

1                   **Identification of Disease and Efforts to Protect Natural Orchid Plants Against**  
2                   **Mixed Infections in the Liwa Botanical Garden**

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10                   **ABSTRACT**

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12                   Liwa Botanical Garden is located in Balik Bukit Subdistrict, West Lampung Regency, is a mountainous area surrounded by hills with a  
13                   slope of 10% - 40%. This botanical garden has one of its superior collections namely native orchids. Based on the results of previous  
14                   studies, it is known that several native orchid individuals at Liwa Botanical Garden show symptoms of being infected with a mixture of  
15                   viruses, bacteria and fungi. Mixed infections will show more severe symptoms than single infections. This study was conducted to  
16                   determine the identification of diseases and efforts to protect natural orchids against mixed infections at Liwa Botanical Garden through  
17                   a collection of samples that showed symptoms of infection, analysis of disease symptoms, and analysis of disease resistance levels. The  
18                   results showed the response of natural orchids at Liwa Botanical Garden to mixed infections showed symptoms in the form of necrosis,  
19                   streak, chlorotic, soft rot, and fungal spots on the orchid *Ascideria longifolia*, *Polisthacia* sp., *Bulbophyllum* sp., *Calanthe tripicata*, and  
20                   *Eria* sp. The type of orchid that shows the most symptoms is *Bulbophyllum* sp. a total of 8 samples. The type of natural orchid that is  
21                   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  
22                   information in efforts to protect plants against diseases to support the application of conservation of native orchids at Liwa Botanical  
23                   Garden.

24                   **Key words:** identification of diseases, mixed infections, orchid viruses, orchid fungi, orchid bacteria, liwa botanical  
25                   garden

26                   **INTRODUCTION**

27                   Liwa Botanical Garden located in Balik Bukit Subdistrict, West Lampung Regency, is a mountainous area  
28                   surrounded by hills with a slope of 10% - 40%. This botanical garden has an Indonesian ornamental plant  
29                   concept with its superior collection of native orchids. Until November 2013, the total number of natural orchid  
30                   collections was 805 specimens which were included in 425 collection numbers (60 genera, identified to species  
31                   level of 214 collection numbers). Most collections are *Dendrobium*, *Eria* and *Bulbophyllum* with 48, 40 and 38  
32                   numbers, respectively (Saniyatun, 2015). Mahfut et al. (2019) reported several natural orchid individuals at  
33                   Liwa Botanical Garden showing symptoms of being infected with a mixture of viruses, bacteria and fungi.  
34                   Mixed infections will show more severe symptoms than single infections. Each infection of this disease can  
35                   inhibit plant growth and resilience, and decrease the aesthetic value of orchids periodically Mahfut (2019).

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

## MATERIALS AND METHODS

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

47  
48 **Analysis of Disease Symptoms.** This analysis is done by matching the sample documentation with the  
49 literature that has been previously reported. The literature used is Muharam et al., 2013; Mahfut and Daryono,  
50 2014; Mahfut et al., 2017; Fery et al., 2018.

51  
52 **Disease Intensity Analysis.** This stage is done to determine the severity of the disease (disease severity).  
53 Disease intensity is the proportion of infected hosts to the total observed surface area of the host. Calculation of  
54 disease intensity analysis is done using the method of Rahardjo and Suhardi (2008) and the scale of disease  
55 intensity refers to Mahfut et al. (2019).

56  
57 **Plant Resistance Level.** Determination of the level of resistance of orchids to disease follows the method of  
58 Mahfut et al. (2019).

## RESULTS AND DISCUSSION

### 60 Sample Collection

61 Sample collection stage is conducted in the second week of January to the second week of February 2020.  
62 Samples are randomly selected from individuals who show mixed symptoms of infection at Liwa Botanical  
63 Garden green house. Based on the results of the collection obtained 24 samples from 5 types of orchids namely  
64 *Ascideria longifolia*, *Polisthacia* sp., *Bulbophyllum* sp., *Calanthe tripicata*, and *Eria* sp. The type of orchid that  
65 has the most infection is *Bulbophyllum* sp. as many as 8 samples, then *Calanthe tripicata* orchids as many as 5  
66 samples. Overall data collection results are shown in **Table 1**.

67  
68 **Table 1.** Collection of orchid leaf samples at Liwa Botanical Garden which shows a mixed infection

No	Species of Orchid	Number of Samples	Symptoms of Infection
1	<i>Ascideria longifolia</i>	3	All parts of the leaf wither and turn yellow, fungal spots, and chlorotic
2	<i>Polisthacia</i> sp.	1	Dry leaves and necrosis
3	<i>Bulbophyllum</i> sp.	8	Soft rot, chlorotic, and necrosis
4	<i>Calanthe tripicata</i>	5	Necrosis, streak, fungus spots
5	<i>Eria</i> sp.	4	Soft rot, chlorotic, fungal spots

70 Selection of sampling locations is based on previous research (Mahfut et al., 2019), who reported mixed  
71 disease infections in the collection of native orchids at Liwa Botanical Garden in August and December 2019.  
72 The type of orchid that was mixed was *Calanthe* sp. and *Flicking eria* with a mixture of viruses, fungi and  
73 bacteria. The study (Mahfut et al., 2019) also reported a mixed infection on *Cymbidium* sp. with symptoms of  
74 full necrosis, streak, and fungal spots (back), in the *Corynborsis* sp. with symptoms of necrosis, mosaic, fungal  
75 spots, and on orchid *Flicking eria*. with symptoms of large necrosis and mosaics at Liwa Botanical Garden.

76 In this study, *Bulbophyllum* sp. being the most mixed type of orchid. This shows that this type of orchid is  
77 the most vulnerable host infected with pathogens. Besides that are *Ascideria longifolia*, *Polisthacia* sp.,  
78 *Calanthe tripicata*, and *Eria* sp. Mahfut et al., (2019) also reported that on *Bulbophyllum* sp. showed a fairly  
79 severe mixed infection at Liwa Botanical Garden.

80 Other studies have also reported viral infections with *Phalaenopsis amboinensis* (KRB2) and *Phalaenopsis*  
81 *amabilis* (KRB12) from Bogor Botanical Gardens, *Phalaenopsis amabilis* (KRP18) and *Dendrobium salacence*  
82 (KRP20) from the Purwodadi Botanical Gardens, and *Phalaenopsis modesta* J. Sm. (KRBp5) from Balikpapan  
83 Botanical Gardens (Mahfut et al., 2017). Muharam et al. (2013) reported the presence of CymMV infection on  
84 *Phalaenopsis* sp. and *Dendrobium* sp. in Java and Bali, there are symptoms of necrosis.

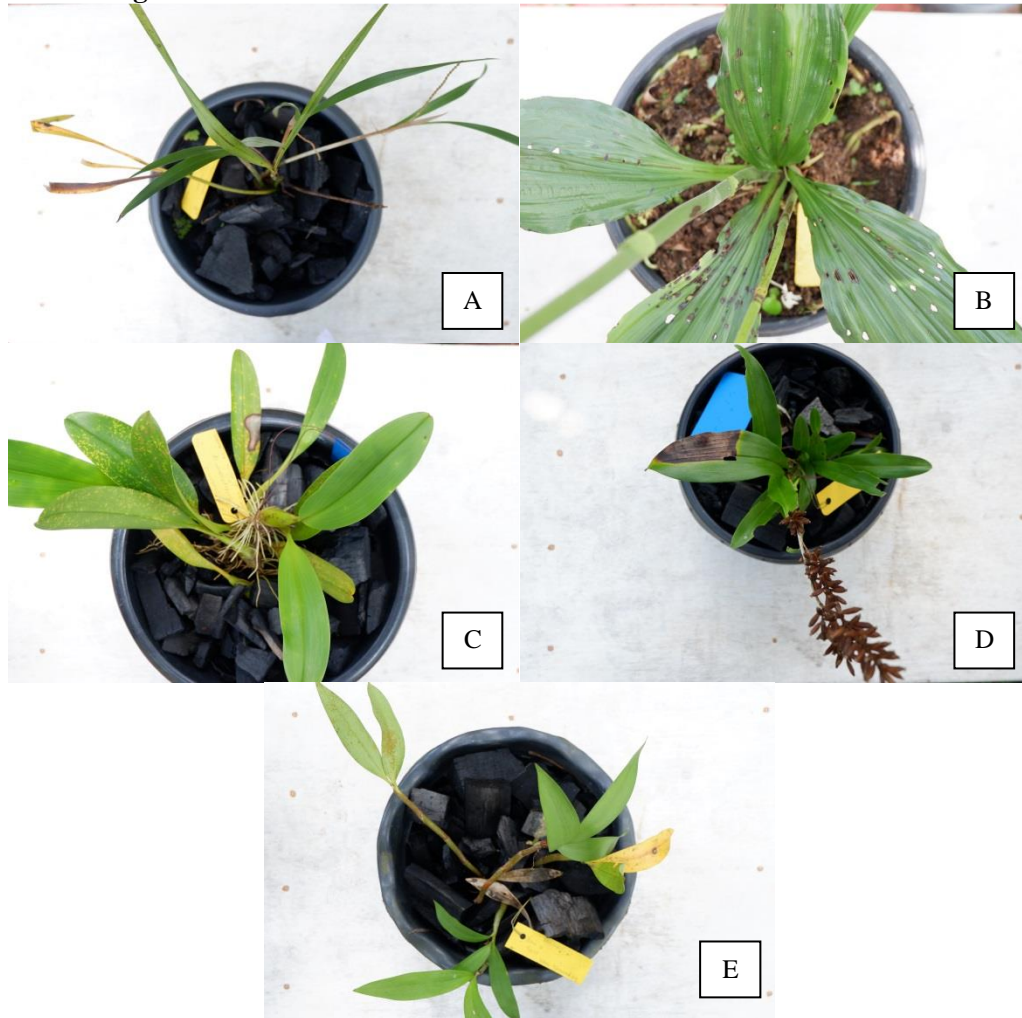
85 Other studies have also reported bacterial infections with symptoms of soft rot on *Onchidium* sp. and  
86 *Paphiopedilum* sp. in Bogor, West Java (Hanudin et al., 2011), *Grammatophyllum*, *Dendrobium*, and *Catleya*

87 sp. in D. I. Yogyakarta (Joko et al., 2010). Research on fungal infections with symptoms of wilting was also  
88 reported to infect 3 types of *Dendrobium stratiotes*, *Dendrobium moschatum*, *Dendrobium concinnum*, and  
89 *Pteroceras unguiculatum* at Bogor Botanical Gardens (Anita, 2017); spot on *Phalaenopsis* sp. in Lembang,  
90 West Bandung (Devi, 2018).

91

### 92 Analysis of Disease Symptoms

93 Based on the analysis of mixed infectious diseases in orchid samples of *Ascideria longifolia*, *Polisthacia* sp.,  
94 *Bulbophyllum* sp., *Calanthe triplicata*, and *Eria* sp. Overall samples showing symptoms of mixed infections  
95 namely necrosis, streak, chlorotic, soft rot, and fungal spots that infect native orchids at Liwa Botanical Garden  
96 are presented on **Fig 1**.

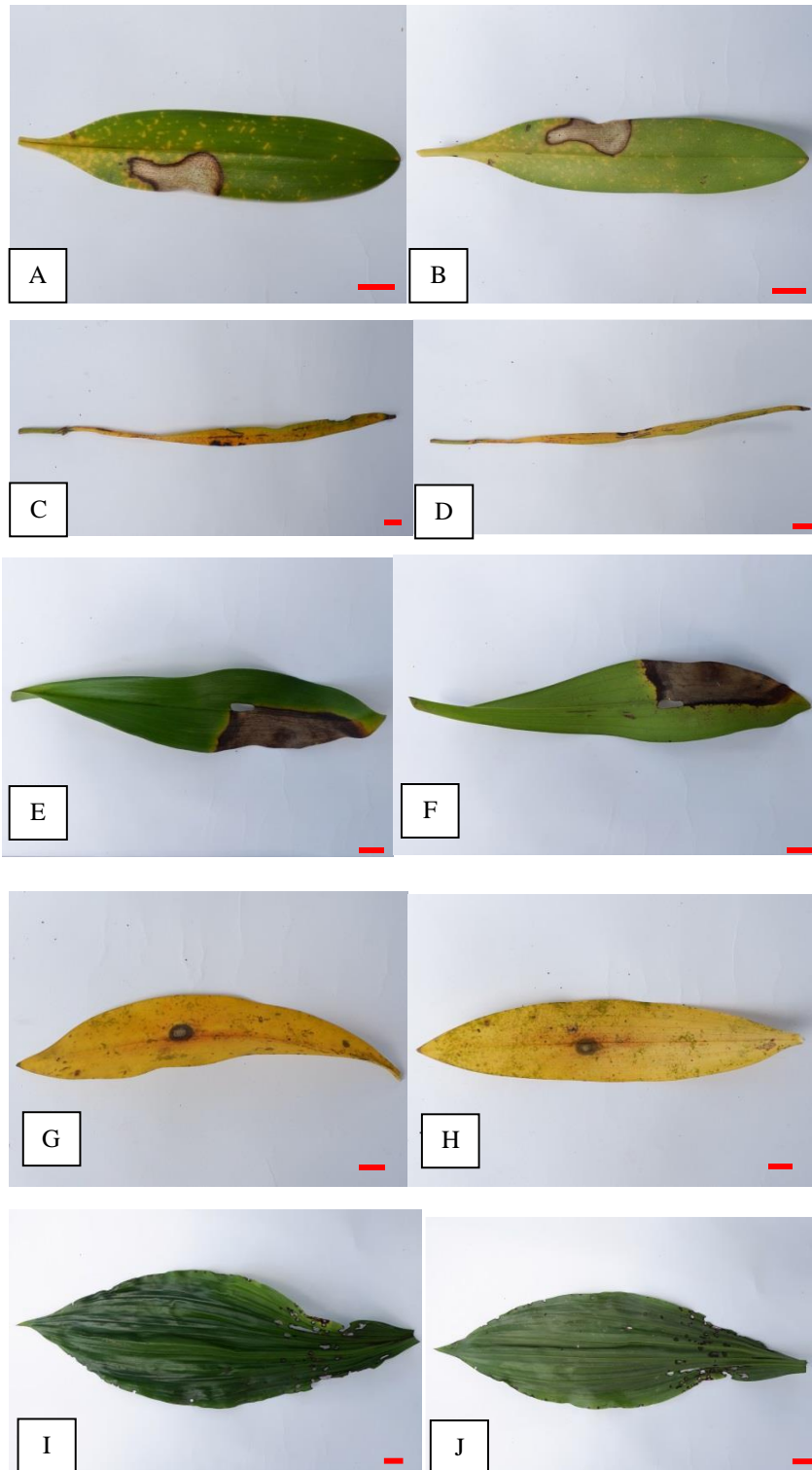


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98 **Figure 1.** Symptoms of mixed infections with natural orchids at Liwa Botanical Garden: (A) *Ascideria*  
99 *longifolia* (B) *Calanthe triplicata* (C) *Bulbophyllum* sp. (D) *Polisthacia* sp. (E) *Eria* sp.

100

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



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**Figure 2.** Collection of 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

117 Necrotic is characterized by physical damage or death to cells or tissues. Some of the symptoms that include  
 118 necrotic types are necrose, rot, die back, and cancer (dead bark dries with a clear border) (Purnomo, 2006).  
 119 Chlorotic symptoms are symptoms caused by damage to chloroplasts which results in parts of the plant which  
 120 are normally green to yellow. Chlorotic symptoms are often associated with necrotic where chlorotic surrounds  
 121 necrotic called "hello". Whereas curling leaf is a leaf symptom that shows changes in the shape of the edges in  
 122 the form of curling (Mahfut and Daryono, 2014).

123

### 124 **Disease Intensity**

125 The results of disease intensity analysis of mixed infection in native orchids in the Liwa Botanical Garden,  
 126 obtained the percentage of intensity as showed on **Table 2**.

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128 **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%

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130 *Eria* sp. has the highest disease intensity. This is caused by several factors, including: the percentage of  
 131 disease events is quite high at 90%, and as many as 4 samples from a total of 11 leaves show mixed infection  
 132 symptoms. Three samples of *Ascideria longifolia* showed mixed infection symptoms and the percentage of  
 133 disease was 90%, but the intensity of the disease in this orchid was smaller when compared with *Eria* sp. The  
 134 lower intensity of disease on *Ascideria longifolia* is caused by the total number of leaves in one plant that is 12  
 135 leaves, so that the ratio of the number of diseased and healthy leaves in this orchid is lower when compared to  
 136 *Eria* sp. The lower the total ratio of the number of diseased and healthy leaves and the lower percentage of  
 137 disease events will cause the results of the analysis of disease intensity to be lower (Rahardjo and Suhardi,  
 138 2008). Then the percentage of disease intensity of 20% occurred in *Bulbophyllum* sp., 16.6% in *Calanthe*  
 139 *tripicata*, and the lowest percentage was 8.57% on *Polisthacia* sp.

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### 141 **Plant Resistance**

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

144

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

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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 tripicata</i>	Resistance
5	<i>Eria</i> sp.	Resistance

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148 Overall the sample has a level of resistance rather resistant to mixed infections because it has a disease  
 149 incidence of less than 40%. Mahfut et al. (2019) explain that the level of resistance is somewhat resistant is the  
 150 level of resistance in orchids infected with the disease but the incidence of the disease is not more than 40%.  
 151 This shows that the overall collection of natural orchid samples at Liwa Botanical Garden is capable of being  
 152 infected by pathogens but a small portion of the cells supports the growth and development of pathogens so that  
 153 they cause less disease.

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