The Intensity of Leaf Rust Disease, Growth and Yield of Chrysanthemum on Concentration and Time of Giving *Paenibacillus Polymixa* Biological Agent

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Abstract. This study aims to determine the intensity of leaf rust disease, growth and yield of chrysanthemum at the concentration and time of application of *Paenibacillus polymixa* biological agent (PPBA). The study used a randomized block design with a factorial pattern. The first factor, the concentration of PPBA with 4 levels (1%, 2%, 3%, 4%). The second factor, time of PPBA application with 3 levels (1, 2, 3 wap). The results showed that the concentration of PPBA and the interaction between concentration and time of PPBA application was not significant for all observed variables, but the time of PPBA application had a significant effect on the number of infected leaves and the intensity of the attack. The lowest intensity of attacks acquired in 1 wap 19.58% is still considered low category because previous PPBA applications will be able to suppress the pathogen of *Puccinia horiana* Hen and reduce the number of infected leaves on chrysanthemum. Applications 2 wap (32.68%) and 3 wap (32.94%) indicate that the intensity of the attack is caused by the delay of PPBA, microclimate, and high rainfall intensity. Therefore, the attack intensity is lower with faster PPBA application time and increases when application time of PPBA is slower.

1. Introduction

The high demand for ornamental plants to make business in the field of ornamental plants procurement is very promising big profits, one of the popular ornamental plants are chrysanthemum flowers. High market demand makes chrysanthemums have a bright prospect to develop now and in the future [1]. Chrysanthemum production in Bali has not been able to meet market needs due to low productivity and planting area. Farmers working on chrysanthemum in Bali are concentrated in only two districts in Buleleng and Tabanan. Pancasari Village, Buleleng Regency has a potential location for the development of chrysanthemum cultivation as a mainstay commodity [2]. Intensive cultivation due to high demand for chrysanthemum flowers, because chrysanthemum farmers are faced with the potential for widespread leaf disease if not controlled quickly and precisely.

Important diseases in chrysanthemums that are very harmful are rust disease. In Indonesia is known two types of rust disease, which is brown rust caused by *Puccinia chrysanthemum* and white rust caused by *Puccinia horiana* [3]. This disease is most important in chrysanthemum plants because this disease damages the leaves and decreases the quality of the flowers. Damage results due to rust disease are especially on vulnerable plants can reach 100% or harvest failure [4]. In addition to rust disease, which causes the low quality of chrysanthemum flowers according to [5] is a poor production process, the provision of planting materials (Cuttings) coming from outside Bali does not guarantee continuity and
quality so that the impact is less good on consumer confidence. The problems of chrysanthemum seedlings above can be overcome by using cuttings from parent plants or production plants from yellow or white varieties of Fiji.

Currently, pesticides play an important role in improving agricultural productivity. The threat of crop damage due to rust disease as well as reduced yields can be overcome by the use of pesticides, especially chemical pesticides, these efforts provide quick and effective results in controlling pests and diseases. On the other hand, the price of chemical pesticides in Indonesia is quite high, thus burdening the cost of agricultural production. In the count of farmers, the cost of pesticide components reaches 25-50% of the total cost of agricultural production. High chemical pesticide prices are due to active ingredients are still imported. Thus, gradually efforts should be made to reduce the use of chemical pesticides and begin to shift to the cheaper and safer types of pesticides for the environment. One alternative to environmentally friendly pesticides is paenibacillus polymixa.

Paenibacillus polymyxa is a biological agent that has antagonistic properties against the development of plant pathogens and also has induced plant resistance properties. These bacteria have been reported to be effective in decreasing the level of fungal attack of P. horiana on chrysanthemum plants. The recommended dose for PPBA solution is 400 liters per hectare. In Bali, no one has examined the concentration and timing of the appropriate PPBA application. Therefore, research is done to know the intensity of leaf rust disease, growth and yield of chrysanthemum plants at the concentration and time of PPBA application.

2. Materials and methods
The materials used in this research are PPBA, chrysanthemum seeds reagent white, chicken manure, and black silver plastic mulch. Tools used include scales, hoses, scissors, pushers, lights and enforcement nets. The research was conducted in Pancasari Village, Sukasada District, Buleleng Regency with the altitude of 1,247 meters above sea level and average temperature 17°C to 20°C. This study runs from July to October 2016.

This study used a randomized block design (RBD) with a factorial pattern consisting of two factors: concentration (C) paenibacillus polymixa biological agent (PPBA) and time of giving of PPBA (T). The first factor was the treatment of concentration (C) of paenibacillus polymixa biological agent (PPBA) consisting of 4 level: C1 = 1% PPBA, C2 = 2% PPBA, C3 = 3% PPBA, C4 = 4% PPBA. The second factor is the time of giving PPBA consisting of 3 level i.e. T1 = 1 week after planting (wap), T2 = 2 weeks after planting (wap), and T3 = 3 weeks after planting (wap). Each treatment was repeated 3 times so that there were 36 combined treatment experiments.

3. Results
The significance of the concentration of biological agents of paenibacillus polymixa (C) and the time of giving (T) and its interaction (CT) on the observed variables are presented in Table 1.

From Table 1 it can be seen that the interaction between concentrations with time of application PPBA (CT) was not significant (P≥0.05) to all observed variables. The treatments of the concentration of PPBA were not significant (P≥0.05) on all observed variables, while the treatment time had a very significant effect (P <0.01) on the number of affected leaves and the intensity of the attack, but not significant (P ≥0.05) to plant height, leaf number, economical fresh weight, and economical dry weight.

| Variable                  | Treatment | C  | T  | CT |
|---------------------------|-----------|----|----|----|
| 1. Plant height           | ns        | ns | ns | ns |
| 2. Number of leaves       | ns        | ns | ns | ns |
| 3. Number of leaves affected | ns       | ** | ns | ns |
| 4. Intensity of attack    | ns        | ** | ns | ns |
| 5. Economical fresh weight| ns        | ns | ns | ns |
| 6. Economical dry weight  | ns        | ns | ns | ns |
3.1. Plant height (cm)
The result of the statistical analysis was found that the treatment of concentration PPBA (C) and time of giving (T) and its interaction (CT) had no significant effect (P ≥ 0.05), to maximum plant height (Table 1). The average height of the plants in the concentration treatment and the time of giving was presented in Table 2.

The highest average height of the plants obtained by the concentration treatment of 4% PPBA (C4) of 114.89 cm was not significant with the treatment of 1% PPBA (C1), 2% PPBA (C2), and 3% PPBA (C3) respectively 114.43 cm, 113.57 cm, and 113.04 cm. The average of plant height was highest in treatment time (T) 3 wap (T3) that was 114.94 cm which was not significant with treatment 1 wap (T1) and 2 wap (T2) respectively 113.57 cm and 113.43 cm.

3.2. Number (strands)
The result of the statistical analysis was found that the treatment of concentration of PPBA (C) and time of giving (T) and its interaction (CT) had no significant effect (P ≥ 0.05) on the number of leaves (Table 1). The average number of leaves on the concentration and the time of giving were presented in Table 2.

The highest mean leaf number obtained at the 1% PPBA (C1) concentration treatment of 14.56 was not significantly different with the 2% PPBA (C2) 3% PPBA (C3), 4% PPBA (C4) each 14.46, 13.24, and 13.62 strands. The average number of leaves (strands) was obtained at the highest treatment time of giving (T), 1 wap (T1) i.e. 14.17 different strains not significant with treatment 2 wap (T2) and 3 wap (T3) respectively 14.10 and 13.64 strands.

| Treatment | Plant height (cm) | Leaves number (strands) |
|-----------|------------------|-------------------------|
| Concentration PPBA |                   |                         |
| 1% (C1)   | 114.43 a         | 14.56 a                 |
| 2% (C2)   | 113.57 a         | 14.46 a                 |
| 3% (C3)   | 113.04 a         | 13.24 a                 |
| 4% (C4)   | 114.89 a         | 13.62 a                 |
| LSD 5%    | -                | -                       |
| Time of giving |                  |                         |
| 1 wap (T1) | 113.57 a         | 14.17 a                 |
| 2 wap (T2) | 113.43 a         | 14.10 a                 |
| 3 wap (T3) | 114.94 a         | 13.64 a                 |
| LSD 5%    | -                | -                       |

The average value followed by the same letter in the same row, different not significant at 5% LSD test level.

3.3. Number of leaves affected
The result of the statistical analysis was found that the treatment of concentration of PPBA (C) and its interaction (CT) was not significant (P ≥ 0.05), while the effect of time of giving (T) had the significant effect (P<0.01). The average number of leaves infected with concentration treatment and time of giving was presented in Table 3.

The highest number of leaves affected by the highest was obtained in the treatment of 4% PPBA concentration of 4.28 strands, not significantly different with the treatment concentration of 1% PPBA (C1), 2% PPBA (C2), 3% PPBA (C3) respectively, i.e. 3.91, 3.70, and 3.68 strands. The average number of leaf stricken (strands) obtained highest at a treatment time of 3 wap (T3) that is 4.53 significantly
different with a treatment time of 1 wap (T1) that is 2.67 strands and not significantly different with the time of giving 2 wap (T2) that is 4.49 strands.

3.4. Intensity of attack
The result of the statistical analysis showed that the treatment of concentration of PPBA (C) and its interaction (CT) was not significant (P≥0.05), while the effect of time of giving (T) had the significant effect (P<0.01). The average intensity of the attack on the concentration and the time of giving was presented in Table 3. The highest average intensity of attack was obtained in the concentration of 4% PPBA (C4) of 32.19% was not significantly different with the treatment of 1% PPBA (C1), 2% PPBA (C2), and 3% PPBA (C3) respectively, i.e. 26.35%, 26.96%, 28.10%. The highest average of attack intensity was obtained at time 3 wap (T3) that is 32.94% significantly different with the treatment time of 1 wap (T1) that is 19.58% and different, not significant with 2 wap (T2) of 32.68%.

Table 3. Average number of leaves affected and intensity of the attack on concentration and time of giving PPBA.

| Treatment          | Number of leaves affected (strands) | Intensity of attack (%) |
|--------------------|-------------------------------------|-------------------------|
| Concentration PPBA |                                     |                         |
| 1% (C1)            | 3.91 a                              | 26.35 a                 |
| 2% (C2)            | 3.70 a                              | 26.96 a                 |
| 3% (C3)            | 3.68 a                              | 28.10 a                 |
| 4% (C4)            | 4.28 a                              | 32.19 a                 |
| LSD 5%             | -                                   | -                       |
| Time of giving     |                                     |                         |
| 1 wap (T1)         | 2.67 b                              | 19.58 b                 |
| 2 wap (T2)         | 4.49 a                              | 32.68 a                 |
| 3 wap (T3)         | 4.53 a                              | 32.94 a                 |
| LSD 5%             | 1.34                                | 9.69                    |

The average value followed by the same letter in the same row, different not significant at 5% LSD test level.

3.5. Economical fresh weight
The result of statistic analysis showed that the treatment of concentration PPBA (C) and time of giving (T) and its interaction (CT) had no significant effect (P ≥ 0.05), to economical fresh weight (Table 2). The average economic weight of the concentration treatment and delivery time is presented in Table 4. The highest average economic fresh weight obtained in the treatment of 2% PPBA (C2) concentration of 261.67 g is not significantly different with 1% PPBA (C1) and 3% PPBA (C3) with values of 245.56 g, 237.78 grams respectively but significantly different with 4% PPBA (C4) with a value of 213.89 g. The highest average economic weight (g) obtained at treatment time (T), 2 wap (T2) is 255.00 g was not significant with 1 wap (T1) i.e. 239.17 gram and 3 wap (T3) that is 225.00 g.
Table 4. Average economical fresh weight and economical dry weight on concentration and time of giving PPBA.

| Treatment | Economical fresh weight (g) | Economical dry weight (g) |
|-----------|----------------------------|---------------------------|
| **Concentration PPBA** |                           |                           |
| 1% (C1)   | 245.56 ab                   | 50.67 a                   |
| 2% (C2)   | 261.67 a                    | 51.36 a                   |
| 3% (C3)   | 237.78 ab                   | 48.56 a                   |
| 4% (C4)   | 213.89 b                    | 50.13 a                   |
| LSD 5%    | 64.69                       | ns                        |
| **Time of giving** |                           |                           |
| 1 wap (T1)| 239.17 a                    | 48.93 b                   |
| 2 wap (T2)| 255.00 a                    | 49.35 ab                  |
| 3 wap (T3)| 225.00 a                    | 52.25 a                   |
| LSD 5%    | ns                          | 3.95                      |

The average value followed by the same letter in the same row, different not significant at 5% LSD test level.

3.6. Economical dry weight
The result of the statistical analysis showed that the treatment of concentration of PPBA (C) and time of giving (T) and its interaction (CT) had no significant effect (P≥0.05), on the dry weight of economic yield (Table 2). The average dry weight of the economic yield on the concentration treatment and the time of giving is presented in Table 4.

The average dry weight of the economic yield obtained at the treatment of concentration (C) 2% PPBA (C2) of 51.36 g is not significantly different with the concentration of 1% (C1), concentration of 3% PPBA (C3) and 4% PPBA (C4) with values of 50.67 g, 48.56 g and 50.13 g, respectively. The mean dry weight of the economic result was obtained at the treatment time of PPBA 3 wap (T3) that was 52.25 g different was not significant with the effect of 2 wap (T2) 49.35 g and significantly different from the treatment of 1 wap (T1) is 48.93 g.

3.7. The number of infected leaves
The average number of leaves infected 1 wap until 8 wap in the treatment time of giving *paenibacillus polymixa* (T) is presented in Table 5. The average number of leaves attacked on observation of 1 wap highest obtained in the time of giving 3 wap (T3) that is 4.47 strands significantly different with a time of 1 wap (T1) that is 3.61 strands and not significantly different with a time of 2 wap (T2) that is 4.22 strands.

The average number of leaves attacked on observation of 2 wap highest obtained in the time of 3 wap (T3) is 4.63 significantly different with a time of 1 wap (T1) 3.80 strands that are different and not significant with a time of 2 wap (T2) that is 4.35 strands. The average number of leaves attacked on observation of 3 wap highest obtained in treatment time 3 wap (T3) is 4.92 different strands not significant with a time 2 wap (T2) that is 4.63 strands but different real with the time of giving 1 wap (T1) that is 4.13 strands. The average number of leaves infected at the highest 4 wap observation was obtained at a treatment time of 3 wap (T3) in 5.30 different strands not significant with a time 2 wap (T2) that are 4.93 strands but different real with the time of giving 1 wap (T1) is 4.46 strands. The average number of leaves attacked on observation of 5 wap highest was obtained at the time of 3 wap (T3) that is 5.72 different strands not significant with time 2 wap (T2) that is 5.37 strands but different real with a time of giving 1 wap (T1) that is 4.84 strands. The average number of leaves infected at the highest 6 wap observation was obtained at treatment time of 3 wap (T3) that was 4.94 strands significantly different with time of 1 wap (T1) that was 4.35 strands and different, not real with the time of giving 2 wap (T2) that is 4.92 strands.
The average number of leaves infected at the highest 7 wap observation was obtained at a treatment time of 3 wap (T3) that was 4.72 strands was significantly different with treatment time of 1 wap (T1) is 3.98 strands and different not real with the time of 2 wap (T2) that is 4.61 strands. The average number of leaves infected at the highest 8 wap observation was obtained at treatment time 3 wap (T3) that is 4.53 significantly different with the treatment time of 1 wap (T1) that is 2.67 piece and different not real with time giving 2 wap (T2) that is 4.49 strands.

Table 5. The average number of leaves affected from observation week 1 to week 8 on time treatment of PPBA.

| Observation week to | 1 wap (T1) | 2 wap (T2) | 3 wap (T3) |
|---------------------|------------|------------|------------|
| 1                   | 3.61 b     | 4.22 a     | 4.47 a     |
| 2                   | 3.80 b     | 4.35 a     | 4.63 a     |
| 3                   | 4.13 b     | 4.63 ab    | 4.92 a     |
| 4                   | 4.46 b     | 4.93 ab    | 5.30 a     |
| 5                   | 4.84 b     | 5.37 ab    | 5.72 a     |
| 6                   | 4.35 b     | 4.92 a     | 4.94 a     |
| 7                   | 3.98 b     | 4.61 a     | 4.72 a     |
| 8                   | 2.67 b     | 4.49 a     | 4.53 a     |

The average value followed by the same letter in the same row, different not significant at 5% LSD test level.

4. Discussion

The influence concentration of PPBA and its interaction was not significant to all observed variables (Table 1). This is because the application of biological agents after application is highly dependent on the ecosystem, at the time of giving if the conditions support the growth of biological agents will be maximal [6]. This is also supported by the opinion of [4] saying that a humid, rainy environment will affect the response of biological agents.

The effect of time of giving of PPBA had a very real effect on the variables of attacked leaf count and intensity of the attack. From the result of the analysis, the number of leaves attacked got that the treatment time of giving of PPBA 1 wap give the lowest number of stricken leaves that is 2.67 strands compared with treatment 2 wap that is 4.49 strands and increased at the time of giving 3 wap 4.53 strands (Figure 1). This is due to the time of early PPBA giving is able to suppress the development of pathogen *Puccinia horiana Henn* and this is supported by the opinion [3] that *Puccinia horiana Henn* rust disease has been brought from the planting material (seeds) that have spread to the location. The results of observation at the research site showed that *Puccinia horiana Henn* disease appeared on 7 wap, the time of PPBA 1 wap was 3.61 strands of PPBA 2 wap time was 4.22 strands and the time of giving biologically 3 wap i.e. 4.47 strands increased the number of leaves affected by observation 5 wap then decreased at observation 6 wap until 8 wap (Table 5). Plant resistance to disease will change at different age levels [7]. Chrysanthemum plants are infected by the pathogen *Puccinia horiana Henn* by showing the symptoms of white patches on the bottom or top surface of the leaves which will then be followed by the formation of rust pustules on the lower surface of the leaf.
Figure 1. Relationship PPBA concentration and time of giving to the number of leaves affected.

The lowest intensity of attacks acquired during 1 wap treatment, i.e. 19.58%, is still considered by the application of biologic agents of *paenibacillus polymixa* previously capable of suppressing the pathogen of Puccinia horiana Hen and being able to reduce the number of affected leaves. On chrysanthemum, 2 wap i.e. 32.68% and 3 wap time 32.94% indicates that the intensity of moderate attack (Figure 2) is the biological agent of *paenibacillus polymixa* and supported by the microclimate that occurs during the study, the intensity of high rainfall is very supportive of growth rust disease *Puccinia horiana* Henn. The extent of crop damage in the field is caused by environmental conditions [8], i.e. with high humidity (96-100%) and low temperatures. The results of the attack intensity analysis found that the care time of 1 wap with the lowest attack intensity value of 19.58%. Based on the results of time-intensity analysis of PPBA provision, and increased when the time of giving of PPBA is slower. Biological control is a powerful way to reduce damage caused by plant pathogens [9, 10]. Identifying, understanding and utilizing microbial products to control plant diseases and increasing crop production is an integral part of sustainable agriculture [11], so the application of PPBA has the potential to control plant diseases without causing adverse environmental impacts.

Figure 2. Relationship PPBA concentration and time of giving to the number of the intensity of attacks.

5. Conclusion
The interaction between concentration and time of application of PPBA (*paenibacillus polymixa* biological agent) did not have a significant effect on all observed variables. Similarly, the treatment of PPBA concentration has no significant effect on all observed variables. However, the treatment time of
application has a very significant effect on the number of affected leaves and the intensity of the attack. Treatment from a time of giving of PPBA at 1 week after planting gave the lowest number of leaves attacked 2.67 strands compared with treatment 2 weeks after planting that is 4.49 strands and increased at the time of giving 3 weeks after planting that is 4.53 strands. The most appropriate time of giving is at the time of giving 1 week after planting with the lowest attack intensity value of 19.58%.

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References
[1] Balai Penelitian Tanaman Hias 2000 Deskripsi Klaster Klones Unggul Krisan Tipe Spray dan Standar pp. 30.
[2] Arjana I G M, Situmeang Y P and Suaria I N 2015 International Journal on Advanced Science, Engineering and Information Technology 5 (5) 350-354.
[3] Suhardi 2009 J. Hort. 19 (2) 207-209.
[4] Hanudin, W Nuryani, E Silvia, I Djaminka and B Marwoto 2010 J. Horti. 20 (3) 247-261.
[5] Arjana I G M, Situmeang Y P, Suaria I N and Mudra N K S 2015 International Journal on Advanced Science, Engineering, and Information Technology 5 (6) 407-409.
[6] FAO 1998 Guidelines for the Registration of Biological Pest Control Agents (Food and Agriculture Organization of the United Nations, Rome. 7 pp).
[7] Agrios G 2005 Plant Pathology 5th Edition (Elsevier Academic Press, Amsterdam, 26-27, 398-401).
[8] Haryono S 1996 Pengantar Ilmu Penyakit Tumbuhan (Gadjah University Press, Yogyakarta).
[9] Jeyarajan R and Nakkeeran S 2000 In: R. Upadhyay et al. (eds.). “Biocontrol Potential and Its Exploitation in Sustainable Agriculture,” Crop Dis. Weeds and Nemat. 1 95-116.
[10] Haggag W M 2002 Arab Journal of Biotechnology 5 (2) 151-164.
[11] Haggag W M and Mohamed H A A 2007 American-Eurasian Journal of Sustainable Agriculture 1 (1) 7-12.