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Original Article

Impaact of fungal uptretreatment of palm kernel cake on the growth and development of black soldier fly (*Hermetia illucens* L.) larvae in a total feeding system

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ABSTRACT

Black soldier fly (BSF) larvae (*Hermetia illucens* L.) are recognized as effective agents for the bioconversion of organic waste, including agricultural residues. The bioconversion efficiency of BSF larvae is highly dependent on the substrate and is considerably reduced when the substrate is rich in cellulose. Palm kernel cake (PKC), a by-product of palm oil processing with considerable potential for upcycling, contains high levels of lignocellulose. Its lignin content can impede nutrient release, making pretreatment essential before its use as a feed substrate. This study evaluated the growth and developmental performance of BSF larvae reared on PKC subjected to biological pretreatment with *Rhizopus oligosporus* (RO), *Phanerochaete chrysosporium* (PC), and *Aspergillus niger* (AN), in combination with a total feeding system (TFS) where feed was provided only once at the start of rearing. Fungi were cultured on PKC for 10 days before being fed to six-day-old BSF larvae; non-fermented PKC and commercial chicken feed served as controls. Larval performance was assessed by measuring dimensional growth (length and weight) and survival rate (SR) across five replicates per treatment. All treatments exhibited high survival rates (85.3 ± 3.02-91.4 ± 5.69%), with the highest values observed in the positive control (91.4 ± 5.69%) and the AN treatment (91.4 ± 3.25%), and the lowest in the negative control (85.3 ± 3.02%). Among the fermented treatments, AN resulted in the highest larval growth rate (6.42 ± 0.28 mg/larva/day), which was significantly higher than that of the non-fermented negative control (4.81 ± 0.21 mg/larva/day) ($p < 0.05$). These results suggest that PKC pretreatment via *A. niger* fermentation is a suitable strategy for improving PKC quality as a feed substrate for BSF larvae.

Key words: biological pretreatment, black soldier fly larvae, lignocellulose, palm kernel cake

INTRODUCTION

The agricultural sector accounts for approximately 23% of global annual greenhouse gas (GHG) emissions, primarily due to the degradation of organic residues (Golasa et al. 2021). In 2016, GHG emissions from agriculture reached 1.6 billion tons and are projected to rise to 2.6 billion tons by 2050 without major improvements in organic waste management (Kaza et al. 2018). The increasing demand for agricultural products, coupled with estimates that 30–40% of agricultural output is lost as waste during production, underscores the need for innovative solutions throughout the supply chain. Transforming this waste into value-added products within a circular system, in a process known as upcycling, can reduce reliance on anaerobic decomposition, a major source of GHG emissions (Willett et al. 2019), while also generating additional revenue.

One approach to upcycling organic waste involves the use of saprophagous animals, which accelerate decomposition and diversify the range of end products. Black soldier fly (BSF) larvae (*Hermetia illucens* (Linnaeus)) are among the most widely utilized saprophages in organic waste upcycling. This species can consume a wide variety of organic materials, including food waste, agricultural residues, industrial by-products, and animal or human manure. Waste degradation by BSF larvae reduces GHG emissions more efficiently than conventional composting (Kinasih et al. 2018; Smetana et al. 2019; Seyedalmoosavi et al. 2022; Siddiqui et al. 2024). Through bioconversion, BSF larvae produce biomass rich in carbohydrates, protein, and fat,

as well as residues that can serve as raw materials for food and feed production (including feed ingredients and fertilizer) or for other industrial applications such as bioenergy and medical uses (Gold et al. 2018; Liu et al. 2019; Chavez 2021; Sidauruk et al. 2024).

Although BSF larvae are generalist feeders with a broad dietary range, the efficiency of their bioconversion process is strongly influenced by the availability as well as digestibility of agricultural waste substrates. A major category of agricultural waste produced in large quantities is lignocellulose-rich material, including straw, palm kernel cake, oil palm fronds, and other crop residues. These wastes possess a crystalline structure that necessitates delignification to disrupt the cell wall and remove structural barriers such as lignin, thereby enabling access to cellulose as the primary nutrient source (Kurniaty et al. 2017). Consequently, lignocellulosic waste is relatively resistant to biological degradation, including by BSF larvae (Liu et al. 2019).

Improving the digestibility of organic waste for macroorganisms can be achieved through physical, chemical, or biological pretreatment (Isibika et al. 2019). Biological pretreatment is notably effective for lignocellulose degradation, as it requires less energy, avoids hazardous chemicals, and does not produce toxic by-products (Agbor et al. 2011). However, this process is relatively slow and strongly affected by environmental factors such as temperature, humidity, and substrate pH (Sharma et al. 2019; ur Rehman et al. 2025).

In practice, biological pretreatment relies on microorganisms capable of producing lignocellulolytic enzymes that break down complex components such as lignin, cellulose, and hemicellulose (Isibika et al. 2019; Rahmati et al. 2020; Khalil et al. 2021). Fungi such as *Rhizopus oligosporus*, *Phanerochaete chrysosporium*, and *Aspergillus niger* have been used to degrade lignocellulosic agricultural waste (Fitriana et al. 2022; Liew et al. 2022). However, when applied to lignocellulose-rich organic waste treatment systems, these fungi have generally produced only modest bioconversion efficiency. Combining this method with BSF larvae bioconversion may improve conversion efficiency and yield a more varied range of end products.

Two feeding methods are commonly used in BSF larvae waste-processing systems: the daily feeding system (DFS), in which larvae are fed every day, and the total feeding system (TFS), in which a large quantity of feed is provided only once at the start of rearing, with no additional feeding thereafter. TFS is a desirable option because it reduces both feeding time and labor requirements (Meneguz et al. 2018). However, its successful use depends heavily on the consistency of the organic waste used as feed, a major challenge for agricultural waste, which tends to be inconsistent in composition and low in nutrient content (Gold et al. 2018). Pretreatment can help achieve the substrate consistency this system requires.

This study aimed to evaluate the effect of pretreatment on a lignocellulose-rich organic waste, palm kernel cake (PKC), used as a model substrate. BSF larvae have potential as decomposers of fermented PKC, with varying degrees of success in decomposition and larval growth reported in previous studies (Liew et al. 2022; Damanik et al. 2024; Nugroho et al. 2024; Bajra et al. 2025).

This study differs from earlier work in its use of three fungal species capable of producing lignocellulolytic enzymes, combined with a total feeding system in which BSF larvae receive a single large feeding calibrated to the amount of substrate they can consume within a set period. The main objective was to determine how pretreatment of lignocellulosic waste affects larval growth, productivity (final larval weight), and survival rate as indicators of substrate suitability for BSF larvae. The findings are projected to inform alternative strategies for using BSF larvae to decompose lignocellulose-rich waste.

MATERIALS AND METHODS

Study time and location

The study was conducted at the Toxicology and Instructional Testing Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia, during March 2025. The average ambient temperature during rearing was 26.76 °C (daily range 21–28 °C), and the average relative humidity was 68.1% (range 59–82%).

Preparation of BSF larvae and palm kernel cake feed

BSF eggs used in this study were obtained from a BSF breeder (Abah), Cigadung, Bandung, West Java. The feed substrate, palm kernel cake (PKC), was sourced from Kapijus Farm, Tulungagung Regency, East Java. Before use, the PKC was grounded to a particle size of less than 2 mm using a Philips HR2115/00 blender.

Preparation of fungi and fungal culture on PDA medium

Preparation of PDA medium. Potato dextrose agar (PDA) medium was prepared by dissolving 39 g of dehydrated PDA powder in 1 l of distilled water and heating the mixture while stirring until homogeneous. The medium was then sterilized by autoclaving at 121 °C for 15 minutes. The sterilized medium was poured aseptically into petri dishes in a laminar airflow (LAF) cabinet and left to solidify (Wong et al. 2021).

Fungal culture on PDA medium. Fungi were aseptically inoculated onto the prepared PDA medium. The fungal isolates used, *R. oligosporus*, *P. chrysosporium*, and *A. niger*, were obtained from PT. Agritama Sinergi Inovasi (Agavilab) and the Mycology Laboratory, PAU Building, ITB, Bandung, West Java. Cultures were incubated at 30 °C for 7 days until sporulation occurred. Spores were harvested by adding a harvesting solution to the medium surface and gently scraping it with a sterile loop. The resulting spore suspension was filtered and quantified using a hemocytometer to obtain a concentration of 1×10^6 spores/ml (Nelofer et al. 2018).

Pretreatment of PKC waste

A total of 150 g of PKC waste was placed in a closed plastic container (17.5 cm × 12 cm × 7.5 cm) for biological pretreatment via fungal fermentation using *R. oligosporus* (RO), *P. chrysosporium* (PC), and *A. niger* (AN). Each treatment was inoculated with 5 mL of spore suspension (1×10^6 spores/ml), then 100 ml of distilled water was added and mixed carefully. Fermentation proceeded for 10 days in containers covered with lids that were not sealed airtight (Liew et al. 2022). Non-fermented PKC served as the negative control, and commercial chicken feed (Hi-Pro-Vite 511-BRAVO, PT. Charoen Pokphand Indonesia Tbk.) served as the positive control feed for BSF larvae.

BSF larvae rearing

BSF eggs were hatched using chicken feed as the initial diet. Six-day-old larvae (6 DOL) were used as the starting point for rearing, following the rearing standard also used by Kinasih et al. (2018). Each replicate container contained 158 larvae, with five replicate containers per treatment. Eight larvae were randomly selected from each replicate container for daily measurements of larval weight and length. Larvae were reared under a total feeding system (TFS). Data on larval weight and larval length were recorded daily. The rearing period ended when approximately 40% of the larval population had reached the prepupal stage, at which point the total number of surviving larvae in each container was counted to calculate the survival rate for each treatment (Diener et al. 2009; Meneguz et al. 2018).

Observed parameters

Proximate analysis was conducted following the methods of the Feed Quality Testing and Certification Center (Balai Pengujian Mutu dan Sertifikasi Pakan, (BPMSP)). Moisture and ash contents were determined gravimetrically using MP 01 BPMSP and MP 05 BPMSP, respectively. Crude protein and crude fat were determined using AOAC 2001.11 (2019) and AOAC 2003.06 (2019), respectively. Crude fiber was determined gravimetrically using MP 06 BPMSP, while carbohydrate content was determined volumetrically using MP 20 BPMSP.

Observations of BSF larval growth performance focused on four components:

Weight change. The fresh body weight of larvae was measured daily from the start of rearing until they reached the prepupal stage, to track growth dynamics. Eight fresh larvae were sampled and weighed from each replicate container, then returned to their containers.

Length change. Larval body length was measured daily as an additional parameter to track growth dynamics. Eight fresh larvae were sampled and measured from each replicate container, then returned to their containers.

Survival rate. Survival rate (SR) was calculated as the number of larvae surviving to the end of rearing relative to the initial number of larvae, as follows:

$$SR = \frac{\text{Number of larvae alive at the end}}{\text{Initial number of larvae}} \times 100\%$$

Growth rate. The growth rate (GR) of BSF larvae was calculated from larval dry weight data using the following formula (Bava et al. 2019):

$$GR\ (\%) = \frac{\text{Final larval biomass weight (mg) - Initial larval biomass weight (mg)}}{\text{Time (days)}} \times 100\%$$

Data analysis

Before statistical analysis, the normality of the data was assessed using the Shapiro-Wilk test. Normally distributed data for growth rate and length change were analyzed using one-way ANOVA; where ANOVA showed significant differences, Tukey's HSD test was used for post hoc comparisons among treatments. Weight change and survival rate data, which were not normally distributed, were analyzed using the Kruskal-Wallis test; where significant differences were found, Dunn's test was used for post hoc comparisons. All statistical tests used a 95% confidence level ($\alpha = 0.05$). Statistical analyses were performed using RStudio version 2024.12.1.

RESULTS

Proximate composition of PKC after fungal pretreatment

Fungal inoculation of PKC produced several fundamental changes in the substrate, including changes in texture

and in proximate composition, most notably a fourfold increase in moisture content and up to a 50% increase in carbohydrate content. Fungal treatment reduced crude fiber content by up to 22%, crude protein by up to 23%, crude fat by up to 21%, while ash content remained relatively unchanged (Table 1). Because the proximate analysis was performed on a single composite sample per treatment, these differences are reported descriptively and were not statistically compared.

Fermentation also produced visible differences in substrate appearance on day 10 (Figure 1). Non-fermented PKC (NF) remained crumbly, dry, and homogeneous, with no visible hyphal growth. PKC fermented with *R. oligosporus* (RO) developed a denser texture and was covered by a thick white mycelial mat, with some areas appearing greyish. PKC fermented with *P. chrysosporium* (PC) had a comparatively looser texture, with thin white hyphae sparsely covering the substrate surface. PKC fermented with *A. niger* (AN) retained a crumbly texture, with fine greyish-white hyphae and dark-coloured colonies distributed more uniformly across the substrate surface.

Growth and development of BSF larvae

Under the TFS strategy, BSF larvae exhibited a sigmoidal growth pattern, with an exponential phase of rapid weight gain from approximately day 0 to day 5 after the start of rearing. Larvae reached peak weight on day 7, after which growth slowed as the larvae approached the prepupal stage on day 9 of rearing (Figure 2).

The AN treatment showed the most stable weight-gain pattern, closely approaching that of the control, whereas the RO and PC treatments exhibited slower growth rates. Despite these differences in growth patterns, Figure 2B showed that AN group achieved the highest final (fresh) weight (290 mg), and Figure 2A the control group achieved the greatest length (18.9 mm). However, these differences were not statistically significant among treatments ($P > 0.05$). The NF (negative control) treatment showed the lowest final larval weight among all groups (Figure 2).

The larvae were 6 days old at the beginning of the treatment and were subsequently reared on the treatment substrates for 10 days until reaching the prepupal stage, resulting in a total development period of 16 days from egg hatching to the prepupal stage in all treatments.

Growth rate and survival rate

Overall, the growth rate (GR) of BSF larvae in the treatment groups was significantly lower than in the control group (7.14 ± 0.14 mg/larva/day) (ANOVA, $P < 0.05$) (Figure 3). Among the treatment groups, the non-fermented (NF) treatment had the significantly lowest growth rate, at 4.81 ± 0.21 mg/larva/day (ANOVA, $P < 0.05$). Among the fermented substrates, AN produced

Table 1. Proximate composition of substrate treatments used in this study

Substrate pretreatment	Moisture (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Carbohydrate (%)
Non-fermented (NF)	6.97	3.82	13.01	5.64	38.69	13.20
Fermented with *Rhizopus oligosporus* (RO)	26.11	3.24	10.74	5.38	30.32	17.24
Fermented with *Phanerochaete chrysosporium* (PC)	28.81	3.02	10.05	4.79	30.42	15.97
Fermented with *Aspergillus niger* (AN)	23.98	3.17	10.44	4.48	32.71	19.79

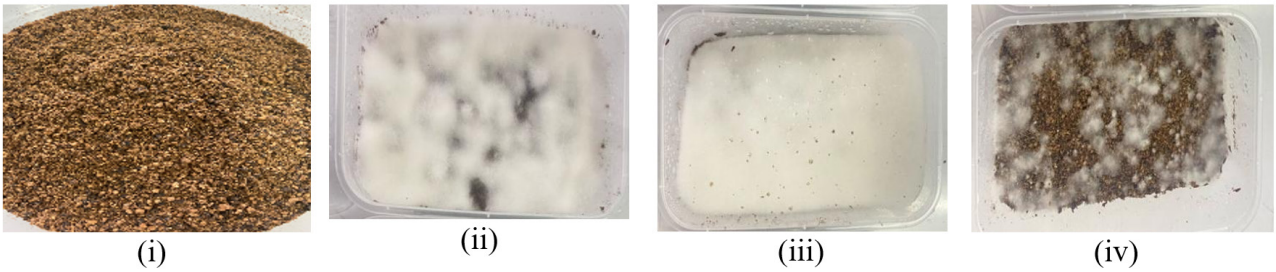

Figure 1. Substrate appearance under different fermentation treatments on day 10. i: non-fermented PKC (NF); ii: PKC fermented with *Rhizopus oligosporus* (RO); iii: PKC fermented with *Phanerochaete chrysosporium* (PC); iv: PKC fermented with *Aspergillus niger* (AN).

the highest growth rate (6.42 ± 0.28 mg/larva/day), followed by PC (6.30 ± 0.16) and RO (6.02 ± 0.21) mg/larva/day; the growth rate of the AN treatment was significantly higher than that of the non-fermented control (Tukey's HSD, $P < 0.05$).

Survival rate was high in all treatments and ranged from 85.3 ± 3.02% to 91.4 ± 5.69% (Table 2). The highest values were recorded in the positive control (91.4 ± 5.69%) and the AN treatment (91.4 ± 3.25%), followed by RO (90.6 ± 1.64%) and PC (90.3 ± 2.56%), while the non-fermented negative control (NF) showed the lowest survival rate (85.3 ± 3.02%). Differences in survival rate among treatments were not statistically significant (Kruskal-Wallis, $P > 0.05$).

DISCUSSION

The nutritional quality of the feed strongly determines BSF larval growth performance and survival. One key feed component is carbohydrate availability, the primary energy source for larval metabolism, activity, and biomass growth, which is likely derived from the breakdown of crude fiber (Cohn et al. 2022).

The relatively low carbohydrate content of the non-fermented (NF) substrate may explain the correspondingly low final larval weight in this treatment (Table 1). In contrast, fermentation increased carbohydrate content to 17.24–19.79% through breaking down starch into simple sugars, thereby providing an energy source more readily accessible to the larvae (Rizal et al. 2022).

The availability of free sugars supports the proliferation of gut microbiota, which assist in breaking down food particles into nutrients that can be absorbed and used for body biomass production (Jiang et al. 2019).

Nonetheless, protein and fat content decreased across all fermentation treatments: crude protein

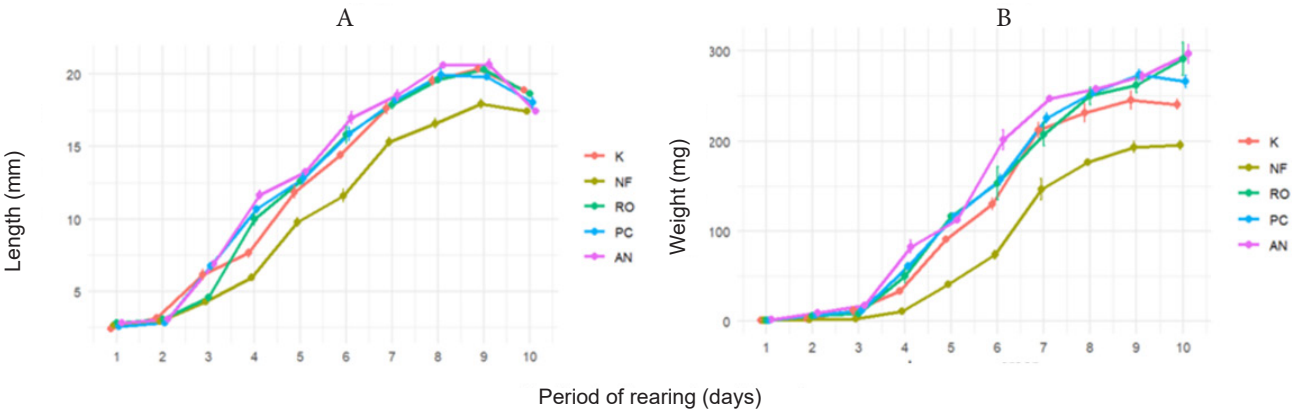

Figure 2. Changes in mean black soldier fly larval (A) length and (B) weight by treatment. Treatments: K: positive control; NF: negative control; RO: *Rhizopus oligosporus*; PC: *Phanerochaete chrysosporium*; AN: *Aspergillus niger.*

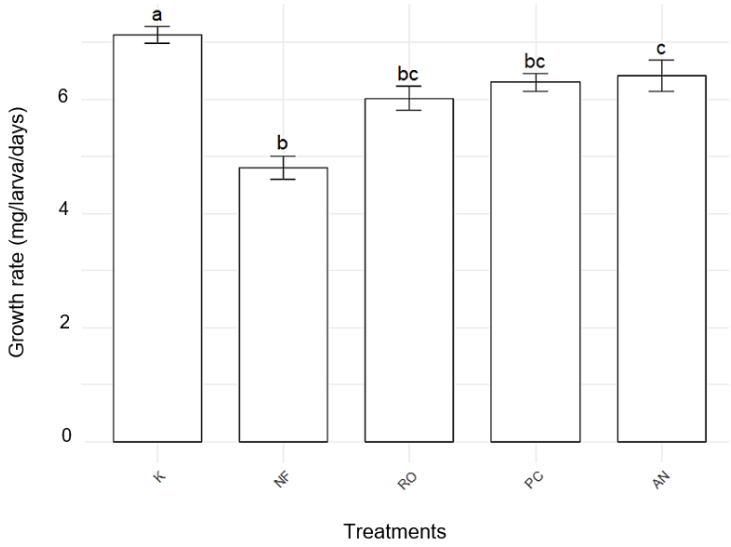

Figure 3. Growth rate of black soldier fly larvae under different treatments. Values are presented as mean ± SE. Treatments K: positive control; NF: negative control; RO: *Rhizopus oligosporus*; PC: *Phanerochaete chrysosporium*; AN: *Aspergillus niger.* Different letters indicate significant differences among treatments based on one-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$).

Table 2. Comparison of the developmental period to prepupa and survival rate of black soldier fly larvae reared on fungus-fermented substrates versus other lignocellulose-rich substrates (non-fermented PKC (NF), PKC fermented with *Rhizopus oligosporus* (RO), PKC fermented with *Phanerochaete chrysosporium* (PC), and PKC fermented with *Aspergillus niger* (AN))

Feed type	Developmental period (days)	Survival rate (%)	Reference
Chicken feed	16	91.4 ± 5.09	This study
NF	16	85.3 ± 3.02	This study
RO	16	90.6 ± 1.64	This study
PC	16	90.3 ± 2.56	This study
AN	16	91.4 ± 3.25	This study
Brewery waste (non-TFS)	14.97	98.00	Liu et al. 2018
Fermented soybean hull (non-TFS)	15.00	98.80	Permana et al. 2022
PKC fermented with EM4 and molasses (non-TFS)	17.00–28.00	96–99.88	Damanik et al. 2024
Fermented banana peel (non-TFS)	19.20	98.30	Permana et al. 2022
Fermented corn waste (non-TFS)	24.20	93.00	Gao et al. 2019
Fermented barley waste (non-TFS)	31.00	98.67	Permana et al. 2021
Straw (non-TFS)	38.20–54.20	51.21–98.27	Manurung et al. 2016

ranged from 10.05–10.74% (compared with 13.01% in the non-fermented substrate), and crude fat ranged from 4.48–5.38% (compared with 5.64% in the non-fermented substrate) (Table 1).

According to Nelofer et al. (2018), fungal fermentation such as with *Rhizopus oligosporus* involves enzymatic degradation in which the fungus utilizes substrate resources for its growth. Secreted enzymes break down lignocellulosic components into simple sugars, while native proteins and lipids are hydrolyzed into amino acids and fatty acids. These simple nutrients are absorbed and utilized to synthesize cellular components for fungal biomass. The rapid consumption of substrate proteins and lipids for metabolic energy during this process may explain the observed decline in total protein and fat content.

Another notable change was the increase in PKC moisture content after fermentation, from 6.97% in the non-fermented substrate to 23.98–28.81% in the fermented treatments. This increase reflects water retention from aerobic biochemical activity and results in more favorable substrate conditions, particularly with respect to water availability. Although the resulting moisture content was still below the commonly cited minimum threshold of 30% (Broeckx et al. 2021), it did not appear to impair larval growth. This may be because the substrate did not release excess water, which can otherwise cause larvae to become waterlogged, as reported in other BSF applications on organic waste (Lalander et al. 2020; Frooninckx et al. 2024). Further study is needed to test the hypothesis that fungal treatment of the substrate can provide an appropriate moisture level for BSF larvae.

Among the fermentation agents tested, *A. niger* produced the highest carbohydrate content. This fungus is known to produce various cellulase enzymes that break down cellulose - the main component of lignocellulose - into simpler, more digestible forms in lignocellulose-rich waste. These enzymes include endoglucanase (CMCase), which cleaves glycosidic bonds within the cellulose chain, and exoglucanase (FPase), which acts on the chain ends to release smaller oligosaccharides (Santos et al. 2022). The lignocellulose-degrading capacity of *A. niger* has also been demonstrated in a study using rapeseed meal - a by-product of oil extraction from rapeseed - as BSF larval feed, which showed better growth performance than other treatments (Shi et al. 2016).

The 16-day development period recorded in this study was longer than that reported for BSF larvae reared on several other lignocellulose-rich substrates, but shorter than that reported for PKC fermented with EM4 and molasses (17.00–28.00 days; Damanik et al. 2024) and for several other fermented substrates (Table 2). The growth pattern noted for BSF larvae in this study was similar to that reported in comparable rearing systems (Meneguz et al. 2018) and (Arnone et al. 2022). However, growth rate and final larval weight differed. These differences probably stem from variation in substrate protein or carbohydrate content, which affects larval development (Scala et al. 2020). This study also shows that appropriately pretreated PKC can

support larval growth approaching that achieved with standard control feed.

Substrate quality as larval feed can be assessed by the balance between nutrient content and crude fiber levels, which is a key determinant of larval developmental performance. Substrates with relatively high fiber content tend to slow development, whereas substrates with more readily accessible nutrients accelerate growth and shorten the developmental period (Hosseindoust et al. 2023). In this study, the AN treatment showed a faster growth rate than the other treatments, likely because *A. niger* fermentation more effectively degraded the recalcitrant lignocellulosic components of PKC into more basic compounds, thereby making nutrients such as sugars, proteins, and lipids more readily available for larval use.

Beyond substrate nutrient availability, this study also demonstrates the influence of feeding strategy on BSF larval development, since the total feeding system (TFS) provides a large quantity of feed at the outset of rearing. This approach helps ensure feed availability for BSF larvae, particularly during the early growth stages (Singh et al. 2021; Yuan & Hasan 2022). However, a key drawback of this system is the potential for early-instar larval mortality due to rising substrate temperature driven by metabolic activity (Schon et al. 2024). Substrate temperature was not measured in this study, so this hypothesis warrants testing in future research.

Other factors that may influence larval growth but were not measured in this study, which represent a limitation and a potential route for future research, include substrate moisture, larval density, and the presence of antinutritional compounds (Nayak & Klüber 2025). Substrate moisture can affect larval development to the prepupal stage; a moisture ratio of 45–75%, with an optimum around 45%, supports larval development (Bekker et al. 2021). Larval density also affects development: low-to-moderate substrate density is associated with shorter developmental periods, whereas high density tends to prolong development due to increased competition for resources among larvae (Barragan-Fonseca et al. 2018). In addition, antinutritional compounds in lignocellulosic substrates, such as tannins and other inhibitory compounds, can reduce nutrient availability, thereby reducing growth and prolonging larval development (Isibika et al. 2019). Biological pretreatment through fermentation may help by supporting tannase activity from *A. niger*, which can reduce tannic acid levels, a major antinutrient (Sharma et al. 2014). These optimal conditions could increase nutrient availability and feed digestibility (unpublished data) and possibly accelerate larval development.

The survival rates recorded in this study (Table 2) were lower than those reported in other studies that employed similar substrates but different feeding systems. Most larval mortality likely occurred early in the rearing period, when the large initial feed quantity may trigger fermentation processes that adversely affect young larvae.

Aspergillus niger is a fungus capable of growing across a wide temperature range and under low-carbohydrate conditions. In this study, AN is thought to have produced shorter hyphae, leaving parts of the substrate surface uncovered. This likely resulted in comparatively lower competition for substrate and space than in substrates inoculated with *R. oligosporus* and *P. chrysosporium*, where the entire substrate surface was covered by mycelium (Figure 3). Such competition can reduce the final weight achieved by the larvae (Liew et al. 2022). Further study is needed to precisely determine the limiting factors imposed by fungal-inoculated substrates on BSF larval growth.

Overall, the final weight of BSF larvae fed fermented PKC was higher than that of larvae fed the control diet (chicken feed) - a pattern not observed in other studies using chicken feed as a control. This may relate to the feeding method used here, which ensured that feed was continuously available, a condition that can benefit larvae living within the substrate. This is consistent with Meneguz et al. (2018), who found that BSF larval growth is affected by factors beyond the initial nutrient content of the substrate, such as feed quantity and availability, both of which play an important role in determining larval growth. This also supports the hypothesis that substrate pretreatment can create more uniform nutrient conditions, making large single feedings more beneficial for BSF larval growth. The final weight was measured as fresh weight, whereas the GR was calculated from dry weight. The significantly higher GR of the positive control therefore reflects faster dry-matter accumulation, while the higher final fresh weight in the AN treatment may partly reflect the higher water content of larvae reared on the fermented substrate.

Each fungal fermentation treatment produced visually distinct substrate textures and colonization patterns. Non-fermented PKC (NF) retained a crumbly, dry, and homogeneous texture with no visible hyphal growth. In contrast, PKC fermented with RO developed a denser texture, with a thick white colony and hyphae covering the substrate surface, with some hyphal areas appearing grayish. This is consistent with Mitchell et al. (1990), who reported that *R. oligosporus* in solid-state fermentation develops through wall formation, increased mycelial density, and partial hyphal

penetration into the substrate - a growth pattern that allows *Rhizopus* to improve the digestibility of fiber-rich substrates such as PKC through partial degradation of complex polysaccharides.

PKC fermented with *P. chrysosporium*, meanwhile, showed a comparatively looser texture, with thin white hyphae sparsely covering the substrate surface. This growth pattern is consistent with the known role of *P. chrysosporium* as a white-rot fungus, which produces lignin peroxidase, manganese peroxidase, and laccase enzymes that effectively degrade lignin in lignocellulosic substrates. Its degradation strategy begins with the use of more readily assimilable substrate components, followed by degradation of solid lignocellulosic material once lignocellulolytic enzymes are induced, allowing PC's enzymatic activity to soften and decompose substrates with high lignocellulose content (Hassan et al. 2021).

PKC fermented with *A. niger* had a crumbly texture, with fine, grayish-white hyphae and dark-colored colonies distributed more uniformly across the substrate surface. This growth pattern is consistent with *A. niger*'s characteristic development, characterized by fine, branching hyphae that form a dense mycelial network (van Leeuwen et al. 2013).

The differences in hyphal growth patterns and substrate textures among the three fungi reflect distinct strategies for substrate degradation. *R. oligosporus* tends to form dense colonies with limited hyphal penetration; *P. chrysosporium* produces thin hyphae but has strong enzymatic capacity for delignification; and *A. niger* displays fine hyphae with dark conidia that support PKC degradation. These results show that the choice of fermentation fungus produces distinct patterns of substrate modification, which ultimately influence the digestibility and nutrient utilization of the substrate by BSF larvae.

The finding that the AN treatment produced a BSF larval growth rate close to that of the positive control (K) indicates that *A. niger* shows potential as a biological pretreatment agent for processing lignocellulose-rich organic waste. *Aspergillus niger* fermentation of PKC may generate cellulase enzymes effective at breaking down lignocellulosic structures and increasing nutrient availability for the larvae, although this study did not include such data, which is a limitation of the study. The novelty of this study lies in it being, to our knowledge, the first research in Indonesia to test the effectiveness of PKC pretreatment using three different fungal species within a TFS system. These outcomes not only broaden understanding of the role of fungi in optimizing bioconversion but also point to potential industrial-scale applications, particularly for processing the abundant palm oil solid waste generated by Indonesia's plantation and agro-industrial sectors. A limitation of this study is that substrate moisture was not measured during larval rearing; since moisture can influence BSF larval activity and growth, this parameter should be included in future studies to strengthen interpretation of results.

CONCLUSION

Pretreatment of PKC with *A. niger* produced the best growth performance among all treatment groups. The larval growth rate under the *A. niger* treatment increased by approximately 35% compared with the non-fermented treatment, showing the effectiveness of biological pretreatment in improving the availability of substrate nutrients. Fermentation by *A. niger* produced a substrate with higher nutrient content, which had a positive effect on both larval weight gain and survival rate under the TFS method. Beyond improving larval growth performance, *A. niger* pretreatment also opens opportunities to use lignocellulose-rich waste as an alternative feed or fertilizer source in Indonesia, supporting eco-friendly waste management and providing environmentally friendly resources.

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