

Identification of Mixed Infection on Native Orchids in Liwa Botanical Garden

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Abstract. Liwa Botanical Garden is located in Balik Bukit Subdistrict, West Lampung Regency, is a mountainous area surrounded by hills with a slope of 10% - 40%. This botanical garden has one of its superior collections namely native orchids. Based on the results of previous studies, it is known that several native orchid individuals at Liwa Botanical Garden show symptoms of being infected with a mixture of viruses, bacteria and fungi. Mixed infections will show more severe symptoms than single infections. This study was conducted to determine the identification of diseases and efforts to protect native orchids against mixed infections at Liwa Botanical Garden through a collection of samples that showed symptoms of infection, analysis of disease symptoms, and analysis of disease resistance levels. The results showed the response of native orchids at Liwa Botanical Garden to mixed infections showed symptoms in the form of necrosis, streak, chlorotic, soft rot, and fungal spots on the orchid *Ascideria longifolia*, *Polisthacia* sp., *Bulbophyllum* sp., *Calanthe triplicata*, and *Eria* sp. The type of orchid that shows the most symptoms is *Bulbophyllum* sp. a total of 8 samples. The type of native orchid that is most susceptible to mixed infections is *Eria* sp. with a disease intensity of 32.7%. The results of this activity are expected to be basic information in efforts to protect plants against diseases to support the application of conservation of native orchids at Liwa Botanical Garden.

Keyword: identification of diseases, mixed infections, orchid viruses, orchid fungi, orchid bacteria, liwa botanical garden

1. Introduction

Liwa Botanical Garden located in Balik Bukit Subdistrict, West Lampung Regency, is a mountainous area surrounded by hills with a slope of 10% - 40%. This botanical garden has an Indonesian ornamental plant concept with its superior collection of native orchids. Until November 2013, total number of native orchid collections was 805 specimens which were included in 425 collection

numbers (60 genera, identified to species level of 214 collection numbers). Most collections are *Dendrobium*, *Eria* and *Bulbophyllum* with 48, 40 and 38 numbers, respectively [1;11]. Mahfut et al. (2019) reported several natural orchid individuals at Liwa Botanical Garden showing symptoms of being infected with a mixture of viruses, bacteria and fungi. Mixed infections will show more severe symptoms than single infections. Each infection of this disease can inhibit plant growth and resilience, and decrease the aesthetic value of orchids periodically [3;4;5;6;7;8;9;10].

To facilitate disease control, an inventory of data is first carried out as preliminary information about the infection. Recognition of symptoms as an infection response is the first data in disease identification. The data is used to determine disease intensity, plant resistance, and disease control efforts. Research on the identification of diseases and efforts to protect natural orchids against mixed infections at Liwa Botanical Garden has never been done. The results of this study are expected to be fundamental data in efforts to protect orchids to support the application of conservation of natural orchids at Liwa Botanical Garden. In addition, the results of this activity serve as a medium of good communication between researchers and managers of Liwa Botanical Garden in their disease control efforts.

2. Materials and Methods

2.1. Sample collection

Sample collection was carried out on orchid leaves in Liwa Botanical Garden which showed symptoms of being infected with a virus. All samples were tabulated and documented with photos.

2.2. Analysis of Disease Symptoms

This analysis is done by matching the sample documentation with the literature that has been previously reported. The literature used is [3;4;5;6;7;8;9].

2.3. Disease Intensity Analysis

This stage is done to determine the severity of the disease (disease severity). Disease intensity is the proportion of infected hosts to the total observed surface area of the host. Calculation of disease intensity analysis is done using the method and the scale of disease intensity refers to [7].

2.4. Disease Intensity Analysis

Determination of the level of resistance of orchids to disease follows the method of [7].

3. Results and Discussion

3.1. Sample collection

The sample collection was conducted from January 2nd week to February 2nd week 2020. Samples were randomly selected from orchid individuals who showed virus infection. Based on the results of the collection obtained 24 samples from 5 types of orchids namely *Ascideria longifolia*, *Polisthacia* sp., *Bulbophyllum* sp., *Calanthe tripicata*, and *Eria* sp. The type of orchid that has the most infection is *Bulbophyllum* sp. as many as 8 samples, then *Calanthe tripicata* orchids as many as 5 samples. Overall data collection results are shown in **Table 1**.

Table 1. Collection of orchid leaf samples at Liwa Botanical Garden which shows a mixed

infection.

Species of Orchid	Number of Samples	Symptoms of Infection
<i>Ascideria longifolia</i>	3	All parts of the leaf wither and turn yellow, fungal spots, and chlorotic
<i>Polisthacia</i> sp.	1	Dry leaves and necrosis
<i>Bulbophyllum</i> sp.	8	Soft rot, chlorotic, and necrosis
<i>Calanthe tripicata</i>	5	Necrosis, streak, fungus spots
<i>Eria</i> sp.	4	Soft rot, chlorotic, fungal spots

The selection of sampling locations is based on previous research by [7] who reported mixed infection in the collection of natural orchids at Liwa Botanical Garden in August and December 2019. The types of infected orchids were *Calanthe* sp. and *Flicking eria* with a mixture of viruses, fungi and bacteria. Other studies have also reported mixed infection on *Cymbidium* sp. with symptoms of full necrosis, streak, and fungal spots (back), in the *Corynboris* sp. with symptoms of necrosis, mosaic, fungal spots, and on orchid *Flicking eria*. with symptoms of large necrosis and mosaics at Liwa Botanical Garden.

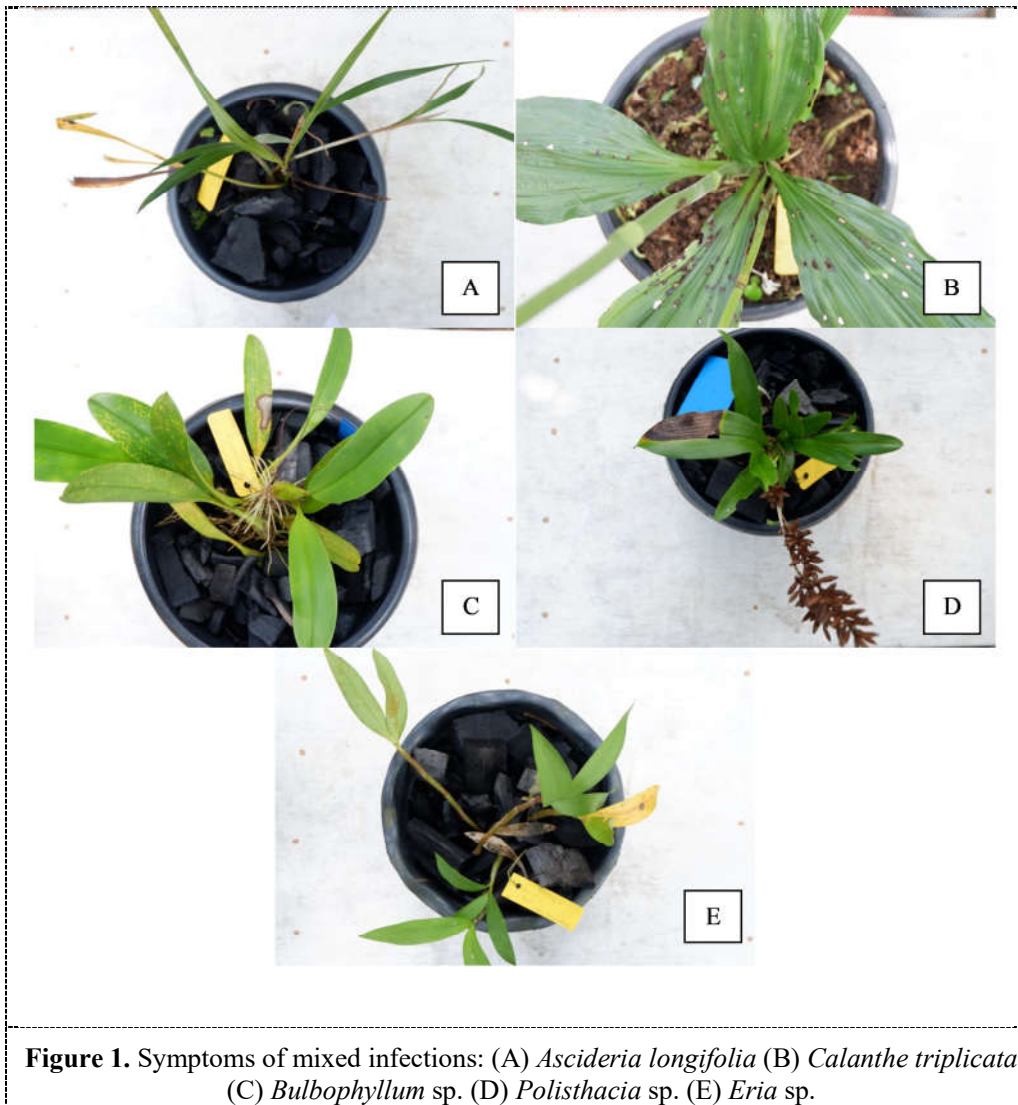
In this study, *Bulbophyllum* sp. being the most mixed type of orchid. This shows that this type of orchid is the most vulnerable host infected with pathogens. Besides that are *Ascideria longifolia*, *Polisthacia* sp., *Calanthe tripicata*, and *Eria* sp. [7] also reported that on *Bulbophyllum* sp. showed a fairly severe mixed infection at Liwa Botanical Garden.

[3] were reported viral infections with *Phalaenopsis amboinensis* (KRB2) and *Phalaenopsis amabilis* (KRB12) from Bogor Botanical Gardens, *Phalaenopsis amabilis* (KRP18) and *Dendrobium salacence* (KRP20) from the Purwodadi Botanical Gardens, and *Phalaenopsis modesta* J. Sm. (KRBp5) from Balikpapan Botanical Garden. Previous study also reported the presence of CymMV infection on *Phalaenopsis* sp. and *Dendrobium* sp. in Java and Bali, there are symptoms of necrosis.

Other studies reported bacterial infections with symptoms of soft rot on *Onchidium* sp. and *Paphiopedilum* sp. in Bogor, West Java *Grammatophyllum*, *Dendrobium*, and *Catleya* sp. in D. I. Yogyakarta [2]. Research on fungal infections with symptoms of wilting was also reported to infect 3 types of *Dendrobium stratiotes*, *Dendrobium moschatum*, *Dendrobium concinnum*, and *Pteroceras unguiculatum* at Bogor Botanical Gardens (Anita, 2017); spot on *Phalaenopsis* sp. in Lembang, West Bandung.

3.2. Analysis of Disease Symptoms

Based on the analysis of disease symptoms, viral infections in orchid samples of *Ascideria longifolia*, *Polisthacia* sp., *Bulbophyllum* sp., *Calanthe tripicata*, and *Eria* sp. showing symptoms of mixed infections namely necrosis, streak, chlorotic, soft rot, and fungal spots. Overall samples showing symptoms of mixed infection are presented on **Fig 1**.



On *Ascideria longifolia* sample, a mixed infection causes the entire leaf to wilt and turn yellow, fungal and chlorotic spots. In the *Polisthacia* sp. Sample, the symptoms caused by a mixed infection were dry leaves and necrosis. Symptoms on *Bulbophyllum* sp. were soft rot, chlorotic and necrosis. On *Calanthe triplicata* were necrosis, streak and fungal spots, whereas on *Eria* sp. were soft rot, chlorotic and fungal spots. A collection of samples of mixed disease symptoms that infect native orchids at Liwa Botanical Garden is presented on **Fig. 2**.

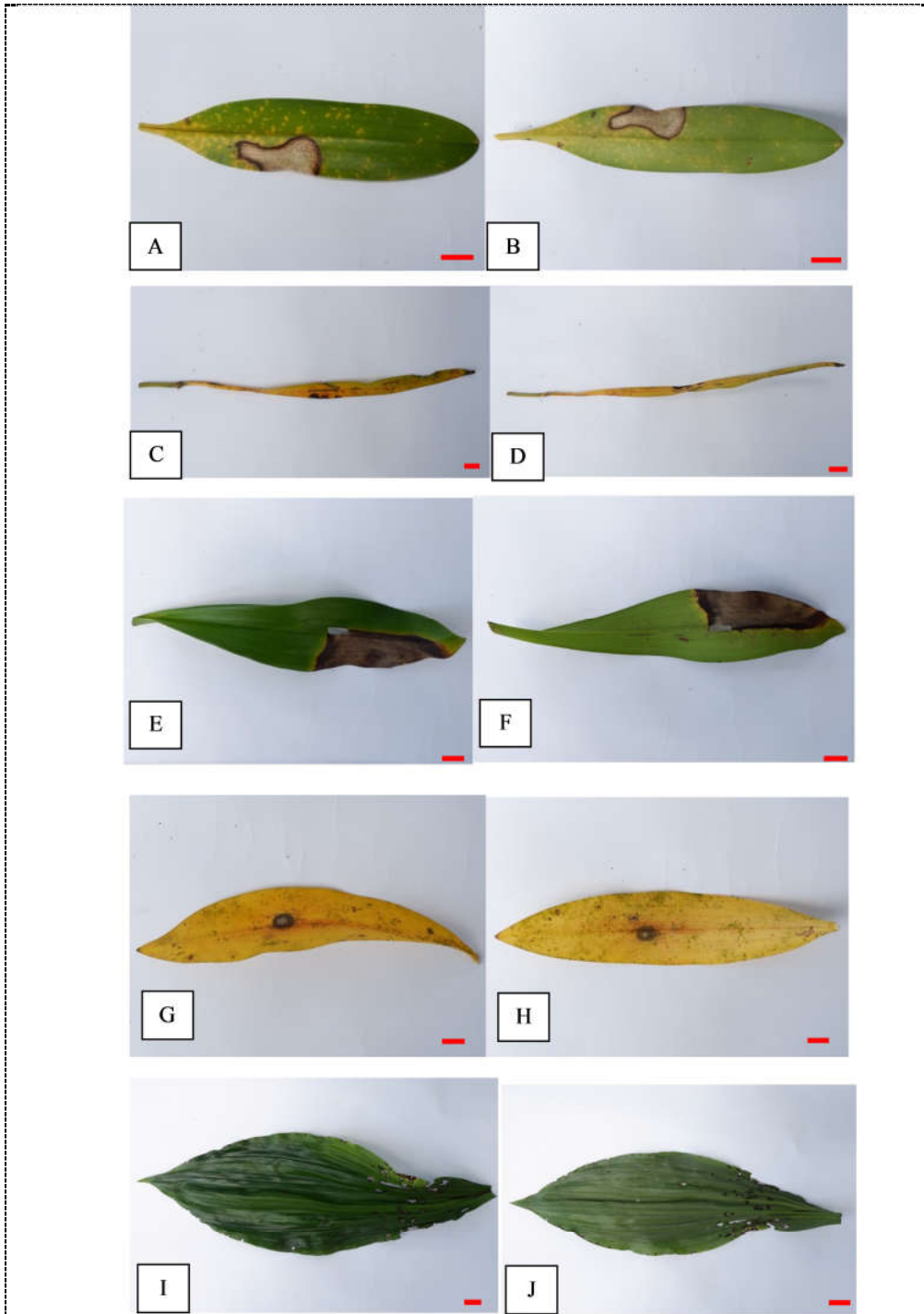


Figure 2. Collection samples of mixed infection symptoms in native orchids at Liwa Botanical Garden:

(A, B): Soft rot, chlorotic, and necrotic symptoms on *Bulbophyllum* sp.

(C, D): Symptoms of fungus, chlorotic spots and all parts of the leaves wither and change color turn yellow on *Ascideria longifolia*.

(E, F): Symptoms of necrosis and leaf drying on *Polisthacia* sp.

(G, H): Symptoms of soft rot, chlorotics, and mold spots on *Eria* sp.

(I, J): Symptoms of necrosis, streak, and mold spots on *Calanthe triplicate*.

(A, C, E, G, I): Top surface of the leaf, (B, D, F, H, J): Surface under the leaf. Bar: 1 cm

Necrotic is characterized by physical damage or death to cells or tissues. Some of the symptoms that include necrotic types are necrose, rot, die back, and cancer (dead bark dries with a clear border) [3;4;5;6;9]. Chlorotic symptoms are symptoms caused by damage to chloroplasts which results in parts of the plant which are normally green to yellow. Chlorotic symptoms are often associated with necrotic where chlorotic surrounds necrotic called "hello". Whereas curling leaf is a leaf symptom that shows changes in the shape of the edges in the form of curling [9].

3.3. Disease Intensity

Disease intensity analysis results obtained as a percentage as showed on **Table 2**.

Table 2. Analysis of mixed diseases in natural orchids on Liwa Botanical Garden.

No.	Species of Orchid	Disease Intensity
1	<i>Ascideria longifolia</i>	22,5%
2	<i>Polisthacia</i> sp.	8,57%
3	<i>Bulbophyllum</i> sp.	20%
4	<i>Calanthe tripicata</i>	16,6%
5	<i>Eria</i> sp.	32,7%

Eria sp. has the highest disease intensity. This is caused by several factors, including: the percentage of disease events is quite high at 90%, and as many as 4 samples from a total of 11 leaves show mixed infection symptoms. Three samples of *Ascideria longifolia* showed mixed infection symptoms and the percentage of disease was 90%, but the intensity of the disease in this orchid was smaller when compared with *Eria* sp. The lower intensity of disease on *Ascideria longifolia* is caused by the total number of leaves in one plant that is 12 leaves, so that the ratio of the number of diseased and healthy leaves in this orchid is lower when compared to *Eria* sp. The lower the total ratio of the number of diseased and healthy leaves and the lower percentage of disease events will cause the results of the analysis of disease intensity to be lower [9]. Then the percentage of disease intensity of 20% occurred in *Bulbophyllum* sp., 16.6% in *Calanthe tripicata*, and the lowest percentage was 8.57% on *Polisthacia* sp.

3.4. Plant Resistance

The results of the analysis of the level of resistance to mixed infection on native orchids in Liwa Botanical Garden are showed on **Table 3**.

Table 3. Analysis of the level of plant resistance to mixed infections on Liwa Botanical Garden.

No.	Species of Orcid	Plant Resistance Level
1	<i>Ascideria longifolia</i>	Resistance
2	<i>Polisthacia</i> sp.	Resistance

3	<i>Bulbophyllum</i> sp.	Resistance
4	<i>Calanthe triplicata</i>	Resistance
5	<i>Eria</i> sp.	Resistance

Overall the sample has a level of resistance rather resistant to mixed infections because it has a disease incidence of less than 40%. [6;9] explain that the level of resistance is somewhat resistant is the level of resistance in orchids infected with the disease but the incidence of the disease is not more than 40%. This shows that the overall collection of natural orchid samples at Liwa Botanical Garden is capable of being infected by pathogens but a small portion of the cells supports the growth and development of pathogens so that they cause less disease.

4. Acknowledgments

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5. References

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