

Bioconversion and growth performance of *Hermetia illucens* in single fruit by-products

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Abstract

As agricultural production increases due to population growth, agricultural by-products that are generated at all production stages increase commensurately. Black soldier fly (BSF) (*Hermetia illucens*) treatment has potential as an environmentally friendly process to combat the environmental pollution caused by agricultural by-products. This study examined the utilization of BSF larvae in the decomposition of the by-products of apples and mandarins, fruits commonly produced in South Korea. The BSF test larvae were fed apple pomace or mandarin waste, and the control larvae were fed calf feed. Larval weight and size were measured at 4-day intervals until larvae reached the pre-pupal stage. Larval development time, survival rate, and BSF fecundity rate were calculated for all three substrates. Waste reduction and bioconversion ratios were also calculated. The developmental time of larvae fed with apple pomace and mandarin waste was greater than that of the control larvae. The average weight of larvae fed with the fruit by-products was less than that of the control. There was no significant difference in the survival rate of BSF larvae or the fecundity rate of BSF between the substrates used in this study. BSF larvae decomposed 48.0% and 61.5% of apple pomace and mandarin waste, respectively. The bioconversion efficiency rates of BSF larvae fed with apple pomace and mandarin waste were 9.1% and 12.1%, respectively. These results indicated that decomposition of single fruit by-products by BSF larvae is an environmentally friendly and effective bioconversion process.

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Int. J. Indust. Entomol. 46(2), 34-40 (2023)

Received : 20 Mar 2023
Revised : 31 Mar 2023
Accepted : 31 Mar 2023

Keywords:

Black Soldier Fly
(*Hermetia illucens*),
apple pomace,
mandarin waste

Introduction

The global population is continuously increasing and is expected to reach 9.7 billion in 2050 (United Nations, 2022). Due to increasing global population, agricultural production is projected to increase by 49% from 2013 to 2050, to meet demand (Food and Agriculture Organization of the United

Nations, 2017). Agricultural production will increase rapidly, and the number of agricultural by-products will increase correspondingly. Waste agricultural by-products are generated at all stages of production including pre-harvest, harvest, post-harvest, processing, packaging, retail, and consumption. Global food loss in 2016, amounting to 13.8%, was generated from post-harvest to distribution. This amounts to US \$400 billion

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per year (United Nations, 2020). Although agricultural by-products are used as fertilizer, compost, and a source of biogas, most by-products are left untreated and underutilized. Many are disposed of by burning, dumping, or unplanned landfilling. It is necessary to efficiently treat agricultural by-products and convert them into sustainable resources. Treatment with black soldier fly (BSF) (*Hermetia illucens*) could be an efficient system to process these agricultural by-products.

There are several advantages to using BSF treatment to convert agricultural by-products into usable biomass. First, the BSF life cycle is shorter than that of the other farming insects such as mealworms, superworms, and white-spotted flower chafers (Kwon, 2009; Ludwig, 1956; Ludwig and Fiore, 1960; Rumbos and Athanassiou, 2021), spanning approximately 45 days from egg to adult under optimal conditions. The larval stage of BSF at which it consumes substrates lasts approximately 13–18 days (Liu *et al.*, 2021; Surendra *et al.*, 2020). Second, BSF larvae can decompose various organic wastes such as food waste, agricultural by-products, livestock manure, and even human feces (Banks *et al.*, 2014; Cai *et al.*, 2018; Lim *et al.*, 2019; Mohd-Noor *et al.*, 2017; Nguyen *et al.*, 2015; Oonincx *et al.*, 2015; Zheng *et al.*, 2012). BSF larvae have an efficient digestive system in terms of physical, chemical, and biological aspects; mouthparts resembling a “tunnel boring machine” (Bruno *et al.*, 2020), and a varying pH range in the midgut (Bonelli *et al.*, 2019). Third, the BSF can convert organic waste into protein- and fat-rich biomass in the larval, pre-pupal and pupal stages (Chun *et al.*, 2019; Meneguz *et al.*, 2018; Salomone *et al.*, 2017; Spranghers *et al.*, 2017; Wang *et al.*, 2020). The biomass from BSF bioconversion can be used as a sustainable source of livestock feed and biofuels. BSFs are also considered a non-vector of human and animal diseases (Diener, 2010; Furman *et al.*, 1959).

Recently, many studies have been conducted on BSF treatment of various by-products and waste products (Giannetto *et al.*, 2020; Lalander *et al.*, 2019). To the best of our knowledge, limited studies exist on BSF decomposition of single fruit by-products such as apple pomace and waste mandarin. In South Korea, 515,931 tons of apples and 635,835 tons of mandarin were produced in 2021 (Korean Statistical Information Service, 2022), ranking them first and third in South Korea’s total fruit production, respectively. Each of these fruits are produced in large quantities in certain regions, therefore processing of by-products generated during

production poses a serious issue. In particular, approximately 99.8% of South Korea’s total mandarin production for 2021 was cultivated in Jeju Island, Republic of Korea (Korean Statistical Information Service, 2022), and due to the geographical characteristics of the island, by-products are processed by inefficient methods such as storage and reclamation.

Therefore, this study aims to investigate the processing of a single agricultural by-product, either apple pomace or mandarin waste, by BSF larvae, and examine the resulting decomposition and bioconversion of each substrate.

Materials and Methods

1. Insect culture

Black soldier fly (*Hermetia illucens*) is utilized in this study. BSFs were sourced from a colony that was maintained year-round in a glasshouse at the National Institute of Agricultural Sciences, RDA, Wanju, Republic of Korea. Eggs were harvested in traps made of floral foam (Economy Oasis K-1117, Smithers-Oasis, Republic of Korea). The traps were placed on the oviposition substrate which was mixed with 50% food waste from the cafeteria of the National Institute of Agricultural Sciences and 50% calf feed (Newtech Premium, Daehan feed, Republic of Korea) (Park *et al.*, 2016). Eggs were held in a laboratory and hatched in food waste at 26°C and 60% relative humidity (RH).

2. Growth performance of BSF larvae

Six-day old BSF larvae were used in this experiment. Five hundred BSF larvae per experimental group were kept in a plastic container (23 cm × 12.5 cm × 16.5 cm), and test larvae were fed with either apple pomace or mandarin waste. Control BSF larvae were kept under the same conditions, but were fed with calf feed. The apple pomace as the rest after squeezing apple juice, mostly comprised of peel was obtained from the apple farm, Jangsu, Republic of Korea and the mandarin waste as a semi-liquid consisting of peel and pulp was obtained from the mandarin farm, Jeju, Republic of Korea. Before use as substrates for BSF larvae, substrates were stored at 4°C. The experiment was conducted at 26°C and 60% RH. Thirty BSF larvae per experimental group were randomly collected and the weight and the length of BSF larvae were measured one by one with an electronic scale (BP190S, Sartorius AG, Germany)

and an electronic calipers (CD-10CP, Mitutoyo Corp., Japan). The measurements were conducted every 4 days until over 40% of larvae reached the pre-pupal stage. The number of BSF pre-pupae per experimental group were counted for calculating larval survival rate. Fecundity rate was conducted by checking the number of BSF adults after one hundred BSF pre-pupae in each experimental group were randomly selected.

3. Calculations and statistical analysis

The dry matter, fruit wastes, and frass from the BSF larvae were measured to calculate the feeding performance, by drying at 105° for 3h in the dry-oven (SOF-155, Daihan Science, Republic of Korea) (Shreve *et al.*, 2006). The feeding performances were calculated for different substrates using equations (1), and (2) (Liu *et al.*, 2018). *W* is the total dry weight of the substrates supplied, *R* is the total dry weight of the residue after the experiment, *G* is the difference between the last instar of BSF larvae (40% of larvae became pre-pupae) and initial BSF larvae (6-d old BSF larvae from eggs).

$$\text{Ratio of reduced waste dry matter(\%)} = \frac{W - R}{W} \times 100 \quad (1)$$

$$\text{Bioconversion efficiency (\%)} = \frac{G}{W - R} \times 100 \quad (2)$$

Statistical analyses were performed using IBM SPSS Statistics 27.0.0.0 software. Data obtained were analyzed using one-way analyses of variance (ANOVA) at a significance rate of $p < 0.05$, followed by Tukey's Honestly Significant Difference post-hoc test.

Results and Discussion

Larval Developmental time, larval survival rate and fecundity rate

The larval developmental times of BSF larvae fed with apple pomace and mandarin waste were 26 and 23 days, respectively. The larval developmental time for the control larvae was 12 days. These results were expected, because calf feed is more nutritious than the two fruit by-products (Table. 1). The developmental time of BSF is correlated with many factors such as larval density, feeding rate, pH, and the texture of substrates (Liu *et al.*, 2018; Meneguz *et al.*, 2018; Parra Paz *et al.*, 2015; Scala *et al.*, 2020). The quality of nutrients in the substrate is an

Table 1. The larval developmental time, larval survival rate, and fecundity rate of BSF adults fed with different substrates. After 40% of larvae fed with each different substrates developed into pre-pupae, larval developmental time was measured and survival rate was calculated. After the pupae of BSF became adults, the fecundity rate of adults was calculated. Larval survival and fecundity rate data are shown as the mean values \pm SD (larval survival $n = 500$, fecundity rate $n = 100$).

Parameter	Control	Apple pomace	Mandarin waste
Larval developmental time (d)	12	26	23
Larval survival rate (%)	97.1 \pm 2.9	90.6 \pm 11.5	88.13 \pm 5.1
Fecundity rate (%)	100 \pm 0	99 \pm 1	99.33 \pm 0.6

Table 2. Analysis of feeding performance of BSF larvae after feeding with different substrates. Ratio of reduced waste (%) and bioconversion efficiency (%) were analyzed using dry matter of BSF larvae and BSF larval frass for different substrates. Data are shown as the mean values \pm SE ($n = 30$). Different superscript letters indicate significant differences at $p < 0.05$.

Parameter	Control	Apple pomace	Mandarin waste
Ratio of reduced waste (%)	50.4 \pm 0.4 ^a	48.0 \pm 1.3 ^a	61.5 \pm 1.5 ^b
Bioconversion efficiency (%)	16.5 \pm 0.5 ^a	9.1 \pm 0.4 ^c	12.1 \pm 0.5 ^b

important factor affecting the developmental time of BSF (Gobbi *et al.*, 2013; Jucker *et al.*, 2017; Oonincx *et al.*, 2015). Larvae are also affected by hormones when they develop into pupae. The production of these hormones is dictated by the nutrients available, therefore if the larvae are supplied with insufficient nutrients they are slower to develop into pupae, and the larval developmental time is prolonged (Nijhout, 2003). The survival rate of larvae fed with apple pomace and mandarin waste were 90.6% and 88.1%, respectively. The survival rate of the control larvae was 97.1% higher than that of the larvae fed with two fruit by-products. However, there was no significant difference between the survival rates of the two fruit substrates. Some studies show that if nutrient levels are sufficient, the survival rate of BSF larvae are not affected by the substrate (Bohm *et al.*, 2022; Gao *et al.*, 2019; Lalander *et al.*, 2019). The test substrates are therefore presumed to have sufficient nutrients if there is no decrease the survival rate of BSF larvae. According to Jucker *et al.* (2017), the survival rate of BSF larvae were significantly

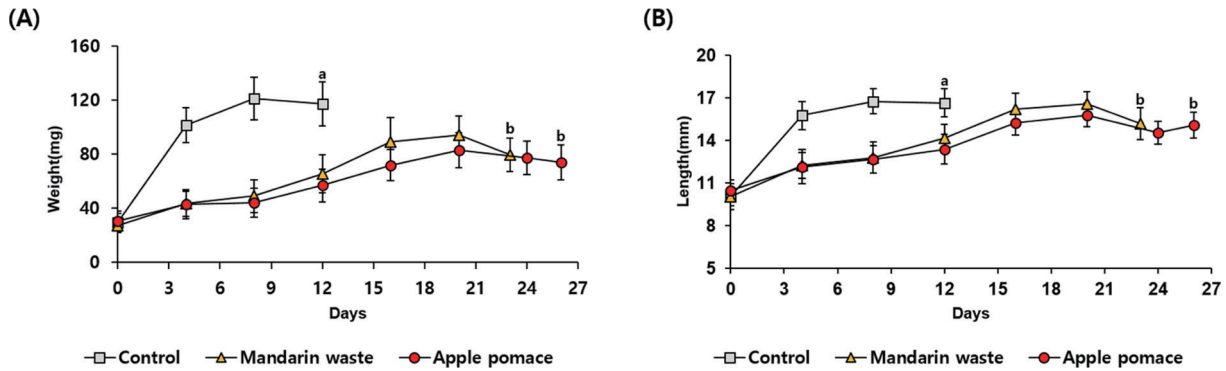


Fig. 1. The growth performance of BSF larvae fed with different substrates. BSF larvae (7 d old from eggs) were fed on cattle feed as a control, test larvae were fed either apple pomace or mandarin waste. (A) The change in weight of BSF larvae fed with different substrates. (B) The change in length of BSF larvae fed with different substrates. Data are shown as the mean values \pm SE (n = 30). Different lowercase letters indicate a significant difference in the weight or the length of control and test animals ($p < 0.05$, *t*-test)

affected by vegetable and fruit substrates. In particular, low levels of carbohydrates in the substrate resulted in lower survival rate of BSFs. Although the carbohydrate content in apple pomace and mandarin waste are more than 3 times lower than the carbohydrate content of the control calf feed, no significant differences between the survival rates of test and control larvae were observed in this study. This result showed that apple pomace and mandarin waste can be used as a food source for BSF larvae without affecting their survival rate. The fecundity rate of adults fed with the three different substrates was at least 99.0% with no significant differences observed.

The growth performance of BSF larvae fed with different substrates

After 40% of BSF larvae developed to the pre-pupal stage, the average weights of BSF larvae fed on mandarin waste and apple pomace were measured at 79.49 mg \pm 12.61 mg and 73.99 mg \pm 12.79 mg, respectively (Fig. 1A). There were no significant weight differences between BSF larvae fed with mandarin waste and apple pomace. The average weight of the control BSF larvae was significantly greater at 117.17 mg \pm 16.34 mg. The average lengths of BSF larvae fed on mandarin waste and apple pomace were 15.20 mm \pm 1.12 mm and 15.06 mm \pm 0.91 mm, respectively. The test larvae were significantly shorter than the control larvae (16.66 mm \pm 1.01 mm). These results suggest that the nutrient profiles of the test substrates were poor compared to the control. The fruit by-products mainly comprise moisture, and levels of nutrients such as proteins, fats, and carbohydrates are relatively insufficient for growth of BSF larvae compared to

the calf feed. Some studies reported that the quantity and quality of protein in substrate was a significant factor for the growth performance of BSF larvae (Gobbi *et al.*, 2013; Nguyen *et al.*, 2013; Oonincx *et al.*, 2015; Tinder *et al.*, 2017). In addition to protein content, the balance of nutrients present in the substrate is also critical to growth performance of BSF larvae (Jucker *et al.*, 2017; Nguyen *et al.*, 2013).

Analysis of feeding performance of BSF larvae fed with different substrates

The rate of waste reduction was significantly different in the different substrates. The rates of waste reduction for BSF larvae fed with apple pomace, mandarin waste, and control were 48.0 \pm 1.32%, 61.5 \pm 1.45%, and 50.4 \pm 0.41% respectively. Several studies reported that BSF larvae can reduce various organic waste including human feces, vegetable, fruit, food waste, and sludge by 25–72% (Banks *et al.*, 2014; Beskin *et al.*, 2018; Cai *et al.*, 2018; Diener *et al.*, 2011; Lalander *et al.*, 2013; Lalander *et al.*, 2015; Mazza *et al.*, 2020; Nguyen *et al.*, 2015; Nyakeri *et al.*, 2017; Sarpong *et al.*, 2019; Surendra *et al.*, 2016). Gold *et al.* (2020) reported that the rate of waste reduction for BSF larvae fed with fruit and vegetable waste was 46.7–60.0%. This result is similar to the waste reduction rate of BSF larvae observed in this study. The BSF larvae decomposed mandarin waste at a rate at least 1.2 times greater than the rate of decomposition of the cattle feed or apple pomace. This result could be correlated to the physical properties of the substrates. The mandarin waste was semi-liquid compared to the other substrates. The apple pomace substrate was physically more solid than the mandarin

waste because it mostly contained peel. According to Bruno *et al.* (2020), BSF larvae can process a wide range of organic substrates because of the structure of its digestive system. The mouthparts resemble a “tunnel boring machine” which seems suitable for processing semi-liquid substrates. Chemical composition of the substrate is another reported factor that explains the ratio of reduced waste of BSF larvae. Fibers such as acid detergent fiber, neutral detergent fiber, cellulose, and lignin were negatively correlated with the waste reduction ratio (Liu *et al.*, 2018). The fiber content of the fruit by-products could have affected the waste reduction rate for BSF in our study.

The bioconversion efficiency of BSF depended on different substrates. The bioconversion efficiencies of BSF larvae fed with apple pomace and mandarin waste were $9.14 \pm 0.43\%$ and $12.05 \pm 0.53\%$, respectively. These bioconversion efficiencies were significantly lower than those of the control ($16.54 \pm 0.51\%$). Moreover, the BSF larvae had significantly better bioconversion efficiency for mandarin waste than apple pomace. According to Surendra *et al.* (2020), BSF fed with different fruit and vegetable waste exhibited bioconversion efficiencies ranging from 4.1–10.8%. The range observed in these results could be due to the different compositions of the fruit and vegetable substrates. In the current study, the mandarin waste was richer in crude proteins and fats than the apple pomace. These results indicate that the nutrient content of the test substrates affected the bioconversion efficiency of the BSF larvae. The 4.1% bioconversion efficiency of BSF larvae was measured on a substrate comprising 50% lettuce waste (Lalander *et al.*, 2019), whereas, the 10.8% bioconversion efficiency of BSF larvae was measured on a substrate including only 10% leafy vegetable waste (Giannetto *et al.*, 2020). The results of this study are similar in showing that the nutrient composition of the substrate is important for the biomass conversion efficiency of BSF larvae.

Conclusion

The growth and feeding performances of black soldier fly larvae were analyzed to validate their use in the sustainable management and recycling of fruit waste such as apple pomace and mandarin waste. There were no significant differences in larval survival rate or BSF fecundity rate between the BSF larvae fed with the two different test substrates. The BSF larvae fed with mandarin waste had shorter larval developmental time

and more efficient bioconversion rates than the BSF fed with apple pomace. This may be due to the greater crude protein and fat contents of the mandarin waste than the apple pomace. The BSF fed with mandarin waste had the highest waste reduction ratio compared to the two other substrates. Because the mandarin waste is semi-liquid compared to the more solid apple pomace, it indicates that the higher ratio of waste reduction of BSF larvae on the mandarin waste is related to the larvae being better equipped to process a semi-liquid substrate. BSF larvae could decompose at least 48.0% of apple pomace and mandarin waste. Moreover, the bioconversion efficiency of BSF larvae for the fruit by-products tested was at least 9.14%. According to these results, it is possible that BSF larvae can be used to sustainably decompose and recycle fruit by-products. However, to optimize the waste reduction and bioconversion potential of BSF larvae, further studies of the growth and feeding performance depending on culture conditions such as feeding rate, larval density, and moisture content will be needed.

Acknowledgments

This research was supported by the ‘Research Program for Agriculture Science and Technology Development’ (Grant No. PJ0159602023), National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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