Talk (Table 2). Among the previously stated cultivars, cv. Reagent Purple showed the best performance in terms of the rust infection under plastic house condition.

The different white rust intensities among the chrysanthemum cultivars reflected the different degree of tolerances from each cultivar to the pathogenic rust incidence. These characteristic was indigenously exhibited depending on the genetic construction of the genotypes and their interaction with the specific environment where the cultivars were planted (Francl, 2001). Previous study by Suhardi (2009) on these three cultivars in relation to white rust was also in line with the findings, where cv. Reagent Purple and Puma White were more tolerant to this white rust pathotype than Town Talk.

#### Effect of Rust Symptom at Seedling Stage, Fungicide Application on the Growth of Three Chrysanthemum Varieties under Plastic House Conditions

Analysis of variance revealed that the growth performance of chrysanthemum plants was influenced by fungicide application and cultivars. On the other hands, rust symptom at seedling stage gave insignificant effects on the chrysanthemum growth under plastic house condition. No interaction was detected among the treatments being applied on the plant height and number of leaves of chrysanthemum plant as far as these concerns.

The different genetic constructions among the chrysanthemum cultivars were not reflected only on the tolerant/resistant characteristic to a certain pest and disease. The complex interaction of these genotypes to a specific environment also exhibited in the capability of the cultivar to take maximum advantages of a defined circumstance and establish their potential growth rates and performances. Higher values of plant height and number of leaves of cv. Reagent Purple than Town Talk and Puma White reflected the capability of this genotype to establish greater growth rates (Table 3). The growth involved the new formations of plant organs such as nodes, internodes and leaves.

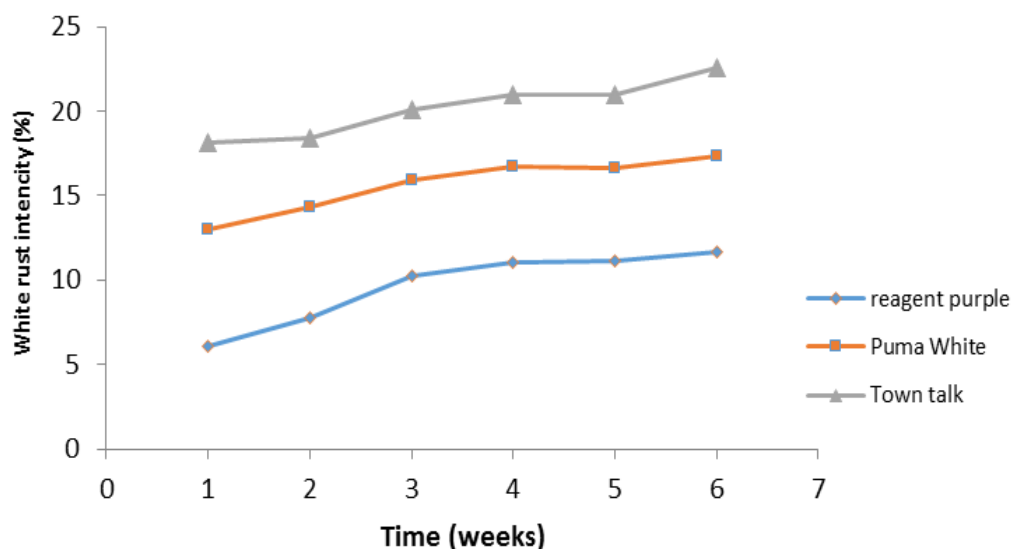


Figure 3. Development white rust disease intensity on three chrysanthemum cultivars

Final plant height and number of leaves of chrysanthemum were not affected by the sources of cuttings i.e. cuttings with rust symptom and symptomless cuttings (Table 3). After these two kinds of cuttings were planted under plastic house conditions, the plants were attacked by the rust pathogen in the same degree of intensity until harvest period (Figure 2), thus impacted on the plant growth performance.

Fungicide application, on the other hands, gave significant impact on the plant height and number of leaves of chrysanthemum plants under plastic house conditions. These conditions were predicted to have relation with the mode of action of dithiocarbamate as the active ingredient of the fungicide used in this experiment. Though the application of dithiocarbamate was mainly dedicated to counteract the rust infection, this fungicide was known to control wide pathogen ranges including *P. horiana* (Yang, Hamel, Vujanovic, & Gan, 2011; Palmer, Nester, Revell, & Bonde, 2015). Fungicide application then, prevented the attack of other potential fungal pathogens hosted in chrysanthemum that might reduce the growth quality of plants. In contrary, several fungal pathogens were found attacking the non-fungicide chrysanthemum plants in which not all of them could be reported in this paper.

Resistance characteristic of a plant to certain disease was determined by the interaction between the genetic structures of the plant host

and the pathogen (Wojdyla, 2004). In general, white rust intensity on Reagent Purple was lower than those attacked on Puma White and Town Talk. The increase of disease intensity in line with the time of observations on the three varieties can be depicted on linear model as  $y = 1.1037x + 5.7887$  ( $R^2 = 0.8637$ ,  $P=0.69$ ) for Reagent Purple;  $y = 0.828x + 12.892$  ( $R^2 = 0.8384$ ,  $P=0.69$ ) for Puma White and  $y = 0.9263x + 17.231$  ( $R^2 = 0.7693$ ,  $P=0.69$ ) for Town Talk (Figure 3). These involved coordinated expression of a multitude of virulence factors and genes of both plant and pathogen that would contribute of the compatibility of host plant and pathogen interrelation, establishment of pathogen colonization and severity degree of infection (Killiny & Almeida, 2011). These inferred that the resistance characteristic of a genotype might be expressed in different degrees to different pathogens or pathotypes. Chrysanthemum cv. Puma White had shown less rust infection (Table 2), Chrysanthemum cv. Reagent Purple, on the other hands, exhibited more stable performances which showed the least infection to both pathogenic fungal diseases.

## CONCLUSION

The study of the effects of white rust symptom at seedling stage and fungicide application on three chrysanthemum cultivars revealed the absent of interaction among



pertinent factors on further development of the rust disease under plastic house conditions, and plant growth on three chrysanthemum cultivars. The three chrysanthemum cultivars have showed different growth performances and resistance capacities to white rust in which cv. Reagent Purple exhibited least disease intensities compared to Town Talk and Puma White. The chrysanthemum plants derived from rust-symptomless cuttings were infected during plastic house forcing thus, indicating the absent of rust symptom (pustule) based on visual observation has not satisfactorily determined the cuttings were free from rust inoculum.

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