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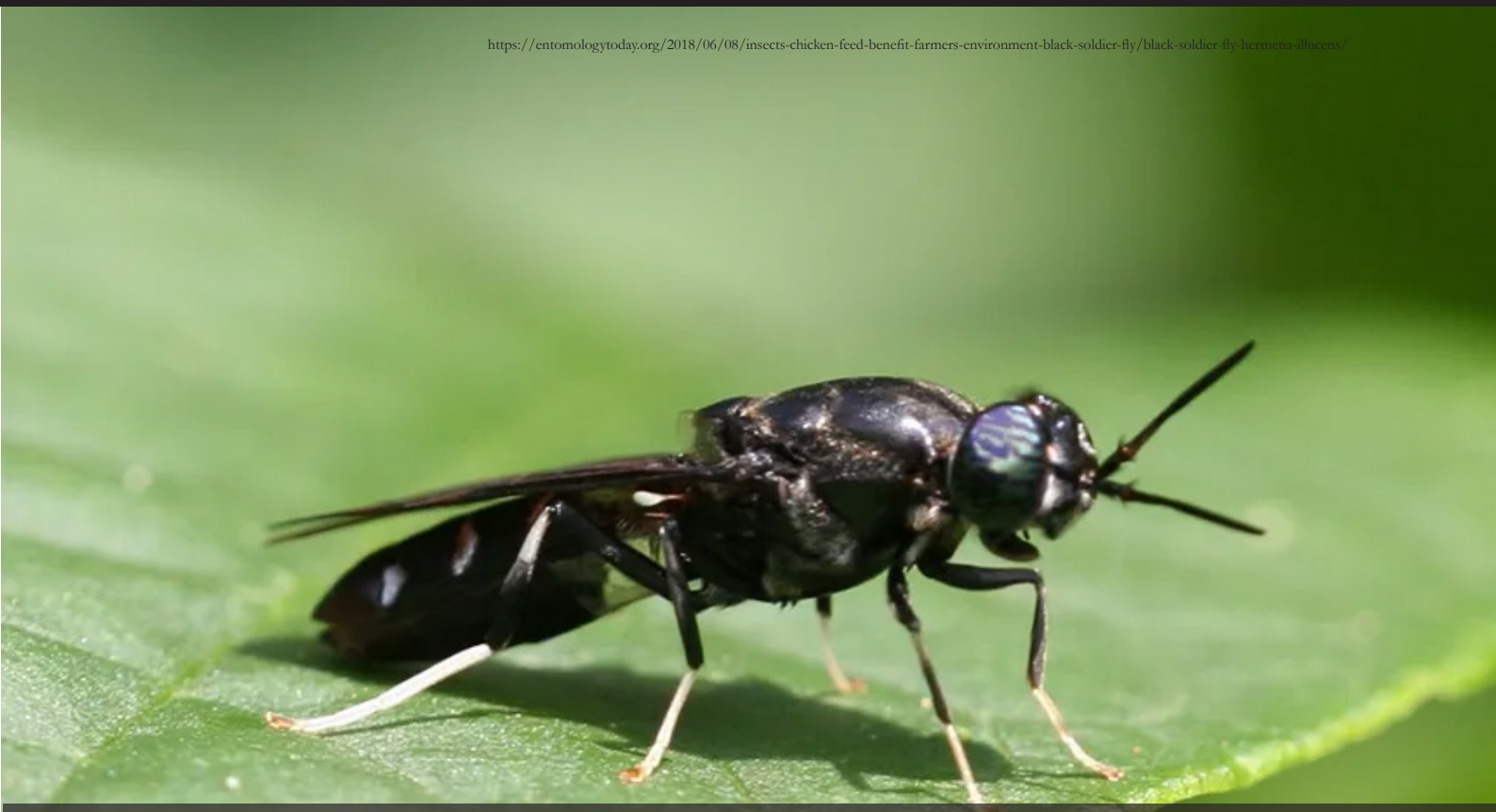

Bioconversion of Oil Palm Waste into Protein-Based Bioplastic Using BSF (*Hermetia illucens*) Larvae

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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-bersembunyi-di-telapak-tangan-petani-gm1503707093-523176742

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.

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

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%

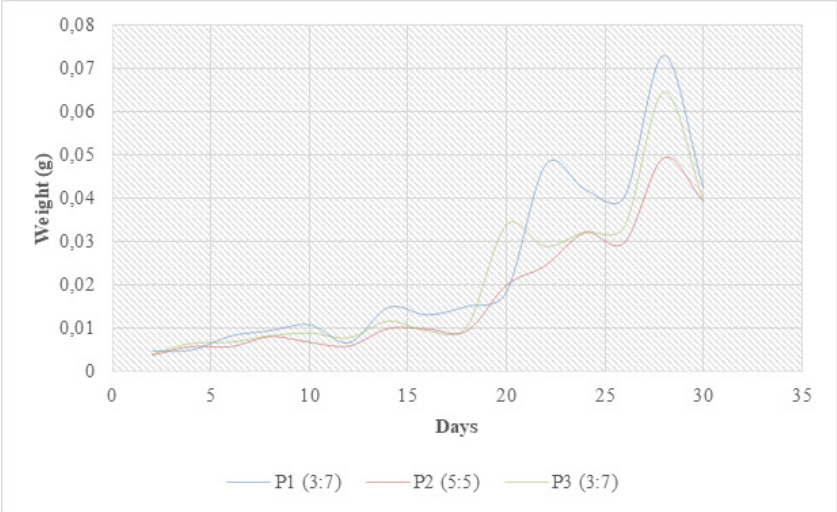

Figure 1 Mean body weight gain of BSF larvae over time across treatments

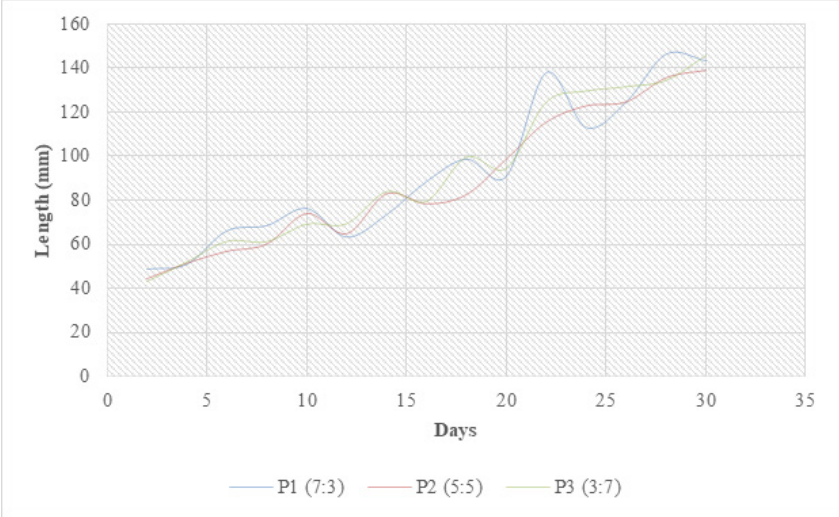

Figure 2 Mean body length gain of BSF larvae over time across treatments

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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SHORT AUTHOR BIOGRAPHY

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Muhammad Gunawan is a Biology graduate from the Faculty of Mathematics and Natural Sciences, Universitas Mulawarman. His academic interests center on environmental biotechnology, particularly the bioconversion of organic waste using Black Soldier Fly (Hermetia illucens) and the development of protein-based bioplastics. Passionate about sustainable solutions for industrial waste, Muhammad explores how biological systems can support circular bioeconomy approaches. His recent work focuses on transforming palm oil residues into biodegradable materials that offer both environmental and economic value.

Prof. Rudy Agung Nugroho, M.Si, Ph.D

Rudy Agung Nugroho is a Professor of Animal Physiology at the Faculty of Mathematics and Natural Sciences, Universitas Mulawarman. He is an active researcher, book author, and a Fulbright Visiting Scholar at the University of Arkansas at Pine Bluff. He obtained his PhD in Animal Physiology from Curtin University, Australia. His current research focuses on biotechnology applications, aquaculture nutrition, and the use of natural products to improve animal health and productivity. His recent work includes Black Soldier Fly–based feed innovation, wound healing biomaterials, and biodiversity conservation research in the Nusantara Capital Region (IKN).

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.