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BIODIVERS is a bio-science general audience magazine launched in December 2021 by the Southeast Asian Ministers of Education Organization Regional Centre for Tropical Biology (SEAMEO BIOTROP). As a scientific publication, it is designed to increase awareness and literacy on issues related to saving Tropical Biodiversity from the "Mountain to the Ocean" (MOTO). The scope of BIODIVERS includes the following (1) Ecosystem Restoration and Conservation, (2) Sustainable Use of Biodiversity, Bioenergy, Biotechnology, Food Security, and (3) Ecosystem Resilience in the face of global climate change

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Foreword from the Director of SEAMEO BIOTROP

Biodiversity forms the foundation of ecological stability, human well-being, and sustainable development. Across Southeast Asia, rich biological resources sustain food systems, regulate climate, support livelihoods, and shape the cultural landscapes of our societies. Yet these ecosystems are increasingly under pressure from deforestation, land-use change, pollution, and climate-related disturbances. In the face of these challenges, scientific knowledge and regional collaboration become essential to ensure that biodiversity continues to support resilient ecosystems and communities.

It is therefore a pleasure to present BIODIVERS Vol. 5 No. 1, which brings together a collection of scientific contributions highlighting the important role of biodiversity research in addressing contemporary environmental challenges. The studies featured in this issue demonstrate how science can bridge conservation, innovation, and sustainable development across diverse ecological and socio-economic contexts.

The articles in this edition reflect the expanding scope of biodiversity research. They explore topics ranging from wildlife conservation and ecosystem restoration to advances in biotechnology, sustainable materials, and environmentally responsible cultivation systems. These studies show that biodiversity is not only a subject of conservation concern but also a valuable source of scientific insight and innovation for sustainable solutions.

Several contributions highlight how research can translate into practical approaches for sustainability. Efforts to conserve threatened species illustrate the importance of combining scientific research with education and community engagement to strengthen conservation outcomes. At the same time, innovations derived from biological resources demonstrate how biodiversity can inspire environmentally friendly technologies and the development of sustainable materials through circular bioeconomy approaches.

This issue also underscores the growing importance of sustainable cultivation systems and urban ecological research. As cities expand and agricultural systems evolve, innovative approaches are needed to ensure food security while maintaining ecological balance. Studies on urban green spaces and alternative cultivation methods highlight how biodiversity can continue to support ecosystem services such as pollination, climate regulation, and resource efficiency even within highly modified landscapes.

Taken together, the contributions in BIODIVERS Vol. 5 No. 1 remind us that biodiversity is not only a natural heritage that must be protected but also a source of knowledge, innovation, and resilience. Through interdisciplinary research, collaboration, and knowledge sharing, we can strengthen our collective efforts to safeguard ecosystems while advancing sustainable development.

On behalf of SEAMEO BIOTROP, I extend my sincere appreciation to all authors, reviewers, and collaborators who have contributed to this publication. Their dedication helps strengthen the scientific community and supports ongoing efforts to promote biodiversity conservation and sustainability in Southeast Asia and beyond.

We hope that this issue of *BIODIVERS* will inspire continued dialogue, collaboration, and action in protecting the region's rich biodiversity for present and future generations.

Prof. Dr. Edi Santosa, S.P., M.Si.
Director of SEAMEO BIOTROP

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https://www.pexels.com/photo/macro-photography-of-butterflies-perched-on-lavender-flower-675313/

OVERVIEW OF BIODIVERS

BIODIVERS is a bio-science general audience magazine launched in December 2021 by the Southeast Asian Ministers of Education Organization Regional Centre for Tropical Biology (SEAMEO BIOTROP). It is designed as a scientific publication to increase awareness on issues related to Tropical Biodiversity from the Mountain to the Ocean (MOTO) and increase biodiversity literacy.

BIODIVERS is a bi-annual publication that focuses on the Restoration and Conservation of Unique and Degraded Ecosystems, Sustainable of Management and Proper Utilization of Biodiversity, Bioenergy, Biotechnology to Support Food Security and on Strengthening Ecosystem Resilience in Facing Global Climate Change. This magazine also envisions becoming a popular-scientific magazine for promoting and publishing research findings of scientists from SEAMEO BIOTROP and overseas. The articles will come from writers worldwide.

Vision

To become a reputable center for sustainable biodiversity management in Southeast Asia

Mission

To deliver innovative products and technologies in science-education for saving biodiversity in transforming best practices for the betterment of Southeast Asia societies.

To promote applied science education on sustainable use of biodiversity for the well-being of society in Southeast Asia.

To build highly competent human resources for managing sustainable biodiversity in Southeast Asia.

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Improving the Nutritional Quality (Protein, Fat, Ash and Water Content) of Water Spinach (*Ipomoea reptans* Poir) Through Aquaponics and Hydroponics

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SYNOPSIS

Soilless cultivation systems such as hydroponics and aquaponics are increasingly developing in modern agriculture. Land kale (Ipomoea reptans Poir) is widely cultivated, but information regarding the influence of cultivation techniques on proximate composition is still limited. This research aims to compare the water, protein, fat, ash and carbohydrate content of land kale cultivated hydroponically, aquaponically and conventionally. The research includes the cultivation process to harvest as well as proximate analysis based on SNI 01-2891-1992. The results showed that hydroponic kale had the highest levels of protein (3.02%), fat (3.04%), and ash (1.95%); Aquaponic kale has the highest water content (92.62%); while conventional kale has the highest carbohydrate content (6.02%).

INTRODUCTION

Hydroponic and aquaponic systems have emerged as modern cultivation methods due to their efficiency in water and land use and their suitability for limited agricultural space (Roidah, 2014; Marbun et al., 2022). Aquaponics integrates fish culture and plant production by utilizing fish metabolic waste as a nutrient source, while hydroponics relies on controlled artificial nutrient solutions such as AB mix (Darsudi et al., 2008). Differences in nutrient sources and availability may influence plant growth and nutritional composition.

Water spinach (*Ipomoea reptans* Poir) is a widely consumed leafy vegetable that can be cultivated using hydroponic, aquaponic, and conventional systems. Several studies have reported that cultivation methods affect proximate composition, including moisture, protein, fat, ash, and carbohydrate contents (Falaha et al., 2015; Prasetyo et al., 2019). Water-based systems tend to enhance nutrient uptake efficiency and tissue moisture content, whereas soil-based conventional systems often result in higher carbohydrate accumulation due to increased dry matter formation (Maf'ulah, 2008).

Proximate analysis is an essential approach for evaluating food quality and functional food potential. Moisture content is associated with freshness and shelf life, protein reflects nutritional quality and physiological function, fat contributes to energy value, ash represents mineral content, and carbohydrates serve as the primary energy source (Adrian, 2012; Pargiyanti, 2019). Water spinach is also recognized as a functional food with potential health benefits (Dewi & Mardiana, 2023). However, comparative data on the proximate composition of water spinach grown under different cultivation systems remain limited.

Therefore, this study aims to compare the proximate composition of water spinach cultivated using hydroponic, aquaponic, and conventional methods to provide scientific references for nutritionally oriented cultivation strategies.

ARTICLE CONTENT

The study was conducted in May–June 2025 at the aquaponic and hydroponic units of SEAMEO BIOTROP, Bogor, and at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Pakuan University. Primary data were obtained from water spinach cultivated under hydroponic and aquaponic systems using an NFT installation with rockwool media, V1 kale seeds, AB Mix nutrient solution, and tilapia (Oreochromis niloticus) for aquaponics. Seeds were germinated for approximately 7 days prior to transplanting. In aquaponics, nutrients originated from fish pond water (pH 6.5–7.5; temperature 25–30 °C), while hydroponics used AB Mix solution (TDS 400–1200 ppm; pH 6.5–7.5). Plants were harvested at 25–30 days after planting.

At harvest, plant samples from hydroponic and aquaponic systems were collected randomly and combined into one composite sample per system. Proximate analysis was conducted following BSN (1992), including moisture, ash, fat, protein, and carbohydrate (by difference). Proximate data for conventionally grown water spinach were obtained as secondary data from published references included in this study. Due to differences in data sources and the absence of biological replication, data were analyzed using a descriptive quantitative approach to compare proximate profiles across cultivation systems.replicates.

The highest water content was found in aquaponics (92.62%) and the lowest in hydroponics (90.58%). The difference in water content in each cultivation system is closely related to the characteristics of the growing medium and the efficiency of the root system. The aquaponic system, which utilizes nutrient water from fish waste, is able to increase water absorption, resulting in the highest water content. Hydroponics showed slightly lower water content, but still better than conventional methods. Meanwhile, the use of soil as a growing medium tended to produce the lowest water content, possibly due to lower evaporation and water absorption efficiency. This finding confirms that the structure of the growing medium greatly determines water retention capacity and ultimately affects plant growth. (Asis, 2024).

Hydroponics showed the highest ash content (1.95%), while conventional methods showed the lowest ash content (1.09%). The relatively low ash content indicates better product quality compared to the other two methods. This finding aligns with research by Setyawati *et al.* (2024) which suggests that increasing ash content often leads to a decrease in product quality, while lower ash content actually improves product quality.

Table 1 Comparison of Proximate Content of Water Spinach

Indicator	Hydroponics (%)	Aquaponics (%)	Conventional (%)
Water	90,58	92,62	90,00
Ash	1,95	1,55	1,09
Fat	3,04	2,74	0,55
Protein	3,02	1,98	2,35
Carbohydrates (by difference)	1,40	1,11	6,02

Soure: Author's Undergraduate Thesis

The highest fat content was found in hydroponics (3.04%), followed by aquaponics (2.74%). Water spinach fat content tends to be higher in hydroponic and aquaponic systems due to more optimal nutrient availability and nutrient absorption efficiency. This is due to differences in cultivation systems and media used. This statement aligns with research by Islammiah (2023), which shows that variations in growing media do not significantly affect several growth parameters, but do affect plant nutrient content. In this study, carbohydrate levels tended to be lower than in typical water spinach, while protein levels showed variable results, with some lower and others higher. Fat levels were slightly higher in some treatments. These findings reinforce the idea that growing media composition has the potential to influence plant chemical quality, although not always impacting physical growth.

Hydroponically grown water spinach showed the highest protein content (3.02%), followed by conventional (2.35%) and aquaponic systems (1.98%). These differences are associated with variations in nutrient availability and uptake efficiency across cultivation systems rather than being solely caused by the systems themselves. The hydroponic system provides a stable supply of macro- and micronutrients, which is associated with more efficient nitrogen uptake and enhanced protein synthesis. In contrast, nitrogen availability in conventional cultivation is strongly influenced by soil conditions and fertilizer management, while aquaponic systems depend on fish-derived organic nutrients, which may fluctuate when ecosystem balance is not optimal (Maf'ulah, 2008)

https://roboguru.ruangguru.com/question/perhatikan-gambar-tumbuhan-di-bawah-ini-ada-berapa-banyak-kelompok-yang-dapat_QU-PAVVLE5P

The observed protein pattern is inversely associated with carbohydrate content, where conventionally grown water spinach exhibited the highest carbohydrate level (6.02%). This trend reflects differences in plant metabolic allocation, where higher dry matter accumulation in conventional systems is associated with increased carbohydrate content. Since carbohydrates are calculated by difference, variations in water, protein, fat, and ash contents contribute to the observed carbohydrate values (Adrian, 2012). These results indicate that differences in proximate composition are interrelated and associated with nutrient dynamics and plant metabolism under different cultivation conditions rather than individual parameters acting independently.

FUTURE DIRECTIONS

Further research is recommended to analyze in more detail the differences in carbohydrate content between cultivation methods and the factors that influence them. Optimizing nutrition in hydroponics and fish waste composition in aquaponics is necessary to increase protein content and control ash content. Furthermore, analysis of bioactive compounds such as vitamins A and C, antioxidants, and dietary fiber, organoleptic tests (taste, texture, aroma), and shelf life are also important to complete the picture of quality and commercial potential. A study on the effect of fish species in aquaponics on the nutritional content of kale is also necessary.

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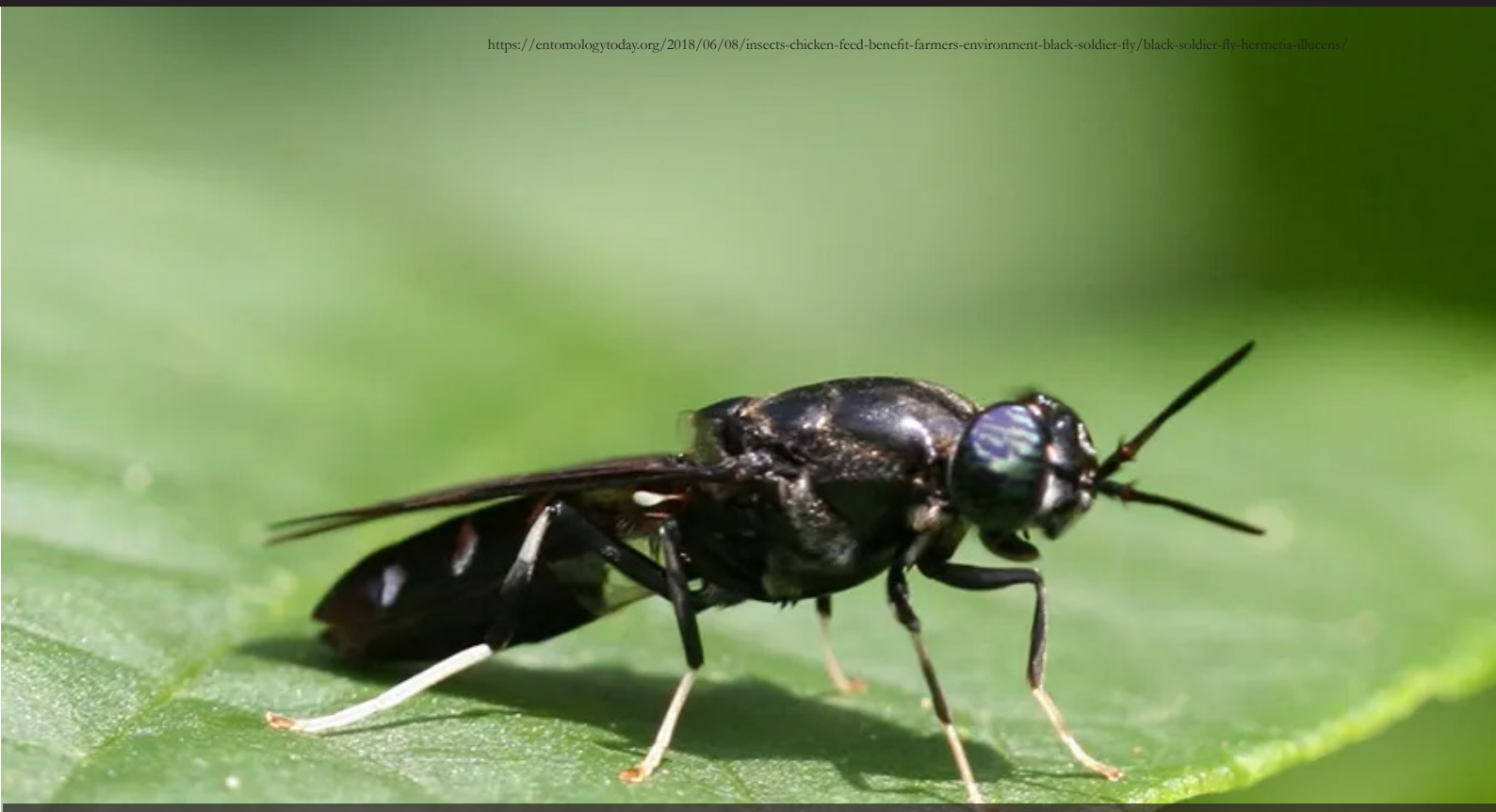

Bioconversion of Oil Palm Waste into Protein-Based Bioplastic Using BSF (*Hermetia illucens*) Larvae

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SYNOPSIS

This study investigated the bioconversion of Oil Palm Empty Fruit Bunches (OPEFB) and Oil Palm Kernel Cake (OPKC) using Black Soldier Fly (BSF; *Hermetia illucens*) Larvae as a strategy for palm oil waste valorization. OPEFB was pretreated through milling, alkaline hydrolysis, and fungal saccharification with Aspergillus niger to enhance digestibility. Protein extracted from BSF larvae was utilized as a precursor for protein-based bioplastics. Larval performance was consistent across treatments, with relative consumption rates (RCR) averaging 0.00498 mg larva-1 day-1, while total waste reduction (D) ranged from 0.85-0.98% and the waste reduction index (WRI) from 2.83.3.27%, with the highest value observed at 7:3 (OPEFB: OPKC) ratio. The resulting bioplastics exhibited biodegradability ranging from 37.76% to 64.34%, along with improved mechanical integrity and reduced water absorption at higher protein content. Overall, this study demonstrates the feasibility of BSF-derived protein as a bioplastic precursor within an integrated palm oil waste management framework.

INTRODUCTION

The palm oil industry constitutes a major pillar of Indonesia's economy, contributing substantially to non-oil and gas exports. In 2023, palm oil and its derivatives generated approximately USD 29.45 billion, accounting for a significant proportion of the national export revenue (BPS-RI, 2024). This economic benefit, however, is accompanied by the large-scale generation of agro-industrial waste. Among the primary by-products are Oil Palm Empty Fruit Bunch (OPEFB), palm Kernel Shell (PKS), and Oil Palm Kernel Cake (OPKC). OPEFB represents a considerable fraction of solid waste streams, posing persistent environmental management challenges when inadequately utilized (Teh, 2016).

Concurrently, Indonesia faces an escalating plastic pollution crisis. In 2023, the country generated 25.22 million tons of waste annually, of which only 66.32% was properly managed, while plastic waste accounted for 18.52%, ranking in 2nd after food waste (SIPSN, 2024). Conventional plastics, including Polypropylene (PP), Polystyrene (PS), and Polyvinyl Chloride (PVC), are derived from fossil-based polymers and exhibit long environmental persistence due to their non-biodegradable nature (Lopez, 2023). In response, increasing attention has been directed toward bioplastics derived from renewable, biodegradable natural polymers, particularly cellulose and proteins.

Previous studies have predominantly focused on cellulose extraction from OPEFB for bioplastic production through intensive chemical treatments such as delignification, bleaching, and acetylation (Saputri and Sukmawan, 2020). While effective, these approaches are often associated with high chemical consumption, environmental risk, and elevated production cost. Moreover, existing research on the black soldier fly (*Hermetia illucens*) has largely emphasized its role in waste reduction and the production of protein-rich biomass, without extending its application to downstream bioplastic synthesis. A critical gap in previous studies is the lack of integrated approaches that couple oil palm waste bioconversion using *Hermetia illucens* with downstream valorization pathways, particularly for the development of high-value biomaterials such as protein-based bioplastics (Bajra *et al.*, 2025; Surendra *et al.*, 2020). To date, the integration of BSF-mediated bioconversion of oil palm waste with the utilization of BSF-derived protein as a bioplastic precursor has not been sufficiently explored.

https://hermetiabioscience.com/page/hermetia-illucens-the-black-soldier-fly

Therefore, this study proposes an alternative, environmentally sustainable strategy that employs BSF larvae to bioconvert OPEFB and OPKC into protein-based bioplastics. Given the recalcitrant lignocellulosic nature of OPEFB, fungal fermentation using *Aspergillus niger* was applied to enhance substrate digestibility and nutrient accessibility before larval feeding (Diyanilla *et al.*, 2020; de Figureiredo *et al.*, 2024). This integrated approach aims to simultaneusly addressed palm oil waste management and plastic pollution by valorizing organic residues into biodegradable, protein-based polymer materials. The resulting bioplastics are expected to provide a cost-effective and environmentally friendly alternative to conventional petroleum-derived plastics, thereby supporting circular bioeconomy and sustainability goals.

https://www.istockphoto.com/id/foto/tampilan-dekat-larva-lalat-tentara-hitam-atau-perhitung-di-telapak-tangan-petani-gm1503707093-523176742

https://www.mdpi.com/2073-4360/17/11/1582

ARTICLE CONTENT

Material and Methods

This study was conducted at the Laboratory of Molecular Biology, the Laboratory of Animal Physiology and Molecular Development, and the Laboratory of Chemistry, all within the Faculty of Mathematics and Natural Sciences, University of Mulawarman, Samarinda, East Kalimantan, Indonesia. Oil Palm Empty Fruit Bunch (OPEFB) and Oil Palm Kernel Cake (OPKC) were obtained from PT REA Kaltim Plantation, Kembang Janggut, Kutai Kartanegara, East Kalimantan, Indonesia. The fungal strain *Aspergillus niger* was obtained from the culture collection of the Laboratory of Microbiology and Molecular Genetics, University of Mulawarman. Black Soldier Fly/BSF (*Hermetia illucens*) larvae were reared from eggs supplied by CV AHASA Larva Group, Samarinda, East Kalimantan, Indonesia. All chemicals used for hydrolysis and protein extraction were of analytical grade.

Fresh OPEFB was thoroughly cleaned, oven-dried at 60°C for 24 hours, and milled into fine fibers (powder). Alkaline hydrolysis was performed using 17.5% (w/v) NaOH at a solid-to-liquid ratio of 1:10, followed by heating at 90°C for 90 minutes to disrupt the lignocellulosic matrix. The resulting hydrolysate was neutralized with distilled water and filtered. The hydrosylates OPEFB and OPKC were subsequently subjected to enzymatic saccharification via fermentation with *Aspergillus niger* at 30°C for 5 days. This pretreatment aimed to enhance cellulose depolymerization and improve nutrient accessibility for larval consumption.

BSF eggs were incubated under controlled conditions until hatching, after which approximately 200 larvae were introduced into each experimental tray. Three fermented substrate formulations were prepared by combining OPEFB and fermented OPKC at different ratios of OPEFB: OPKC, was: 3:7; 5:5; and 7:3. Each treatment was conducted in triplicate (n=3). The substrate was homogenized, adjusted to 10% moisture content, and allowed to stabilize prior to larval introduction. Larval rearing was conducted under controlled environmental conditions (28 ± 2°C; 70% relative humidity) for 12 days, during which substrate consumption and larval weight gain were recorded at two-day intervals.

The substrate consumption rate (SCR) and waste reduction index (WRI) were calculated following the method described by Diener *et al.* (2011).

$$SCR = \frac{W - R}{W} \quad (1)$$

$$WRI(\%) = \frac{D}{t} \times 100 \quad (2)$$

$$D = \frac{W - R}{W} \quad (3)$$

where:

SCR : substrate consumption rate (%)
WRI : waste reduction index (%)
D : total waste reduced
t : reduction time (days)
W : waste before degradation process (gr)
R : waste after degradation process (gr)

All quantitative data were first tested for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. Differences among substrate treatments were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's honestly significant difference (HSD) post hoc test was applied to identify pairwise differences between treatments. Statistical significance was established at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics version 22.

RESULT AND DISCUSSIONS

Effect of Substrate Composition on the Consumption Rate and Growth of BSF Larvae

In this study, the feeding behavior of Black Soldier Fly (BSF) larvae on mixed substrates composed of OPEFB-OPKC exhibited a dynamic pattern characterized by an initial adaptation phase followed by progressive substrate degradation, as shown in Table 1.

In this study, the feeding behavior of BSFL reared on mixed OPEFB-OPKC substrates exhibited a dynamic pattern, characterized by an initial adaptation phase followed by gradual substrate degradation. During the first day of rearing, larvae showed minimal feeding activity, indicating physiological acclimatization to the substrate matrix. A noticeable increase in consumption was observed on the second day, with the average relative consumption rate (RCR) reaching approximately 0.00498 mg larva-1 day-1 across all treatments, suggesting comparable feeding efficiency regardless of substrate ratios.

Despite similar RCR values, differences were observed in total waste reduction (D) and the waste reduction index (WRI), with treatment P3 exhibiting the highest WRI (3.27%) and P1 the lowest (2.83%). Overall, the waste reduction values obtained in this study (<4%) were markedly lower than those reported in previous studies employing nutrient-rich or protein-dominant substrates (Wicaksono *et al.*, 2017; Silalahi *et al.*, 2022). This discrepancy can be attributed primarily to the high lignocellulosic content of OPEFB, which inherently limits digestibility, even after alkaline hydrolysis and fungal fermentation. The recalcitrant structure of cellulose and residual lignin fractions likely constrained larval assimilation efficiency, thereby reducing overall substrate conversion.

Larval growth patterns further reflected these limitations. Although treatment P1 yielded the highest average larval weight and length (Figures 1 and 2), P3 demonstrated a more stable growth trajectory throughout the rearing period. Larval growth increased markedly between days 17 and 23, coinciding with the transition to the fourth instar. However, accelerated substrate depletion observed in P3 may not solely reflect enhanced bioconversion efficiency. Instead, this trend was likely influenced by oviposition events during the early experimental phase, leading to the emergence of additional larvae and a subsequent increase in larval density.

Oviposition introduces a potential confounding factor, as uncontrolled increases in larval population can intensify feeding pressure independently of substrate quality. Elevated larval density may promote faster substrate depletion while simultaneously inducing intra-specific competition, leading to reduced individual larval weight and suppressed feeding activity among larvae entering the pre-pupal stage. Consequently, waste reduction and growth metrics should be interpreted cautiously, as they may reflect combined effects of substrate composition and population dynamics rather than substrate digestibility alone.

Differences between the present findings and earlier reports can also be explained by variations in substrate nutritional balance and moisture content. Wicaksono *et al.* (2017) reported waste reduction rates exceeding 48% using substrates dominated by OPKC, which contains higher protein and lipid fractions favorable for BSF metabolism. In contrast, excessive lignocellulosic content and suboptimal nutrient ratios in OPEFB-based substrates have been shown to constrain larval performance (Silalahi *et al.*, 2022). Additionally, substrate moisture plays a critical role in larval development; excessive moisture can impair oxygen diffusion and promote microbial competition, ultimately inhibiting larval feeding efficiency (Nurkomariyah & Rahmanto, 2023). Therefore, maintaining optimal moisture levels to support effective substrate aeration and larval metabolism.

Statistical analysis revealed no significant differences among treatments ($p > 0.05$), indicating that variations in substrate composition did not exert a statistically significant effect on larval growth or waste reduction under the experimental conditions used in this study.

Table 1 The Relative Consumption Rate (RCR), Total Waste Reduction (D), and Waste Reduction Index (WRI) of BSF larvae

Larvae Treatment	Relative Consumption Rate (RCR) (mg/larvae/day)	Total Waste Reduction (D)	Waste Reduction Index (WRI)
P1	0.004981	0.85%	2.83%
P2	0.004986	0.93%	3.10%
P3	0.004980	0.98%	3.27%

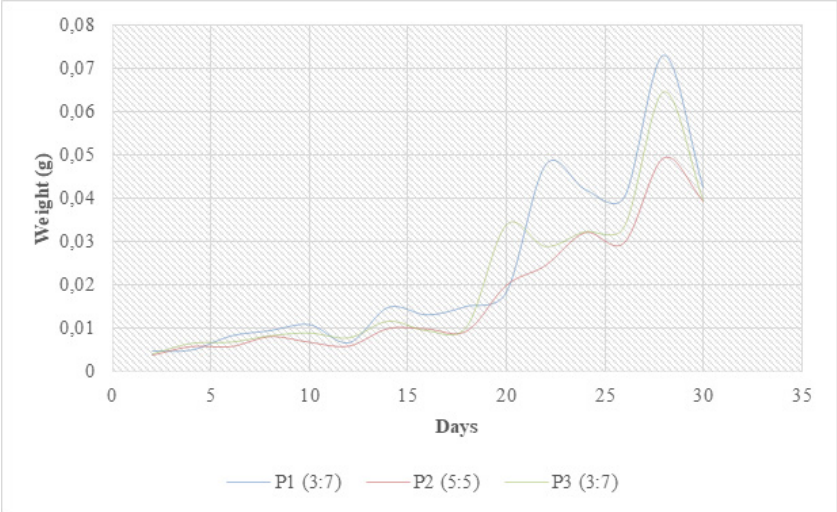

Figure 1 Mean body weight gain of BSF larvae over time across treatments

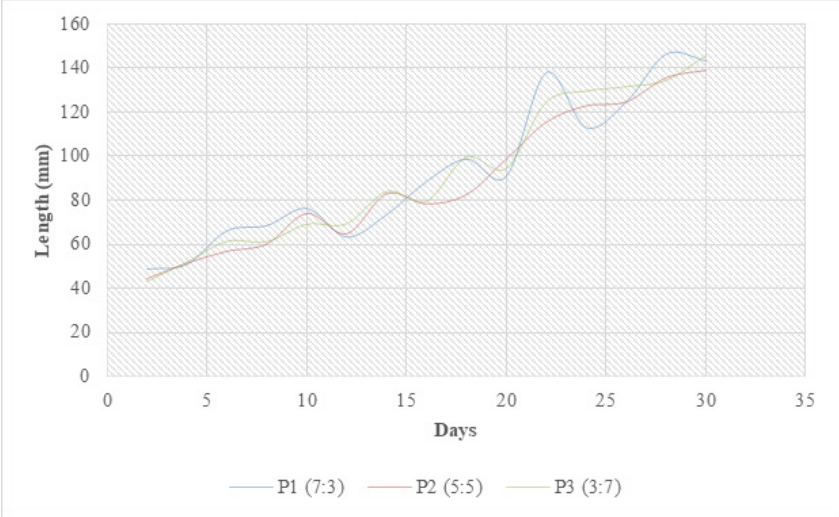

Figure 2 Mean body length gain of BSF larvae over time across treatments

Physical Characteristics and Biodegradability of Protein-Based Bioplastics

At the pupal stage, larvae were harvested, rinsed, and oven dried at 60°C for 48 hours. Protein extraction was performed using a one-step protein extraction (NaOH 0.1 M) followed by isoelectric precipitation at pH 4.5. The resulting protein isolate was dried and stored for bioplastic synthesis. Protein-Based Bioplastics (PBB) were synthesized using the solution-casting method. The isolated protein was dissolved in distilled water containing 2 mL of glycerol as a plasticizer. The solution was stirred at 80°C for 30 minutes until homogeneous, then cast onto glass plates and air-dried.

All bioplastic films exhibited a transparent appearance and comparable elasticity (Table 2). However, distinct differences were observed in tensile strength and structural integrity. Films produced under treatment S1 (3:5:2) appeared thinner and more brittle, whereas those under treatment S3 (5:3:2) displayed superior durability and mechanical cohesion. The enhanced strength of S3 suggests improved polymer matrix formation at higher protein concentrations. The presence of surface granules across samples indicates incomplete protein dispersion or residual non-protein impurities, which may have disrupted polymer homogeneity.

Biodegradability assessment using a soil-burial test revealed substantial variation among formulations. Films with lower protein content (S1 and S2) exhibited higher degradation rates (64.34% and 62.03%, respectively), whereas S3 showed markedly lower biodegradability (36.76%). These findings suggest that increasing protein concentration enhances mechanical stability while reducing susceptibility to microbial degradation, likely due to an increased cross-linking density within the polymer matrix (Lamp *et al.*, 2022; Shah *et al.*, 2023).

https://www.britannica.com/plant/oil-palm

Water absorption analysis further supported these observations. c. Reduced water absorption suggests enhanced hydrophobicity and a more compact polymer packing, thereby limiting pore formation and water diffusion (Gurbuz *et al.*, 2024). Given the amphipathic nature of proteins, the balance between hydrophilic functional groups (-OH, -CO) and hydrophobic interactions is critical for determining film stability and degradation behavior (Purewal *et al.*, 2023).

Overall, these results demonstrate that increasing BSF-derived protein concentration improves the mechanical robustness and water resistance of protein-based bioplastics, albeit at the expense of biodegradation rate. Optimizing this trade-off is essential for tailoring bioplastic formulations toward specific applications that require both functional durability and environmental degradability.

Table 2 Characteristics of dope-solution, biodegradability, and water absorption of bioplastics

Ratio (protein: water: glycerol)	Characteristics of dope-solution			Biodegradability (%)	Water Absorption (%)
	Visual	Elasticity	Strength		
S1 (3:5:2)	Transparent	Elastics	Delicate	64.34	76.04
S2 (4:4:2)	Transparent	Elastics	Sufficiently strong	62.03	69.30
S3 (5:3:2)	Transparent	Elastics	More durable	36.76	64.50

FUTURE DIRECTIONS

Future Direction on Protein Isolation and Bioplastics Characterization

Future studies are recommended to focus on optimizing protein extraction techniques to achieve higher yield and improved purity, as these parameters directly influence the structural integrity and performance of protein-based bioplastics. Advanced extraction strategies, including enzymatic hydrolysis, membrane separation, and multi-step purification, should be explored to enhance protein homogeneity and functionality within the polymer matrix. Improved protein purity is expected to minimize the presence of residual non-protein components that may compromise film uniformity.

https://materialdistrict.com/article/redefining-palm-oil-turning-waste-into-bioplastic/

In addition, subsequent research should incorporate a comprehensive instrumental characterization of bioplastic properties. In the present study, mechanical performance was assessed qualitatively based on visual appearance and handling characteristics; therefore, future investigations should employ standardized mechanical testing methods to obtain quantitative parameters such as tensile strength, elongation at break, and Young's modulus. Furthermore, the occurrence of surface granules observed in several film formulations represents a material limitation that warrants further investigation. Future work should address this issue by improving protein dispersion techniques, optimizing casting conditions, or applying homogenization and filtration steps.

Advanced analytical techniques, including Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM), are essential for elucidating molecular interactions, thermal stability, crystallinity, and microstructural features of protein-based bioplastics. Integrating these analyses will provide deeper insight into structure-property relationships and facilitate the rational refinement of formulation strategies tailored to specific functional and environmental performance requirements.

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Intan Rohyana Devi

Intan Rohyana Devi is a Biology graduate from the Faculty of Mathematics and Natural Sciences, Universitas Mulawarman. She has a strong interest in the utilization of Black Soldier Fly larvae (BSFL) derivatives, particularly protein and non-protein flours as well as saturated oils. Passionate about sustainable bioresources, Intan explores how BSFL-based products can be optimized for environmental and industrial applications. Her work reflects a growing interest in transforming organic waste into value-added biological materials that support circular bioeconomy initiatives.

Maharana

Maharana is a Biology graduate from the Faculty of Mathematics and Natural Sciences, Universitas Mulawarman, with a keen interest in microbiology and bacterial ecology. Her academic work focuses on exploring the potential of bacteria to degrade organic compounds and inhibit microbial activity. She has also investigated the diversity of gut bacteria associated with Black Soldier Fly larvae, aiming to better understand microbial interactions that influence bioconversion efficiency and environmental sustainability.

Maharani

Maharani is a Biology graduate from the Faculty of Mathematics and Natural Sciences, Universitas Mulawarman, with a strong passion for plant biotechnology. Her primary interest lies in plant tissue culture, particularly within the Orchidaceae family. Through her academic work, she explores in vitro propagation techniques as tools for plant conservation, breeding, and sustainable horticulture, with an emphasis on preserving plant diversity through biotechnological approaches.

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Innovative Utilization of Agricultural Waste for Sustainable Bio-Based Materials Development

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SYNOPSIS

Agricultural waste poses serious environmental challenges while offering untapped opportunities for bio-based innovation. This aim study is to evaluate an integrated process for converting agricultural residues (rice husks, corn cobs, and sugarcane bagasse) into sustainable bio-based composites. We applied pyrolysis (450–500 °C), biocomposite synthesis, and green-chemistry techniques to produce and characterize novel biomass-derived materials. The resulting composites exhibited high thermal stability (≈280 °C) and tensile strength (15–28 MPa), highlighting their potential as alternatives to petroleum-based plastics. This approach also reduced open burning and carbon emissions by about 35%, supporting biodiversity, bioenergy development, and SDG 12 (responsible consumption and production). These findings demonstrate a viable pathway for valorizing agricultural waste in a circular bio-based economy.

https://www.homebiogas.com/blog/agricultural-waste-management/?srsltid=AfmBOooOUkO0c8CS-H1DjLDp8DavUrE74IvuiHnlAWTHRQwc3kf-nPWx

INTRODUCTION

Indonesia produces over 55 million tons of agricultural waste annually, much of which remains underutilized or openly burned, contributing to severe air pollution and greenhouse gas emissions (FAO, 2022; UNEP, 2021). Common residues such as rice husks, corn cobs, and sugarcane bagasse represent more than 40% of total agricultural biomass in Southeast Asia (Zulkifli, Rahim, & Noor, 2020). Improper waste management not only depletes environmental quality but also neglects a valuable source of renewable materials and energy, creating a critical research gap in developing efficient conversion technologies and sustainable utilization strategies for agricultural residues (Setyawan, Rahayu, & Nugraha, 2021).

The sustainable utilization of agricultural residues aligns with global commitments to the Sustainable Development Goals (SDGs), particularly Goals 7 (Affordable and Clean Energy), 9 (Industry, Innovation, and Infrastructure), 12 (Responsible Consumption and Production), and 13 (Climate Action) (United Nations, 2015). By integrating biotechnology, bioenergy, and material innovation, agricultural waste can be transformed into high-value bioproducts that contribute to both environmental protection and economic resilience (Singh & Tan, 2022; Jumaah, Ali, & Hassan, 2020).

This study explores how circular material technology specifically pyrolysis, biocomposite synthesis, and green chemistry can convert waste biomass into bio-based materials. The objectives are to (1) reduce environmental pollution from waste burning, (2) improve material circularity, and (3) strengthen sustainable industrial ecosystems through renewable resources (Mansor, Hassan, & Yahya, 2019; Rahman, Ismail, & Ahmad, 2021).

ARTICLE CONTENT

Integration of Bioenergy and Biotechnological Innovations

The transformation of agricultural waste into circular materials fundamentally depends on the integration of bioenergy systems and biotechnological processes. As global energy demand continues to increase, agriculture remains both a major consumer of energy and a substantial generator of biomass residues. Agricultural by-products such as rice husks, corn cobs, and sugarcane bagasse—which together account for nearly 40% of total biomass residues in Southeast Asia are now recognized as valuable resources rather than waste (Zulkifli, Rahim, & Noor, 2020). Through pyrolysis conducted at controlled temperatures of 450–500°C, these lignocellulosic materials can be thermochemically converted into biochar, bio-oil, and syngas, all of which are precursors for renewable energy and composite materials (Mansor, Hassan, & Yahya, 2019; Demirbas, 2020).

Pyrolysis represents a cornerstone of circular bioenergy technology. The resulting biochar can be applied as a soil amendment to improve fertility, increase cation exchange capacity, and enhance water retention, ultimately contributing to carbon sequestration and climate change mitigation (Lehmann & Joseph, 2015). Meanwhile, bio-oil can substitute for fossil-based fuels in industrial applications or serve as a feedstock for the synthesis of biopolymers, while syngas can be

used to generate electricity (UNEP, 2021). This system embodies the zero-waste principle, ensuring that each product and by-product from the process contributes to sustainable resource loops. Moreover, this approach significantly reduces net greenhouse gas emissions by mitigating the need for open-field burning of biomass, which is a major source of air pollutants and carbon dioxide (Singh & Tan, 2022).

In addition to environmental benefits, integrating bioenergy with biotechnology creates cross-sectoral synergies between agriculture, waste management, and industrial manufacturing. Biotechnological tools such as enzymatic catalysts can be used to improve the efficiency of pyrolysis by breaking down recalcitrant lignin structures before thermochemical processing. This integration enables not only energy recovery but also material valorization, making the process both ecologically and economically viable (Gupta & Verma, 2015). Consequently, the convergence of bioenergy and biotechnology establishes a scientific and technological foundation for advancing sustainable material innovation. This connection naturally leads to the next stage-transforming the products of bioenergy processes into functional materials through the development of lignocellulosic biocomposites.

Development of Lignocellulosic Biocomposites

The development of lignocellulosic biocomposites represents a promising innovation in sustainable material science. These composites are derived from natural fibers with high cellulose, hemicellulose, and lignin content, imparting excellent strength and biodegradability. Agricultural residues such as rice husks, corn stalks, and sugarcane bagasse serve as abundant renewable fiber sources (Rahman, Ismail, & Ahmad, 2021). Processing these fibers into polymer matrices yields lightweight yet durable composites that can replace petroleum-based plastics (Jawaid & Abdul Khalil, 2019).

Research shows that incorporating natural fibers enhances tensile strength and elasticity while maintaining thermal stability. For example, sugarcane bagasse composites achieve tensile strengths of 15–28 MPa and remain stable up to 280°C, comparable to commercial thermoplastics (Mansor *et al.*, 2019; Rahman *et al.*, 2021). Beyond performance, these composites are biodegradable, recyclable, and emit up to 35% less lifecycle CO_2 than synthetic polymers (Singh & Tan, 2022).

The chemical composition of lignocellulosic fibers, with strong hydrogen bonding between cellulose chains, improves adhesion between fiber and polymer matrices (Dungani, Sari, & Abdul Khalil, 2020). This strengthens mechanical properties and reduces microplastic pollution from synthetic material degradation. Thus, developing bio-based composites extends the value chain of bioenergy products while promoting eco-functional materials that unify industrial innovation and environmental stewardship.

https://balkangreenenergynews.com/energy-production-from-biomass-still-large-part-of-mix-in-region-undp/

Biotechnological Enhancement in Biomass Conversion

Biotechnology is an essential enabler in converting agricultural residues into high-value bio-based materials. Key approaches include enzymatic hydrolysis, microbial fermentation, and genetic modification of lignocellulosic feedstocks. These methods enhance cellulose and hemicellulose accessibility by breaking down lignin barriers (Jumaah, Ali, & Hassan, 2020). Enzymes such as cellulases, hemicellulases, and laccases hydrolyze complex polysaccharides into fermentable sugars, which can be converted into bioethanol, biogas, or biosynthetic polymers (Zhang, Li, & Chen, 2021).

Microbial fermentation also transforms biomass-derived sugars into bio-based chemicals like polylactic acid (PLA) and polyhydroxyalkanoates (PHAs), widely used in biodegradable plastics (Kim, Lee, & Park, 2022). These biological processes complement thermochemical methods by ensuring full biomass utilization and reinforcing the circular bioenergy loop. Modern genetic engineering improves microbial strains for higher enzymatic activity and stress tolerance, optimizing production under industrial conditions (Gupta & Verma, 2015). For instance, CRISPR-based genetic editing enables microorganisms to process mixed biomass waste more efficiently, reducing input costs and maximizing yields (Zhang *et al.*, 2021). Collectively, these innovations link agriculture, energy, and manufacturing, positioning biotechnology as a key catalyst for circular resource systems.

Environmental and Ecological Implications

The sustainable valorization of agricultural residues contributes to biodiversity conservation, ecosystem restoration, and climate resilience. Open-field burning-still common in many developing countries-emits high levels of $PM_{2.5}$, CO_2, and NO_x, causing air pollution,

global warming, and health problems (Setyawan, Rahayu, & Nugraha, 2021; UNEP, 2021). Converting this biomass into usable materials mitigates these impacts and supports the recovery of degraded lands. Using residues as raw materials also reduces the need for land-intensive energy crops, maintaining balance between food and energy systems (FAO, 2022). This integration prevents competition for arable land and preserves soil fertility. Biochar application enhances microbial activity, nutrient retention, and crop productivity while sequestering carbon in stable forms (Lehmann & Joseph, 2015).

From a broader sustainability perspective, agricultural waste valorization aligns with the United Nations Sustainable Development Goals (SDGs)-particularly SDG 12 on responsible consumption and SDG 13 on climate action (United Nations, 2015). It sustains resource cycles within planetary limits while promoting green industrialization and ecosystem protection.

Industrial Applications and Economic Feasibility

Pilot-scale initiatives in Indonesia, Malaysia, and Thailand demonstrate the feasibility of industrializing agricultural waste-based composites. These projects show promising results, with biocomposites achieving tensile strengths of 15–28 MPa and thermal stability up to 280°C (Mansor *et al.*, 2019; Zulkifli *et al.*, 2020). Their applications range from construction materials and lightweight furniture to packaging and automotive components (Dungani *et al.*, 2020).

Economically, this industry offers competitive advantages through low feedstock costs and the added value of circular production. Global demand for bio-based composites is projected to grow 12% annually to 2030, supported by green policies and market preference for sustainable materials (Rahman *et al.*, 2021). Challenges remain, including supply inconsistency, quality standardization, and limited financing (Singh & Tan, 2022). Addressing these issues through public–private collaboration and certification systems is key to scaling production. These industrial insights highlight the need for stronger alignment between technology, policy, and markets-laying the foundation for a sustainable circular industry.

Toward Sustainable Circular Industry

In conclusion, the development of a sustainable circular industry fundamentally requires strong synergy among academia, industry, and government. Academic institutions play a crucial role in advancing research on bioenergy, biopolymers, and environmental biotechnology, while governments provide enabling policies and access to green financing to support implementation (BAPPENAS, 2023). The Indonesia Circular Economy Roadmap 2023–2040 further reinforces the strategic importance of renewable energy development, efficient manufacturing systems, and waste valorization.

The adoption of eco-design principles, renewable resource management, and digital monitoring systems will substantially enhance production efficiency and transparency. The integration of bioenergy utilization, biotechnology, and biodiversity conservation establishes a resilient bio-based economy that balances societal welfare, food security, and environmental sustainability (FAO, 2022; United Nations, 2015). Ultimately, the advancement of this circular industry will not only reduce ecological footprints but also promote green employment opportunies and strengthen national economic and environmental resilience.

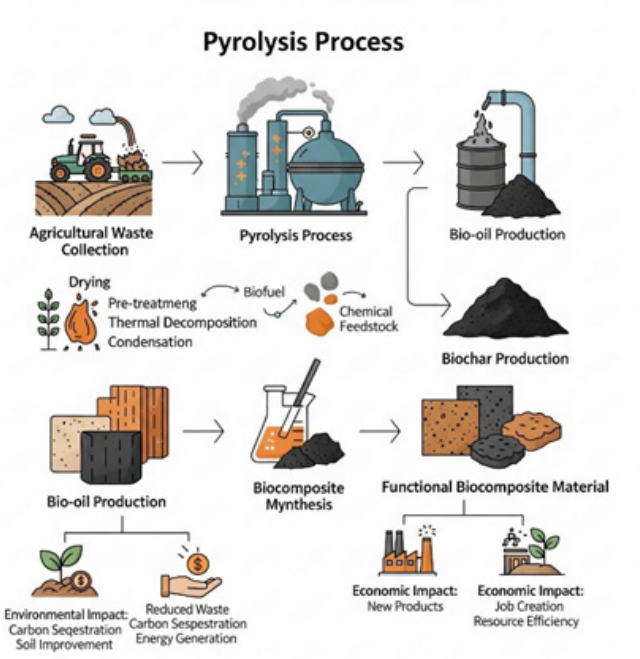

Figure 1 Integrated Pyrolysis Pathway for Transforming Agricultural Waste into Functional Biocomposites
Source: Author's own documentation (personal document, 2025).

FUTURE DIRECTIONS

To advance sustainable bio-based innovation, several actions are recommended:

1. Policy Integration: Governments should strengthen circular economy policies linking agriculture, energy, and industry. Incentives for waste-based material production can accelerate adoption and support green industrial transformation.
2. Technological Innovation: Further research in bio-catalysis, nanobiotechnology, and enzyme-assisted synthesis can enhance material properties, optimize conversion efficiency, and reduce production costs.
3. Community Involvement: Local farmers must be involved in biomass collection and processing cooperatives to ensure inclusive participation and equitable economic benefits.
4. Education and Awareness: Sustainable waste utilization should be integrated into agricultural curricula, vocational training, and public outreach programs to foster knowledge transfer and behavioral change.
5. Biodiversity Protection:. Promoting waste valorization can reduce deforestation, mitigate land degradation, and preserve ecosystems, thereby supporting global biodiversity and climate resilience targets.

Ultimately, achieving a sustainable bio-based future requires more than technological progress- it demands collective vision, interdisciplinary collaboration, and shared responsibility. Every stakeholder, from policymakers and scientists to farmers and consumers, holds a vital role in shaping a circular economy that respects nature's boundaries. By transforming agricultural waste into valuable resources, all stakeholders are strongly encouraged to take immediate and concrete action in adopting sustainable practices, accelerating innovation, and actively supporting policies that drive the transition toward a regenerative development model capable of sustaining both present and future generations.

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Ecosystem Restoration and Conservation in Singkarak Lake Protection Area: Impacts of Reclamation

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SYNOPSIS

Reclamation and landfilling activities within protected zones pose serious threats to ecosystem sustainability by altering natural land–water interactions and violating the essential functions of Singkarak Lake's riparian buffer. This study aims to map the extent of reclamation, examine legal violations related to spatial and environmental permits, and assess ecological and economic losses. A mixed-methods approach was used, including geospatial analysis, normative legal review, and ecocentric valuation of environmental damage. Results show that 2,976 m^2 of land was illegally reclaimed within the protected lake boundary. Overlay with the regional spatial plan confirms non-compliance with zoning regulations and the absence of required permits. The total estimated loss amounts to Rp 3,383,291,152, largely driven by disrupted hydrological functions and reduced land utility.

https://www.galerisumba.com/pariwisata/87610306784/destinasi-liburan-yang-mengagumkan-danau-singkarak-pesona-alam-dan-misteri-kuno-di-sumatera-barat?page=2

INTRODUCTION

Lake degradation has become a sensitive national issue requiring urgent government intervention. Presidential Regulation No. 60 of 2021 on the Rescue of National Priority Lakes highlights severe pressures on many lakes in Indonesia, including watershed degradation, shoreline damage, declining water quality, reduced biodiversity, increased erosion and sedimentation, and the disappearance of endemic species. Uncontrolled reclamation in the Lake Singkarak buffer zone simultaneously degrades ecological integrity and causes significant economic losses for local communities. Coordinated efforts across ministries, local governments, and stakeholders are required to restore and safeguard these vital ecosystems.

Lake Singkarak is the largest tectonic lake in West Sumatra, located within the administrative boundaries of Tanah Datar and Solok Regencies. The lake receives water from the Sampur River in the north and the Sumani River in the south. It supports multiple community needs, including fisheries, irrigation, hydropower generation, domestic use, and tourism. Despite its ecological and economic significance, the lake's condition has deteriorated due to poor management and increasing human pressure. One major issue is illegal reclamation along the shoreline, particularly in the Singkarak Dock area in X Koto Singkarak District. Such reclamation activities are suspected to have caused environmental damage by altering natural land–water interactions and disrupting the ecological balance of the lake. Based on this context, the objectives of this study are: (1) to map the extent of the reclamation area, (2) to identify legal violations related to land use and environmental regulations, and (3) to assess the environmental impacts arising from the reclamation. The research was conducted in Jorong Kaluku, Nagari Singkarak, X Koto Singkarak District.

ARTICLE CONTENT

Research Methods

This study is multidisciplinary research that combines various approaches to data collection and assessment in order to address the problem. The study uses several methods, including geo-information mapping techniques, legal study methods, and environmental impact evaluation methods. The data used in this study consist of primary and secondary data. Primary data include interviews with local community activities and policy stakeholders, as well as aerial photographs of the reclaimed area. Secondary data include satellite images from Google Earth in 2016 and 2021, legal spatial planning maps in the form of regional spatial planning regulations (RTRW), inventories of legal products from the government, and literature related to the problem.

Mapping of Reclamation Areas

This study used on-screen digitization techniques to collect and analyze spatial data on reclamation areas along the lakeshore. The reclaimed zones were delineated from Google Earth satellite imagery and verified by comparison with aerial photographs obtained from drone surveys (Hidayat and Hanif, 2020).

Normative Legal Assessment

For the identification of legal violations in spatial utilization in this study, a normative legal study method was used. In this method, which is fundamental in positive law, a secondary data approach was applied through a literature study by reviewing related legislation and literature relevant to the problem being researched. At this stage, deductive reasoning was used, in which jurisprudence and doctrine were placed as the major premise (Benuf and Azhar, 2020).

Extensive Economic Analysis (Ecocentric)

The calculation of losses resulting from pollution and/or environmental damage considers the overall value of nature and living systems. Extensive economics is characterized by an economic perspective based on ecocentrism, which recognizes the intrinsic value of ecosystems and natural resources (Wasis 2006; Wasis et al. 2018b).

Environmental economic valuation of environmental damage generally consists of three main components: (1) ecological losses, (2) economic losses, and (3) environmental recovery costs. The valuation process involves assigning a monetary value to pollution and environmental degradation in order to estimate the total losses caused by environmental damage.

Table 1 Standard calculation mark loss consequence damage environment

A	Loss Ecology	Rupiah/Ha (Rp)
1	Cost Turn on Water Management Function	40,500,000,
2	Cost Soil Formation	500,000
3	Recycling Costs Nutrient	4,610,000
4	Cost Water Management	22,810,000
5	Cost Erosion and Runoff Control	6,000,000
B	**Economic Loss**	**Rupiah/M² (Rp)**
1	Lost Land Use Life	32,000,000
C	**Recovery Environment**	**Rupiah/M² (Rp)**
1	Recovery Water Management Function	40,500,000,
2	Land Recovery	500,000
3	Recovery Nutrient	4,610,000
4	Recovery Water Management	22,810,000
5	Erosion and Runoff Recovery	6,000,000

Source: (1) Regulations Minister of Finance No. 7 of 2014 concerning change make a loss consequence pollution and damage environment , (2) Law No. 17 of 2003 concerning State finances , (3) Law No. 1 of 2004 concerning State treasury

Because this study uses multiple methods and approaches, including technical, legal, and environmental data analyses, the research process is presented in the following flowchart to facilitate understanding.

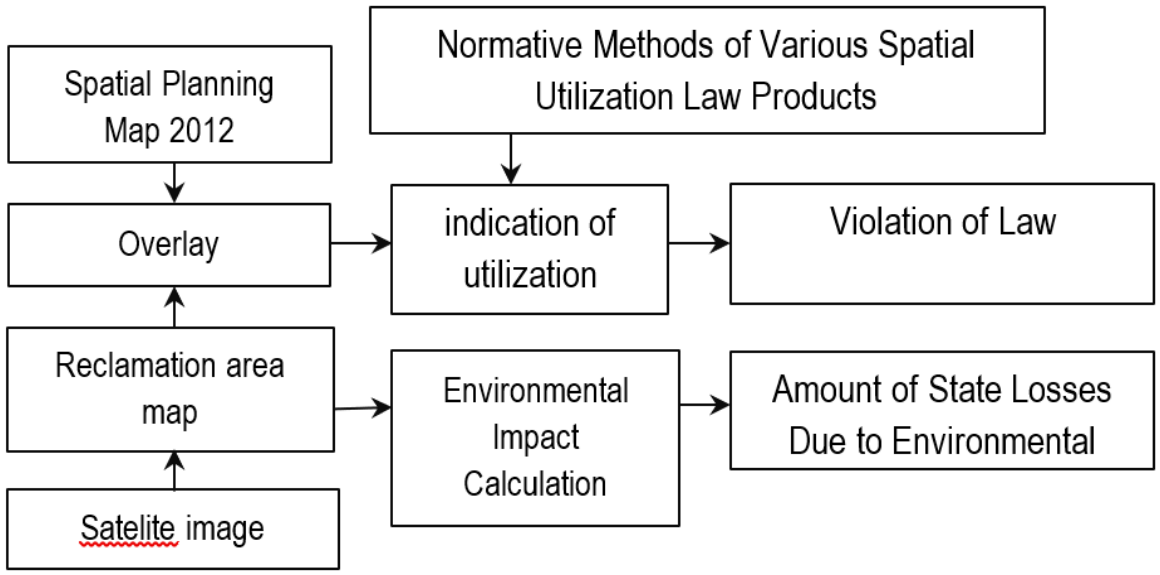

Figure 1 Flowchart study

RESULTS AND DISCUSSION

Dynamics of Reclamation Areas

This study used pre-reclamation satellite imagery from 2015 and post-reclamation imagery from 2021, verified with aerial photographs from 2022, to digitize and identify reclaimed areas along the shoreline of Lake Singkarak. Spatial analysis shows that 2,976 m² of the lakeshore has been reclaimed, causing significant disruption to ecosystem structure and energy flow processes.

Activity Status Identification Reclamation

Overlay analysis between reclamation activities and the Solok Regency spatial planning map identified both legal and illegal reclamation within the Lake Singkarak buffer zone, located less than 100 meters from the highest lake water level and designated as a locally protected area. The RTRW map shows no allocation for infrastructure or settlement development around the lake, confirming that the entire lakeshore is zoned as a protected area.

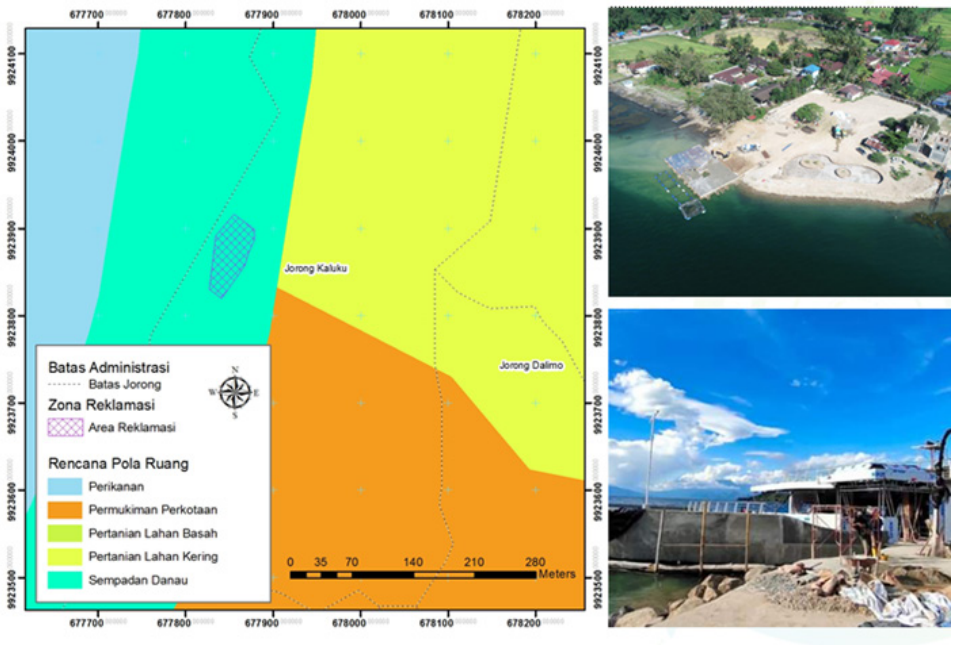

Figure 2 Location area map reclamation lake with area of 2,976 m 2 and RTRW Map of Regional Regulation No. 1 of 2013, Solok Regency , Singkarak Lake Area

Table 2 Calculation impact loss consequence reclamation of Lake Singkarak

A	Loss Ecology	Rupiah (Rp)
1	Cost Turn on Water Management Function	1,205,280,000
2	Cost Soil Formation	148,000
3	Recycling Costs Nutrient	1,364,560
4	Cost Water Management	6,788,256
5	Cost Erosion and Runoff Control	1,776,000
	Total	**1,215,485,576**

	Loss Consequence Reclamation	Rupiah (Rp)
1	Ecological Loss	1,215,485,576
2	Economic Loss	952,320,000
3	Kergian Environment	1,215,485,576
	Total	**3,383,291,152**

B	Economic Loss	Rupiah/M² (Rp)
1	Lost Land Use Life	952,320,000
	Total	**952,320,000**

C	Recovery Environment	Rupiah/M² (Rp)
1	Recovery Water Management Function	1,205,280,000
2	Land Recovery	148,000
3	Recovery Nutrient	1,364,560
4	Recovery Water Management	6,788,256
5	Erosion and Runoff Recovery	1,776,000
	Total	**1,215,485,576**

Source : Results of data analysis for 2025

Under Solok Regency Regulation No. 1 of 2013 and the ATR/BPN Ministerial Regulations Nos. 14 and 12 of 2021, lake buffer zones are designated as protected areas with strictly limited land-use functions. Legal analysis and official confirmation from environmental authorities indicate that the reclamation activity lacked permits, did not meet public or national strategic needs, and is therefore illegal and contrary to applicable spatial planning regulations.

Referring to Article 69 (1) of the Undang-Undang Cipta Kerja, any person who conducts business and/or activities that utilize spatial areas without possessing a spatial utilization conformity permit, as referred to in Article 61 letter (a), resulting in changes to spatial functions, shall be subject to criminal sanctions of imprisonment for a maximum of three years and a fine of up to IDR 1,000,000,000 (one billion rupiah). In addition, reclamation activities carried out without an environmental permit are illegal and are further regulated in Government Regulation No. 5 of 2021 concerning Risk-Based Business Licensing and Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management.

The valuation results show total state losses of Rp 3,383,291,152, consisting of ecological losses of Rp 1,215,485,576, economic losses of Rp 952,320,000, and environmental recovery costs of Rp 1,215,485,576, with the largest impact related to water resource restoration and land-use change. Environmental economic valuation assigns monetary values to pollution and environmental damage as a basis for compensation, restoration, and the sustainable management of natural resources.

CONCLUSION AND SUGGESTIONS

Spatial analysis using pre-reclamation satellite imagery (2015) and post-reclamation data (2021), validated with aerial photographs (2022), identified 2,976 m² of reclaimed land within the Lake Singkarak buffer zone. This reclamation has significantly disrupted lakeshore ecosystems and energy flow processes. Environmental economic valuation indicates total losses of Rp 3,383,291,152, comprising ecological losses of Rp 1,215,485,576, economic losses of Rp 952,320,000, and environmental recovery costs of Rp 1,215,485,576, with the highest impact related to water resource restoration. These findings highlight the need for stricter enforcement of lake protection regulations and the prioritization of ecosystem restoration in reclaimed buffer areas.

FUTURE DIRECTIONS

The findings of this study demonstrate that reclamation activities within the Lake Singkarak buffer zone have caused measurable ecological and economic losses, highlighting the urgent need for stronger governance and restoration efforts. Future actions must prioritize ecosystem recovery while preventing further degradation of lake protection areas.

First, local and regional governments should strengthen law enforcement and spatial planning compliance by strictly regulating land use within lake buffer zones. Reclaimed areas that violate existing regulations should be subject to ecological restoration obligations, including shoreline rehabilitation, reforestation with native vegetation, and the recovery of hydrological functions. Integrating environmental economic valuation into licensing and monitoring processes is also essential to ensure that environmental damage is fully accounted for in decision-making.

Second, long-term ecological monitoring should be established to assess changes in water quality, biodiversity, and sedimentation dynamics in Lake Singkarak. The use of remote sensing, GIS-based monitoring, and community-based reporting systems can enhance transparency and enable the early detection of illegal or harmful activities.

Third, researchers are encouraged to expand interdisciplinary studies that combine ecological assessment, socio-economic valuation, and legal analysis to better understand the cumulative impacts of reclamation and other land-use changes in lake ecosystems. Comparative studies across different lake protection areas in Indonesia would also contribute to stronger national policy frameworks.

Finally, public participation plays a critical role in lake conservation. Local communities, civil society organizations, and the broader public are encouraged to actively engage in monitoring, advocacy, and sustainable lake management initiatives. Raising public awareness about the ecological functions of lake buffer zones and the long-term consequences of reclamation is vital for fostering collective responsibility. Protecting Lake Singkarak requires coordinated action from government institutions, researchers, and society. Immediate and sustained efforts are necessary to safeguard freshwater ecosystems and ensure their ecological and socio-economic benefits for future generations.

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We gratefully acknowledge the support of all institutions and individuals who contributed to the data collection, analysis, and fieldwork for this study. Special thanks are extended to the local communities around Lake Singkarak for their cooperation and valuable insights during the research process.

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Guardians of the Forest Floor: Learning Conservation from the Asian Forest Tortoise (*Manouria emys*)

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SYNOPSIS

Deep within the tropical forests of Southeast Asia, the Asian forest tortoise (*Manouria emys*) moves with the quiet memory of millions of years. Yet today, its path is threatened by deforestation, illegal trade, and a warming climate. In Bengkulu, Indonesia, the University of Bengkulu's ex-situ program has successfully increased the survival and breeding success of rescued tortoises, providing controlled habitats where health, behavior, and reproduction can be monitored. Supported by collaboration with the University of North Carolina Greensboro, the program rehabilitates confiscated individuals and prepares them for long-term population recovery. Beyond protecting a species, this initiative transforms conservation into education, where the tortoise becomes a living teacher of patience, empathy, and coexistence.

INTRODUCTION

The Ancient Guardian Under Threat

The Asian forest tortoise (*Manouria emys*), also known in Indonesia as kura-kura baning coklat, is the largest terrestrial tortoise in Southeast Asia and one of the region's most ancient reptiles. Once abundant across tropical forests from Myanmar to Sumatra, this species now faces a perilous decline. It is currently listed as Critically Endangered by the International Union for Conservation of Nature (IUCN), with populations estimated to have dropped by as much as 80% over the last three generations due to illegal trade and habitat loss. Expanding agriculture, deforestation, and wildlife trafficking have pushed *M. emys* to the brink of extinction, making conservation efforts both urgent and complex.

In Indonesia, the University of Bengkulu has emerged as a pioneering institution addressing these challenges through innovative ex-situ conservation. Its program is built on three pillars: (1) animal care and breeding, (2) international collaboration, and (3) community-based education. Within the university's captive facilities, individuals of *M. emys* are monitored for growth, behavior, and reproductive success. Although advanced genetic studies have yet to be fully implemented, the initiative has already shown promising outcomes, particularly in improving breeding success and maintaining population stability.

A crucial factor supporting this progress is the university's partnership with the University of North Carolina at Greensboro (UNCG). This collaboration strengthens technical capacity in animal health management, husbandry, and monitoring, illustrating how scientific exchange can elevate conservation practice.

Figure 1 *Manouria emys* at The University of Bengkulu (UNIB)
Source: Turtle Learning Center (TLC) University of Bengkulu, 2025.

Yet the fight against illegal trade remains formidable. Between 2000 and 2015, CITES recorded more than 500 *M. emys* individuals seized from illegal markets, many originating from Sumatra. In response, the University of Bengkulu has expanded its role beyond the laboratory, engaging local communities through environmental education and legal awareness programs. These efforts reflect a broader transformation: from species protection to integrated conservation learning, where research, education, and advocacy merge to safeguard one of Southeast Asia's last living forest guardians. This article highlights how Bengkulu's ex-situ program, supported by international collaboration and community engagement, provides a practical and educational pathway for sustaining the future of *Manouria emys*.

https://palmoildetectives.com/2021/01/25/asian-giant-tortoise-manouria-emys/

ARTICLE CONTENT

Ex-Situ Conservation: A Beacon from Bengkulu

In Bengkulu, on the western coast of Sumatra, conservationists have found new purpose in protecting *Manouria emys*, the Asian forest tortoise. Once roaming freely beneath the rainforest canopy, the species now finds refuge within the care of researchers and students. The University of Bengkulu's ex-situ conservation program has become a small but vital sanctuary where this ancient reptile is studied, bred, and nurtured under controlled yet naturalistic conditions. The program follows a clear conservation method: rescued or confiscated individuals are placed in semi-natural enclosures, monitored daily for health and behavior, supported with regulated diet and microclimate, and gradually rehabilitated to ensure stability before potential reintroduction.

The facility focuses on three major aspects: animal maintenance, breeding management, and behavioral observation. Through careful daily monitoring, researchers track growth, diet composition, soil temperature, and microclimate, ensuring that captive conditions mimic the tortoise's natural habitat. Reproductive biology and hormonal cycles are studied to optimize egg production and hatching rates, while health screening helps prevent common reptilian diseases that could threaten population recovery.

Recent research underscores that understanding the tortoise's physiology is key to sustaining a viable captive population. Studies worldwide (such as those by Cigler *et al.* and Gallardo-Alvarez *et al.*) have shown how hormonal analysis and genetic monitoring can maintain reproductive health and genetic diversity, reducing the risk of inbreeding. While such advanced technologies are still developing in Indonesia, the University of Bengkulu is laying the groundwork for their implementation, bridging local fieldwork with global scientific standards.

Although Indonesia's current ex-situ initiatives remain limited compared to large-scale programs in Madagascar or Mexico, Bengkulu's efforts stand out for their integration of science, ethics, and education. As one researcher remarked, "Each hatchling born here is more than a number, it's a story of survival, hope, and human responsibility". This section presents how the program operates and highlights its significance as a model for community-based conservation in Indonesia.

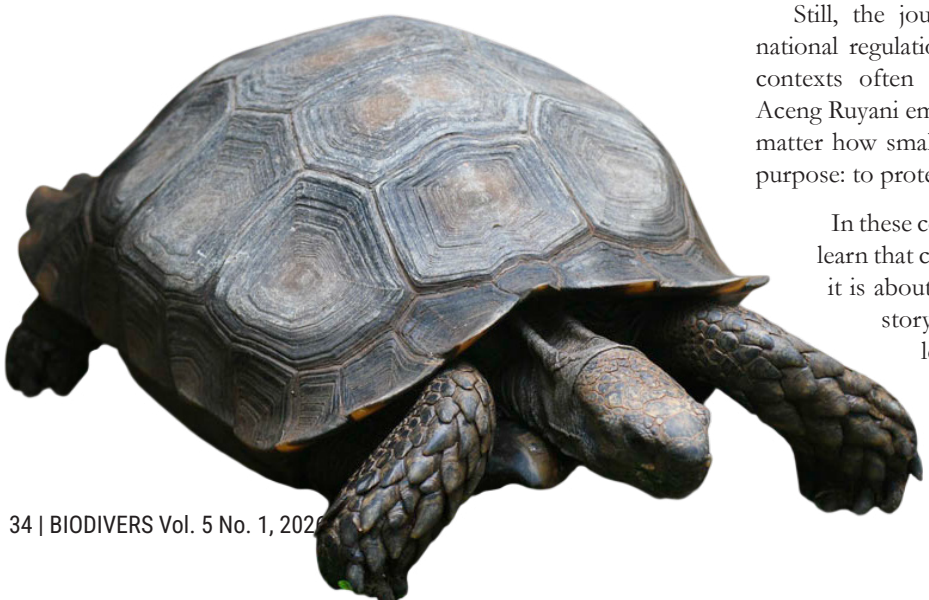

Figure 2 The International Project Team for the Conservation of Sumatran Turtles, The University of Bengkulu
Source: Ruyani et al., 2022.

Science Without Borders: Collaboration Across Continents

Behind every successful conservation program lies collaboration. The University of Bengkulu's partnership with the University of North Carolina Greensboro (UNCG) represents an exemplary model of knowledge exchange and international solidarity. This partnership has opened opportunities for joint studies on animal health management, breeding techniques, and conservation education.

Through this collaboration, staff and students have gained access to modern tools and methods, ranging from genetic data analysis to standardized animal care protocols. International researchers, in turn, learn from Bengkulu's experience in managing tropical ecosystems and engaging local communities. These reciprocal exchanges demonstrate how conservation thrives through cooperation, not isolation.

Global networks such as the Global Species Management Plan (GSMP) and ASEAN Wildlife Enforcement Network (ASEAN-WEN) have also played a role in supporting these local efforts. They facilitate the sharing of data, training resources, and conservation policies across nations. Researchers like Roberts and Mo have noted that such transnational collaboration strengthens ecological resilience and enhances species management across borders.

Still, the journey is not without obstacles. Differing national regulations, funding limitations, and sociocultural contexts often challenge implementation. Yet, as Prof. Aceng Ruyani emphasizes, "Every step we take together, no matter how small, connects our science to a shared moral purpose: to protect life itself."

> In these collaborations, students and researchers alike learn that conservation is not just about saving species; it is about connecting people, ideas, and values. The story of *M. emys* becomes a story of collective learning of science that crosses oceans and builds bridges between cultures.

Figure 3 *Manouria emys*, Asian forest tortoise - Kaeng Krachan National Park
Source: Rushenb, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, 2016.

Fighting the Silent Trade

Illegal wildlife trade remains one of the most persistent threats to *M. emys* across Indonesia and Southeast Asia. Despite its full protection under national law and CITES Appendix II, black markets continue to supply forest tortoises as exotic pets or food. Between 2000 and 2015, CITES reported over 500 individuals confiscated from illegal trade networks, with many cases traced back to Sumatra. The demand for *M. Emys*, often driven by curiosity, status, or traditional beliefs, has led to unsustainable harvesting and the loss of countless wild individuals.

Weak law enforcement, overlapping authorities, and insufficient public awareness allow the trade to persist. However, in Bengkulu, change begins with education. The University of Bengkulu has expanded its conservation mission beyond the pen enclosures into classrooms and villages. Law and biology students collaborate to design community outreach programs that promote wildlife awareness and legal literacy.

Public campaigns, local school visits, and conservation workshops are held to teach why the tortoise belongs in the forest, not in cages. These activities, often led by university volunteers, have reached hundreds of students and local residents, turning awareness into advocacy. Strengthening enforcement through inter-agency coordination and cross-border monitoring remains vital, but as the program's motto reminds: "Knowledge is the first defense against extinction."

When communities understand the ecological and cultural importance of *M. emys*, they become allies, not adversaries. Empowered by science and empathy, local people begin to see the tortoise not as a commodity, but as a companion in preserving the forest's balance.

Table 1 Key Threats and Conservation Responses

Major Threats to *Manouria emys*	Impacts	Conservation Responses
Illegal wildlife trade	Rapid population decline, loss of genetic diversity	Strengthened law enforcement, awareness campaigns
Deforestation and habitat fragmentation	Loss of nesting and feeding sites	Reforestation, habitat protection
Climate change	Disrupted breeding cycles	Monitoring and adaptive management
Low public awareness	Weak conservation participation	Education and outreach programs by universities

Table 2 Educational Integration of *M. emys* in Conservation Learning

Learning Aspect	Activity Example	Intended Outcome
Inquiry-based learning	Field observation of *M. emys* behavior and microhabitat	Strengthen scientific inquiry and data literacy
Local wisdom integration	Connecting traditional forest knowledge with ecological principles	Deepen ethno-STEM understanding
Community engagement	School-based conservation and awareness projects	Foster environmental empathy and shared responsibility

When Conservation Becomes a Classroom

At the heart of Bengkulu's initiative lies a conviction that conservation is not only a scientific responsibility, it is an educational journey. Within the university's field station, rows of semi-natural enclosures provide students with living laboratories. Here, future teachers and researchers observe *M. emys* as it feeds, digs, and rests beneath the shade of tropical trees.

This hands-on approach transforms theory into experience. Students measure environmental variables, document behavioral patterns, and design small experiments under supervision. They learn about adaptation, energy flow, and ecological balance, not from textbooks, but from life itself. Through inquiry-based learning, *M. emys* becomes more than a study object; it becomes a mentor.

The program also embraces ethno-STEM, connecting scientific concepts to local wisdom. Traditional ecological knowledge from Bengkulu's surrounding communities, such as recognizing forest microclimates or understanding soil fertility, is integrated into coursework. In this way, conservation becomes both culturally grounded and scientifically rigorous.

For many students, these encounters mark their first real connection to biodiversity. They begin to see the forest not as a distant concept, but as a living classroom, an ecosystem in which humans are active participants. "The tortoise teaches us patience," says one education student. "It moves slowly, but every step has purpose. That's how we should approach conservation, slowly, carefully, but never stopping."

Through this fusion of conservation and education, the University of Bengkulu demonstrates how a single species can ignite a movement of learning, empathy, and environmental stewardship. In saving *Manouria emys*, they are also nurturing the next generation of eco-literate citizens, proof that science and compassion can indeed share the same shell.

Altogether, these findings illustrate why ex-situ conservation, collaboration, and education form the backbone of Bengkulu's efforts to protect *Manouria emys*. This section underscores the article's purpose: to show how science and community partnerships can sustain the future of this ancient tortoise.

Figure 4 The Area of Turtle Learning Center (TLC)
Source: The University of Bengkulu, 2025.

FUTURE DIRECTIONS

The road to safeguarding *Manouria emys* is long and challenging, but its journey mirrors the patience and resilience of the tortoise itself. As conservationists, educators, and policymakers look ahead, the future of this ancient species depends on how well science, policy, and education can unite in purpose.

1. Advancing Ex-Situ Science and Genetic Research

   Future conservation must deepen understanding of *M. emys* biology especially genetics, hormonal cycles, and behavioral ecology to improve captive breeding. Integrating molecular tools like microsatellites or mtDNA analysis will help maintain genetic diversity, vital for reintroduction success. This approach also supports alignment with international conservation standards and fills regional research gaps.

2. Expanding International Collaboration and Policy Harmonization

   Collaborations, such as between the University of Bengkulu and UNCG, show how shared knowledge boosts husbandry and species management. Going forward, harmonizing regulations through platforms like ASEAN-WEN and GSMP is key. Strengthening regional enforcement and securing long-term funding will transform temporary partnerships into sustainable conservation networks.

3. Fostering Environmental Literacy through Education

   Beyond labs and field stations, *M. emys* can catalyze biodiversity-based science learning. Using living species in curricula promotes empathy and ecological awareness. Universities must expand inquiry-based education and partnerships with communities to localize conservation messages. When people learn to care, they are more likely to act.

Ultimately, the survival of *Manouria emys* symbolizes a broader human responsibility. Conservation cannot be achieved by scientists alone; it requires communities that understand, governments that support, and learners who are inspired. As we look toward the future, every new hatchling and every educated mind represents hope, slow, steady, and enduring, just like the tortoise itself. "True conservation begins when knowledge finds its heart in compassion." Many of us still see turtles as creatures whose presence does not complete us and whose absence does not diminish us. This article calls on readers to help shift that mindset by refusing to purchase wildlife, reporting suspected illegal trade, supporting community-based conservation and rehabilitation programs, participating in environmental education efforts, and promoting habitat restoration initiatives that keep *Manouria emys* in the forests where it belongs.

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We wish to express our gratitude to SEAMEO BIOTROP for facilitating this publication and to the University of Bengkulu for its continuous support in ex-situ conservation research and education programs.

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https://www.pexels.com/photo/common-grass-yellow-butterfly-on-a-flower-12653675/

Jakarta's Urban Green Spaces as Butterfly Habitats and Its Implications for Climate Mitigation

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SYNOPSIS

Jakarta's urban green spaces are more than city parks. They are small ecosystems alive with pollinators that keep urban nature functioning. To understand how vegetation and human activity influence butterfly diversity, this study surveyed five parks across the city using standardized observation methods, namely the Pollard Walk and Point Count. A total of 157 butterflies representing 22 species were recorded, with *Eurema* sp. appearing most frequently. Parks with lush, layered vegetation like Penjaringan Urban Forest Park hosted the most species, while heavily urbanized areas had fewer. The findings reveal that preserving diverse plants and reducing disturbance can make cities more resilient to climate change, one butterfly at a time.

INTRODUCTION

As Jakarta grows upward and outward, its remaining green spaces carry responsibilities far greater than their size suggests. These pockets of urban nature cool overheated neighborhoods, store carbon, filter polluted air, and offer refuge for both people and wildlife (Aulia *et al.*, 2023). Yet rapid land-use change threatens to erode the very ecological functions that help the city withstand rising temperatures and worsening environmental stress (Maheng *et al.*, 2021). Understanding whether these green areas can continue performing their role is becoming increasingly urgent for climate-vulnerable megacities.

Pollinators are a crucial but often overlooked part of this picture. While discussions on urban resilience typically focus on trees and infrastructure, far less attention is given to the small organisms that keep urban vegetation alive. Butterflies, in particular, are highly sensitive to shifts in vegetation structure, temperature, and disturbance. Their presence often reflects deeper ecological conditions beneath the surface. Because they contribute to pollination and plant regeneration, their patterns also reveal how effectively urban green spaces can sustain the ecosystem processes that support climate mitigation (Azahra *et al.*, 2022; Aulia *et al.*, 2023).

Jakarta's parks vary widely in size, vegetation complexity, and levels of human activity, creating a mosaic of habitats with differing ecological potential. Some parks host dense canopies and flowering shrubs that support diverse butterfly assemblages, while others-smaller, more exposed, or heavily disturbed-struggle to maintain even common generalist species. These contrasts raise important questions about which green spaces still function effectively and what this means for urban climate strategies.

This study documents butterfly diversity across five representative parks in Jakarta, analyzes how vegetation structure and human disturbance shape species richness and abundance, and explores the ecological implications for climate-related ecosystem services. By linking butterfly ecology with urban climate resilience, the study provides empirical evidence to inform biodiversity-aware planning and strengthen nature-based solutions in dense urban environments.

ARTICLE CONTENT

Challenges in Maintaining Urban Ecological Functionality amid Rapid Urbanization

Rapid urbanization in Jakarta continues to fragment and degrade green spaces, threatening the ecosystem functions that support urban climate resilience. Parks help regulate temperature, store carbon, and purify air, but these benefits weaken when vegetation becomes sparse or isolated. Although these services are well recognized, the role of biodiversity-especially pollinators-remains underexplored in Southeast Asia. Butterflies are particularly informative because they respond quickly to vegetation structure, disturbance, and local microclimate-small-scale temperature and humidity conditions that can differ from the broader city environment. Their pollination activities also contribute to plant regeneration and ecological stability.

Butterfly communities in Jakarta vary widely across parks depending on floral resources, canopy cover, and habitat configuration. Parks with layered vegetation support richer assemblages, while smaller or heavily disturbed parks lose species more rapidly, especially specialists. These patterns illustrate how fragmentation and human pressure shape ecological resilience in dense urban settings. Yet biodiversity considerations remain largely absent from municipal climate plans, reflecting a persistent policy gap. This study addresses that gap by documenting butterfly diversity across five Jakarta parks and examining how vegetation and disturbance influence their distribution and ecological functions. To contextualize these patterns, the next section outlines the field observations conducted to capture how butterflies move, feed, and interact with different urban habitats.

https://www.biomes.be/product/eurema-hecabe-tamiathis-2

Chasing Wings in the Concrete Jungle: How We Tracked Jakarta's Urban Butterflies

For ecologists, spotting butterflies in a megacity is not just leisure; it is detective work. Each fluttering wing tells a story about how the city breathes, greens, and heals itself. But finding these fragile clues in Jakarta's maze of glass and traffic takes more than luck; it takes method and patience. That is why, from November to December 2024, this study explored five of Jakarta's green lungs, from the quiet Langsat Park to the lively Taman Ismail Marzuki. A simple but careful rhythm was followed during the survey. The Pollard Walk method was used by walking at a steady pace along predetermined transects and recording butterflies that appeared within a fixed observation width. In addition to this, the Point Count technique was applied by standing still at designated locations and noting all butterflies seen within a defined radius over a short, timed interval. Together, these approaches ensured that both mobile and more cryptic species were detected throughout the parks. Armed with cameras and the KupuKita citizen-science app, each sighting was captured and verified, from the bright *Eurema* to the shy skippers. Every photograph became part of a living database that connects citizens, scientists, and policymakers in understanding urban biodiversity.

Who's Thriving, Who's Hiding: The City's Butterfly Census

We recorded 157 butterflies representing 22 species across five Jakarta urban green spaces. Even this simple tally reveals clear patterns. Nymphalidae dominated with 10 species and 50 individuals, reflecting their adaptability to human-modified habitats. Pieridae, although represented by only seven species, had the highest number of individuals (94), with *Eurema* emerging as the most widespread urban generalist, appearing at every site. In contrast, Lycaenidae and Hesperiidae appeared only rarely; their small size and discreet behavior make them harder to detect, and they are among the first to disappear when habitat quality declines.

Location strongly influenced diversity. Penjaringan Urban Forest Park hosted the highest counts (58 individuals and 13 species), followed by Langsat Park (40 individuals, 12 species) and Ismail Marzuki Park (36 individuals, 11 species). The smallest and most exposed parks, Pulir Park and Ria Rio Park, supported only 13 and 10 individuals respectively. These contrasts function as ecological signals, showing where urban nature remains resilient and where it is most vulnerable. Understanding why such differences occur requires a closer look at the vegetation structures that shape habitat quality across sites.

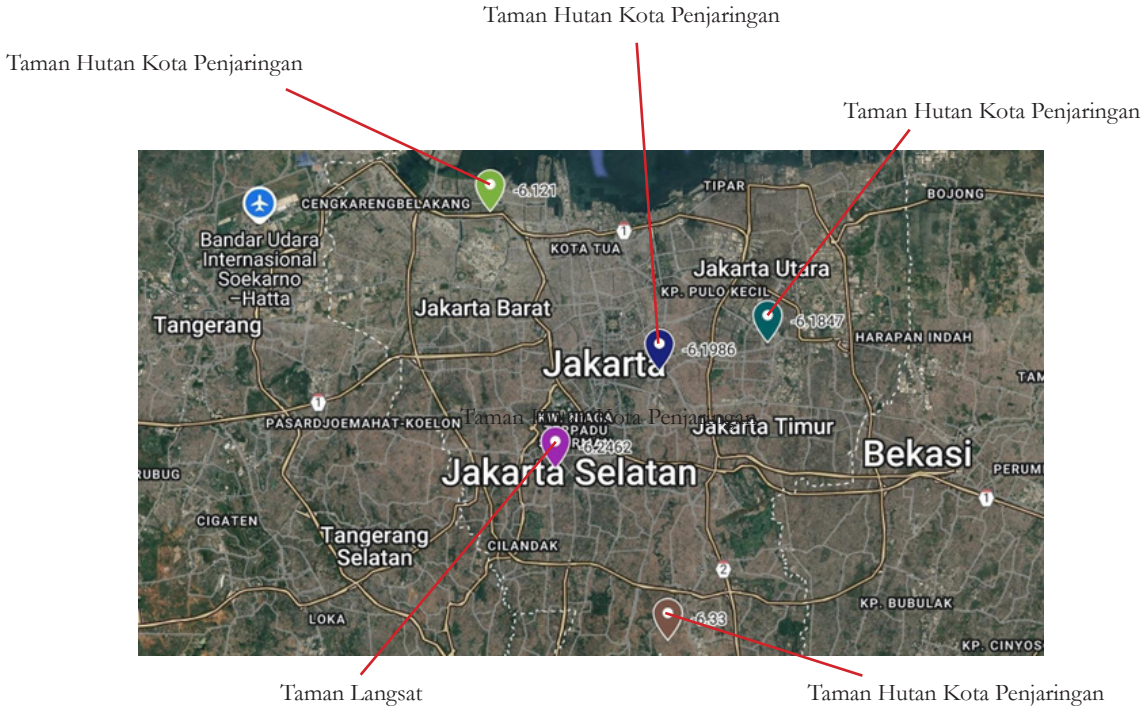

Figure 1 Geographic Distribution of Surveyed Parks in Jakarta
Source: Author, base map from Google Earth

Table 1 Summary of Butterfly Families, Representative Species, and Total Individuals Recorded Across Five Jakarta Parks.

Family	Representative species	Total individuals	Total species	Most common sites
Pieridae	*Eurema* sp. *Catopsilia pomona*	94	7	Penjaringan Urban Forest Park
Nymphalidae	*Ypthima philomela* *Hypolimnas bolina*	50	10	Langsat Park
Papilionidae	*Graphium doson* *Papilio demoleus*	5	2	Penjaringan Urban Forest Park Langsat Park
Hesperiidae	*Suastus gremius*	5	1	Langsat Park
Lycaenidae	*Euchrysops cnejus* *Zizina otis*	2	2	Langsat Park Ismail Marzuki Park
Total	-	**22**	**157**	-

Source: Data obtained from field observations (November – December 2024).

Table 2 Butterfly Richness and Abundance Recorded in Five Jakarta Parks.

Research site	Total species	Total individuals	Dominant family/species
Penjaringan Urban Forest Park	13	58	Pieridae (*Appias olferna*)
Langsat Park	12	40	Pieridae (*Eurema* sp.)
Ismail Marzuki Park	11	36	Nymphalidae (*Ypthima philomela*)
Pulir Park	8	13	Pieridae (*Catopsilia pomona*)
Ria Rio Park	7	10	Pieridae (*Eurema* sp.)
Total	**22**	**157**	-

Source: Data obtained from field observations (November – December 2024).

Plants Set the Rules: Why Vegetation Structure Shapes Butterfly Life

Butterflies are ecological minimalists: they need nectar for adults and host plants for larvae. Parks that offer a canopy, shrub layers, and flowering herbs meet these needs, which is why Penjaringan Urban Forest Park and Langsat Park, with tall flowering trees and stratified understorey-layers of vegetation ranging from shrubs to herbs beneath the canopy-supported more species and more individuals. Open, grassy parks with few flowers provide neither shade nor food, and butterflies respond instantly.

Vegetation structure shapes more than resource availability. Vertical complexity, the layering of plants from ground cover to canopy, helps cool the microclimate and reduce heat stress. Horizontal heterogeneity, the varied arrangement of plant patches across a park, creates diverse microhabitats for species with specialized requirements. Species such as *Appias olferna*, *Catopsilia pomona*, and *Eurema* were closely associated with sites rich in floral and host plants, and were scarce in parks lacking these resources. These patterns make one point clear: planting is not ornamental, it is essential ecological infrastructure. They also raise a broader question: how can cities translate these insights into practical actions that strengthen climate resilience?

From Wings to Climate Action: Practical Fixes that Matter

Butterflies may be small, but they are powerful allies for urban climate resilience. Through pollination and plant regeneration, healthy butterfly communities help sustain vegetation that cools neighborhoods, stores carbon, and filters air. This creates a clear opportunity for urban planners: protecting pollinators strengthens many ecosystem services at once.

What to do, specifically and site by site: at Penjaringan Urban Forest Park, protect existing canopy and fix management failures: improve access, stop illegal dumping and burning, and maintain regular waste services so habitat quality is not eroded. At Langsat Park, increase native flowering species and protect calm weekday windows from disturbance. At Ismail Marzuki Park, use underutilized corners for nectar planting and regulate events that fragment habitat. For Pulir Park and Ria Rio Park, urgent greening is required: add vertical layers, flowering shrubs, and roadside buffer strips to reduce exposure to traffic emissions and noise. Across all parks, reduce frequent mowing, establish pocket pollinator gardens, and integrate citizen science with expert verification so data can inform management.

Several site-specific actions can move this forward. Penjaringan Urban Forest Park should prioritize canopy protection and improve basic management by preventing illegal dumping, reducing burning, and maintaining waste services. Langsat Park would benefit from more native flowering plants and reduced disturbance during quieter periods. At Ismail Marzuki Park, unused corners can be converted into nectar-rich pockets while event activities are managed to avoid habitat fragmentation. Pulir Park and Ria Rio Park require urgent greening through added vegetation layers, flowering shrubs, and buffer strips to reduce pollution exposure. Across all parks, limiting frequent mowing, expanding small pollinator gardens, and strengthening citizen-science programs with expert support will help ensure decisions are grounded in reliable ecological data.

CONCLUSION

A final caveat: these patterns come from a short, season-limited survey, so the fine details of rarity and temporal trends remain uncertain. Even so, the spatial contrasts are robust enough to justify immediate, low-cost interventions. In other words, we do not need perfect data to act; we need the right planting, better maintenance, and smarter park design so butterflies can keep reading the city's health for us.

Future Direction for Nature-Based Solutions in Urban Areas

Urban biodiversity research is only just beginning to reveal how much pollinators contribute to climate-relevant ecosystem services. To strengthen this understanding, future studies should extend butterfly monitoring across multiple seasons and expand observations to a wider range of parks, including informal green pockets that often fall outside formal surveys. Integrating microclimate data, vegetation mapping, and pollution metrics will help uncover how urban environmental gradients shape butterfly

communities and the ecological services they support. Strengthening citizen-science platforms can turn everyday observers into active contributors to urban conservation. Experimental restoration, such as planting native flowering shrubs and larval host plants in degraded parks, offers a promising pathway to test how vegetation enrichment can boost pollinator resilience and enhance climate-supporting functions.

These efforts should not be left to researchers alone. Urban residents, park managers, students, and policymakers all have a role in shaping greener, more climate-resilient cities. Every planted flower bed, every protected green patch, and every butterfly record helps build a shared ecological foundation for Jakarta's future. As the city grows hotter and denser, the real question becomes whether we choose to let urban nature fade or help it flourish. The path forward is in our hands-small actions today can shape the resilient city we hope to inherit tomorrow.

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https://indiabiodiversity.org/species/show/235706

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Comparative Review of Bioinformatics and Wheat Protoplast Assays for Effector Identification in Pathogenic Fungi

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SYNOPSIS

Fungal effectors are key pathogenicity factors, but their identification in wheat pathogens is challenging due to lack of conserved motifs and rapid evolution. This review compares two complementary approaches for effector discovery: bioinformatics pipelines for large-scale in silico prediction and wheat protoplast assays for functional validation in a homologous host system. We analyze the strengths, limitations, and complementarity of each method, and propose an integrated, iterative workflow that sequentially leverages computational prediction and experimental screening. This synergistic strategy accelerates the accurate identification of effectors and their cognate resistance genes, providing a critical foundation for breeding durable disease-resistant wheat varieties and enhancing global food security.

https://www.nium.bio/bioinformatics-unlocking-the-secrets-of-gut-health/

INTRODUCTION

Challenges in Effector Identification in Wheat Fungal Pathogens

Wheat (*Triticum aestivum* L.) is a vital global food crop, but its harvests are under constant threat from damaging fungal diseases like rusts. These pathogens put almost 90 percent of the world's wheat-growing regions at risk, leading to major losses in yield and challenging food security. A key factor in fungal infection is the release of small proteins called effectors, which help the pathogen attack the plant by weakening its defenses. Finding these effectors is difficult because they change quickly, lack common identifying features, and vary widely between different pathogen strains. Standard methods based on genetic similarity often fail to detect them.

Therefore, new and combined methods are urgently needed. This review examines and compares two main strategies used to find fungal effectors in wheat: (1) bioinformatics, which uses computer analysis of genetic data to predict candidate effectors, and (2) wheat protoplast assays, which are lab tests using wheat cells to check if predicted effectors actually work. Our goal is to highlight the advantages and drawbacks of each approach, create a clear comparison framework, and recommend a combined step-by-step process. This integrated workflow aims to efficiently move from computer-based prediction to real-world testing, speeding up the discovery of new effectors. This knowledge is essential for breeding stronger, disease-resistant wheat varieties to protect global food supplies.

ARTICLE CONTENT

Comparative analysis of protoplast-based assays for functional characterization of plant-pathogen interactions

Based on the review's analysis, protoplast-based assays have emerged as powerful and versatile tools for rapidly dissecting molecular plant-pathogen interactions. Table 1 provides a comparative overview of key protoplast-based assays used to study wheat-pathogen interactions. It details the methodological diversity across studies, including the biological and genetic inputs, analytical workflows, and primary outputs for effector and R gene validation. The table illustrates the progression from assays measuring specific interactions to recent high-throughput, multiplexed screens capable of testing hundreds of candidates. While these methods are faster than stable transformations, common limitations include experimental variability and dependence on specific phenotypic readouts.

Table 1 Comparison of Protoplast-Based Assays for Studying Plant-Pathogen Interactions (Table compiled by the author)

References	Data Input	Analytical Workflow	Key Output	Scalability	Limitations
Saur *et al.*, 2019. *Plant Methods*. (Max Planck Institute, Germany)	Biological: Barley/ wheat mesophyll protoplasts. Genetic: NLR and AVR plasmids.	Protoplast isolation → PEG-mediated co-transfection → Luciferase assay → Statistical analysis.	Functional evidence of a specific NLR/ AVR interaction and quantitative cell death measurement. Statistically significant results ($p < 0.05$).	**Medium.** Faster than stable transgenics but limited by manual protoplast isolation.	High variability among replicates; requires high-quality DNA and viable protoplasts.
Su *et al.*, 2021. *International Journal of Molecular Sciences*. (Sichuan Agricultural University, China)	Biological: Wheat mesophyll protoplasts. Genetic: Effector plasmids from *Puccinia striiformis*.	Protoplast transfection → PTI induction by chitin → qRT-PCR of marker genes.	Identification of PTI-suppressing effectors (PSEC2, PSEC17, PSEC45) . Significant suppression of marker genes ($p < 0.05$).	**Medium.** Limited by qRT-PCR throughput. Suitable for dozens of candidates.	Relies on a single read-out; requires prior knowledge of PTI markers; does not measure R gene interaction.
Wilson, Salome., 2024. (Australian National University, Australia)	Biological: Wheat protoplasts with endogenous R genes. Genetic: Pools of effector plasmids, dual-luciferase reporters.	Protoplast isolation → PEG-transfection with effector pool → Ratiometric luminescence measurement.	List of recognized effector candidates from a multiplexed pool without a cloned R gene. High sensitivity and specificity.	**High.** Multiplexes up to 25+ effectors. Low DNA requirement enables high throughput.	May miss effectors that actively suppress defense; outcome dependent on endogenous R gene expression.
Wilson *et al.*, 2024. *New Phytologist*. (The Australian National University, Australia)	Biological: Wheat mesophyll protoplasts. Genetic: AVR effector plasmids; uses endogenous R genes.	AVR/R-induced promoter-driven ratiometric dual-luciferase assay.	Normalized luminescence signal indicating early defense activation without cell death. Significant induction ($p < 0.05$) in matching pairs.	**High.** Allows multiplexed screening of up to 50 AVR candidates. Very low DNA requirement.	Relies on a conserved early defense pathway; may miss unique AVR/R pairs; does not measure cell death.
Arndell *et al.*, 2024. *Nature Plants*. (CSIRO Agriculture and Food, Australia).	Biological: Wheat mesophyll protoplasts. Genetic: Pooled library of 696 predicted effectors and individual R genes.	PEG-transformation → Cell death measurement via flow cytometry → RNA-seq and DESeq2 analysis.	Found known Avr genes (AvrSr50, AvrSr27-2) and new ones (AvrSr13, AvrSr22). Strong statistical confirmation ($p < 0.0001$).	**Very High.** Tests of thousands of R-Avr combinations simultaneously in a pooled approach.	Limited to R-Avr interactions inducing measurable cell death in protoplasts; relies on efficient transformation and RNA-seq.

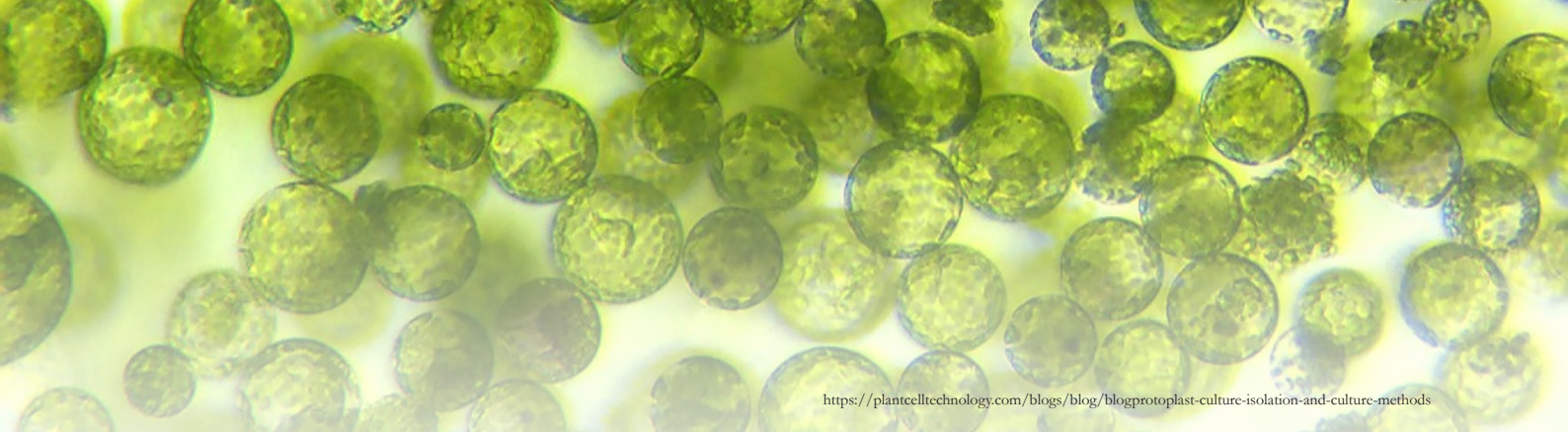
Overview of *in silico* and bioinformatics pipelines for the prediction and prioritization of candidate effector proteins

The discovery and prioritization of fungal effector proteins have been greatly accelerated by sophisticated *silico* pipelines. These computation approaches leverage genomic and transcriptomic data to predict secreted proteins likely to function as effectors. Table 2, compiled by the author, provides a comparative overview of various bioinformatics strategies, detailing their typical workflows, outputs, scalability, and inherent limitations. These methods enable genome-wide screening but remain dependent on prediction algorithms and require downstream experimental validation.

This comparative analysis illustrates a standard workflow for computational effector discovery, moving from genomic data to a shortlist of high-priority candidates. This approach is powerful for initial screening across entire pathogen genomes. However, the final list of predicted effectors remains a set of hypotheses. True biological function, such as suppressing plant immunity or triggering a resistance response, must be confirmed through direct laboratory experiments in plants or plant cells.

Table 2 Comparison of *In Silico* and Bioinformatics-Based Approaches for Effector Discovery (Table compiled by the author)

References	Data Input	Analytical Workflow	Key Output	Scalability	Limitations
Mirzadi Gohari *et al.*, 2015. Molecular Plant Pathology. (Wageningen University, The Netherlands)	Biological: Wheat plants, *Zymoseptoria tritici* isolates. Genetic: Fungal genome sequence, predicted secreted proteins.	Bioinformatics → Expression profiling → Knock-out mutagenesis → Quantitative Trait Locus mapping.	Identified SSP15 and SSP18 as highly expressed but dispensable for pathogenicity. A major virulence QTL on chromosome 5 contained distinct, lowly expressed candidates.	**Medium**. Complex workflow, low throughput for functional validation.	Classic effector criteria (size, cysteine, expression) are biased. QTL candidates are hard to validate due to low expression.
Prasad *et al.*, 2023. Frontiers in Microbiology. (ICAR-Indian Institute of Wheat and Barley Research, India)	Biological: Resistant (Lr28) and susceptible wheat infected with *Puccinia triticina*. Genetic: RNA-seq data.	Transcriptome analysis → Effector prediction (EffectorP) → Homology/ annotation → qRT-PCR → Molecular Docking & MD Simulation.	Found 6 promising candidate effectors. Their expression peaked earlier in resistant plants. One candidate (c14094) showed a strong, stable interaction with the Lr28 protein model.	**Medium**. The bioinformatics approach can be used for other diseases, but detailed lab validation is slow and complex.	No direct experimental proof that these candidates are real effectors; heavy reliance on prediction software and modeled protein structures.
Kamajian *et al.*, 2024. Physiological and Molecular Plant Pathology. (Isfahan University of Technology,	Biological: Wheat plants, *B. sorokiniana* isolate. Genetic: *B. sorokiniana* genome sequence, public RNA-Seq data.	Bioinformatics pipeline (size, secretion, cysteines) → RNA-Seq analysis → qRT-PCR validation → Structural modeling.	Identified 81 putative effector candidates (ECs); Validated expression of several ECs during infection; Found structural similarity of key ECs to known effectors from other fungi.	**Medium**. Pipeline is robust but relies on pre-existing genome and validation is low-throughput (qRT-PCR).	Heavy reliance on *in silico* predictions; limited functional validation (no gene knockout or pathogenicity tests).
Zhang *et al.*, 2020. Frontiers in Microbiology. (Hebei Agricultural University, China)	Biological: *Puccinia triticina* isolates, wheat plants, *N. benthamiana*. Genetic: RNA-Seq data from three fungal life stages.	Bioinformatics pipeline (SignalP, EffectorP) → Motif & domain analysis → Functional validation (BAX-PCD suppression, HR induction in wheat).	Identified 635 candidate effector proteins. 30 effectors suppressed plant cell death; 6 triggered defense responses in wheat. Candidates showed stage-specific expression.	**Medium to High**. Efficient prediction pipeline; functional assays allow medium-throughput validation.	Relies on transcriptomic data, not the full genome; functional validation is indirect (heterologous system) and not all candidates were tested.
Zhao *et al.*, 2020. Frontiers in Microbiology. (Hebei Agricultural University, China)	Biological: Wheat plants, *Puccinia triticina* uredospores. Genetic: RNA from germ tubes and infected leaves.	Transcriptome sequencing → Gene annotation & expression analysis → Secreted protein prediction → Motif-based identification → Protein interaction test & transient expression in tobacco.	Identified 719 potential effectors; 4 of the potential effectors were shown to bind to a key wheat defense protein.	**High.** Bioinformatics pipeline allows for genome-wide effector candidate identification.	Majority of candidates showed no phenotype in cell death assays; transient expression in *N. benthamiana* may not reflect native function in wheat.



https://plantcelltechnology.com/blogs/blog/blogprotoplast-culture-isolation-and-culture-methods

Integrated Methods Recommendation

We propose an integrated, iterative pipeline to bridge computational effector prediction and empirical validation. Initial silico screening of genomic and transcriptomic datasets (Figure 1) yields candidate secreted proteins, which are refined by motif, homology and structural analyses (Figure 2). Multi-omics integration and genomic context filters then generate a prioritized shortlist suitable for functional assays (Figure 3). Shortlisted candidates are tested using wheat protoplast assays, transient expression systems and recombinant protein approaches (Figure 4). Validated effectors are subsequently incorporated into training datasets and curated databases to improve prediction accuracy and standardize workflows.

Genome-to-Secretome Prediction Pipeline for Candidate Effector Discovery

The initial, large-scale identification of candidate effectors begins with a computational genome-to-secretome pipeline. This *in silico* workflow systematically filters whole-genome or transcriptome sequences of pathogenic fungi to generate a refined list of candidate secretory effector proteins (CSEPs). The process initiates with gene prediction and open reading frame identification. Predicted proteins are screened for secretion signals and absence of transmembrane domains (with SignalP, TargetP, TMHMM), then filtered by size and cysteine content to define the putative secretome. Downstream effector-likelihood scoring (EffectorP or equivalent machine-learning classifiers) highlights candidate secretory effector proteins (CSEPs) for further analysis. The output is a curated list of CSEPs, annotated with core sequence features and supported by any available expression evidence, which serves as the primary resource for downstream bioinformatic refinement and experimental prioritization.

Bioinformatic Refinement: Motif, Homology and Structural Screening

Following initial CSEP identification, candidate sequences undergo a multi-layered bioinformatic refinement to prioritize those with the strongest functional potential. This involves domain and motif annotation, homology searches against known effector databases, and prediction of secondary and tertiary structure. Furthermore, analysis for evolutionary signatures, such as diversifying selection or presence/absence polymorphisms, helps identify rapidly evolving loci typical of effector genes. Structural modeling and *in silico* molecular docking against known wheat resistance proteins can provide functional plausibility scores. The integration of these diverse lines of evidence transforms a broad candidate list into a ranked, high-confidence shortlist tailored for experimental validation.

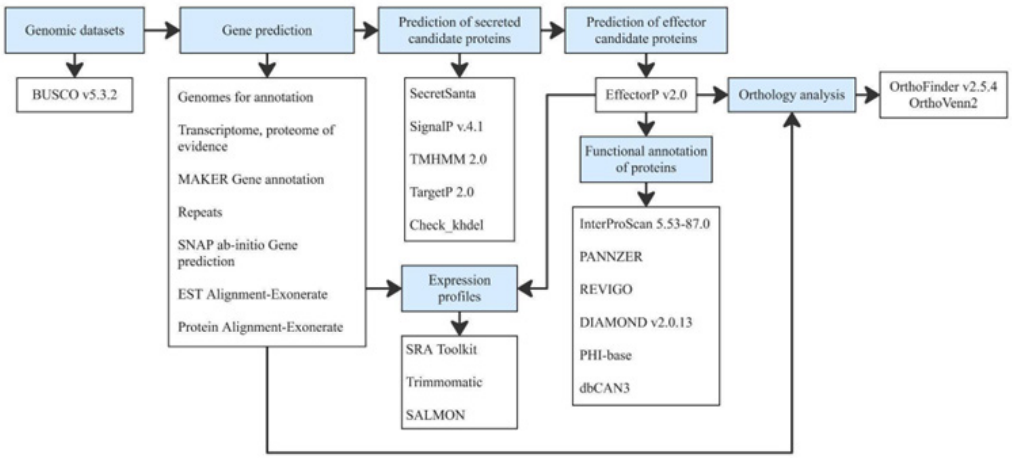


Figure 1 The workflow shows the steps for genome annotation and in-silico analysis of the secretome and effectorome of *Ceratocystis* species and the different bioinformatics tools used.
(Ramos-Lizardo *et al.*, 2023)

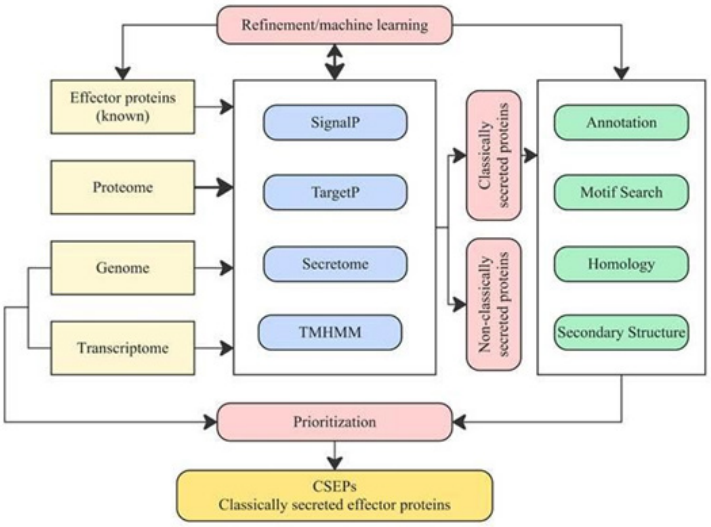


Figure 2 Flowchart of analytical tools that can be used for the prediction of secretome and candidate secretory effector proteins (CSEPs) in fungi.
(Sonah *et al.*, 2016)

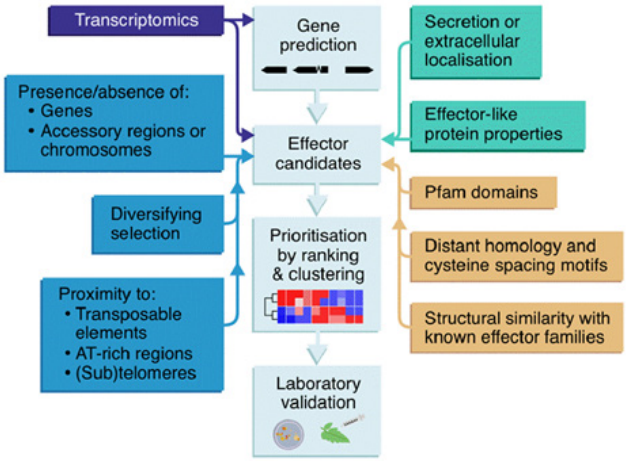


Figure 3 Integrative Prioritization: Multi-omics and Genomic Context for Candidate Selection.
(Jones *et al.*, 2018)

Integrative Prioritization: Multi-omics and Genomic Context for Candidate Selection

Figure 3 illustrates the integrative prioritization strategy that combines multi-omics data and genomic context to refine a list of high-confidence effector candidates. Moving beyond sequence-based predictions, the integration of multi-omics data and genomic context provides a powerful strategy to prioritize effector candidates with higher biological relevance. This step combines transcriptomic, proteomic, and genomic features to filter and rank candidates, focusing experimental efforts on those most likely to function during infection. Transcriptomic data and proteomic evidence highlight candidates actively expressed during host colonization. Additionally, genomic features, including proximity to transposable elements, localization within AT-rich compartments, or presence/absence variation across pathogen isolates, serve as hallmarks of rapidly evolving effector loci. Clustering by sequence or structural similarity further reduces redundancy and groups candidates into families suitable for panel screening. The outcome of this integrative analysis is a concise, high-confidence shortlist tailored for medium- to high-throughput functional assays, typically ranging from 20 to 200 genes depending on available resources.

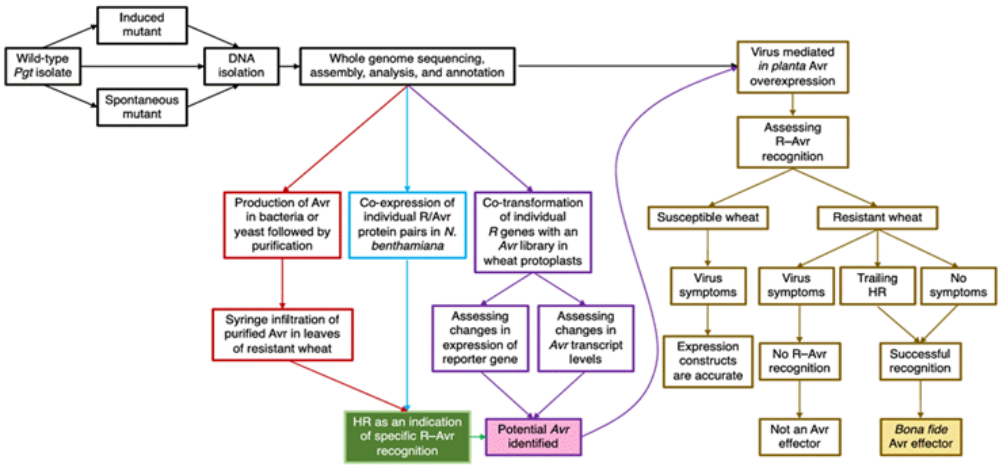


Figure 4 Experimental Validation: Wheat Protoplasts, Transient Expression and Protein Assays. (Lubega *et al.*, 2024)

Experimental Validation: Functional Characterization of Candidate Effectors

Figure 4 shows that the comprehensive wet-lab validation strategies required to functionally characterize candidate Avr effectors after their computational prioritization. Following prioritization, candidate effectors must undergo rigorous functional validation to confirm their role in pathogenicity and plant immunity. A multi-pronged experimental approach is typically employed, leveraging complementary assays to assess activity across different biological contexts. Validation strategies include: (A) in planta expression via virus-mediated systems or agroinfiltration in Nicotiana benthamiana or wheat to test whether candidates trigger a hypersensitive response (HR) in plants carrying the corresponding resistance (R) gene; (B) wheat mesophyll protoplast assays, which offer a medium- to high-throughput platform for screening defense activation or suppression using sensitive reporters such as dual-luciferase systems; and (C) recombinant protein production in systems like E. coli or Pichia pastoris, followed by purification and infiltration into wheat leaves to assess protein-level activity, particularly for larger effectors or to confirm direct biochemical function. A positive HR or reporter activation in an R-dependent manner confirms a candidate as a genuine Avr effector, while negative results prompt re-evaluation or alternate hypotheses. Importantly, validation outcomes feed back into computational models and curated databases, refining future predictions and closing the discovery loop.

CONCLUSION

This comparative review clearly demonstrates that bioinformatics pipelines and wheat protoplast assays are not competing but fundamentally complementary approaches for effector identification. *In silico* methods provide an unparalleled, cost-effective means for genome-wide candidate prediction, leveraging computational power to sift through vast genomic and transcriptomic datasets. However, they ultimately generate hypotheses that require functional confirmation. In contrast, wheat protoplast assays offer a homologous, medium-to-high-throughput experimental system to validate these predictions directly in the relevant host cellular environment, confirming biological activity, be it PTI suppression or ETI activation.

The primary conclusion of this analysis is that a sequential, integrated pipeline moving from broad computational prediction to targeted functional validation represents the most efficient and reliable strategy for fungal effector discovery. The proposed workflow, which progresses from *in silico* candidate prioritization to high-throughput protoplast screening and finally to mechanistic characterization, systematically leverages the strengths of each method while mitigating their individual limitations. This synergistic approach minimizes the high rate of false positives common in computational prediction and the low-throughput nature of whole-plant assays.

The importance and relevance of this integrated model are profound for both basic science and applied agriculture. By accelerating the accurate identification of effector proteins and their cognate R genes, this pipeline directly fuels the discovery of new resistance determinants. This, in turn, is critical for informed resistance breeding programs, enabling the development of durable, disease-resistant wheat varieties. Ultimately, standardizing and adopting such a multi-phase workflow is essential for safeguarding global wheat production and enhancing food security against the persistent and evolving threat of fungal pathogens.

FUTURE DIRECTIONS

Integrating Bioinformatics and Protoplast Assays for Accelerated Effector Discovery in Wheat

Moving beyond methodological comparisons, the most promising strategy for fungal effector discovery lies in a synergistic integration of computational prediction and functional validation. This integrated, iterative workflow capitalizes on the complementary strengths of each approach, leveraging the scalability and hypothesis-generating power of bioinformatics pipelines and the biological fidelity and medium-throughput capacity of wheat protoplast assays. A virtuous cycle is established where validated effectors refine the training of predictive algorithms, which in turn generate more accurate candidate lists for subsequent experimental screening.

To advance this paradigm and accelerate the path from pathogen genome to functionally characterized effector, future efforts should prioritize several key areas:

1. **Standardization and Optimization of Protoplast Assays:** Reproducibility across laboratories requires the development and open sharing of optimized, standardized protocols for wheat mesophyll protoplast isolation, transformation, and high-throughput phenotypic readouts (dual-luciferase reporters, cell death quantification). This will facilitate broader adoption and reliable comparison of results.
2. **Development of Next-Generation *In Silico* Predictors:** Computational pipelines must evolve to incorporate multi-dimensional evidence beyond primary sequence. This includes integrating structural predictions (AlphaFold), evolutionary signatures such as positive selection or presence/absence variation, and genomic context features like association with transposable elements or AT-rich compartments. Such integrative models will significantly improve the prioritization of high-confidence candidates.
3. **Establishment of Centralized, Curated Effector Databases:** The creation of publicly accessible, expertly curated databases for experimentally validated effectors and their associated metadata (phenotypes, host interactions, genomic coordinates) is imperative. These resources will prevent redundant validation efforts and provide the high-quality, standardized datasets necessary for training and benchmarking increasingly sophisticated machine learning models.

A coordinated call to action is essential to realize this vision. The research community must embrace and publish within this integrated framework, prioritizing open science, data sharing, and protocol transparency. Strengthening collaborative networks between computational biologists and plant pathologists will be crucial to bridge methodological expertise. Importantly, funding agencies and agricultural research institutions must recognize and strategically invest in this interdisciplinary nexus as a critical component of sustainable and climate-resilient crop improvement. By championing a closed-loop cycle of prediction, validation, and model refinement, we can systematically decode the molecular dialogue between wheat and its pathogens. The resulting knowledge is the cornerstone for engineering durable genetic resistance, a vital step toward securing global wheat production and nutritional security.

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She would like to extend her deepest gratitude to her academic supervisors at the School of Life Sciences and Technology, Bandung Institute of Technology, for their invaluable guidance, insightful discussions, and unwavering support throughout the preparation of this review. Special thanks to SEAMEO BIOTROP for the invaluable support and facilitation.

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