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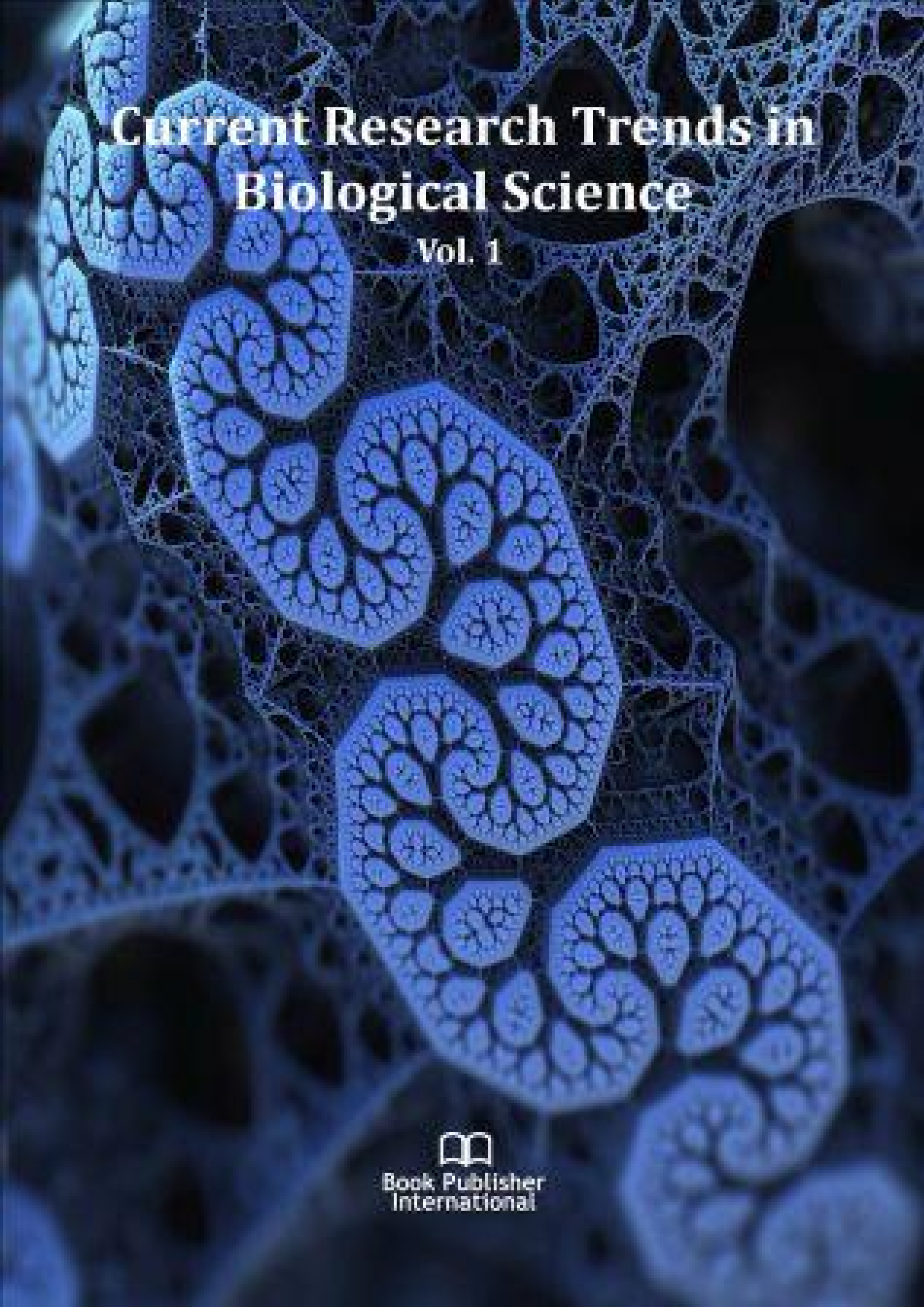

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Contents

Preface

This book covers all areas of biological research. The contributions by the authors include Maloideae; pathogenic fungi; polyphenols; fruits; rhizosphere; carboxylate; PGPR; Escherichia fergusonii; Musca domestica; Anopheles spp. Aedes spp; Periplaneta americana; alkaloids; callus induction; daffodils; Narcissus tazetta; cystic fibrosis; cytokines; Pseudomonas aeruginosa; postharvest; mycotoxin; tomato; inhibition; cashew; pigmentation; sporulation; pycnidia; monosodium glutamate; liver; kidney; Moringa oleifera; bacterial pathogenicity; adhesions; invasions; toxins; bio-molecules; infectious diseases; prokaryotic and eukaryotic organisms; medicinal plants; essential oil; linalool; intercropping; male reproductive system; marine hydrobionts; protease inhibitors; collagenolytic and trypsin-like activities; Orchid mycorrhizal; ceratorhiza; trichoderma; Phalaenopsis amabilis etc. This book contains various materials suitable for students, researchers and academicians in the field of biological research.

Chapter 14
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Effectiveness Test of Orchid Mycorrhizal Isolate (*Ceratorhiza* and *Trichoderma*) Indonesia

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ABSTRACT

Aim: The existence of Orchid Mycorrhizal Fungi (OMF) has a role to stimulate growth and support the supply of orchid nutrition as a biofertilizer agent. This study aimed to determine the association of mycorrhizal with *Phalaenopsis amabilis* (L.) Blume which was carried out through the effectiveness test of two Indonesian orchid mycorrhizal isolates i.e. *Ceratorhiza* and *Trichoderma*.
Study Design: This study consisted of 4 treatments. Each treatment was repeated 3 times, each repetition of 5 plantlets, so that the total plantlet used was 60.
Place and Duration of Study: Laboratory of Plant Biotechnology, Department of Biology, Universitas Gadjah Mada, Indonesia, between June 2017 and April 2018.
Methodology: The method of inoculating orchid mycorrhizal by placing a plantlet in a petri dish containing orchid mycorrhizal for 1, 2, 3 and 4 days. Then plantlets are grown on sterile moss growing media and acclimatized in a greenhouse. Observation of each treatment is carried out every day for the next month. Observation variables include the number of initial and final roots, the number of live and dead roots, and the number of living and dead plants.
Results: The results of the orchid mycorrhizal induction test showed that the *Ceratorhiza* inoculation treatment showed a fluctuation in the mean increase in the number of final roots, live roots, dead roots, and dead plantlets that were higher than the *Trichoderma* inoculation treatment. The results also showed that the best inoculation time on *Ceratorhiza* and *Trichoderma* was day 3 and 4. The adaptation process had the effect of increasing the number of dead roots in weeks 1 and 2. The adaptation process stopped at the beginning of week 4 with the number of new roots appearing a lot.
Conclusion: Orchid mycorrhizal *Ceratorhiza* shows the value of effectiveness test compared with *Trichoderma*. The results of this study are expected to be basic information in efforts to cultivate natural orchids in Indonesia.

Keywords: Orchid mycorrhizal; ceratorhiza; trichoderma; Phalaenopsis amabilis (L.) blume; Indonesia.

1. INTRODUCTION

Phalaenopsis is a genus of orchids, which some of its members have important role as parent crosses. Approximately 30 of the total 62 species are spread throughout Indonesia. The presence of this genus in its native habitat (nature) has been reported to have greatly diminished, even some of the members have been recorded to the IUCN red list version 2013.2 due to excessive exploration and forest degradation. Thus, it is very necessary to conserve the existence of native *Phalaenopsis* orchids in Indonesia through the efforts of preservation and protection of plants.

The presence of endophytic mycorrhizal as Orchid Mycorrhizal Fungi (OMF) in orchid plants is known to play an important role in stimulating orchid seed germination [1], supporting the supply of plantlet nutrition [2,3], helping the formation of more buds and flower buds [2] and control biological agents by

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inducing resistance to bacterial infection of *Erwinia chrysanthemi* [4] and inhibiting the replication of ORSV and CymMV [5]. But its presence in different plants can be as a disease-causing agent (pathogen).

Previous studies have identified the isolates of *Ceratorhiza* and *Trichoderma* isolated from the roots of the orchid *Phalaenopsis amabilis* L. (Blume) in Yogyakarta, Indonesia. Identification of the two is distinguished by observing morphological and molecular characters [6]. Molecular analysis was carried out based on the identification of the rDNA-ITS sequence, while morphological analysis was done by observing the surface colour, appearance and colony growth rate, hyphae colour and diameter, shape and size of monilioid cells, and the number of nuclei [7].

Ceratorhiza isolate in this study were reported Mahfut et al. [6] as one group of Ceratobasidium were isolated from the roots of orchid plants in Yogyakarta. The results of molecular analysis showed 600-750 bp of DNA products located on the ITS1-5.8S-ITS4 region. The sequenced products showed insertion and substitution occurances, which may result in strain diversity and possible variation. Reconstruction of phylogenetic trees using *Maximum Parsimony* and *Bootstrap*-1000 approach showed showed the Indonesian isolate is at the basal clade and already far apart from the other isolates.

Soelistijono et al. [8] reported that induction of *Ceratorhiza* and *Tricoherma mycorrhizal* are as a biofertilizer (organic fertilizer). Mycorrhiza works to improve the structure of the soil around plant roots by breaking down organic substances in the soil. The presence of organic substances in the soil is abundant but the shape and size that cannot be absorbed by plants. Besides saving costs, the use of mycorrhizal is very safe for the environment. In the sequel, the mycorrhizal application can accelerate the growth and development of orchid plantlets. Based on this, further research needs to be carried out on the effectiveness of both Indonesian isolate endophytic mycorrhizal and their role as biofertilizer. This research is expected to be important information in the cultivation and development potential of *Phalaenopsis amabilis* L. (Blume) in Indonesia.

2. MATERIALS AND METHODS

2.1 Source of Orchid Mycorrhizal Isolates

The isolates used were Ceratorhiza and *Trichoderma* isolate collected from the root of *P. amabilis* L. (Blume) in Yogyakarta. Pure isolates were bred on Potato Dextrose Agar (PDA) media. Inoculum incubation is carried out in a dark room at room temperature until the age of 7 days.

2.2 Source of Orchid Plantlet

The orchid plantlet used was 12 months old *P. amabilis* L. (Blume) cultured from seeds on Murashige and Skoog (MS) media. Plantlets are removed from culture bottles and soaked in a fungicide solution (2 g/l water) for 20 minutes. Plantlet is then planted on sterile moss media. Orchids are grown properly for 1 month before treatment. Watering is done twice a week using a spray tool.

2.3 Orchid Mycorrhizal Inoculation

Orchid mycorrhizal inoculation in this study used the method of Nuangmek et al. [9]. Orchid mycorrhizal are grown in Petri dishes 9 cm in diameter. Plantlet is placed in a petri dish containing orchid mycorrhizal for 1, 2, 3 and 4 days. Then the plantlets are regrown on sterile moss growing media and acclimatized in a greenhouse. This study consisted of 4 treatments. Each treatment was repeated 3 times, each repetition of 5 plantlets, so that the total plantlet used was 60.

2.4 Observation of the Orchid Mycorrhizal Effectiveness Test

The effectiveness of orchid mycorrhizal on plantlets was carried out *in vivo*. Observation of each treatment is carried out every day for the next month. Observation variables include the number of initial and final roots, the number of live and dead roots and the number of living and dead plants.

3. RESULTS AND DISCUSSION

3.1 Inoculation of Orchid Mycorrhizal

The results of orchid mycorrhizal inoculation showed hyphae would envelop the roots of the plantlet. The hyphae were getting thicker with the length of treatment (Fig. 1).

3.2 Observation of Orchid Mycorrhizal Effectiveness Test

The effectiveness of orchid mycorrhizal was carried out through observations of growth and survival ability of post-inoculation plantlet shown in Tables 1-4.

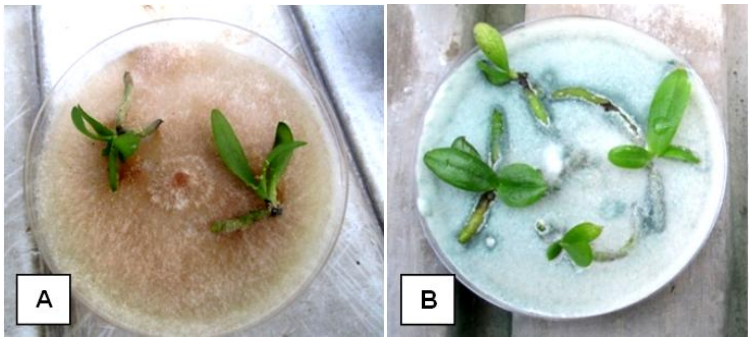

Fig. 1. Orchid mycorrhizal inoculation by placing plantlets on (A) *Ceratorhiza* and (B) *Trichoderma* isolates in a petri dish at 4 days treatment

Table 1. Mean effectiveness of orchid mycorrhizal test results at 1st week

Orchid mycorrhizal isolates	Duration of incubation (Day)	Observation of effectiveness Test (1st week)					
		∑ Root (Initial)	∑ Root (End)	∑ Root (Life)	∑ Root (Dead)	∑ Plantlet (Life)	∑ Plantlet (Dead)
Control	-	7	7	7	0	3	0
Ceratorhiza	1	11	7	7	4	3	0
	2	10	10	10	0	3	0
	3	10	7	7	3	3	0
	4	8	9	3	6	2	1
Trichoderma	1	8	8	8	0	3	0
	2	5	5	5	0	3	0
	3	11	10	10	1	3	0
	4	7	6	7	0	3	0

The test results showed that the *Ceratorhiza* inoculation treatment showed a fluctuation in the mean increase in the number of final roots, live roots, dead roots, and dead plantlets that were higher than the *Trichoderma* inoculation treatment. The highest observation of the highest final root number in *Ceratorhiza* inoculation was at incubation time of 1 day which was 3 at week 2 (Table 2) and 2 at week 4 (Table 4), whereas at *Tricodherma* inoculation was at incubation time at 3 day namely 4 at week 3 (Table 3) and -2 at week 4 (Table 4).

The highest number of life roots in *Ceratorhiza* inoculation was at 4 days incubation ie 1 at week 3 (Table 3) and -3 at week 4 (Table 4), whereas at *Trichoderma* inoculation was at 4 day incubation time at 4 at week 3 (Table 3) and 4 weeks (Table 4). The highest mean number of dead roots in *Ceratorhiza* inoculation was at incubation time of 1 day which was 3 at week 3 (Table 3) and 6 at week 4 (Table 4), whereas at *Trichoderma* inoculation was at incubation time of 2 days namely 3 at week 3 (Table 3) and 1 at week 4 (Table 4). Finally, the mean observation the highest number of dead plantlets in *Ceratorhiza* inoculation was at an incubation time of 1 day which is 1 at week 4 (Table 4), whereas in *Trichoderma* inoculation no dead plantlets were found.

Table 2. Mean effectiveness of orchid mycorrhizal test results at 2nd week

Orchid mycorrhizal isolates	Duration of incubation (Day)	Observation of effectiveness test (2nd week)					
		∑ Root (Initial)	∑ Root (End)	∑ Root (Life)	∑ Root (Dead)	∑ Plantlet (Life)	∑ Plantlet (Dead)
Control	-	7	7	7	0	3	0
Ceratorhiza	1	7	7	7	0	3	0
	2	7	5	5	2	3	0
	3	8	9	9	1	3	0
	4	3	3	3	3	3	0
Trichoderma	1	8	7	7	1	3	0
	2	5	5	5	0	3	0
	3	10	10	9	3	3	0
	4	6	5	5	1	3	0

Table 3. Mean effectiveness of orchid mycorrhizal test results at 3rd week

Orchid mycorrhizal isolates	Duration of incubation (Day)	Observation of effectiveness test (3rd week)					
		∑ Root (Initial)	∑ Root (End)	∑ Root (Life)	∑ Root (Dead)	∑ Plantlet (Life)	∑ Plantlet (Dead)
Control	-	7	7	7	0	3	0
Ceratorhiza	1	7	7	7	0	3	0
	2	8	8	8	0	3	0
	3	10	7	7	3	3	0
	4	3	3	3	0	3	0
Trichoderma	1	7	7	7	0	3	0
	2	5	5	5	0	3	0
	3	11	11	11	0	3	0
	4	5	5	5	0	3	0

Table 4. Mean effectiveness of orchid mycorrhizal test results at 4th week

Orchid mycorrhizal isolates	Duration of incubation (Day)	Observation of effectiveness test (4th week)					
		∑ Root (Initial)	∑ Root (End)	∑ Root (Life)	∑ Root (Dead)	∑ Plantlet (Life)	∑ Plantlet (Dead)
Control	-	7	7	7	0	3	0
Ceratorhiza	1	7	8	8	0	3	0
	2	8	10	7	3	3	0
	3	7	9	9	1	3	0
	4	3	5	5	0	3	0
Trichoderma	1	7	5	5	2	3	0
	2	5	7	5	2	3	0
	3	9	9	9	3	3	0
	4	5	6	6	0	3	0

The results of this study showed that the inoculation of *Ceratorhiza* gave more effect on the number of dead roots in week 1 and 2 compared to *Trichoderma* inoculation. Although at weeks 3 and 4 a large number of new roots appear (Fig. 2). The results also showed that the best inoculation time for *Ceratorhiza* and *Trichoderma* was day 3 and 4. This adaptation process stopped at the beginning of week 4. As of week 4, the mean number of dead roots decreased and the number of root increases increased. Based on visual observations, the root undergoes decay as a process of adaptation to the orchid my corrhizal inoculation treatment. This is due to the faster growth of *Ceratorhiza* hypha so that it can associate with plants faster. The faster the orchid mycorrhizal is associated with the host plant, the higher the capacity to absorb nutrients and cause increased growth. Muslim et al. [10] explained that the main principle of orchid mycorrhizal work is to infect the root system of the host plant and produce hyphae intensively so that it can increase the capacity of plants to absorb nutrients.

Fig. 2. Development of plantlet results from mycorrhizal inoculation per week; (1) week 1; (2) week 2; (3) week 3; (4) week 4. The arrows indicate the emergence of new roots

Orchid plants require orchid mycorrhizal infections to complete their life cycle. An important role of orchid mycorrhizal in plant growth is its ability to absorb nutrients both macro and micro. The treatment of orchid mycorrhizal inoculation on orchids is known to be able to increase the efficiency of inhibition of N nutrient absorption to increase plant growth, such as increasing length, width and the number of leaves and roots. Element N is a building material for amino acids/ proteins, enzymes, nucleic acids, nucleoproteins and alkaloids. N deficiency will limit cell division and distribution [11].

4. CONCLUSION

The results of the effectiveness of orchid mycorrhizal isolates in Indonesia and its role as biofertilizer showed that the inoculation treatment of *Ceratorhiza* showed fluctuations in the average increase in the number of final roots, life roots, dead roots, and dead plantlets which were higher than those in the *Trichoderma* inoculation treatment. The results also showed that the best inoculation time on *Ceratorhiza* and *Trichoderma* was day 3 and 4. The adaptation process had the effect of increasing the number of dead roots in week 1 and 2. The adaptation process stopped at the beginning of week 4 with the number of new roots appearing a lot.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author

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He was born on 9 September 1981 in Merbau Mataram, South Lampung. In 2006 he obtained his Bachelor of Biology degree from the Faculty of Mathematics and Natural Sciences, University of Lampung. In 2011 he obtained a Master's degree (Master of Science) and in 2018 obtained his doctorate degree from the Biology Postgraduate Program, Gadjah Mada University, Yogyakarta. The title of the thesis of the author is "The Effect of the Insecticide Made from Profenofros Active on Organ Reproduction and Reversal of Tomatoes (*Lycopersicum esculentum* Mill.)", Entitled Thesis "Detection and Characterization of Odontoglosuum ringspot virus (ORSV) Isolates of Java and Bali, Indonesia", and titled Dissertation "Variation Genetic rbcL Genes and CP Genes, and Induction of Natural Phalaenopsis Resistance in Indonesia Against Odontoglossum ringspot virus with Orchid Mycorrhiza ". Currently the he is actively working as a lecturer in the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung since 2014.

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