Journal of Asia-Pacific Entomology 21 (2018) 836–842

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Full Length Article

Factors affecting the bioconversion of Philippine tung seed by black soldier fly larvae for the production of protein and oil-rich biomass

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ARTICLE INFO

Keywords:
Reutealis trisperma
Hermetia illucens
Biomass productivity
Protein
Cattle feed

ABSTRACT

A systematic study on the use of Philippine tung (*Reutealis trisperma)* seed as a substrate for the cultivation of black soldier fly larvae (*Hermetia illucens*) was performed. The characteristics of *Reutealis trisperma* seed from two different locations: West Java and Papua, were determined. The seed has a relatively high oil (37.6–39.2%, dry weight) and protein content (14.9–28.2%, dry weight). The effect of cake content in the substrate (0–20%, wet weight), moisture content in the substrate (50–70%, wet weight), feeding rate (50–100 mg/larva/d), lighting condition (dark-light) and substrate depth in a rearing container (4–10 cm) was performed. An optimum prepupal biomass productivity of 123.4 g/m^2/d was obtained (20%, wet weight of cake content in the substrate, 60%, wet weight of moisture content in the substrate, 100 mg/larva/d, dark, 6 cm substrate depth). The protein and oil content of the biomass were also determined to evaluate the effect of *Reutealis trisperma* seed as a substrate for the cultivation of black soldier fly larvae to produce protein and oil-rich biomass. The oil content in the biomass was also extracted and the fatty acid composition was identified. The prepupal biomass has a relatively high amount of protein (45%, dry weight) and oil content (26.6%, dry weight) and is suitable for cattle feed application.

Introduction

The Philippine tung tree (*Reutealis trisperma*) is an evergreen tree that is widely scattered in forests at low and medium altitudes in Philippines. This tree is also cultivated in other countries such as Indonesia, China and India for its timber and medicinal use. The tree produces seed which has not yet being valorized. The productivity of Philippine tung (trisperma) seeds is reported to be in the range of 3.8–8.7 ton/ha/yr (Kumar et al., 2015; Agricultural Office of West Java). The identification of added values from trisperma seeds, using a biorefinery concept is highly relevant to increase the overall value of the trisperma tree.

Trisperma seeds consist of 65% kernels by weight (wt%) on a dry basis (d.b) and 35 wt%, d.b. shells. The kernels have an oil content in the range of 58–61 wt% d.b. which corresponds to 38–40 wt%, d.b. oil content on a seed basis (Abduh et al., 2018). The oil, also known as trisperma oil can be isolated from the kernel and may be a valuable source for biodiesel production and may find applications as ingredients in making soap and paints (Holilah et al., 2015; Kumar et al., 2015). The cake obtained after the isolation of oil from the kernels may be utilized as cattle feed because it contains a considerable amount of protein (Manurung et al., 2016). The remaining biomass, particularly the shell, is normally discarded as waste.

The shell containing a relatively high amount of lignocellulose may be mixed with the cake as a substrate for the cultivation of black soldier fly larvae (BSFL) (*Hermetia illucens* L.) to produce higher value added products such as protein and oil-rich biomass. BSFL is a non-pest fly that has a relatively high protein and fat content which lies in the range of 29–55 wt% and 19–39 wt%, respectively. Diener et al. (2009) demonstrated that the protein content of BSFL varied from 28 to 43 wt% when the larvae were fed with chicken feed which depends on the feeding rate. In another study by Abduh et al. (2017), BSFL was fed with rubber seeds and the protein content varied from 29 to 55 wt% whereas the oil content varied from 19 to 28 wt% which depends on whether the rubber seeds were pre-treated with consortium of microbes or not. St-Hillaire reported that BSFL fed with fish offal had on average 30 wt% oil, which was 43 wt% more than the BSFL fed with cow manure only. Another study by Zheng et al. (2011) demonstrated that the oil content of the BSFL reached up to 39 wt% when the BSFL was fed with restaurant solid waste. As such highlights that the protein and oil

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https://doi.org/10.1016/j.aspen.2018.06.007
Received 15 January 2017; Received in revised form 7 June 2018; Accepted 12 June 2018
Available online 18 June 2018
1226-8615/

content of the BSFL highly depends on the substrate consumed by the larvae.

Studies on the conversion of organic material by BSFL to produce insect biomass have been reported in the literature (Diener et al., 2009; Zheng et al., 2012; Li et al., 2011). However systematic studies on factors affecting bioconversion of trisperma seeds for producing protein and oil-rich biomass are not yet available in open literature. Hence, this study aims to determine the factors affecting bioconversion of trisperma seed by BSFL, particularly the cake content in the substrate, moisture content in the substrate, substrate depth in a rearing container, and feeding rate, for producing insect biomass with a high level of protein and oil. The protein and oil content of the biomass were also determined to evaluate the potential of the produced insect biomass to be used as cattle feed.

Materials and methods

Hydraulic pressing of trisperma seeds

Ruetealis trisperma seeds from West Java were first dehulled to separate kernel from the shell. Trisperma oil was isolated from the kernel using a locally constructed laboratory scale hydraulic press (ITB, Bandung). Approximately 500 g of sample was placed in the pressing chamber and pressed at room temperature (27 °C) for 4–5 h (Abduh et al., 2017). The pressed oil was analyzed with GC–MS to determine the fatty acid composition and the pressed cake was used as a substrate for the cultivation of BSFL.

Soxhlet extraction of trisperma oil and BSFL oil

Ruetealis trisperma seeds from Papua were first dehulled to separate the kernels from the shells. Trisperma oil within the kernels was extracted using a Soxhlet extraction procedure. The kernel was dried at dried at 103 °C and ground using a coffee grinder. Approximately 7 g of sample was transferred into a Soxhlet timble and extracted with n-hexane (99 vol%, Bratachem, Bandung) for at least 5 h. The solvent was evaporated using a rotary evaporator (atmospheric pressure, 69 °C) and sample was subsequently dried at 103 °C until constant weight was achieved (Abduh et al., 2016).

The oil within the BSFL sample was also extracted using similar procedures. The oil content in the sample is reported as gram oil per gram sample on a dry basis. The extracted oil was analyzed with GC–MS to determine the fatty acid composition. The analyses were performed in triplicate and reported as an average value.

Cultivation of BSFL with the remaining biomass from trisperma seeds

Black soldier fly eggs were obtained from a local farmer at Sumedang, Indonesia. The eggs were initially hatched and later reared on chicken meal (60 wt% moisture) for 7 d. Twenty 7 d old BSFL were placed inside a rearing container and fed with the remaining biomass obtained after the isolation of oil from the trisperma seeds (Manurung et al., 2016). Due to limited availability of trisperma seeds from Papua, only trisperma seeds from West Java were used as a substrate for the cultivation of BSFL. The pressed cake was mixed with the shell (cake content: 0, 10, 20 wt%) before ground and sieved (12 mesh). The mixed biomass was added with water (moisture content: 50, 60, 70, wt%) and used as a substrate for the cultivation of BSFL. The feeding rate was set at 50, 75 and 100 mg/larvae/d. The depth of substrate provided to BSFL in the rearing container was also varied from 4, 6, 8, and 10 cm. Two types of rearing container were used in this study: i) a cylindrical container (diameter: 7 cm, substrate depth: 10 cm) ii) a rectangular container with (length: 26 cm, width: 16 cm, substrate depth: 4, 6, 8 and 10 cm). The rearing containers were subjected to different lighting conditions: dark and light. A dark condition implies that the rearing containers were covered by a black plastic cover (approximately 0 lx), ii) a light condition implies that rearing containers were covered by a transparent plastic cover (approximately 80–90 lx).

The larvae were weighed every 3 d and moved into a new container and fed with a fresh substrate. The weight of residues in the container that comprises of excretory products and unconsumed feed was recorded before and after drying at 103 °C. Data measurement was carried out until most of the larvae (at least 50% of the total population) in the rearing container had developed into prepupae (approximately 12–13 d). The prepupae were inactivated by drying at 103 °C until constant weight was achieved. All of the analyses were performed in triplicate and expressed as mean ± standard deviation. Differences were tested with one-way analysis of variance (ANNOVA) using MINITAB 17.

Data analysis

The efficiency of BSFL in converting the remaining biomass of *Ruetealis trisperma* seed into protein-rich biomass was accessed by calculating the assimilation efficiency (AE), efficiency of conversion of digested-feed (ECD) and waste reduction index (WRI) as suggested by Scriber and Slansky (1981) and Diener et al. (2009). The equations and description to calculate AE, ECD and WRI are described by Manurung et al. (2016).

Analytical methods

The lignocellulose content of the samples was determined based on the procedures by Manurung et al. (2016). The protein content of the sample was analyzed by Kjedahl method (SNI-01-2891-1992). The analysis was carried out at the Analytical Laboratory, University of Padjajaran, Jatinangor. The fatty acid composition of trisperma and BSFL oil was determined by gas chromatography-mass spectrometry (GC–MS) at the Chemical Laboratory, University of Education of Indonesia, Bandung. The analyses were performed in triplicate and reported as an average value.

Results and discussion

Characteristic of trisperma seeds

Trisperma seeds investigated in this study were obtained from two different locations: West Java and Papua, Indonesia. The initial moisture content of the seeds from West Java and Papua upon receipt was approximately 20.6 wt% and 26.8 wt%, respectively as shown in Table 1. The seeds from West Java consist of approximately 35.1 wt%, d.b. shells and 64.9 wt%, d.b. kernels whereas the seeds from Papua consist of approximately 32.7 wt%, d.b. shells and 67.3 wt%, d.b.

Table 1
Weight fraction, oil content, protein content and moisture content of trisperma seed, kernel and shell from West Java and Papua.

Property	West Java	Papua
Weight fraction, wt% d.b.		
Seed	100	100
Kernel	35.1	32.7
Shell	64.9	67.3
Oil content, wt% d.b.		
Seed	39.2	37.6
Kernel	60.3	56.4
Shell	0	0
Protein content, wt%, d.b.		
Seed	14.9	28.2
Kernel	32.7	67.8
Shell	5.2	9.0
Moisture content, % d.b.		
Seed	20.6	26.8
Kernel	22.0	–
Shell	16.8	–