Resistance varieties and pattern of disease progress of rust (\textit{Pucciana horiana} p. henn) in Chrysanthemum

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Abstract. Rust is a major disease in chrysanthemum, which causes degradation of flower quality and results in up to 100\% yield loss. Out of all the available strategies, breeding for host-plant resistance and knowledge of the pattern of disease progression in each variety is an effective strategy to control rust disease. The disease progress figured out the level of disease severity from time to time. It would be as guidance to control the disease, especially pesticide application. Six national superior and one introduced varieties were tested for their rust resistance. The varieties were studied to know the pattern of disease progression in each variety from vegetative to generative phase. The study was conducted from August 2014 to January 2015 in the plastic house of chrysanthemum plantation at Garung sub-District, Wonosobo regency, one of the centers chrysanthemum production in Central Java. The disease intensity was measured at 30, 60, and 90 days after planting. The results relayed that based on the rate of disease progress and the average of disease intensity, it could be identified that the superior national varieties of chrysanthemum, Kusumaswasti, Marimar, and Yulimar were more resistant to rust than the four other varieties tested. The rate of disease progress and the level of disease intensity of rust by the plant age determined the resistance of the chrysanthemum varieties to rust. Moreover, the result showed that each variety had a different rate and pattern of disease progression and could be used to control rust effectively.

1. Introduction

One of the major diseases on chrysanthemum is white rust, which is caused by the fungus of \textit{Puccinia horriana} P.Henn. The obvious symptom of the diseases is white to orange pustule at the leaves bottom of and white-yellowish spots or blemishes on top of the leaves. In several chrysanthemum production areas, rust has been reported as one of the destructive diseases that can lower the chrysanthemum's quality and productivity. As a major disease, rust can reduce the yield up to 100\% \cite{1}, while in Indonesia, it is around 30 to 100\% \cite{2}\cite{3}. In the other testings carried out at multiple locations and in different climatic conditions, it was reported that rust infection on the susceptible varieties caused the whole part of the plants, including flower, leaves, and stems, to get burn and search, which bring about 100\% yield losses \cite{4}.
Among the available strategies to control rust disease, breeding for host-plant resistance is an effective strategy [5][3]. Resistance varieties are identified as varieties having a slow getting infected and low disease intensity [6][7]. Moreover, resistant varieties can be determined by the rate of infection of the disease on these varieties. It can be explained that the value of rate infection in resistant variety is quite low; even some of them are zero. In contrast, the value of the rate of infection in susceptible variety is high. The study of rust on red bean progress showed that the disease progress of rust in resistant varieties is slower than in susceptible varieties [8].

Furthermore, Vander Plank (1963) stated that the disease progress is a tool to figure out the varieties' resistance, the aggressiveness of pathogen, and the quality of the environment [8]. As an explanation, when \( r \) (disease progress) has a value lower than 0.5 units/day in any variety, the variety considered resistant, the pathogen is not aggressive, and the environment does not support the development of the disease, reversally. By using the same control treatment, each variety will give different response to the disease depend on the level of resistance and can be identified based on the magnitude of the disease intensity and the rate of infection. The disease intensity and the rate of infection of rust in Chrysanthemum cv. Giant is lower than that in Chrysanthemum cv. Cat Eye, and cv. ILK6B1, even though it was controlled using the same methods, i.e. roughing, and application of pesticides [9].

Resistance against the disease is an important character of a variety; however, color flower, shape flower, vase life, sometimes are considered more by the farmer, many susceptible varieties are available in the field. Consequently, chemical pesticides are applied intensively to control the disease. Hence to suppress the excessive use of fungicide, information about the peak of disease progression is needed. Freedman and Mackenzie explain that the magnitude of the disease intensity at a certain period of the plant's age is affected by the rate of infection and the disease intensity of the initial attack[10]. The result suggests that the rate of infection may fluctuate with age. The rate of infection of rust in chrysanthemum cv Swarnakencana from age 1 to 2 week, 2 to 3 week, and 3 to 4 week are different. The infection rate is fastest at the age of 1 to 2 weeks. The infection rate is likely to a slowdown in the period 2-3 weeks and flattens out at 3 to 4 weeks. In this research, the fungicides are applied every week, starting at two weeks after planting for up to 2 weeks ahead of the harvest [11].

Moreover, the infection rate can be used as a reference in determining the types of pesticides that are effective in suppressing the disease. On controlling rust in chrysanthemum, bio fungicides Neem-plus can slow down the infection rate of white rust on the plant from age 1-4 weeks for 1 to 3 times compared to other fungicides three times slower than the control [11]. While biopesticide based on kascing (degraded soil by soil worm), isolates of Bacillus subtilis, Pseudomonas florence, Escherichia coli or biopesticides based potato extract plus kascing, molasses, isolates of Bacillus subtilis, Pseudomonas florence, and Escherichia coli can slower infection rate of rust in chrysanthemum plant effectively[12].

2. Methods
2.1 Location and time of study
The research was carried out in the plastic house in Garung District, Wonosobo Regency, Central Java Province, from August 2014 to January 2015. Garung is located at 1000 m above sea level (a.s.l.); the daily temperature ranges from 20-30°C, during the cold season from July to August, the night temperature is around 10-15°C. Based on climatological data recorded in 2014 at the closest station WadasLintang, the average humidity in Garung was 81%, average rainfall was 3214 ml/day, and rainy days was 160 days. The soil was classified as Regosol.

2.2 Varieties and methods of testing
Six varieties of chrysanthemum, i.e., Marimar, and Yulimar (originated from mutation breeding), Kusumaswasti, Puspita Pelangi, Puspita Nusantara, and Shakuntala which were released by the Indonesian
Ornamental Crops Research Institute, IAARD, and one variety named Bakardi, an introductory variety, were tested for their rust resistance. The experiments were arranged in a randomized block design with seven treatments and three replications, so 21 experimental plots were used. The disease was inoculated naturally since natural inoculum is available in abundance. Disease intensity of rust in each variety was measured periodically at 30, 60, and 90 days after planting (d.a.p.) by taking five systematic, randomly selected plant samples. Each sample was assessed based on the index of rust disease with criteria as follows [13]:

| Categories | Damage Scale |
|------------|--------------|
| 1          | 111 211 311  |
| 2          | 122 222 322  |
| 3          | 132 232 332  |
| 4          | 133 233 333  |

Explanation:

a. The first number indicates the leaf position.
   1 = leaves of 1/3 of the plant at the bottom position
   2 = leaves of 1/3 of the plant in the middle position
   3 = leaves of 1/3 of the plant in the upper position

b. The second number shows the number of pustules on the leaves:
   1 = no pustules, 2 = 1-25 pustules, 3 = 26-50 pustules, 4 => 51 pustules

c. The third number indicates the state of the spore
   1 = not yet formed spores
   2 = the spores have not broken yet
   3 = spores broken

Disease intensity in each plot is calculated based on the following formula:

\[
IP = \frac{\text{Amount} \times n \times v}{(N \times z)} \times 100\%
\]  

(1)

IP = Disease intensity
n = Number of crops per category
v = Scale value of each category
Z = The highest scale value
N = Number of plants observed
Table 2. Category of resistance based on the disease intensity

| Disease Intensity (%) | Category        |
|-----------------------|-----------------|
| 0.00-10.99            | Resistant       |
| 11.00-35.99           | Moderate resistant |
| 36.00-65.99           | Susceptible     |
| 70.00-100.00          | Very susceptible|

The rate of infection is determined based on the Van der Plank formula [8]

\[ R = \frac{2,30259}{t} \times \log \left( \frac{X_t}{X_0} \right) \]  

where:

- \( R \) = Infection rate
- \( t \) = Observation interval
- \( X_t \) = Disease intensity in time of \( t \)
- \( X_0 \) = Disease intensity in the initial observation

The flower's productivity and quality are appraised using parameters: ray floret and disk floret diameter, the length and diameter of the flower stalk measured before harvest, morphological character: flower color, flower type, and flower shape.

2.3 Planting chrysanthemum

Land preparation was done by plowing and rotating the soil until loose, and beds were set up for 2 m length, 1 m width, 25-30 cm height, and 40 cm between beds. Let the soil dry and given basal fertilizer in the form of chicken manure (CM) for 100 kg per 100 m² (10 t.ha⁻¹), compost 105 kg per 100 m²(10 t.ha⁻¹), plant growth-promoting bacteria (PGPR), and red ponska 5 kg 100 m² (500 t.ha⁻¹). The fertilizer was put on the bed surface and stirred evenly. Planting cutting stems was done one week after land preparation. A day before planting, the soil was reprocessed lightly then covered with the net as plant enforcement. The net was purposed to hold the cutting stems and to determine plant spacing. Then the bed is flooded until it reaches the saturation point. Before planting, the holes were made with a spacing of 12.0 x 12.0 cm (66 plants m⁻²). Rooted chrysanthemum cutting stems were then planted on prepared planting holes.

Addition of light: On the day of planting, light TL 18 Watt (yellow ray) was set up with light distance 2.0 x 2.5 m, and height 1.5 m - 2.0 m. The light set in the nite break pattern (20 minutes lights up followed by 10 minutes off for 8 cycles) for 4 hours a day from 10:00 to 02:00 in the morning, arranged using a timer. The addition of light was set for 35-40 d.a.p. or adjust the type of variety or until the plant reaches the height of about 50 cm.

Plant maintenance: Subsequent fertilization was done 2 weeks after planting using NPK fertilizer (16-16-16) as much 50 g m⁻² and 5 weeks after planting using NPK fertilizer (18-20-20) as much 50 g m⁻². Leaf and flower fertilizers were given 4 weeks after planting and continued 2 times per week with the appropriate doses indicated on the packaging label. Roguing has done 2-3 weeks after planting, topping (removing the first flower for spray type) was done 3 weeks after planting, and pinching (dumping the next flower for standard type) was done 7 weeks after planting. Pest was controlled using insecticide
alternately with 7-day intervals, using ½ recommended doses up to 28 days, and using the recommended doses after 28 d.a.p. Rust disease was controlled with Cabrio with 7-day intervals. Weeding was done every 2 weeks, and watering is done by flooding as needed.

3. Results and Discussion
3.1 Resistance against rust disease

The response of seven varieties of chrysanthemum to rust disease differed depending on the level of resistance of each variety and plant age period (Table 2). On this testing, a variety of Shakuntala, Puspita Nusantara, Puspita Pelangi, and Bacardi always showed a high sensitivity level at 30, 60, and 90 d.a.p. Nevertheless, at 90 d.a.p. only in Shakuntala, the disease increased drastically to 100% and significantly different from six other varieties. Kusumaswasti, Marimar, and Yulimar showed more resistance among varieties tested, with the average disease intensity of 31% to 38%.

At 30 d.a.p, the resistance of seven varieties tested to rust had already been recognized (Tabel 2). At that period, the disease intensity level was quite high (31%); even Bacardi was loaded for 63.3%. Together with Puspita Pelangi, Bacardi is the most susceptible variety. It means that intensive control of rust should be applied earlier on those two varieties. In this period, Marimar was categorized as a resistant variety as the disease intensity only 6.67%. The categorization defined [13] reveals that as resistant variety if the disease intensity on that variety as much 0.00-10.99%. Two other varieties Kusuma Swasti and Yulimar, were also quite durable since those varieties' disease intensity was only 13.33% and 20%, respectively, and did not significantly differ from the disease intensity Marimar. Marimar's obvious rust symptom can be described that 20% of the observed plants had the condition, i.e., the leaves in 1/3 bottom part of the plants covered partially with unbroken pustules containing and without spores, and 30% of plants covered with pustules containing broken spores.

Table 3. Disease intensity of rust in seven varieties of chrysanthemum at 30, 60, and 90 days after planting. Garung, Wonosobo district, August 2014 to January 2015.

| Varieties          | Disease intensity of rust (%) |
|--------------------|-------------------------------|
|                    | 30 d.a.p. | 60 d.a.p. | 90 d.a.p. | Average |
| Puspita Nusantara  | 33.33a<sup>ij</sup> | 58.33<sup>a</sup> | 75.0<sup>b</sup> | 55.55 |
| Shakuntala         | 40.00<sup>ab</sup> | 43.33<sup>b</sup> | 100.0<sup>a</sup> | 61.11 |
| Puspita Pelangi    | 46.67<sup>a</sup> | 40.00<sup>b</sup> | 75.00<sup>b</sup> | 53.89 |
| Bakardi            | 63.33<sup>a</sup> | 56.67<sup>a</sup> | 73.00<sup>b</sup> | 64.44 |
| Kusuma Swasti      | 13.33<sup>b</sup> | 13.33<sup>d</sup> | 75.00<sup>b</sup> | 31.11 |
| Marimar            | 6.67<sup>b</sup> | 28.67<sup>c</sup> | 75.00<sup>b</sup> | 36.78 |
| Yulimar            | 20.00<sup>b</sup> | 18.33<sup>d</sup> | 75.00<sup>b</sup> | 37.77 |
| Average            | 31.90 | 25.33 | 77.14 |

<sup>*)</sup> the value followed by the same letter not significantly different at p<0.05.  
**<sup>**)  d.a.p. = days after planting.

At 60 d.a.p., the level of resistance to rust of seven varieties was significantly different (Table 2). Consistently, Kusumaswasti, Yulimar, and Marimar endure to rust until 60 d.a.p. at 60 d.a.p., the disease intensity was likely lower, i.e., 13.33, 18.33, and 28.67%, and significantly different from the disease intensity of susceptible varieties Bacardi, Puspita Nusantara, Shakuntala, and Puspita Pelangi. Moreover,
in these periods, both susceptible and resistant varieties were less suffering from rust, and the average disease intensity was declined. The treatment of leaves roguing on the bottom part of the plants might successfully reduce the disease. The previous study [9], [15] was found that the treatment of roguing on rust infected leaves was effectively reduced the disease intensity of rust in chrysanthemum. However, the treatment should be appropriately done and intensively; otherwise, it can stimulate infection rate. Unfortunately, in 30 to 60 d.a.p., the disease intensity of the resistant variety of Marimar increased drastically by more than three times as a sufficient amount of healthy leaves.

Moreover, roguing is addressed only for leaves with pustules, while at 30 d.a.p. the pustules in Marimar have not come up yet, so it is a kind of disease escape. High disease intensity of rust in Bacardi, Puspita Nusantara, Shakuntala, and Puspita Pelangi at 60 d.a.p. indicated that these varieties susceptible to rust persistently. Disease control, including roguing and pesticide application, turns out to be less useful to control rust in those varieties. The same result was found from several experiments conducted in different locations and times, indicating Shakuntala as a susceptible variety. Some observations also found that resistance of Puspita Nusantara to rust had broken in recent years, although in 2002, Puspita Nusantara is released as a resistant variety. The ability of Puspita Nusantara and Puspita Pelangi against rust in this research corresponds with the result of the former trials. Adaptation trial conducted in Bandungan in 2012 showed that Puspita Nusantara and Puspita Pelangi had been categorized as a rather susceptible and very susceptible variety to rust [16]. Different results were found in the adaptation trial carried out in Magelang (2008) and Bandungan (2014); in these trials, Puspita Nusantara was considered as moderately resistant to rust [17][18]. It reveals that the resistance of Puspita Nusantara to rust is getting weaker by the year.

The following observation was done ahead of harvesting (90 d.a.p). In this observation, the resistance to rust of seven varieties was significantly different (Table 2). Shakuntala to be the most susceptible with a disease intensity of 100%, and significantly higher than the other six varieties (73 to 75%). The symptom showed that most of the leaves and stems of Shakuntala got burn and scorched, and the broken yellowing pustules came out in widely part of the bottom side of every single leaf. At 90 d.a.p., only Kusumaswasti looked less suffer from rust, and rust did not reduce the flower's aesthetic as pustules did not come out on the top of the flower arrangement.

3.2 The disease progress of rust on chrysanthemum

The pattern of disease progression is governed by initial infection and the rate of infection over time. In this observation, the pattern of disease progress of rust in each chrysanthemum variety is different, so do on the period (Figure 1). At 60 d.a.p., the average disease intensity of rust in seven varieties is slightly lower than at 30 d.a.p. from 32.85 to 31.90% (Table 2), which means that the rate of disease progress becoming slower at the period of 30 to 60 d.a.p. (Figure 1). The disease escalation in susceptible varieties Bacardi, Puspita Pelangi, and Yulimar in this period is going down; in contrast, the disease escalation in resistant variety of Marimar, Puspita Nusantara, and Shakuntala going up as much 4.3, 1.75, 1.08 times (disease intensity 28.67, 58.33, and 43.33%). A similar pattern of the disease progress also arises at 60-90 d.a.p. The disease intensity increases sharply in all varieties and mostly occurs in resistant varieties such as Kusumaswasti, Marimar, and Yulimar, unfortunately (Figure 1). The former sensitive variety of Puspita Nusantara has the least amount of disease progress (only 16.67%) at the periods of 60-90 d.a.p., it might due to limited healthy leaves available. The disease intensity in Kusumaswasti at 30 d.a.p is still low (13.33%) and does not change until 60 d.a.p. It means that the disease progression rate in Kusumaswasti at 30-60 d.a.p is zero (r_{30-60}=0.00 unit/day) (Table 3). The situation is extremely changed to 90 d.a.p.

The dynamic of disease progress of rust in chrysanthemum was influenced by technique to control the disease, such as planting resistant varieties and applying natural or chemical pesticides. The application of natural pesticides significantly affected the disease progress of rust in chrysanthemum. According to
previous research, the use of varieties and natural pesticides might affect the degree of disease control. A natural pesticide called Neem-plus can reduce disease progression by up to 60% in 7 to 30 days.

Figure 1. The pattern of rust infection in seven varieties of chrysanthemums. Disease intensity of rust (%) of seven varieties of chrysanthemum at 30, 60, and 90 days after planting (DAP). Garung, Wonosobo district, August 2014 to January 2015.

Disease attack impacts flower size, such as ray floret diameter, which is an important character of the chrysanthemum's standard flower type. Having a standard flower type, Shakuntala's ray floret diameter is usually wide and has a big size. However, in this study, the ray floret diameter was only 5.52 cm, significantly narrower than other standard flower types Marimar and Kusumaswasti (Table 4). Kusumaswasti and Marimar significantly were more resistant to rust than Shakuntala from 60 to 90 d.a.p. or during the generative phase (Table 2). Rust diseases had affected plant growth of chrysanthemum, as shown in the study of the effect of rust in chrysanthemum's vegetative growth. The result showed a significantly negative correlation between disease intensity of rust with plant height with $r = -0.837$ [20].

Table 4. Flower stalk length, flower stalk diameter; ray floret diameter, disk floret diameter of seven varieties of chrysanthemum, planted at Garung, Wonosobo in August 2014 to January 2015.

| Varieties      | Flower stalk length (cm) | Flower stalk diameter (cm) | Ray floret diameter (cm) | Disk floret diameter (cm) |
|----------------|--------------------------|----------------------------|--------------------------|---------------------------|
| Puspita Nusantara | 11.76$^{b*}$            | 5.83 $^b$                  | 5.95$^c$                 | 1.36$^c$                  |
| Shakuntala     | 11.37$^b$                | 5.71$^b$                  | 5.52$^c$                 | 1.33$^c$                  |
| Puspita Pelangi | 13.00$^a$                | 6.13$^{ab}$               | 4.65$^d$                 | 1.65$^b$                  |
| Bakardi        | 13.13$^a$                | 6.42$^a$                  | 5.96$^{bc}$              | 0.50$^d$                  |
| Kusuma Swasti  | 10.20$^c$                | 6.21$^{ab}$               | 6.52$^{ab}$              | 1.71$^b$                  |
| Marimar        | 10.15$^c$                | 6.11$^{ab}$               | 6.87$^a$                 | 1.97$^a$                  |
| Yulimar        | 13.07$^a$                | 5.84$^{ab}$               | 6.13$^{bc}$              | 1.35$^c$                  |

*the value followed by the same letter not significantly different at $p<0.05$. 

Table 5. Flower color, flower type, flower shape of seven varieties of chrysanthemum tested at Garung, Wonosobo in August 2014 to January 2015.

| Varieties          | Flower Colour | Flower type | Flower shape   |
|--------------------|---------------|-------------|----------------|
| Puspita Nusantara  | Yellow        | Spray       | Single         |
| Shakuntala         | Yellow        | Standard    | Decorative     |
| Puspita Pelangi    | White         | Spray       | Semi decorative|
| Bakardi            | White         | Spray       | Single         |
| Kusuma Swasti      | Purple        | Standard    | Semi decorative|
| Marimar            | Yellow        | Standard    | Decorative     |
| Yulimar            | White         | Standard    | Decorative     |

4. Conclusion
The rate of disease progress and the level of disease intensity of rust by the plant age determined the resistance of the chrysanthemum varieties to rust. Based on the disease progression rate and the average of the disease intensity, it can be identified that chrysanthemum varieties of Kusumawasti, Marimar, and Yulimar were more resistant than the four other varieties tested at Garung, Wonosobo. The pattern of disease progress of rust in chrysanthemum can be used as guidance to control that disease.

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