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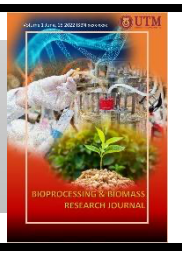

Research Article

Optimization of Crude Biodiesel Production from *Zophobas morio* Larvae using Response Surface Methodology

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ABSTRACT

Biodiesel is a renewable, sustainable, and environmentally friendly alternative fuel that plays a critical role in the automotive and transportation sectors. It is commonly produced through a transesterification process. The growing interest in biodiesel production is driven by the depletion of fossil fuel reserves and the environmental impact associated with fossil fuel combustion. In this study, *Zophobas morio* larvae (supermealworms) were investigated as a novel feedstock for biodiesel production due to their high lipid content. Key process parameters affecting biodiesel yield including the temperature, reaction time and oil-to-methanol volume ratio were initially evaluated using a one-factor-at-a-time (OFAT) approach. Significant variables identified through OFAT were optimized using RSM, yielding a maximum biodiesel output of 0.5719 g at 54.77 °C, 92.05 minutes, and an oil to methanol volume ratio of 1:0.76. This study highlights the potential of insect-based fat as a sustainable alternative to conventional vegetable oils such as corn or sunflower oil, which are edible and economically valuable. The development of biodiesel from non-edible, lipid-rich biomass such as insect larvae represents a promising step toward sustainable biofuel production in anticipation of fossil fuel scarcity.

INTRODUCTION

Biodiesel is a yellowish liquid composed of mono-alkyl esters of long-chain fatty acids, produced via transesterification of vegetable oils or animal fats with alcohol in the presence of a catalyst (Suhara *et al*., 2024). Fat is a saturated oil that solidifies at room temperature. It can be used as renewable energy without modification in diesel engines for on-road vehicles, off-road machinery such as agricultural equipment, naval engines, and locomotives, and stationary units for electricity generation (Mahmudul *et al*., 2017). The process, typically conducted at 60 – 70 °C, yields fatty acid ethyl esters (FAEE) and glycerol as a byproduct (Suhara *et al*., 2024). Biodiesel exhibits several favorable properties comparable to conventional diesel, including non-toxicity, biodegradability, and cleaner combustion (Kumar, 2020). It produces lower emissions of carbon dioxide, unburned hydrocarbons, carbon monoxide, and nitrogen oxides, contributing to reduced environmental impact (Kumar, 2020). Biodiesel also lacks sulphur content, poses minimal storage challenges, and offers excellent lubricity (Graboski & McCormick, 1998; Agarwal *et al*., 2011). Additionally, its combustion odour is less pungent than that of petroleum diesel.

Currently, approximately 95% of global biodiesel production relies on plant-derived oils such as soybean, palm, sunflower, and rapeseed oils (Mahmudul *et al*., 2017). However, the increasing demand for these oils in both the food and fuel industries has led to a significant rise in their

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market prices. This not only places economic pressure on food supply chains but also raises ethical concerns regarding the competition between fuel and food use. Consequently, the use of high-value, edible oils for biodiesel production is becoming economically and socially unsustainable. To address this issue, alternative feedstocks such as waste frying oils, animal fats, and non-edible crude vegetable oils are being explored as cost-effective and sustainable options (Wan Osman *et al.*, 2024). These non-edible and waste-derived sources reduce dependency on food-grade oils and promote the circular use of waste materials.

However, research into alternative lipid sources, particularly insect-based feedstocks, remains limited. The use of insect lipids for biodiesel is a relatively new area of study with substantial potential for development. Although some ethical concerns have been raised regarding the use of insects for fuel production, these must be balanced against the pressing need for alternative, renewable energy sources. In the context of rising global energy demand and the environmental impacts of fossil fuel consumption, exploring insect-derived biodiesel offers a viable path toward sustainable fuel production.

Insects represent one of the most abundant and diverse biological resources globally, with considerable potential as alternative lipid sources for biodiesel production. These lipids comprising both fats and oils vary in content depending on species, developmental stage, and geographical origin (Sajjad *et al.*, 2024). Among insect orders, Coleopterans, which include beetles, are reported to possess some of the highest lipid concentrations (Siddiqui *et al.*, 2025). They often contain higher levels of essential fatty acids compared to conventional animal fats, enhancing their viability for biofuel application (Harsányi *et al.*, 2020).

Zophobas morio or *Zophobas atratus* larvae, commonly known as supermealworms, are the larval stage of a darkling beetle species belonging to the family Tenebrionidae and are native to Central and South America (Tautz, 2002). These larvae are widely used as feed for birds, fish, and reptiles, and can grow up to approximately 50 mm in length. Notably, supermealworms possess a high lipid content, making them a promising candidate for biodiesel production. The oil extracted from the larvae is not only abundant but also rich in fatty acids, both in quantity and quality, which are suitable for conversion into biodiesel via the transesterification process. Nevertheless, studies on biodiesel production and lipid enhancement in *Zophobas morio* larvae remain limited, with only two reported to date, by Leung *et al.* (2011) and Goo *et al.* (2023). Therefore, the present study aims to investigate the potential of supermealworms as a novel and sustainable feedstock for biodiesel production.

MATERIALS AND METHOD

Materials

A total of 3 kg of four-month-old supermealworms (ca. 1000 individuals) were obtained from a local supplier (**Figure 1**). The batch comprised mixed larval instars ranging from the 14th to 16th stages according to Kim *et al.* (2015). Prior to acquisition, the larvae had been reared on a diet consisting of wheat bran, oats, and assorted vegetables.

Husbandry of Supermealworms

The supermealworms were placed in five plastic trays (43 × 29 × 9 cm) containing 500 g of a substrate mixture composed of ground chicken bran, wheat bran, and bubble rice in a 2:1:1 ratio, which served as the food. Each tray housed approximately 200 larvae, with average individual body weights 0f 700 mg. The trays were kept uncovered to allow adequate air circulation at room temperature under a 12 h:12 h light–dark cycle (Srinil *et al.*, 2005). Medium-sized carrot slices were provided as a water source and replenished three times per week. The substrate was replaced every two weeks following cleaning. The larvae were reared on the substrate for a period of four weeks until they reached the 17th and 18th larval instars (Kim *et al.*, 2015) to promote enhanced lipid accumulation.

Figure 1 Supermealworms maintained in a plastic tray with chicken bran, wheat bran and bubble rice

Preparation of Supermealworms

Following a four-week feeding period, a total of 200 larvae (wet basis: 140 g) supermealworms were prepared for oil (fat) extraction. The larvae were exposed to −20 °C for one hour to reduce metabolic activity and ensure mortality prior to processing. The frozen larvae were then homogenized in distilled water using a blender. The homogenates were centrifuged at 10,000 rpm for 30 minutes, and the resulting triglyceride layer was collected, weighed, and transferred into 100 ml conical flasks. The solidified oil layer was subsequently heated at 120 °C for 15 minutes to ensure complete melting into a liquid oil. After heating, the extracted oil was allowed to cool to 50 °C prior to further processing (Math & Irfan, 2007).

Genera Procedure of Transesterification

Methanol (99% purity) was added to the oil in each conical flask at a selected ratio and stirred for 15 minutes, resulting in the formation of a chalky emulsion. Subsequently, 1.5 mL of sulfuric acid was added per 100 mL of oil to catalyze the reaction. The mixture was continuously stirred at 50 °C for 1 hour, followed by an additional hour of stirring without heating. In parallel, a sodium ethoxide solution was prepared by dissolving 0.12 g of sodium hydroxide in 120 ml of methanol per litre of oil. Half of the prepared sodium ethoxide solution was added to the reaction mixture, which was then heated to the selected reaction temperature. The remaining half of the sodium ethoxide solution was subsequently introduced. The reaction was carried out under continuous heating and stirring for different reaction time intervals (Math & Irfan, 2007).

Phase Separation of Biodiesel

Following transesterification, the reaction mixture was allowed to separate into two distinct phases due to the difference in density between biodiesel and glycerol. Under gravitational settling, biodiesel formed the upper layer, while the denser glycerol mixed with water and other impurities settled at the bottom (Wang *et al.*, 2024). This

separation resulted in a clear, golden-coloured biodiesel layer atop a light brown glycerol layer. The bottom glycerol phase was carefully drained, and the remaining biodiesel was washed three times with deionized water to remove residual contaminants. The oil (fat) content of the supermealworms was quantified and expressed as a percentage of total biomass. The biodiesel yield and the conversion efficiency of oil into biodiesel were subsequently calculated.

Screening of Process Parameters for Transesterification

Factors influencing biodiesel production were investigated using a one-factor-at-a-time (OFAT) experimental approach, in which only one parameter was varied while all others were held constant. Each experimental condition was conducted in triplicate to ensure reproducibility. The oil-to-methanol volume ratios (w/v) examined were 1:0.3, 1:0.4, 1:0.5, 1:0.6, and 1:0.7. The reaction temperatures tested were 55, 60, 65, 70, and 75 °C, while the reaction times evaluated were 30, 60, 90, 120, and 150 minutes. These parameter values were previously optimized and used as a basis to determine the ranges of oil-to-methanol ratio, reaction time and temperature for preliminary screening in this study (Naseef & Tulaimat, 2025). The parameter levels that yielded the highest biodiesel output in each set were selected as the starting points for subsequent optimization experiments. Statistical analysis was conducted using SigmaStat (v.3.1), with significance set at $p < 0.05$. Multiple comparisons among means were evaluated using Tukey's Honest Significant Difference (HSD) test.

Statistical Optimization of Biodiesel Production

In this study, optimization of three selected parameters: oil-to-methanol volume ratio, reaction temperature, and reaction time, was carried out using a Box–Behnken Design (BBD) to maximize biodiesel yield. The lower, middle, and upper levels of these design variables were defined based on the findings from the screening experiments. The selected ranges and levels for each process variable are presented in **Table 1**.

Table 1 The upper, middle and lower level of process variables

Symbol	Independent Variables	Units	Level		
			+1	0	-1
A	Volume ratio of oil to methanol (w/v)	%	0.9	0.75	0.6
B	Reaction time	minute	120	90	60
C	Reaction temperature	°C	60	55	50

A total of 17 experimental runs were generated, including five replicated centre points to estimate experimental error and ensure the reliability of the model. Each parameter was studied at three levels: low (−1), medium (0), and high (+1). **Table 2** presents the experimental design matrix based on the BBD used in this study.

A second-order polynomial model is required in such cases to adequately describe the relationship between the variables and the response. For the three variables, the data were fitted to the second-order polynomial equation shown in **Equation (1)**:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad \textbf{Equation (1)}$$

where Y represents the dependent variable (biodiesel yield), and X_1, X_2, and X_3 are the independent variables: oil-to-methanol volume ratio, reaction time, and reaction temperature, respectively. The coefficient β_0 denotes the intercept; β_1, β_2, and β_3 are the linear coefficients; β_{12}, β_{13}, and β_{23} represent the interaction coefficients; and β_{11}, β_{22}, and β_{33} are the quadratic coefficients. The coefficient of determination (R^2) and adjusted R^2 were used to assess the goodness of fit of the model. The statistical significance of the model was evaluated through analysis of variance (ANOVA) using JMP statistical software (SAS Institute Inc., Cary, NC, USA).

Table 2 Experimental design using BBD with five centre points

Run	Variable 1	Variable 2	Variable 3
	Volume ratio oil to methanol (%)	Reaction time (min)	Reaction temperature (°C)
1	0.75 (0)	60 (-1)	50 (-1)
2	0.60 (-1)	120 (+1)	55 (0)
3	0.60 (-1)	90 (0)	50 (-1)
4	0.90 (+1)	90 (0)	50 (-1)
5	0.60 (-1)	60 (-1)	55 (0)
6	0.75 (0)	90 (0)	55 (0)
7	0.75 (0)	120 (+1)	50 (-1)
8	0.75 (0)	90 (0)	55 (0)
9	0.75 (0)	90 (0)	55 (0)
10	0.75 (0)	60 (-1)	60 (+1)
11	0.60 (-1)	90 (0)	60 (+1)
12	0.75 (0)	90 (0)	55 (0)
13	0.75 (0)	120 (+1)	60 (+1)
14	0.75(0)	90 (0)	55 (0)
15	0.90 (+1)	120 (+1)	55 (0)
16	0.90 (+1)	60 (-1)	55 (0)
17	0.90 (+1)	90 (0)	60 (+1)

RESULTS AND DISCUSSION

Extracted Oil Content of Supermealworms

The oil (fat) content obtained in the present study was 78.9 g (56.4%) from 200 supermealworms using an aqueous solution, representing the highest reported value among recent studies on oil extraction from *Z. morio*. The variation in oil content compared to previous reports is likely attributable to two main factors. First, a different extraction approach was employed. In this study, the entire bodies of supermealworms were homogenized without discarding any parts, thereby minimizing oil loss and resulting in a higher oil yield. Second, differences in the behavioural ecology and physiological state of the insects may have influenced lipid accumulation.

Several studies, including those by Rumpold & Schlüter (2013) and Bednářová *et al*. (2013) reported oil contents ranging from 40% to 42%. These studies shared a common trait where larvae were harvested without prior feeding, which may have contributed to lower oil accumulation. In contrast, Leung *et al*. (2011) reported a lower oil content of 33.8%, possibly due to their use of an oil-burning method. This process may have underestimated the total oil content, as some lipids may have remained stored deep within the tissues, hindering complete extraction.

Preliminary Transesterification

A preliminary transesterification was conducted using optimized parameters reported by Alamu *et al*. (2007) for palm kernel oil. These included oil-to-methanol volume ratio of 0.3 (v/w), a reaction time of 90 minutes, and a temperature of 65 °C. Although these conditions were not specifically tailored for supermealworm oil, they provided a rational starting point for evaluating biodiesel conversion from this feedstock. Transesterification of 4.0 g of extracted oil under these conditions yielded 2.3 g of biodiesel, corresponding to a conversion efficiency of 57.5% (**Figure 2**). These preliminary results indicate that the obtained values are suitable as a baseline for biodiesel production from supermealworm oil and can be used to guide further optimization**.**

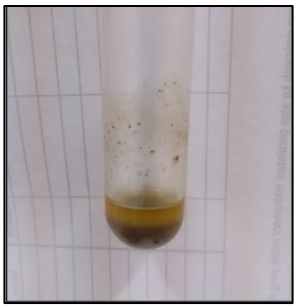


Figure 2 From supermealworm oil (fat), yellowish biodiesel formed on top and dark brown glycerol at the bottom

Effect of Volume ratio of oil to Methanol

Figure 3 illustrates the effect of the oil-to-methanol volume ratio on biodiesel yield using 1 g of oil. As the volume ratio increased from 30% (w/v) to 60% (w/v), a significant increase in biodiesel yield was observed. The maximum yield of 0.70 ± 0.003 g was achieved at a 60% (w/v) oil-to-methanol ratio. Beyond this point, increasing the ratio to 70% (w/v) resulted in a slight but statistically non-significant decrease in yield to 0.65 ± 0.002 g. The oil to methanol ratio strongly influences biodiesel recovery from insect fat because sufficient methanol drives complete transesterification, lowers viscosity for better mixing, and promotes clean glycerol separation, while excessive methanol can hinder phase separation and reduce overall yield (Shi *et al*., 2022; Siddiqui *et al*., 2025). Based on these findings, the 60% (w/v) ratio was selected as the fixed parameter for subsequent OFAT experiments, as it had a statistically significant effect on biodiesel production at $p < 0.05$.

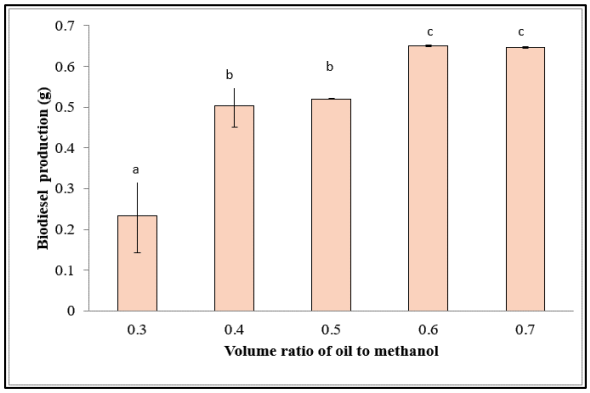


Figure 3 The effect of volume ratio of oil to methanol on biodiesel yield. Same alphabets located above the bar graph indicate no significant difference ($p < 0.05$)

Effect of Reaction Time

At a constant oil-to-methanol volume ratio (v/v) of 0.6 and a temperature of 65 °C, the effect of reaction time on biodiesel production was evaluated by varying the reaction duration at 30, 60, 90, 120, and 150 minutes. **Figure 4** shows the effect of the reaction time on biodiesel production using 1 g of oil. The graph illustrates a significant increase in biodiesel yield as the reaction time increased from 30 to 120 minutes, rising from 0.56 ± 0.018 g to 0.74 ± 0.054 g. However, extending the reaction time to 150 minutes resulted in a non-significant decrease in yield to 0.65 ± 0.026 g. Therefore, a reaction time of 120 minutes was selected as the fixed parameter for subsequent OFAT experiments, as it significantly influenced biodiesel production at $p < 0.05$.

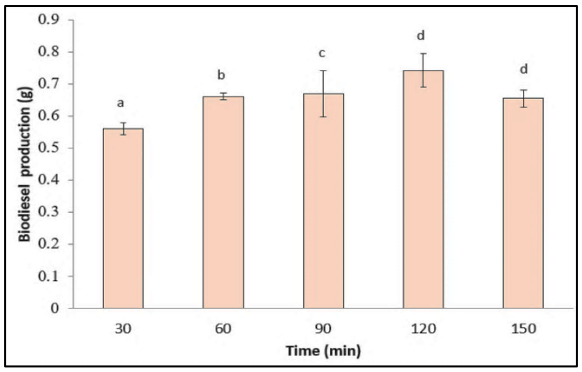


Figure 4 The effect of reaction time on biodiesel yield. Same alphabets located above the bar graph indicate no significant difference ($p < 0.05$)

In the experiment, the highest biodiesel yield was achieved at 120 minutes. This reaction time is considered relatively long, as several studies have reported maximum biodiesel yields at around 90 minutes. According to Alamu *et al*. (2007), once the optimal biodiesel yield is achieved at 90 minutes, extending the reaction time may no longer be cost-effective. A reaction time beyond the optimum point does not result in further yield improvement. This suggests that the effect of reaction time on base-catalysed transesterification using oil (fat) extracted from supermealworms with methanol differs from that reported by Alamu *et al.* (2007), who used palm kernel oil under similar conditions.

The increase in fatty acid ester conversion observed with longer reaction times can be attributed to the gradual progression of the reaction. Initially, the reaction proceeds slowly due to the time required for proper mixing and dispersion of methanol and oil. As the reaction continues, the conversion rate increases until it reaches a maximum at 120 minutes. Extending the reaction time beyond this point does not improve yield and may, in fact, reduce the final biodiesel output. This reduction can be explained by the reversible nature of the transesterification reaction and the potential for side reactions such as soap formation, which reduce the available esters (Jagadale & Jugulkar, 2012).

Effect of Reaction Temperature

The base-catalysed transesterification was conducted at a fixed oil-to-methanol volume ratio of 0.6 and a reaction time of 120 minutes, while varying the reaction temperature at 50 °C, 55 °C, 60 °C, 65 °C, and 70 °C. The effect of reaction temperature on biodiesel yield is presented in **Figure 5**. The graph indicates that biodiesel yield increased significantly

from 0.46 ± 0.025 g to a maximum of 0.60 ± 0.013 g as the temperature increased from 50 °C to 60 °C. However, a significant decline in yield was observed at 70 °C, dropping to 0.51 ± 0.028 g. Therefore, a reaction temperature of 60 °C was selected as it significantly ($p < 0.05$) influenced biodiesel production.

In this experiment, the highest biodiesel yield was obtained at 60 °C. This temperature falls within the optimal range reported by Mathiyazhagan & Ganapathi (2011), who noted that the ideal reaction temperature typically varies between 50 °C and 60 °C, depending on the type of oil or fat used. Many studies recommend conducting the reaction near the boiling point of the alcohol to accelerate conversion efficiency.

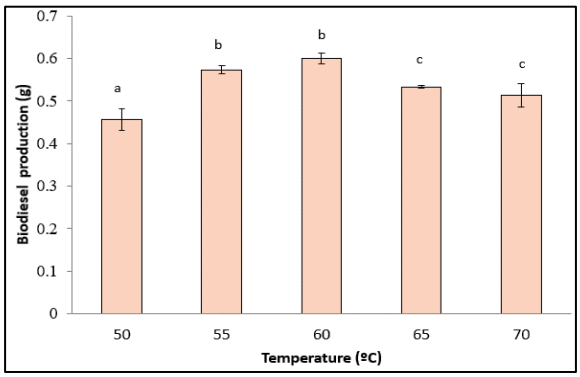


Figure 5 The effect of reaction temperature on biodiesel yield. Same alphabets located above the bar graph indicate no significant difference ($p < 0.05$)

Reaction temperature is a critical factor influencing biodiesel yield. Elevated temperatures can enhance the reaction rate and reduce viscosity, thereby shortening the reaction time. However, exceeding the optimal temperature can reduce biodiesel yield. This reduction is attributed to increased saponification of triglycerides at higher temperatures (Mathiyazhagan & Ganapathi, 2011), as well as alcohol evaporation, which lowers the availability of reactants (Anitha & Dawn, 2010). Therefore, the transesterification temperature is typically maintained below the boiling point of the alcohol to minimize evaporation and optimize yield.

Optimization of Transesterification Process

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques employed for modeling and analysing problems in which a response of interest is influenced by several variables, with the goal of optimizing this response (Montgomery *et al*., 2004). The use of RSM in process optimization aims to minimize the cost and computational burden associated with conventional experimental methods, while also accounting for potential variability or noise in the data.

The significant effects of the individual parameters and their interactions on biodiesel production were analyzed to determine the optimized conditions of transesterification for maximal biodiesel yield. The three key parameters identified through the preceding OFAT analysis: oil-to-methanol volume ratio, reaction time, and reaction temperature, were selected for further investigation.

Using the generated design matrix, the corresponding experimental biodiesel yields in triplicate are presented in **Table 3**. Based on the experimental data, the biodiesel yield ranged from 0.3226 g ± 0.0074 to 0.5719 g ± 0.0068. The highest yield (0.5719 g) was achieved in Run 6, which utilized a volume ratio of oil to methanol of 0.7, a reaction time of 90 minutes, and a reaction temperature of 55 °C. In contrast, the lowest yield (0.3226 g) was recorded in Run 11, conducted at a volume ratio of 0.6, a reaction time of 90 minutes, and a reaction temperature of 60 °C.

Based on the data in **Table 3**, the second-order polynomial equation (**Equation 2**) was fitted to model the relationship between the three independent variables and the biodiesel yield. The coded factor equation is shown in **Equation (2)**, where A, B, and C represent the regression coefficients for the volume ratio of oil to methanol, reaction time, and reaction temperature, respectively.

$$Biodiesel\ yield = 0.57 + 0.017A + 9.575E - 003B - 0.012C + 0.014AC + 0.085A^2 + 0.076B^2 - 0.12C^2$$

Equation (2)

The analysis of variance (ANOVA) was conducted to determine the most appropriate mathematical model for the experimental data. Model selection was based on the statistical significance of the terms, where terms with *p*-values less than 0.05 were considered significant. Among the evaluated models, the quadratic model was identified as the best fit, as it exhibited a highly significant *p*-value (< 0.0001) compared to the two-factor interaction (2FI) model. Therefore, the quadratic model was selected to describe the response of biodiesel production in this experimental design.

Table 3 Experimental results for the three independent variables using Box-Behnken design

Run	Variable 1	Variable 2	Variable 3	Biodiesel production (g)
	Volume ratio oil to methanol (v/v)	Reaction time (min)	Reaction temperature (ºC)	
1	0.75 (0)	60 (-1)	50 (-1)	0.3739 ± 0.0069
2	0.60 (-1)	120 (+1)	55 (0)	0.3947 ± 0.0112
3	0.60 (-1)	90 (0)	50 (-1)	0.3663 ± 0.0092
4	0.90 (+1)	90 (0)	50 (-1)	0.3744 ± 0.0047
5	0.60 (-1)	60 (-1)	55 (0)	0.3857 ± 0.0153
6	0.75 (0)	90 (0)	55 (0)	0.5719 ± 0.0068
7	0.75 (0)	120 (+1)	50 (-1)	0.4008 ± 0.0127
8	0.75 (0)	90 (0)	55 (0)	0.5643 ± 0.0283
9	0.75 (0)	90 (0)	55 (0)	0.5587 ± 0.0068
10	0.75 (0)	60 (-1)	60 (+1)	0.3518 ± 0.0059
11	0.60 (-1)	90 (0)	60 (+1)	0.3226 ± 0.0074
12	0.75 (0)	90 (0)	55 (0)	0.5702 ± 0.0017
13	0.75 (0)	120 (+1)	60 (+1)	0.3586 ± 0.0016
14	0.75(0)	90 (0)	55 (0)	0.5629 ± 0.0045
15	0.90 (+1)	120 (+1)	55 (0)	0.4370 ± 0.0025
16	0.90 (+1)	60 (-1)	55 (0)	0.4031 ± 0.0056
17	0.90 (+1)	90 (0)	60 (+1)	0.3877 ± 0.0111

Note: The shaded rows indicate the maximum and minimum biodiesel production during the experiment

The lack-of-fit test evaluates how well the model fits the experimental data by comparing the variation around the fitted model to the pure error. For the quadratic model, the lack-of-fit *p*-value was 0.2550, indicating that it was statistically insignificant ($p > 0.05$). This suggests that the model does not exhibit a significant lack of fit. With an F-value of 2.01 relative to the pure error, the quadratic model was considered to adequately represent the experimental

data and is thus deemed appropriate for predicting the response.

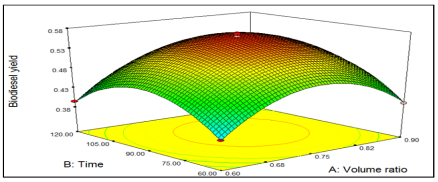
The significance of model parameters was determined using ANOVA. As shown in **Table 4**, the model F-value of 346.29 (p < 0.0001) indicates a highly significant model. Significant terms include A, B, C, AC, A^2, B^2, and C^2, all with p-values below 0.05. The predicted R^2 =0.9949 is in good agreement with the adjusted R^2 = 0.9978, confirming the adequacy of the model. A low coefficient of variation of 1.49% further indicates high reliability and low variability in the experimental data.

Table 4 Analysis of variance for quadratic model

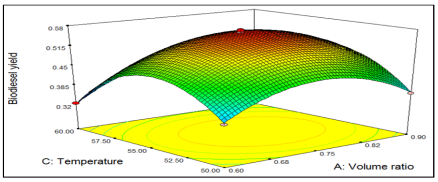
Source	Sum of Squares	F Value	p-value
Model	0.13	346.29	< 0.0001
A-Volume ratio of oil to methanol	2.208E-003	52.49	0.0002
B-Reaction time	7.334E-004	17.44	0.0042
C-Reaction temperature	1.121E-003	26.65	0.0013
AB	1.550E-004	3.68	0.0964
AC	8.122E-004	19.31	0.0032
BC	1.010E-004	2.40	0.1652
A^2	0.030	714.71	< 0.0001
B^2	0.024	577.78	< 0.0001
C^2	0.059	1402.02	< 0.0001

Note: coefficient of determination, R^2 = 0.9978, adjusted coefficient of determination, adjusted R^2 = 0.9949 and C.V. = 1.49.

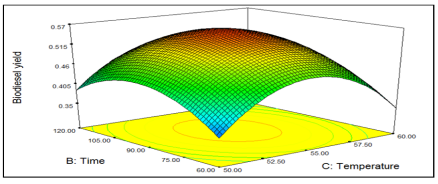
The 3D response surface plots (**Figure 6A-6C**) shows an optimized transesterification condition that yields a maximum biodiesel output of 0.5719 g, with a distinct peak indicating optimal values. The predicted optimum conditions were an oil-to-methanol volume ratio of 0.76 (w/v), 92.05 minutes reaction time, and 54.77 °C reaction temperature. While many studies have optimized biodiesel production using plant oils, few have used insect fat. For comparison, Dwivedi & Sharma (2015) optimized biodiesel from *Pongamia* oil using BBD, reporting optimal conditions of an 11.06:1 methanol-to-oil molar ratio, 81.4 minutes, and 56.6 °C. The reaction temperatures and times were comparable to this study, though the third parameter (volume ratio vs. molar ratio) differs, limiting direct comparison. Nonetheless, their findings partially support the present results.



A. 3D response surface curve of biodiesel production as a function of reaction time and volume ratio of oil to methanol.



B. 3D response surface curve of biodiesel production as a function of reaction temperature and volume ratio of oil to methanol



C. 3D response surface curve of biodiesel production as a function of reaction time and temperature.

Figure 6 Response surface plots showing the interaction effects of two parameters on biodiesel yield

Model validation confirmed the accuracy of the optimization. At the predicted optimum conditions, the software estimated a biodiesel yield of 0.57 g, while the actual yield obtained was 0.56 g, indicating a small and acceptable error of 1.75%. Leung *et al*. (2011) reported that dried *Z. morio* larvae contained 33.80 wt % oil, and biodiesel produced via optimized two-stage acid–alkaline transesterification yielded 92.35 wt % fatty acid methyl esters; in contrast, 200 wet larvae in this study produced 78.9 g of oil (fat), from which only 0.5719 g of biodiesel (0.73 wt %) was obtained. Similarly, the present results were much lower than Wang *et al*. (2017), who reported that 8.5 g of dried biomass, consisting of combined black soldier fly and yellow mealworm larvae, produced 90.3 wt % biodiesel. The lower yield in this study was likely due to the high moisture content in the larvae's fat obtained from aqueous extraction, which was not dried prior to use and consequently interfered with transesterification during biodiesel production (Lin & Ma, 2020; Makareviciene *et al*., 2020). Future studies could retain aqueous extraction while improving yield by drying the fat (oil) to reduce moisture before transesterification.

CONCLUSION

This study successfully achieved its objective of optimizing the transesterification process for biodiesel production from *Z. morio* larvae. Methanol was selected as the alcohol due to its non-toxicity, water immiscibility, high boiling point, and low vapour pressure. At the end of the reaction, two distinct layers were formed: a straw-yellow biodiesel layer at the top and a brown, viscous glycerine layer at the bottom. Reaction temperature, reaction time, and oil-to-methanol volume ratio were identified as the main parameters affecting biodiesel yield. Initial screening was carried out using the OFAT approach, and the statistical significance of each factor was determined using SigmaStat software. Optimization using the Box–Behnken design under response surface methodology identified the optimum conditions for maximum biodiesel yield of 0.5719g at a reaction temperature of 54.77 °C, a reaction time of 92.05 minutes, and an oil-to-methanol volume ratio of 1:0.76.

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Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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