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RESEARCH ARTICLE
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Bioconversion of Palm Oil Mill Wastes as Substrates for *Beauveria bassiana* (Strain B14532): Solid vs Submerged Fermentation for Biocontrol Applications

Wanida Petlamul * and Sawai Boukaew

Faculty of Agricultural Technology, Songkhla Rajabhat University, 160 Khoa-Roob-Chang, Muang District, Songkhla 90000, Thailand
*Corresponding author: wanida.pe@skru.ac.th

ABSTRACT

The growth performance and metabolic capacity of *Beauveria bassiana* B14532 were assessed on various Palm Oil Mill Waste (POMW)-based agar formulated from six residues: decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil-palm frond (OPF), oil-palm trunk (OPT), and palm kernel cake (PKC). Radial growth and conidial yield varied significantly among substrates, correlating with differences in C:N ratio, total sugars, and nutritional compositions. EFB, DC, and OPF facilitated the greatest radial growth (42.45–44.75mm) and the highest conidial concentrations (up to 6.53×10^{9}conidia mL^{-1}), while PKC exhibited the least growth. For lignocellulolytic assays, the fungus was cultured under submerged-state fermentation (SMF) using carbon sources derived from each POMW to assess enzyme activity: CMCase peaked at 4.98U mL^{-1} on EFB and 4.40U mL^{-1} on OPT at 144h, while xylanase reached 48.30U mL^{-1} on OPF at 120h. Principal component analysis indicated that the initial three components accounted for 87.57% of the total variation, correlating nutrient availability and sugar content with growth, and conidiation efficiency. Balanced C:N ratios and sufficient carbon are key for fungal growth, while substrate composition influences enzyme activity and conidiation; *B. bassiana* B14532 efficiently converts agro-industrial wastes into biomass and conidia for sustainable pest management and biomass valorization.

Keywords: Palm Oil Mill Waste, By-Products, Mycoinsecticide, *Beauveria bassiana.*

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INTRODUCTION

The oil palm industry is a significant component of the global agri-food economy. It generates substantial amounts of unused biomass. Over 90% of the biomass from harvested fresh fruit is not oil, resulting in approximately 80-100million tonnes (Mt) of solid waste and significant liquid waste annually (Kaniapan et al., 2021). In 2018, the global production of empty fruit bunches (EFB) exceeded 80Mt. Dolah et al. (2021) asserted that the annual total of oil palm dry biomass, including oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), and palm kernel shells, exceeds 100Mt. The global quantity of palm oil mill effluent (POME) remains uncertain; nevertheless, research indicates that the volume of POME in principal producing nations may be 3–4 times greater than that of crude palm oil (Mohammad et al., 2021). Over 80% of the world's palm oil comes from Southeast Asia. This situation indicates the centralization of both the product and waste streams (U.S. Department of Agriculture, Foreign Agricultural Service, 2025). Palm-mill waste mostly consists of palm kernel cake (PKC), POME, and EFB. From 1,000kg of fresh fruit, around 200kg of oil, 200–300kg of EFB, 50kg of kernels/seeds, 55kg of shells, and around 475kg of sludge are produced (Supriatna et al., 2022). Inadequate management of EFB, fibers/shells, decanter cake (DC), and POME results in methane emissions, environmental acidification, and approximately 1,500kg of CO_2-equivalent per tonne of crude palm oil (CPO) (De Rosa et al., 2022; Tang et al., 2023). These concentrated waste streams are harmful to the ecosystem, so environmentally beneficial utilization strategies must be identified; the present data further underscores the need to develop more effective approaches. Between 2020 and 2025, the valorization of palm residue progressed in bioenergy, composting,

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bioplastics, and biochar. Significant advancements were realized in EFB-derived cellulose composites and POME–EFB co-digestion, resulting in increased biogas outputs (Tan et al., 2022; Suksaroj et al., 2023; Sjahro et al., 2025; Judijanto, 2025). The principles of a circular bioeconomy and Integrated Pest Management (IPM) align with these nutrient-dense streams. In perennial crops, selections are made based on performance, cost, safety, and accessibility of the ingredients (Zahan & Kano, 2018; Gopar et al., 2024; Aldakhil et al., 2025). Simultaneously, methods for utilizing palm waste to manage pests have transitioned from concepts to tangible applications. Solid-state fermentation of palm kernel cake/fiber yields substantial conidial quantities of *Beauveria bassiana* and *Isaria/Cordyceps javanica* (do Nascimento Silva et al., 2018); *Metarhizium* spp. in EFB compost inhibit *Oryctes rhinoceros* (Fauzana et al., 2020); *Trichoderma*-enhanced EFB/POME composts reduce soilborne pathogens (Siddiquee et al., 2017); PKS-derived bio-oil targets *Metisa plana* (Zulkefli et al., 2021); and treated EOPFB fractions exhibit antifungal properties (Akalazu & Duru, 2024). *B. bassiana* is host-specific and has lignocellulase activity (Petlamul & Boukaew, 2019; Sala et al., 2021). EFB, POME, and PKS serve as effective substrates for the production of high-value mycoinsecticides, simultaneously minimizing waste and facilitating circular palm-system management (Gopar et al., 2024; Aldakhil et al., 2025). Because production data remain limited and scattered, this study investigates the growth and conidiation of *B. bassiana* on multiple palm-oil-mill residues under solid-state conditions (SSF) and, in parallel, assesses its lignocellulolytic enzyme activities in submerged culture (SMF) to identify substrate–process combinations that can be scaled for low-cost, circular IPM systems.

MATERIALS & METHODS

Microorganisms

B. bassiana B14532 was purchased from the National Center for Genetic Engineering and Biotechnology in Bangkok, Thailand. It was selected to investigate the use of palm oil mill waste for its development since studies (Petlamul & Prasertsan, 2014; Petlamul & Boukaew, 2019) revealed that it produced significant amounts of the enzymes cellulase and xylanase. According to Petlamul and Boukaew (2019), *B. bassiana* B14532 was cultivated as a stock culture on potato dextrose agar (PDA) at room temperature (30+2°C) and stored in a PDA slant at 4°C until use. Following activation on Czapek Dox Agar (CDA) at ambient temperature (30±2°C), the cultivated *B. bassiana* B14532 was used to prepare the standard inoculum for all investigations. After 7-day, 10mL of sterile distilled water was added to the fungal colonies. The surface was scraped with a spreader, and the solution was gently pipetted to eliminate conidia, which were then homogenized by vortexing. Conidial solutions were standardized to 1.0×10^{8}conidia mL^{-1} utilizing sterile 0.01% (v/v) Tween 80 and calibrated with a hemocytometer (Neubauer enhanced) under light microscopy. The standard inoculum was used immediately.

Palm Oil Mill Wastes

Decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), and palm kernel cake (PKC) are six different types of palm oil mill waste that were generated from various fields. DC, POME, and EFB were collected from Nam Hong Palm Oil Co., Ltd. in Krabi Province's Khao Phanom District (8.0404°N, 99.1281°E). OPF and OPT (palm trees older than 25years) were gathered from a palm garden in Krabi Province's Khao Phanom District (8.26472°N, 99.04917°E). PKC was purchased from JK Industries Import & Export Co., (7.0023°N, 100.5271°E) which is located in Songkhla Province's Hat Yai District.

Prior to use, the obtained wastes were kept in sealed plastic bottles at 4°C to determine their characteristics. Total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) were determined with the Standard Methods for each by-product (American Public Health Association, 1998). The pH was determined with a pH meter. Phosphorus, potassium, magnesium, glucose, and xylose concentrations were determined at the Central Analytical Center of the Faculty of Natural Resources at Prince of Songkhla University.

Substrate

A solid substrate was developed using diverse palm oil mill waste (POMW)-based agar made from six residues: decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil-palm frond (OPF), oil-palm trunk (OPT), and palm kernel cake (PKC). Each dry EFB, OPT, and OPF was individually ground and passed through a 60-mesh sieve, which categorized particle diameters at approximately 0.25mm. Subsequently, they were soaked in distilled water at a 1:1 (w/v) ratio for one hour and then pressed using a screw press to produce EFB, OPT, and OPF solutions. DC and PKC solutions were made by adding distilled water at a 1:1 (w/v) ratio, whereas POME was combined with distilled water at a 1:1 (v/v) ratio. Each POMW extract was centrifuged at 10,000rpm for 5min to obtain a clear supernatant. Clarified supernatants were analyzed for soluble C using a TOC analyzer, and for soluble N by the Kjeldahl digestion–distillation method with colorimetric determination (APHA, 1998). Each type of POMW supernatant was then used as a carbon source at a concentration of 5% (w/v) in an agar medium with a concentration of 1.6% (w/v), which resulted in the formation of POMWA (DCA, POMEA, EFBA, OPTA, OPFA, and PKCA). The pH of each POMW medium was measured directly by a pH meter, followed by sterilization at 121°C for 20min.

Palm Oil Mill Wastes Agar Bioassay: Radial Growth and Conidial Yield

The impact of palm oil mill wastes on *B. bassiana* B14532 was assessed using palm oil mill waste agar (POMWA). Radial growth was evaluated by inoculating 20mL POMWA plates with 10mm radial plugs extracted from 7-day-old cultures. The inoculated plates were thereafter incubated at 30±2°C for 15d under ambient laboratory light and humidity conditions. Radial growth was determined by measuring colony diameters, and conidia were collected using 0.05% Tween80 and quantified with a hemocytometer at 400×magnification. All experiments were performed with five replicates following established protocols (Mwamburi et al., 2015; Humber, 2012; Shah & Pell, 2003).

Carboxymethyl Cellulase and Xylanase Assays under Submerged-state Fermentation on Palm Oil Mill Waste-derived Substrate

B. bassiana B14532 was cultured using carbon sources derived from each POMW under submerged-state fermentation (SMF) to assess enzyme activity. Ariffin et al. (2008) indicated that lignin was removed prior to the manufacture of SMF. Each 5% (w/v) POMW solution substrate was suspended in 50mL within 250mL Erlenmeyer flasks. The materials were carefully blended, and the flasks were subjected to autoclave sterilization at 121°C for 20min. (Petlamul and Prasertsan, 2014). Following sterilization, 10mL of the conidial suspension at a concentration of 1×10^{8}conidia mL^{-1} was inoculated to the medium. The mixture was then incubated at ambient temperature (30±2°C) in a shaking incubator that shook at 150rpm for 168 hours. Subsequently, 5mL of crude enzymes from the SMF cultures were collected at 96, 120, 144, and 168h for analysis. The samples were then filtered and centrifuged at 4000rpm for 10min to obtain a clear supernatant (Kim et al., 2014), from which an aliquot was diluted to determine carboxymethyl cellulase (CMCase) and xylanase activities (Bailey et al., 1992). The activity of CMCase was assessed in a reaction including 1% (w/v) sodium carboxymethyl cellulose (CMC-Na; Sigma-Aldrich, CAS 9004-32-4; medium-viscosity grade) in 50mM citrate buffer at pH 4.8 (0.5mL) and an enzyme dilution (0.5mL). Following a 30-min incubation at 50°C in the incubation, decreasing sugar was measured utilizing the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959), with glucose serving as the reference. One unit (U) of CMCase activity is defined as the quantity of enzyme that releases one mole of glucose per minute. Xylanase activity was assessed using the same methodology as CMCase activity, using xylan as the substrate and a reduced incubation duration of 10min. One unit (U) of xylanase activity corresponds to the quantity of enzyme that releases 1mol of xylose per minute. The studies were performed five replicates.

Statistical Analysis

Conidial yield and radial growth of *B. bassiana* B14532 across POMW substrates were analyzed by one-way ANOVA (α=0.05) followed by DMRT for post hoc grouping (letter superscripts on bars/points). Normality and variance homogeneity were verified by Shapiro–Wilk and Levene's tests. Results are expressed as means ± SD with corresponding effect sizes and 95% confidence intervals where applicable. The principal component analysis (PCA) (autoscaled z-scores) was performed with the following parameters: conidial yield, initial pH, C:N ratio, and total sugar, with sampling adequacy assessed by Kaiser–Meyer–Olkin (KMO) and Bartlett's tests. Analyses were performed in SPSS Statistics 26 (IBM Corp., Armonk, NY, USA) with five replicates per treatment.

RESULTS AND DISCUSSION

Characteristics of the Chemical Composition of Residual Materials from Palm Oil Extraction Plants

The analysis of POMW (DC, POME, EFB, OPF, OPT, and PKC) revealed that these materials included 11.80-47.00% carbon, 10.50-70.10% fiber, 0.34-20.50% lignin, 0.25-3.61% nitrogen, 0.02-1.58% phosphorus, 0.05-0.79% magnesium, and 1.01-12.06g L^{-1} glucose (Table 1). To assess the fraction directly available to fungi, the wastes were centrifuged and the clarified supernatants analyzed. Soluble carbon as dissolved organic carbon (DOC, g C L^{-1}) was 0.50 (DC), 1.07 (POME), 3.42 (EFB), 4.71 (OPF), 5.28 (OPT), and 0.76 (PKC), with corresponding soluble nitrogen (g N L^{-1}) values of 0.34, 0.33, 0.17, 0.08, 0.04, and 0.54, respectively. These gave C:N ratios of 1.47 (DC), 3.29 (POME), 20.70 (EFB), 62.80 (OPF), 140.80 (OPT), and 1.40 (PKC). These data suggest that, although the bulk wastes are nutrient-dense, considering the soluble fraction in the supernatant—i.e., the portion directly accessible to microorganisms—helps identify which residues release nutrients more readily and are therefore more suitable as culture media or bioprocess substrates. Geng (2014) likewise noted that nutrients and carbohydrate-rich dry oil palm biomass and POME have been transformed into fuels, fertilizers, biocomposites, biogas, and other value-added chemicals, with complete valorization expected via a biorefinery strategy. The current study shows that each residual material contains nutrients and minerals—such as carbon, nitrogen, glucose, xylose, and secondary nutrients including sodium, phosphorus, magnesium, and potassium—that can act as substrates for fungal growth (Table 1). Gao et al. (2007) contend that fungal culture medium must contain sufficient macroelements—namely carbon, nitrogen, oxygen, sulfur, and phosphate—as they are vital nutrients for growth and sporulation. Gao (2011) reported that for *B. bassiana* IBC1201, an ideal carbon content of 4g L^{-1} and C:N ratio of 5:1 were achieved utilizing a two-stage culture approach. Other research indicated that rice husk, with a C:N ratio of 22.7, facilitated maximum spore production (Mishra et al., 2016), but Shah et al. (2005) reported the maximum yields at a C:N ratio of 35:1. These results highlight the critical importance of carbon, which forms the basis of organic compounds, and carbon sources typically supply oxygen and hydrogen as well. Magnesium ions are essential divalent cations required for membrane and ribosome integrity, nucleic acid neutralization, and the activity of several enzymes (Groisman et al., 2013). *B. bassiana* produced the highest concentration of conidia mL^{-1} when cultured on Sabouraud dextrose agar supplemented with MgO at 50ppm, followed by concentrations of 6.9 and 6.1 at 20 and 100ppm, respectively, 4.8 at 10ppm, and the lowest concentration of 3.2 at 500ppm (Gayathri et al., 2018), indicating that magnesium promotes sporulation within a defined concentration range.

Table 1: Chemical composition of palm oil mill wastes

Parameters	Decanter cake	Palm oil mill effluent	Empty fruit bunch	Oil palm frond	Oil palm trunk	Palm kernel cake
pH	6.51	6.14	6.23	6.20	6.32	6.81
Carbon (%)	47.00	46.76	45.00	11.80	12.90	36.06
Nitrogen (%)	2.27	2.17	1.10	0.50	0.25	3.61
Fiber (%)	14.08	10.50	70.10	70.02	70.00	35.09
Lignin (%)	12.24	0.34	16.00	13.58	20.50	15.32
Phosphorus (%)	0.33	0.41	0.37	0.02	0.06	1.58
Potassium (%)	0.85	1.72	1.73	0.36	0.40	1.72
Sodium (%)	0.02	0.04	0.02	0.04	0.02	0.01
Magnesium (%)	0.48	0.52	0.07	0.27	0.05	0.79
Glucose (g L^{-1})	1.01	2.07	1.95	5.31	12.06	1.79
Xylose (g L^{-1})	0.26	0.64	6.80	6.50	1.15	0.11
Total sugar (g L^{-1})	1.27	2.71	8.75	11.81	13.21	1.90
C:N ratio	20.70	21.55	40.90	23.60	51.60	9.98

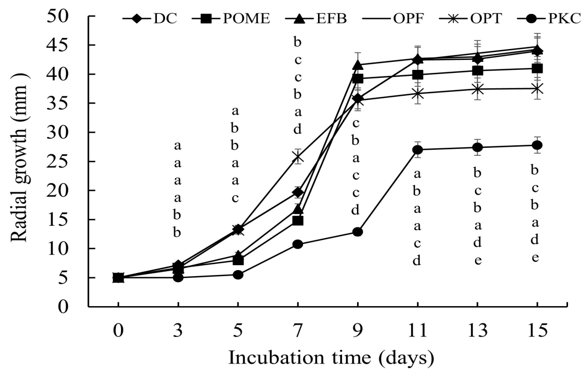

Fig. 1: Radial growth of *Beauveria bassiana* B14532 on various palm oil mill waste–based substrates, including decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), and palm kernel cake (PKC) (n=5). For each incubation day, different lowercase letters beside data points indicate significant differences among substrates represented by the symbols in the legend (one-way ANOVA, DMRT, α=0.05).

Growth Performance of *B. bassiana* B14532 on Palm Oil Mill Wastes

A. Radial Growth of *B. bassiana* B14532

The radial growth of *B. bassiana* B14532 exhibited significant variation across different POMWs (p<0.05), (Fig. 1) indicating the interplay of carbon and nitrogen availability, C:N ratio, and total sugars. The most significant growth was recorded in OPF, EFB, and DC, ranging from 42.45 to 44.75mm. OPF, characterized by a C:N ratio of 62.80 and a sugar content of 11.81g L^{-1}, supplied adequate nitrogen for protein synthesis, thereby facilitating robust radial growth. In a similar manner, DC demonstrated a well-balanced nutrient profile (C:N 1.47; sugars 1.27g L^{-1}), which supports consistent metabolic activity and fungal growth. EFB demonstrated notable growth, maintaining high levels despite its elevated C:N ratio of 20.70 and a low nitrogen content of 0.17g N L^{-1}, factors that typically limit development. The observed anomaly can be attributed to the significant sugar concentration of 8.75g L^{-1} alongside a high fiber content of 70.10%. This combination likely improved aeration and substrate porosity, thereby promoting fungal colonization. In comparison, POME and OPT exhibited only moderate growth. POME (39.25–40.75mm) exhibited a balanced C:N ratio of 3.29; however, it presented relatively low total sugars at 2.71g L^{-1}, which restricts carbon availability for metabolic processes, even with sufficient nitrogen present. OPT (36.50–37.75mm), characterized by high sugar content (13.21g L^{-1}) and low nitrogen levels (0.04g N L^{-1}), along with a highly elevated C:N ratio (140.80), illustrates that an abundance of carbon does not adequately offset nitrogen deficiency, thereby limiting protein biosynthesis and radial growth (Das and Kumar, 2018; Hau et al., 2022). The growth observed on PKC (26.25–27.50mm) was the lowest, significantly lower than that on all other substrates (p<0.05). Although the nitrogen content is high at 0.54g N L^{-1} and phosphorus is at 1.58%, coupled with a low C:N ratio of 1.40, the restricted sugar concentration of 1.90g L^{-1} probably limited carbon metabolism. Additionally, residual oil and possible inhibitory compounds may have contributed to the suppression of fungal colonization (Sahayaraj & Namasivayam, 2008; Ranadev et al., 2023). The findings indicate that the growth of *B. bassiana* is influenced not solely by the C:N ratio but by the intricate interplay between nitrogen availability and the presence of utilizable sugars, which together regulate fungal metabolism and development on agro-industrial substrates (Krasnopolskaya et al., 2021; Gao and Liu, 2010). Fig. 2 illustrates the results obtained from *B. bassiana* B14532 on DC, POME, EFB, OPF, OPT, and PKC. The growth morphology of *B. bassiana* B14532 exhibited variation across palm oil mill waste substrates. DC exhibited robust radial proliferation and beige pigmentation, signifying adequate food availability. POME exhibited heterogeneous growth characterized by darker pigmentation and zones of conidia production, indicating nutritional gradients. EFB and OPF exhibited elevated radial densities and a light coloration. OPT exhibited uneven development accompanied by dispersed sporulation, indicating partial deterioration. PKC demonstrated stunted growth accompanied by dark pigmentation, signifying nutritional deficiency.

B. Conidial Yield of Fungus *B. bassiana* B14532

The conidial yield of *B. bassiana* B14532 exhibited substantial variation across various palm oil mill wastes (POMWs), indicating the impact of substrate composition on fungal growth and reproduction (Fig. 3). Of the evaluated substrates, EFB produced the highest conidial yield (6.53×10^9conidia mL^{-1}), whereas palm kernel cake (PKC) yielded the lowest (4.7×10^9conidia mL^{-1}). The efficacy of EFB can be ascribed to its elevated lignocellulose content and advantageous carbon-to-nitrogen ratio, which facilitate fungal metabolism and conidiation (Alam et al., 2009). DC, which also facilitated significant conidia production, consists of high total organic carbon (47.00%),

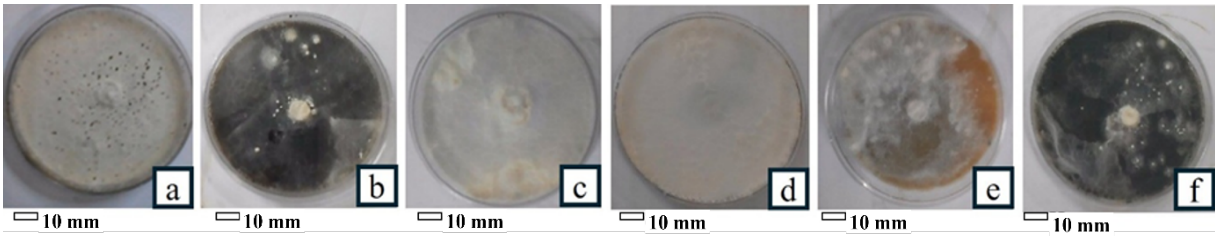

Fig. 2: Plate growth of *Beauveria bassiana* B14532 after 15 days on various palm oil mill waste substrates, including (a) decanter cake (DC), (b) palm oil mill effluent (POME), (c) empty fruit bunch (EFB), (d) oil palm frond (OPF), (e) oil palm trunk (OPT), and (f) palm kernel cake (PKC) (n=5), scale bar = 10mm.

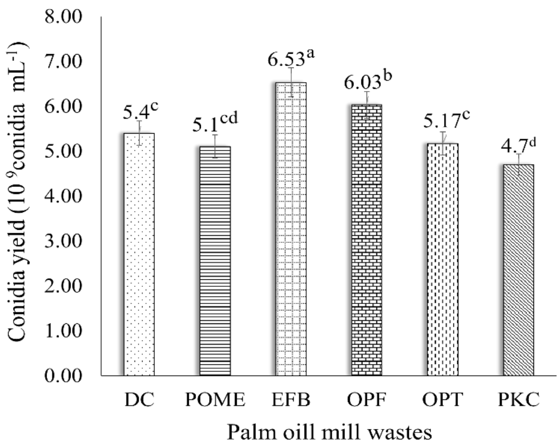

Fig. 3: Fungal conidial yield *Beauveria bassiana* B14532 growth on palm oil mill waste including decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), and palm kernel cake (PKC) (n=5).

moderate nitrogen (2.27%), and vital micronutrients like phosphorus, potassium, and magnesium—conditions favourable for fungal proliferation. The carbon-to-nitrogen (C:N) ratio is crucial, as substrates with excessively low (e.g., PKC, C:N = 1.40) or excessively high values can impede fungal growth. An ideal carbon-to-nitrogen ratio of roughly 20–28 has been documented to promote radial growth and conidiogenesis in *B. bassiana* (Mishra et al., 2016; Wang et al., 2012; Colla et al., 2023). Substrates like OPF and POME produced moderate outcomes, presumably due to inadequate nutritional compositions or moisture-holding capabilities. These findings highlight the potential of nutrient-dense agro-industrial leftovers such as EFB and DC as economical and sustainable substrates for the large-scale cultivation of entomopathogenic fungi in biological control initiatives.

However, when nutrient availability was assessed based on soluble C and N in the clarified supernatant after centrifugation at 10,000rpm for 5min, the interpretation became more precise. Although bulk analyses indicated that DC and POME contained relatively high total C and N (Table 1), *B. bassiana* in this study was exposed only to the soluble fractions. The supernatants contained 0.502–5.281g C L^{-1} and 0.0375–0.5415g N L^{-1}, giving soluble C:N ratios from ~1.4 (DC, PKC) to >60 (OPF) and >140 (OPT). The dissolved ratios aligned closely with the observed radial development and conidial yields, indicating that certain palm-mill residues can effectively release enough accessible nutrients to sustain fungal growth. Assessing the concentrations of carbon and nitrogen post-centrifugation facilitates the differentiation between residues that inherently release nutrients, beneficial for immediate fungal growth, and those requiring prior decomposition. This knowledge facilitates the development of straightforward, low-input methods for farmers to utilize oil palm residues to create favorable circumstances for entomopathogenic and other beneficial fungi in agriculture.

As presented in Table 2, the PCA results show that the first three components explain 87.57% of the total variance (PC1: 53.60%, PC2: 18.21%, PC3: 15.76%), indicating that most of the dataset's variability can be captured by these components, while the remaining components contribute minimally. This supports retaining the first three components for dimensionality reduction and subsequent analyses (Jolliffe, 2011).

Table 2: Total variance explained by principal component analysis

PCA components	Eigenvalue	% of Variance	Cumulative %
1	5.90	53.60	53.60
2	2.00	18.21	71.80
3	1.73	15.76	87.57
4	0.85	7.76	95.32
5	0.51	4.68	100.00

PCA results revealed distinct clustering and interpretable correlations between physicochemical variables and fungal growth parameters (Fig. 4). All variables were mean-centered and standardized to unit variance (z-scores) prior to analysis. The first three components accounted for 87.57% of the total variance (PC1=53.60%, PC2=18.21%, PC3=15.76%). PC1 loaded strongly on K, P, and Mg, together with a moderate contribution from initial pH, indicating a nutrient-availability axis. PC2 co-loaded the C:N ratio with conidial yield and showed a negative loading for Na, which emphasizes the value of nutrient balance for sporulation. PC3 was mainly associated with radial growth along with glucose and total sugar, consistent with carbon-driven colony expansion. Table 3 shows the loading matrix, and Fig. 5 shows the score plot with group separations. The sampling adequacy and factorability were satisfactory (KMO>0.6), and Bartlett's test of sphericity yielded significant results (p<0.001), demonstrating that the dataset was appropriate for PCA. These patterns are consistent with previous reports that nutrient composition shapes fungal metabolism on agro-residues (Cuadrado-Osorio et al., 2022) and highlight the usefulness of multivariate analysis for complex biological datasets (Jolliffe & Cadima, 2016).

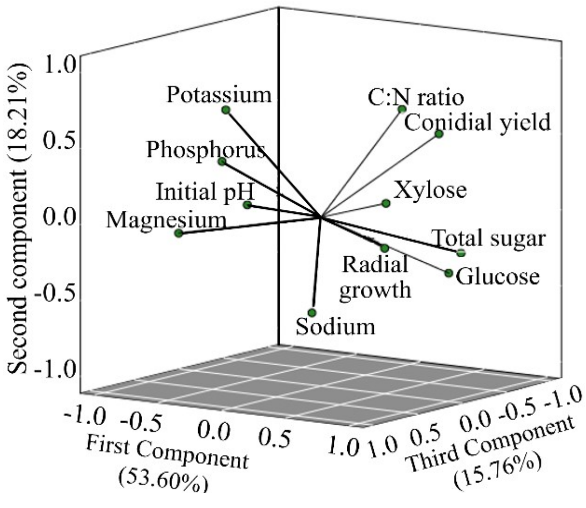

Fig. 4: Principal component analysis loading plot (PC1 53.60%, PC2 18.21%) of physicochemical parameters and their relationships for *Beauveria bassiana* B14532 on Decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), and palm kernel cake (PKC) (*n* = 5).

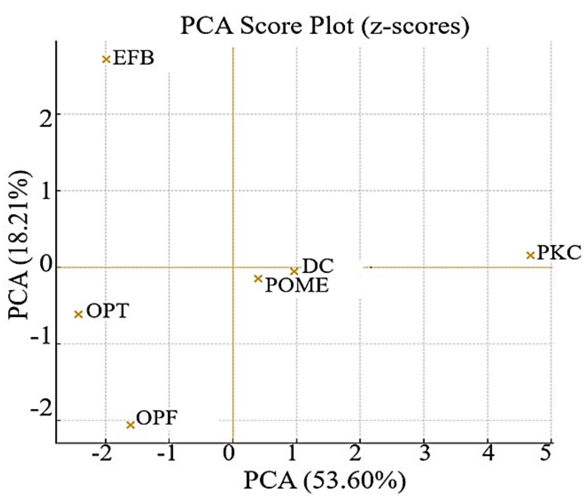

Fig. 5: Principal component analysis score plot (PC1 53.60%, PC2 18.21%) for *Beauveria bassiana* B14532 on Decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), and palm kernel cake (PKC) (*n* = 5).

Table 3: Principal component analysis loadings (PC1–PC3) of palm-oil-mill-waste composition variables

Parameters	PC1	PC2	PC3	Communality
Phosphorus (%)	1.001	0.27	-0.214	1.12
Potassium (%)	0.556	0.773	0.375	1.048
Sodium (%)	-0.478	-0.526	0.793	1.133
Magnesium (%)	1.05	-0.185	0.249	1.198
Glucose (g L^{-1})	-0.618	-0.44	-0.688	1.049
Xylose (g L^{-1})	-0.669	0.191	0.155	0.508
Total sugar (g L^{-1})	-0.889	-0.234	-0.452	1.049
C:N ratio	-0.669	0.841	-0.041	1.157
Initial pH	0.92	-0.062	-0.41	1.018
Radial growth (mm d^{-1})	-0.909	-0.057	0.515	1.094
Conidia yield (conidia 10^9 mL^{-1})	-0.833	0.649	-0.26	1.184

CMCase and Xylanase Activities of *B. bassiana* B14532

B. bassiana B14532 exhibited pronounced cellulolytic and hemicellulolytic activities (Fig. 6) when cultivated on POMWs, confirming its efficiency in degrading lignocellulosic biomass. EFB and OPT were the most effective substrates, with CMCase activity peaking at 144h (4.98 and 4.40U mL^{-1}, respectively), while xylanase activity reached its maximum at 120h (48.30U mL^{-1}), particularly in cultures grown on OPF. In contrast, PKC consistently supported the lowest enzyme production, likely due to its limited organic matter and nutrient availability.

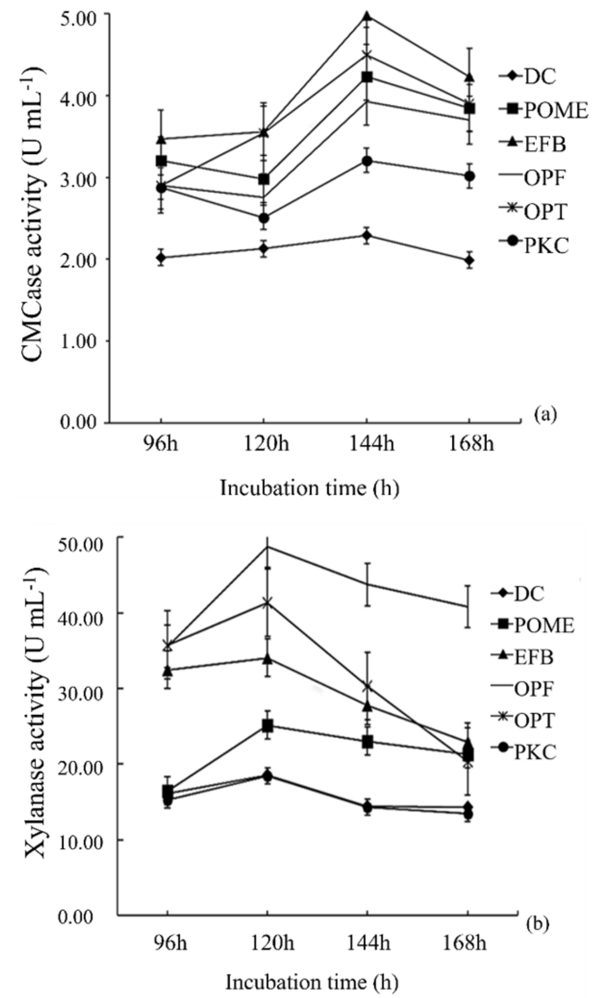

Fig. 6: Enzyme activities produced by *Beauveria bassiana* B14532 during incubation: (a) CMCase activity and (b) xylanase activity measured at 96, 120, 144, and 168h on Decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), and palm kernel cake (PKC) (*n* = 5).

These patterns agree with earlier studies showing that *B. bassiana* secretes inducible hydrolytic enzymes to utilize lignocellulosic substrates (Silva et al., 2005; Alves et al., 2020; Liu et al., 2025). The observed peaks at 4–5d of incubation are consistent with reports by Leopold and Samšiňáková (1970), while comparative studies revealed that *B. bassiana* generally outperforms *Metarhizium anisopliae* in cellulase and xylanase production (Amobonye et al., 2023; Ferreira et al., 2023). Notably, strain B14532 produced higher enzyme titers on POMWs than on defined media, emphasizing the value of agro-industrial residues as cost-effective substrates for enzyme production. The strong enzymatic activity of *B. bassiana* is relevant not only to its entomopathogenic lifestyle—where hydrolytic enzymes facilitate cuticle penetration—but also to biotechnological applications, including biodegradation of agricultural wastes, biofertilizer development, and integrated pest management (Kluczek-Turpeinen et al., 2003; Kaur et al., 2006). These findings support the potential of *B. bassiana* B14532 as a dual-purpose agent for both biological control and sustainable biomass valorization.

The observed enzyme profiles, particularly elevated chitinase and protease activities, were associated with higher conidial yields, influencing downstream production and formulation strategies. Strains with strong hydrolytic activity perform better under SSF, promoting sporulation through optimal moisture and aeration, whereas those adapted to SMF favor biomass accumulation and may require post-process sporulation. Recent studies highlight that SSF-derived *B. bassiana* conidia maintain superior viability and bioefficacy (Rice et al., 2020; Mascarin et al., 2024), and large-scale production must comply with biopesticide standards for viability, purity, and traceability (Food and Agriculture Organization of the United Nations, 2017; U.S. Environmental Protection Agency, 2025).

Conclusion

This study established that POMWs, comprising DC, POME, EFB, OPF, OPT, and PKC, possess vital nutrients including carbon, nitrogen, and sugars, rendering them appropriate substrates for fungal proliferation. EFB, OPF, and DC facilitated the highest radial development and conidial yield of *B. bassiana* B14532, but PKC exhibited the poorest performance owing to its low sugar content and possible inhibitory chemicals. An enzyme study revealed significant cellulolytic and hemicellulolytic activity, especially on EFB and OPT, highlighting the strain's potential for pest control and biomass valorization. PCA emphasized the significant influence of the C:N ratio, sugar availability, and mineral content on fungal growth and enzyme production.

These data suggest that EFB are advisable as economical, sustainable substrates for the large-scale cultivation of *B. bassiana*. Substrate compositions should target an ideal C:N ratio of 20–28 by combining nitrogen- and carbon-rich elements. Furthermore, the recovery of enzymes from these systems presents prospects for industrial applications, including lignocellulose breakdown and biofertilizer manufacturing. Future research should concentrate on substrate pretreatment and process optimization in both solid-state and submerged fermentations to enhance conidial production and enzymatic activity, thereby aiding integrated pest management and circular bioeconomy programs.

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