Proceedings Of

SEAMEO BIOTROP THIRD INTERNATIONAL CONFERENCE ON TROPICAL BIOLOGY

"Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna"

Edited by
Jesus C. Fernandez
Cahyo Wibowo

Bogor, 20-21 September 2018
SEAMEO BIOTROP Convention Hall

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PREFACE

We are pleased to publish the proceedings of our Third International Conference on Tropical Biology held on 20-21 September, 2018 in Bogor, West Java Province, Indonesia. The conference theme, "Conservation, Enhancement, and Sustainable Use of Indigenous Tropical Flora and Fauna", was in response to the urgent need in ensuring a sustainable use of indigenous tropical flora and fauna, as well as the conservation of species facing extinction due to rapid habitat loss caused by rampant deforestation for agricultural purposes and massive infrastructure development, unregulated collection and trafficking of indigenous plants and animals. We believe that there are several past experiences of sustainable use and conservation schemes by governments and non-governmental organizations that have been successful. Lessons learned from these experiences are critical to generate and formulate practical and sustainable conservation strategies for indigenous tropical flora and fauna, determine and prioritize research needs based on current policies and research results, and strengthen participation and contribution of stakeholders in eliminating current problems and at the same time, enhancing the conservation and sustainable use of the region's biodiversity and natural resources.

We were fortunate to convene 227 scientists and practitioners from eight countries during the conference to share useful lessons, address challenges, and generate commitments to strengthen policy decisions and work collaboratively towards conservation and sustainable use of indigenous tropical flora and fauna, especially in the Southeast Asia region.

This volume of our conference proceedings contains the full papers and abstracts of the keynote addresses, panel discussion, and parallel session oral and poster presentations. The keynote addresses attempt to illustrate the gains and challenges, the diversity and resiliency as well as the approaches, technologies and innovations in conservation, enhancement and sustainable use of indigenous tropical flora and fauna. The panel discussions focus on the policies and other legal frameworks as well as the future directions in conservation, enhancement and sustainable use of indigenous tropical flora and fauna. The parallel session papers provide actual experiences on the four conference subthemes, namely: (1) Diversity and Resiliency of Indigenous Tropical Flora and Fauna and Their Ecosystem; (2) Approaches, Technologies and Innovations in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna; (3) Socio-economic, Cultural and Ethical Aspects in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna; and (4) Policies and Other Legal Frameworks in Conservation and Sustainable Use of Indigenous Tropical Flora and Fauna. As much as we would have wanted full papers included in this publication, we respect the presenters' decision to just submit the abstracts of their presentations. We thank all of them for their contributions in making this publication possible. We hope that the papers and abstracts, much more the synthesis and recommendations as well as future agenda generated from the conference, could spark new and continuing efforts to pursue conservation, enhancement, and sustainable use of indigenous tropical flora and fauna in the region.

Our deepest appreciation goes to Southeast Asian Ministers of Education Organization (SEAMEO), Ministry of Environment and Forestry of the Republic of Indonesia, National Committee for Indonesian Germ Plasms, Ministry of Agriculture of the Republic of Indonesia, Lembaga Ilmu Pengetahuan Indonesia (Indonesian Institute of Science/LIPI), Institut Pertanian Bogor (IPB University), Universiti Putra Malaysia, National University of Singapore, Forest Stewardship Council (FSC) Indonesia, Central Luzon State University Philippines, Burung Indonesia, Pampanga State Agricultural University Philippines, Cagayan State University

Philippnes, PT Sinarmas Tbk. Indonesia, Bank Mandiri, Bank Mandiri Syariah and PT Garuda Food for supporting us to hold this conference. We highly value the time and effort of the Scientific Committee members for reviewing all the submitted abstracts and helping us finalize the list of paper and poster presenters. We recognize the valuable contributions of SEAMEO BIOTROP staff members for ensuring the smooth implementation of the conference and in packaging this publication.

We look forward to our Fourth International Conference on Tropical Biology in 2020.

Conference Coordinator and Proceedings Editors

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Dr Irdika Mansur
SEAMEO BIOTROP Director

- Dr Kirsfianti Linda Ginoga, Director of Forest Research Centre, Forest Research Development and Innovation Agency, Ministry of Environment and Forestry of the Republic of Indonesia,
- Governing Board Members, Deputy Directors and Staff of Southeast Asian Ministers of Education Organization (SEAMEO) for Tropical Biology (BIOTROP),
- Dr Maria Ulfah, Chairperson of the 3rd International Conference on Tropical Biology,
- Our Colleagues from Indonesian universities, research institutes, schools and private companies,
- Representatives of local governments from all over Indonesia,
- Distinguished speakers and participants,
- Ladies and Gentlemen,

Assalamu'alaikum warahmatullaahi wabarakatuh,

I am pleased to welcome you all to Bogor City and to SEAMEO BIOTROP for our 3rd International Conference on Tropical Biology starting today until 21 September 2018 which we are now conducting in our Convention Hall. We expect this conference to be a venue for sharing knoweldge, perspectives, and experiences among the participants and speakers on the theme "Conservation, Enhancement, and Sustainable Use of Indigenous Tropical Flora and Fauna." As SEAMEO BIOTROP Director, I am honored for our Centre to host this conference.

First, allow me to briefly introduce our Centre to all of you. SEAMEO BIOTROP is one of 24 specialist centres of the Southeast Asian Ministers of Education Organization (SEAMEO). Our Centre was established on 6 February 1968 and is mandated to conduct research, capacity building, and information exchange toward addressing biology-related problems in Southeast Asia. Since 2012 up to now, SEAMEO BIOTROP's vision is to be "A leading Centre in enriching and promoting the real values of tropical biology in Southeast Asia". Our mission is to provide scientific knowledge and build capacities of institutions and communities in conserving and managing tropical biology sustainably for the well-being of communities and the environment of Southeast Asia. For the next five years, we will be focusing our activities on three program thrusts, namely: (1) Restoration of Degraded Landscapes/Ecosystems, (2) Sustainable Management of Intensively Used Landscapes/Ecosystems, and (3) Conservation and Sustainable Use of Unique Ecosystems/Landscapes of High Biodiversity. We believe that organizing an international conference on tropical biology (ICTB) is one of the ways through which we can realize our vision and mission and address our program thrusts.

Ladies and Gentlemen,

In recent years, we have witnessed an increasing concern on ensuring a sustainable use of indigenous tropical flora and fauna, as well as the conservation of species facing extinction due to rapid habitat loss caused by rampant deforestation for agricultural purposes and massive infrastructure development, unregulated collection and trafficking of indigenous plants and animals. This scenario led us to focus on "Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna" as the theme of this year's conference.

I am very pleased to see delegates from various countries in and outside the Southeast Asian region as well as representatives from many Indonesian institutions. I believe that with the various expertise of the participants and speakers present here, we would have interesting and enthusiastic discussions during our conference. I sincerely hope that this conference will be able to generate consensus among participants to formulate practical and sustainable ways, based on current policies and research results, to strengthen participation and contribution of stakeholders in eliminating currents problems and, at the same time, enhancing the conservation and sustainable use of the region's biodiversity and natural resources.

Ladies and Gentlemen,

I would like to express my gratitude to the Southeast Asian Ministers of Education Organization (SEAMEO) and partner-institutions for supporting us to hold this conference. Let me take this opportunity to acknowledge them here, namely: the Ministry of Environment and Forestry of the Republic of Indonesia, National Committee for Indonesian Germ Plasms, Ministry of Agriculture of the Republic of Indonesia, Indonesian Institute of Science, Institut Pertanian Bogor, Universiti Putra Malaysia, National University of Singapore, Forest Stewardship Council (FSC) Indonesia, Central Luzon State University Philippines, Burung Indonesia, Pampanga State Agricultural University Philippines, Cagayan State University Philippines, PT Sinarmas Tbk Indonesia, Bank Mandiri, Bank Mandiri Syariah and PT Garuda Food. I would also like to express my heartfelt appeciation to all the members of our Conference organizing and scientific committees for their hard work and dedication in making sure that all things are in place and running well. And to all of our speakers and participants, thank you so much for your presence and interest to be a part of this important conference, because without you this event could not be realized.

I wish everyone a productive conference and I hope that you will find your stay in SEAMEO BIOTROP a pleasurable one. Once again, I extend our warm welcome to all of you.

Thank you very much.

Wassalamu'alaikum warahmatulaahi wabarakaatuh.

POTENTIAL OF INDONESIA'S INDIGENOUS DARK SEPTATE ENDOPHYTIC FUNGI TO CONTROL *Fusarium* WILT IN VITRO

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ABSTRACT

Research on Dark Septate Endophytic (DSE) fungi in Indonesia is still limited. Therefore, efforts should be made to investigate the potential of indigenous DSE fungi from Indonesia that can be used as a biological control. The isolation of DSE fungi from tomato root samples was performed using direct isolation and soil baiting methods. Tomato root samples were obtained from tomato farms in Cisarua, Citeupuh, Cianjur, Sukabumi, and Garut, West Java. The soil baiting method was conducted using organic forest soil and cabbage farm soil to grow the tomato plants. DSE fungi from those tomato roots were isolated. The best DSE fungi isolates was selected using *in-vitro* treatments by conducting pathogenicity test of DSE fungal candidates to tomato plant, hemolytic reaction test of selected DSE fungi, antagonistic test to *F. oxysporum* f.sp. *lycopersicii*, and metabolite test of volatile compound. There were 49 DSE fungal candidates isolated from tomato root samples. The pathogenicity test showed that 20 DSE fungal isolates had ability to promote tomato seed germination by 97%-100%. Hemolytic reaction test of 20 DSE fungal isolates using blood agar showed that all isolates were negative. Antagonistic test performed on DSE fungal isolate DS08 ic and DS08 ib showed a significant different ability to inhibit *F. oxysporum* f.sp. *lycopersicii* with percentage of inhibitory zone of 76.11% and 71.07%, respectively. The results of metabolite test showed that the highest volatile compound was obtained by DS08 ib (23.70%), followed by DS06 iib (22.96%) and DS06 iiia (22.96%). This research indicated that DSE fungi isolates from tomato roots can be used as Indonesia's indigenous dark septate endophyte fungi in suppressing *Fusarium* wilt disease.

Keywords: Dark septate endophytic fungi, dual culture, *Fusarium oxysporum* wilt, tomato

INTRODUCTION

Dark septate endophytic fungi (DSE) are a fungal group capable of colonizing plant roots without causing any disease symptoms. The characteristics of DSE is dark colonies in agar media, have melanized hyphae, septate hyphae, conidial or sterile ascomycetous fungi (Jumpponen & Trappe 1998) and sometimes forming microsclerotia (Gomes 2017). DSE can be found in all ecosystems and wide range of plants species. There are 144 families and 587 plant species reported to have been colonized by DSE (Jumpponen & Trappe 1998). The symbiosis between DSE fungi and its host plants includes mutualism. Potential DSE is known to improve plant performance and promoting plant growth under biotic or abiotic stress. Several studies have reported that DSE fungi have the potential as a biological control agent for pathogenic fungi, such as DSE fungi that supressed *Verticillium dahlie* in *chinese cabbage* (Narisawa *et al.* 2004), and *Fusarium* on *chinese cabbage* (Khastini *et al.* 2012). Surono and Narisawa (2018) reported *Phialocephala fortinii* suppressed *Fusarium* disease in *Asparagus officinalis*.

Researches on the DSE fungi have been conducted and developed in many sub-tropical countries, while in tropical countries especially in Indonesia, research on DSE fungi is still limited, whereas Indonesia is one of the countries with a very high biodiversity in the world. Therefore, it is necessary to explore indigenous DSE fungi from Indonesia that have the potency to be used as biological control to plant disease. The aim of this research were to obtain DSE fungal isolate from tomato plant root and to investigate the ability of selected DSE fungal isolate to inhibit *Fusarium oxysporum* f.sp. *lycopersicii* growth under *in vitro* assay. *F. oxysporum* f.sp *lycopersicii* was used as a pathogen target in this study because this pathogen can cause wilt disease in tomato plants, and as one of which is the most important diseases in tomato, both in nursery and field in Indonesia.

MATERIALS AND METHODS

Isolation and Cultivation of DSE Fungi

In this study, isolation of DSE fungi from tomato root samples collected from field was performed using direct isolation and soil baiting methods. Tomato root samples were obtained from tomato farms in Cisarua, Citeupuh, Cianjur, Sukabumi, and Garut, West Java. Soil baiting method was conducted using organic forest soil and cabbage farm soil to grow the tomato plants. DSE fungi from those tomato roots were isolated. Surface sterilization of root samples was performed using tween 20, NaOCl and sterile distilled water. Root samples were surface-sterilized, air-dried with sterile tissue, and then plated into 50% corn meal agar (CMA) medium in 9 cm plastic petri dishes. DSE fungi in this study were considered to be dark and slow-growing fungal isolate. The growing DSE fungal isolates were cultivated on potato dextrose agar (PDA) media.

Early Detection Test of DSE Presence in Tomato Roots Samples

Early detection test was carried out to observe the presence of DSE in the roots of both intercellular or intracellular. The early detection test was done using staining technique of the DSE colonization method (Zhang 2013).

Selection of Isolate with In Vitro Assay

Pathogenecity Test

Pathogenicity test was carried out to determine whether or not the candidate is pathogenic to the host plant. Pathogenecity test was determined according to methods described by Surono and Narisawa (2017) with modification. Tomato seeds surface sterilization was conducted with 1% NaOCl for 3 minutes, followed by washing with sterile water for three times. The sterilized seeds were soaked for three hours in sterile water. Afterwards, they were dried and placed on candidate DSE fungal isolate that have been grown for 7 days and incubated for two weeks.

Hemolytic Reaction Test

Hemolytic reaction test of selected DSE fungi was performed to make sure that the selected DSE fungi are not pathogen to human and animals. The test was conducted using blood agar media according to Beutin (1991). An agar plug (5 mm diameter) of DSE fungal isolate was placed on blood agar media and incubated for one week.

Antagonistic Test

Antagonistic test was carried out using the dual culture test method referring to Dwiastuti *et al.* (2016) with modifications. Dual culture test was carried out using PDA media in 90 mm plastic

petri dishes. Due to the slow growth of DSE fungi, the fungi were firstly grown for 2 weeks. Subsequently, the fungal pathogen was grown in the same petri dish as the DSE fungal isolates for dual culture testing. After 2 weeks, the inhibition zone and inhibition of the radial growth of the pathogen were measured.

Test of DSE Volatile Isolate Metabolites

Testing of volatile metabolites was carried out by following the methods of Dennis and Webster (1971) i.e. taking pieces of 5 mm diameter of pure culture from each DSE isolate and FOL pathogen, then placed on PDA media in a separate petri dish. The two petri dishes were then cupped against each other, so that the DSE isolate was on top and the FOL isolate was below. Observations were made on the growth of FOL pathogenic colonies by measuring the diameter of colonies 7 days after inoculation (dai).

RESULTS AND DISCUSSION

DSE fungi were isolated from the tomato roots in West Java, Indonesia. Figure 1 shows DSE fungi isolated from tomato roots, stained by coloring as an initial detection.

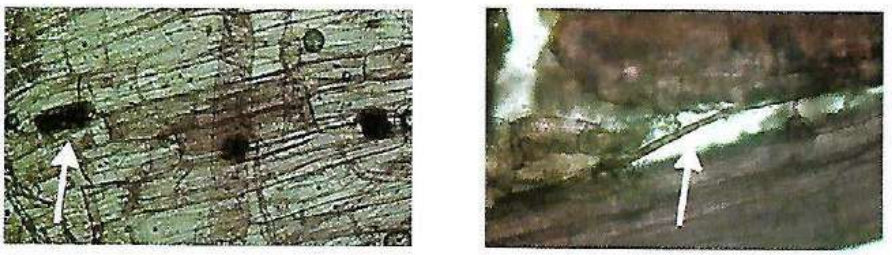

Figure 1 Color stained DSE fungal isolate in tomato root tissue, observed by 400x magnification: (a) microsclerotia; (b) melanin hyphae

Existence percentages and various sample locations of DSE fungi were presented in Table 1. DSE fungal isolates found from this exploration were 49 candidate DSE isolates.

Table 1 Percentage of DSE existence based on sampling location

Isolation Method	Location	Percentage (%)
Direct	Sukabumi	10.23
	Cianjur	7.45
	Citeupuh	4.76
	Garut	4.44
	Cisarua	4.00
Indirect (Soil Baiting)	Organic forest soil	6.67
	Cabbage farm soil	6.19

Selection of successfully isolated DSE fungi was conducted, followed by pathogenicity test. At this stage, isolates selected were those which did not cause death or inhibit the growth of tomato seeds. These isolates were not potential as pathogenic fungi, which would not kill or inhibit seeds' growth. Characteristics of pathogenic fungi in the selection of endophytic fungi included seeds which were not developing, were slow-growing compared to other fungi isolates; were not able to germinate and to grow, and eventually died. Characteristics of non-pathogenic fungi included seeds that were able to germinate well with well-grown roots and stems (Fig. 2).

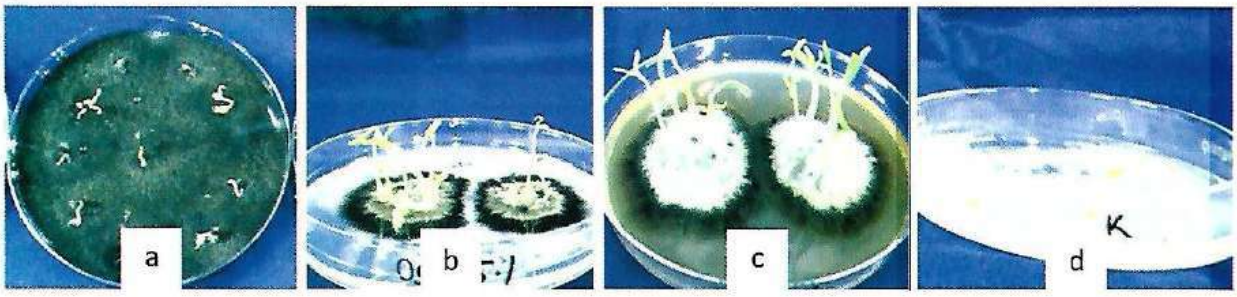

Figure 2 The effect of fungal isolate candidates on tomato seed germination in the pathogenicity test. (a) DSE isolate candidate inhibits seed germination (abnormal germination); (b) DSE isolate candidate shows seed germination (germination 70-85%); (c) DSE isolate candidate shows seed germination (germination 90-100%); (d) control (germination 70%)

Results of pathogenicity test from 49 candidates of DSE isolate obtained 21 DSE fungal isolates having the ability to promote tomato seed germination by 97%-100%.

Hemolytic reaction test of selected DSE fungi was conducted to ensure that the selected DSE fungi are not pathogen to human and animals. Hemolytic reaction is divided into three types (Beutin 1991), i.e. alpha hemolysis (α-hemolysis), beta hemolysis (β-hemolysis), and gamma hemolysis (γ- hemolysis). If Alpha hemolysis (α-hemolysis) is present, the agar under the colony is dark or greenish and the organism is called partial or incomplete hemolysis. Beta hemolysis (β-hemolysis) is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Gamma hemolysis (γ-hemolysis) is unchanged in blood agar media and the organism is called non-hemolytic. α-hemolysis and γ-hemolysis existence on blood agar indicates the growth of normal flora (negative as human or animal pathogen). β-hemolysis existence on blood agar indicates the presence of pathogen (positive as human or animal pathogen). Hemolytic reaction test of 20 selected DSE fungal isolates using blood agar showed that all isolates were negative and 1 positive. Therefore, those isolates are not pathogen to human and animals (Fig. 3).

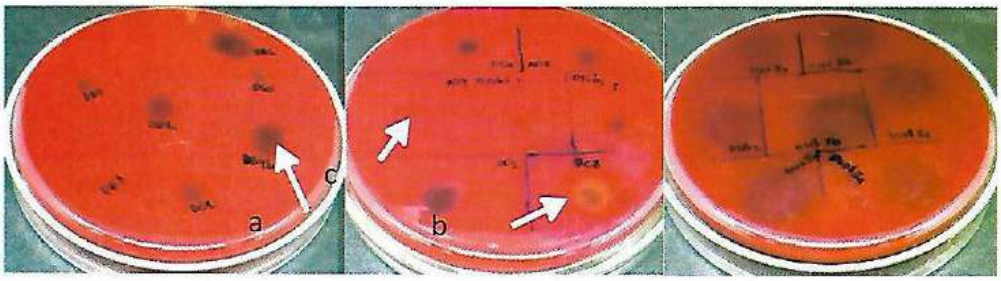

Figure 3 Hemolytic reaction test of selected DSE fungal isolate on blood agar media. (a) Alpha hemolysis (α-hemolysis); (b) beta hemolysis (β-hemolysis); (c) gamma hemolysis (γ-hemolysis)

Nine (9) of the 20 selected DSE fungal isolates inhibited the growth of *F. oxysporum* (Table 2). The inhibition levels by DSE fungal isolate DS08 ic and DS08 ib on *F. oxysporum* were 76.11% and 71.07%. The negative control treatment was not given control formulation, so it did not show inhibitory activity as indicated by the maximum growth of *F. oxysporum* filling the entire surface of the petri dish (Fig. 4).

Tabel 2 Growth diameter and inhibition zone of *F. oxysporum* resulted from antagonism test for 14 days after inoculation (dai) in vitro

DSE Fungal isolate	Inhibition Percentage of pathogen growth rate (%)
DS08-1c	76.11a
DS08-Ib	71.07ab
DS08-Ia	66.67abc
DS06-IIa	66.67abc
AD1	66.04abc
DS06-IIIa	65.00abc
DS06-IIb	64.45abc
DS08-I1a	60.38bc
DG5	56.11c
AD2	36.97d
DB5	18.34e
DB8	16.11e
AD7	15.56ef
DC1	13.33efg
DB6	8.33efg
DB4a	6.33efg
DB4b	1.11gf
DSA	0.00g
DSSB 5.1	0.00g
DSSB 5.3	0.00g
CONTROL	0.00g

Figure 4 Antagonism between *F. oxysporum* and DSE fungal isolates. (a) DS08-Ic; (b) DS08-1b; (c) not inhibit the growth of *F. oxysporum*; (d) control

Result of the volatile organic compounds (VOC) test showed diameter of pathogenic colony that had been inoculated with DSE isolate was smaller than that of control (Fig. 5). This indicated that the presence of volatile organic (VOC) compounds produced by DSE fungi can function as antifungal.

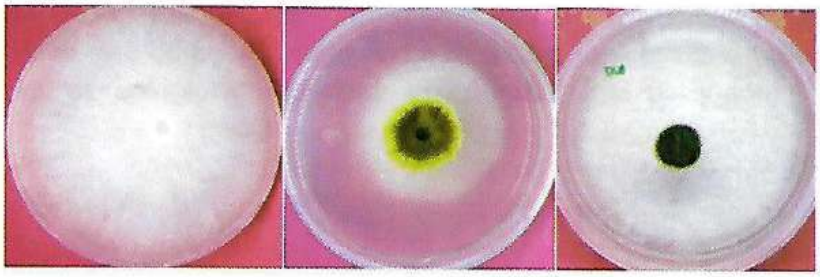

Figure 5 Effect of VOC from DSE fungi isolated from tomatoes plant on *Fusarium* diameter growth: (a) control; (b) showing growth inhibition of *F. oxysporum*; (c) showing no inhibition of growth of *F. oxysporum*

CONCLUSION

DSE fungi can be isolated from the roots of tomato plants in several sample locations on West Java. Twenty (20) candidates of DSE fungi increased tomato seed germination up to 96.67-100% compared to 29 other fungus candidates. Hemolysis reaction test on 20 fungi isolates showed negative and 1 fungi isolate showed positive. DSE isolate DS08-Ic and DS08-Ib had the highest inhibition in suppressing *Fusarium* growth. DSE isolates from Indonesia are potential to be used as biocontrol agents in suppressing *Fusarium* growth.

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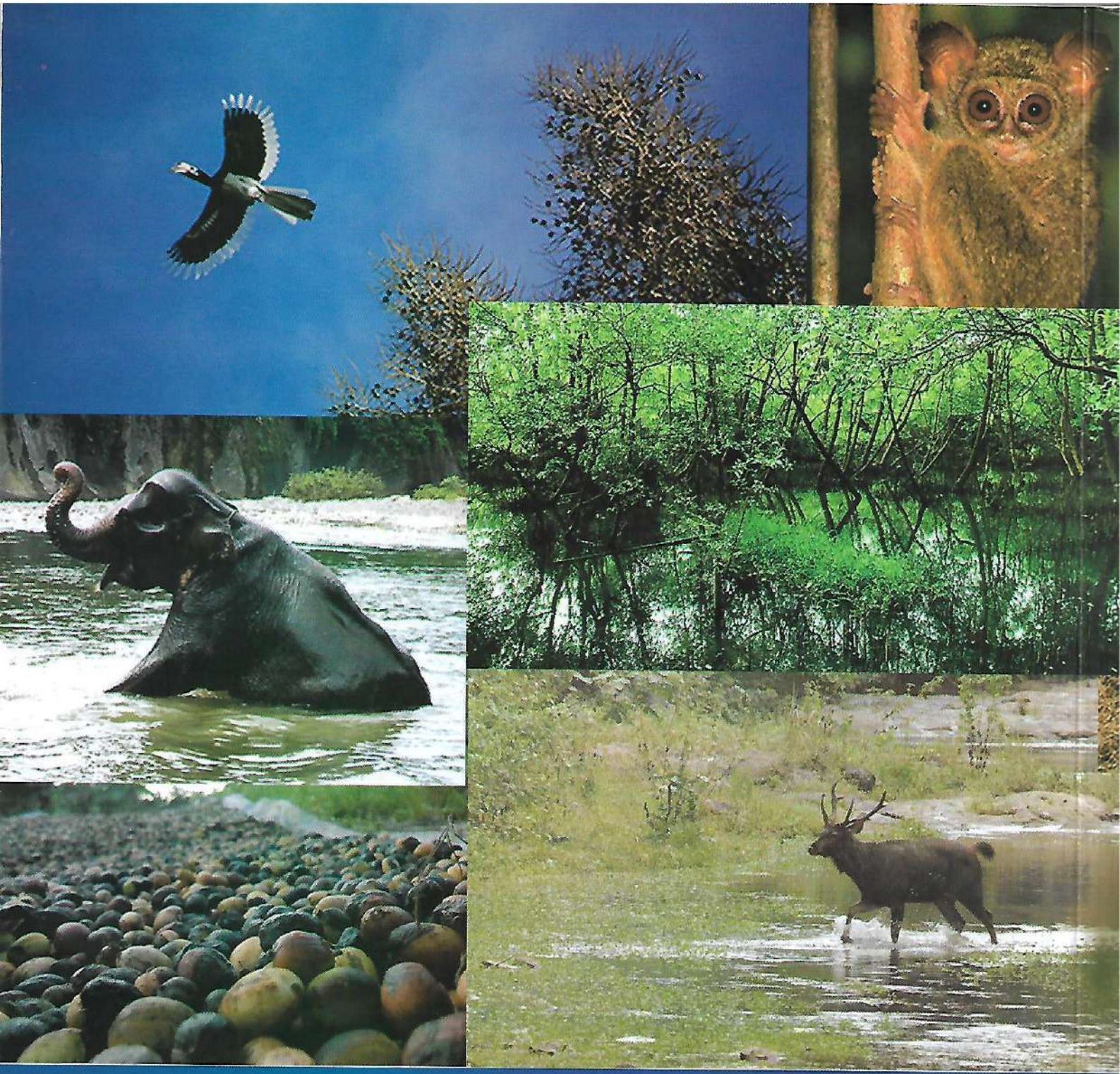

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